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October 30, 2002

Food and Drug Administration
Center for Devices and Radiological Health
Document Mail Center (HFZ-401)
9200 Corporate Blvd.
Rockville, MD 20850

RE: PMA M020018 Mentor Low-Bleed Gel-Filled Mammary Prosthesis:
Module #1 Toxicology

Mentor Corporation is submitting Module #1 of the above-referenced PMA application for Mentor's low-bleed gel-filled mammary prosthesis. This module contains a draft Summary of Safety and Effectiveness and the Toxicology module as outlined in the PMA Shell that was approved by FDA on August 15, 2002.

The existence of the PMA and the data and other information it contains are confidential, and the protection afforded to such confidential information by 18 USC 1905, 21 USC 331(I), 5 USC 552, and other applicable laws are hereby claimed.

If there are questions regarding this submission, I can be reached at (805) 879-6304.

Sincerely,

A handwritten signature in cursive script that reads "Donna A. Crawford".

Donna A. Crawford
Director, Corporate Regulatory Affairs

2005-4101B1-D1-D2-MODULE1-MENTOR

PREMARKET APPROVAL APPLICATION

MENTOR LOW-BLEED
GEL-FILLED MAMMARY PROSTHESIS

M020018

OCTOBER 30, 2002

VOLUME 1 OF 8

Mentor Corporation
201 Mentor Drive
Santa Barbara, CA 93111

LOW BLEED GEL-FILLED MAMMARY PROSTHESES BIOLOGICAL TESTING SECTION

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SUMMARY OF SAFETY AND EFFECTIVENESS DATA

I. GENERAL INFORMATION

Device Generic Name: Silicone Gel-Filled Mammary Prosthesis

Device Trade Name: Mentor Low-Bleed Gel-Filled Mammary Prosthesis

Applicant: Mentor Corporation
201 Mentor Drive
Santa Barbara, California 93111

Premarket Approval (PMA) Application Number: To be determined

Date of Panel Recommendation: To be determined

Date of Good Manufacturing Practice Inspection: To be determined

Date of Notice of Approval to Applicant: To be determined

■ [REDACTED]

[REDACTED]

- [REDACTED]
- [REDACTED]

■ [REDACTED]

[REDACTED]

■ [REDACTED]

[REDACTED]

■ [REDACTED]

[REDACTED]

■ [REDACTED]

[REDACTED]

[REDACTED]

Catalog #	Surface	Shape	Profile	Size
SALINE FILLED MAMMARY PROSTHESIS				
350-7XXXBC	Smooth	Round	Moderate	100-800cc
354-XXX7	Siltex® textured	Round	Moderate	100-800cc
350-XXX1BC	Smooth	Round	Moderate Plus	100-800cc

[REDACTED]

■ [REDACTED]

[REDACTED]

■ [REDACTED]

EXECUTIVE SUMMARY

This Mentor PMA submission for Gel-filled Mammary Prostheses covers three product lines - Moderate Profile Gel (smooth and Siltex styles), Moderate **Plus** Gel (smooth and Siltex styles), and High Profile Gel (smooth and Siltex styles). The product lines differ in their amount of projection and width but are all fabricated using the same materials, basic components, and processes. A generic gel-filled mammary sketch with all components shown has been provided, as well as a list of the raw materials used in each device component, the packaging, and major processing materials.

A review of published pre-clinical testing on the toxicity of silicone is provided. Much of this information was derived from the Institute of Medicine's Committee on the Safety of Silicone Breast Implants report published in 2000. Additional information published since that 2000 report are also provided, as well as information on known extractable materials not mentioned in the report but determined to be present through Mentor's chemical extractables testing on finished devices. The literature not only provided information on the toxicity levels for individual extractable compounds, but also provided an overall conclusion about the toxicological safety of gel-filled mammary implants. The Institute of Medicine's report¹ concluded the following:

"Studies using whole fluids, gels, elastomers, or experimental implant models injected or implanted in ways that are directly relevant to the human experience with implants are also reassuring. These studies show that depots of gel, whether free or in implants, remain almost entirely where injected or implanted. Even low molecular weight cyclic and linear silicone fluids appear to have low mobility. Half-lives of low molecular weight silicones in body fluids and tissues have been measured infrequently, but known values appear to be on the order of 1 to 10 days. In general, there do not appear to be long-term systemic toxic effects from silicone gel implants or from unsuspected compounds in these gels or elastomers detected by these animal experiments."

Mentor uses many standardized acute and longer-term toxicity tests on its raw materials, components, and finished products. Because these tests are used so often, a section of this PMA describes in detail how these individual tests are performed by Mentor's testing vendors. The types of test procedures summarized include cytotoxicity tests, mouse systemic toxicity, rabbit intracutaneous toxicity, hemolysis, material mediated pyrogenicity, guinea pig sensitization (maximization method), bacterial mutagenicity (Ames test), and implantation tests of varying time lengths.

In order to demonstrate that the raw materials used to fabricate Mentor's Gel-filled Mammary Prostheses have no obvious biological incompatibilities, a detailed summary of biological

¹ Bondurant, S., Ernster, V., Herdman, R. ©2000. *Safety of Silicone Breast Implants*. Committee on the Safety of Silicone Breast Implants, Division of Health Promotion and Disease Prevention, Institute of Medicine. (Washington, D.C., National Academy Press) pp 180 - 181.

tests performed on the raw materials which comprise the device, its packaging, and major processing materials has been provided. The results of the testing showed that those materials passed the biological screening tests and were therefore deemed acceptable for use in these devices. The screening tests were performed by either Mentor or the vendors of those materials who then usually included those test results in a FDA Master Access File.

The final part of this PMA submission contains the results of biological tests performed on finished device gel-filled mammary prostheses or components from finished devices when full device testing was not possible. 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. Also, much of Mentor's more sophisticated and longer term biological testing was performed on devices in the early to mid-1990's with silicone materials from different vendors than the current ones. Based upon FDA's Guidance for Manufacturers of Silicone Devices Affected by Withdrawal of Dow Corning Silastic Materials, the newer silicone vendors demonstrated that their replacement materials were not substantially different from the Dow Corning materials and Mentor demonstrated the equivalence of the finished devices made with both sets of materials. For that reason, the finished device biological testing using older silicone materials is still directly applicable to the current Mentor Gel-filled Mammary Prostheses.

Mentor has performed sterile product acute toxicity testing on devices made with current vendor silicone gel material as well as Low Bleed shell material. Extract(s) from the device passed the following tests: ISO elution, ISO agarose overlay, ISO acute systemic toxicity, ISO acute intracutaneous reactivity, hemolysis, material mediated pyrogenicity, bacterial reverse mutation assay, unscheduled DNA synthesis in mammalian cells, and chromosome aberration assay in Chinese hamster ovary cells.

Patch components with [REDACTED] markings for identification purposes have been tested by ISO elution, ISO acute intracutaneous reactivity, ISO acute systemic toxicity, and ISO sensitization (maximization method). Only device patches were tested because only they are marked by [REDACTED]. There were no material toxicity issues.

Device Low Bleed shells made from previous silicone vendor materials have been tested by immunological evaluation (including effects on the mouse immunological system, production of tissue antibodies in mice implanted with the material, and serum cytokine levels in mice implanted with the material), adjuvancy assay using particles partly made from the textured surface material, two generation reproduction/teratogenicity assay, and chronic toxicity/carcinogenicity assay. The results of these tests showed no immunological issues or changes in the mouse immunological system caused by the shell material, no significant adjuvancy potential of the particles, and no reproductive, teratogenic, or chronic toxicity issues due to the shell material.

Silicone gel from devices has been tested by Mentor, the gel vendors and others for acute toxicity, mutagenicity, immunotoxicity, adjuvancy, reproduction/teratogenicity, and chronic toxicity/carcinogenicity. The testing results showed no toxicity issues due to the gel except for an adjuvancy potential when gel is mixed with an antigen. Additional testing showed that gel has no adjuvancy potential when it is not mixed together with the antigen in an emulsion-like state prior to being administered to the animal. This latter state is much more similar to the clinical setting than forming an emulsion with gel and an antigen. For that reason, gel in mammary prosthesis is not believed to be an adjuvant.

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.

INTRODUCTION AND TESTING RATIONALE

This section of the Mentor's Gel-Filled Mammary Prosthesis PMA describes the biological/toxicological testing performed on the sterile finished devices, raw materials, and components used to make these devices, in order to verify their biological safety. The raw materials testing is meant to initially verify that a chosen material has no obvious biological incompatibilities. The finished device testing is meant to verify that the materials in the configuration of the device (after its full manufacturing and sterilization processing) have no biological incompatibilities. Component testing is used in place of full product testing for those biological tests where it is not practical or possible to test the whole device.

Mentor's battery of biological tests is listed in the following table:

BIOLOGICAL TESTING BATTERY

Cytotoxicity
Blood compatibility
Pyrogenicity
Irritation
Implantation reaction
Sensitization
Acute through chronic toxicity
Mutagenicity
Carcinogenicity
Reproduction and teratology
Immunotoxicity
Adjuvancy

The above list of biological testing areas encompasses classical methods of analysis for general materials biocompatibility and more recently developed methods for assessing silicone biocompatibility. The latter state-of-the-art testing methods were developed and employed as a result of the recent silicone materials biocompatibility controversy.

This biological section of the PMA is organized in the following manner. Following a short executive summary, this introductory section details Mentor's testing rationale. This section is followed by a device description section including schematics of the devices along with a list of the raw materials used in each device component (Section III). Section IV reviews the published literature on the toxicity of silicones and gel-filled breast implants as well as those raw materials used to make or extractable materials which come from gel-filled mammary prostheses. Section V contains a description of each standard biocompatibility test method Mentor has historically used. Section VI describes the actual testing and results of each test performed on every major raw material used in the manufacturing of gel-filled devices (including processing aids). Section VII details Mentor's biological testing on sterile finished devices or components taken from sterile finished products. And finally, Section VIII summarizes the toxicology/biocompatibility data, indicates how it addresses the safety issues that have been raised for silicone elastomer materials, and confirms that the gel-filled

mammary prostheses are biologically safe for their intended use. Section IX is the Appendices and contains copies of all reports and documents mentioned in this Biological Section of the PMA

It should be noted that because Dow Corning will no longer allow many long-term implant device manufacturers to access their material master access files, some testing data previously available to the FDA in these files may no longer be referenced. Nevertheless, the extensive biological testing provided in this PMA gives sufficient information to confirm the pre-clinical biological safety of Mentor's Smooth and Siltex Gel-Filled Mammary Prostheses.

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 a number of device styles, and 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 this testing on a selected set of finished devices chosen to include the full range of components and materials. As a result, the testing data on Smooth Moderate Profile Gel-filled devices, for example, are also applicable to High Profile and Moderate Plus Smooth Gel-filled devices because the only major difference in their manufacturing procedures is the shape of the dipping mandrel used to form the smooth shell.

Finally, this biological section of the PMA also addresses the issue of Mentor's replacement of certain materials as a result of Dow Corning and other manufacturers' withdrawal as vendors of long-term implantable materials for use in medical devices. It is important to note that due to this unforeseen emergency need for wholesale replacement of several key materials, the testing results presented in this PMA section often involve devices constructed from Dow Corning and other no longer available materials. In accordance with FDA's "Guidance for Manufacturers of Silicone Devices Affected by Withdrawal of Dow Corning Silastic Materials," the new material suppliers and Mentor have conducted testing of the replacement raw materials to confirm that they are not substantially different from the previously approved material. The biological testing results along with finished device chemical extractables data confirm that the materials are "not substantially different," therefore, the previously conducted testing is directly relevant to the current products with the replacement silicone materials.

DEVICE DESCRIPTION

Mentor's silicone gel-filled mammary prostheses come in three different variations in order to fit individual patient needs. The Moderate Profile Gel product line is available in both smooth and textured round styles and offers a moderate amount of projection. This product works well for patients who have a wider chest wall and is a good choice for most body types. It is currently the most commonly used of these products.

The Moderate **Plus** Gel (available in both smooth and textured round styles) is intended for patients who require more projection and a slightly more narrow base width. This product is an in-between choice if the Moderate Profile Gel is too wide for a given patient and the High Profile Gel is too narrow. The degree of projection is in-between the two other products as well.

The High Profile Gel (available in both smooth and textured round styles) is intended for patients with a narrow chest wall who are seeking more projection. This is especially relevant in reconstruction when the doctor is trying to match the opposite (non-reconstructed breast) and needs a certain volume implant to achieve symmetry.

All three Siltex gel-filled product lines utilize the same materials and processes for the shell, textured surface, gel, patch, and other minor components. All three smooth gel-filled product lines utilize the same materials and processes for the shell, gel, patch, and other minor components. The different device shapes are achieved by using differently shaped mandrels to dip the shells. All styles (both smooth and textured) of all product lines in this PMA contain a basic smooth shell as one of its components (see Figures 1 and 2).

All gel-filled mammary prostheses are sold packaged in double sealed thermoforms each with a Tyvek lid and [REDACTED] sterilized. Devices are shipped to customers in individual boxes.

The following table details the catalog numbers for each style of each product line and provides their range of sizes available:

Catalog Number	Gel-filled Device Style	Size Range
350-7XXXBC	Smooth Moderate Profile	100 - 800cc
354-XXX7	Siltex Moderate Profile	100 - 800cc
350-XXX1BC	Smooth Moderate Plus	100 - 800cc
354-XXX1	Siltex Moderate Plus	100 - 800cc
350-XXX4BC	Smooth High Profile	125 - 800cc
354-4XXX	Siltex High Profile	125 - 800cc

Figure 1 Smooth Round Gel-Filled Mammary Implant

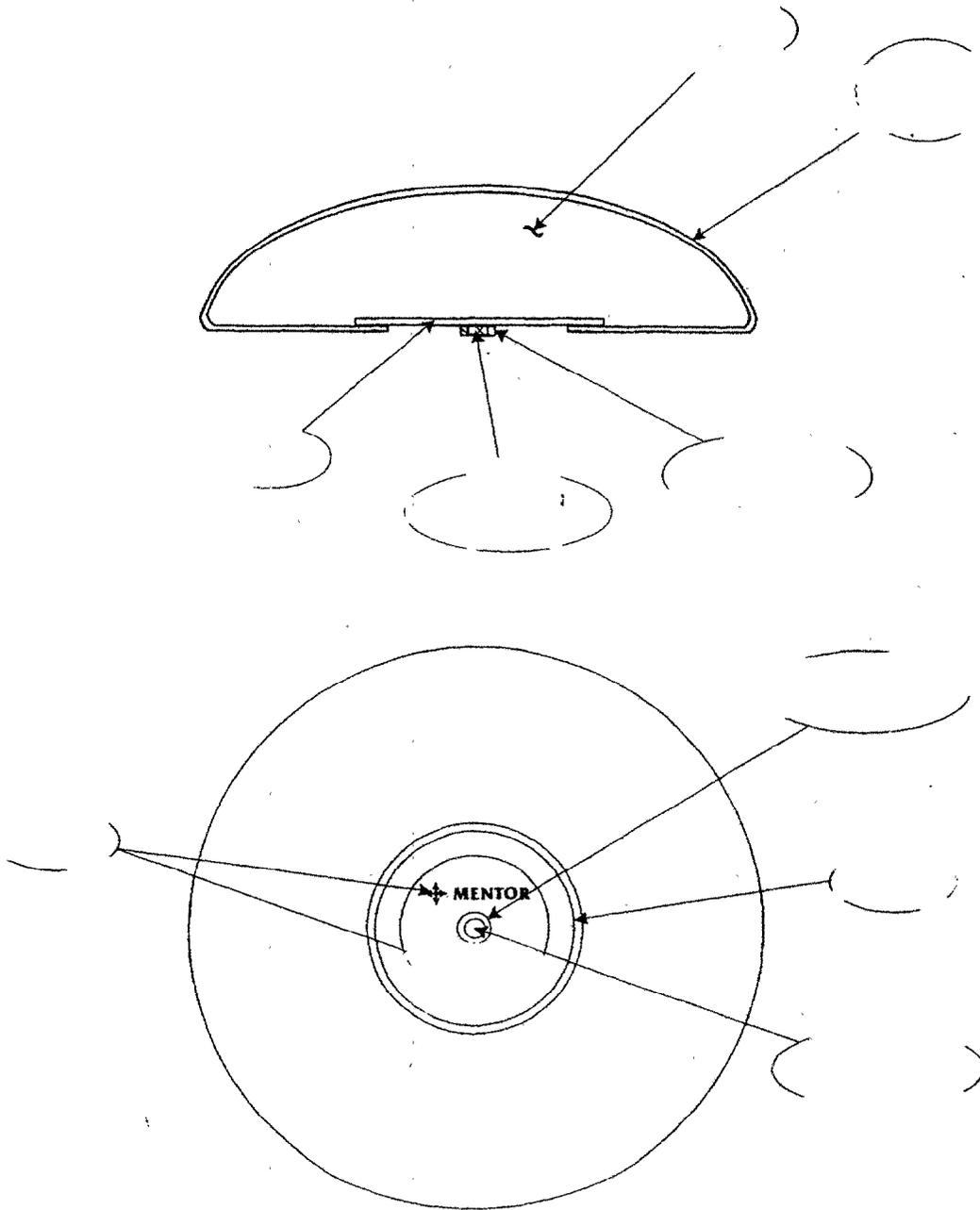
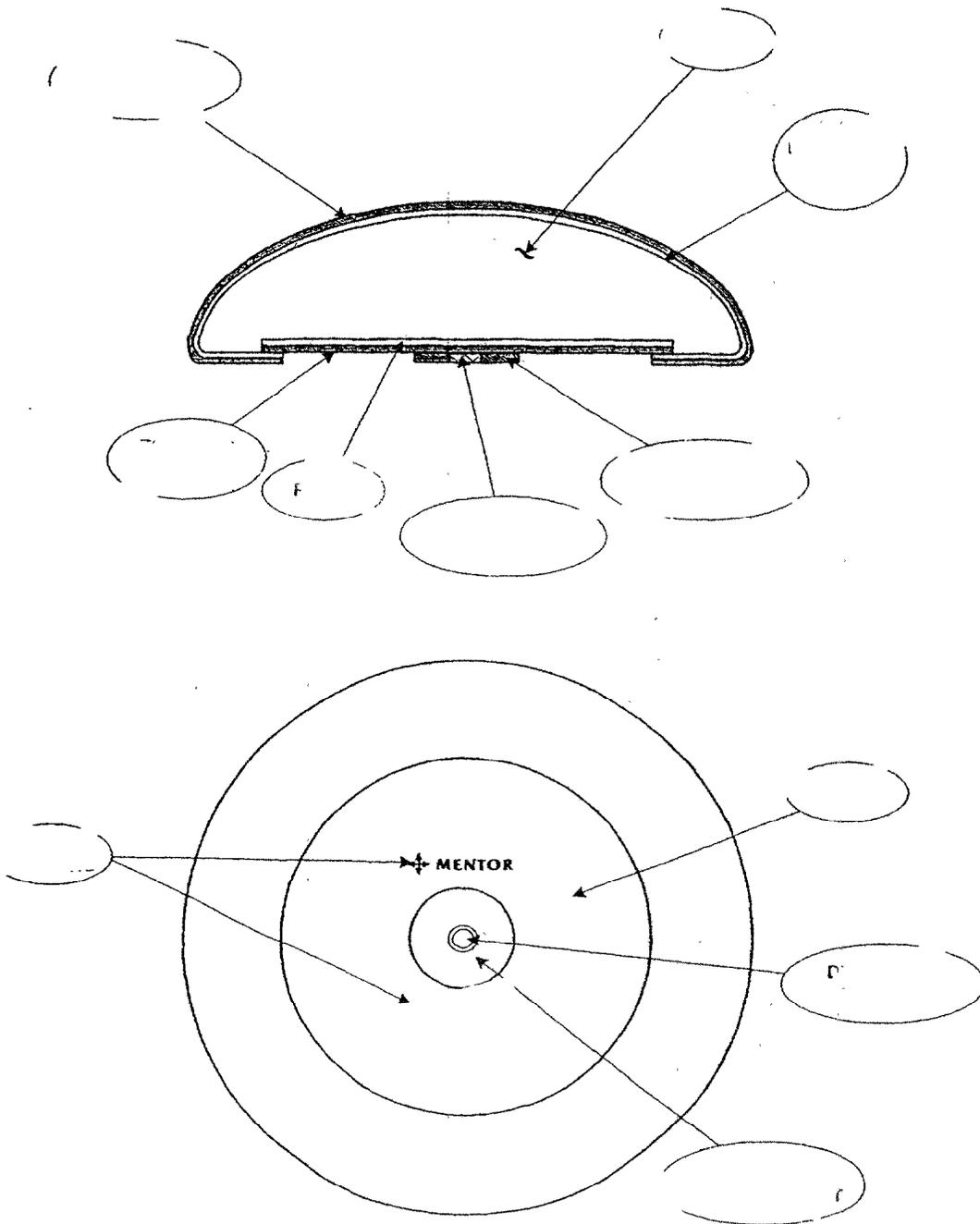


Figure 2 Siltex Round Gel-Filled Mammary Implant



GEL-FILLED MAMMARY PROSTHESES RAW MATERIALS

Component	Material Name(s)	Part Number(s)	Manufacturer/ Supplier	Master Access File
Smooth Shell	<div style="background-color: black; width: 100%; height: 15px; margin-bottom: 5px;"></div> <div style="background-color: black; width: 100%; height: 15px; margin-bottom: 5px;"></div> <div style="background-color: black; width: 100%; height: 15px; margin-bottom: 5px;"></div> <div style="background-color: black; width: 100%; height: 15px; margin-bottom: 5px;"></div> <div style="background-color: black; width: 100%; height: 15px; margin-bottom: 5px;"></div> <div style="background-color: black; width: 100%; height: 15px; margin-bottom: 5px;"></div>	400009-001 400598-001 400601-001 400596-001 400612-001	<div style="background-color: black; width: 100%; height: 15px; margin-bottom: 5px;"></div> <div style="background-color: black; width: 100%; height: 15px; margin-bottom: 5px;"></div> <div style="background-color: black; width: 100%; height: 15px; margin-bottom: 5px;"></div> <div style="background-color: black; width: 100%; height: 15px; margin-bottom: 5px;"></div>	N/A <div style="background-color: black; width: 100%; height: 15px; margin-bottom: 5px;"></div> <div style="background-color: black; width: 100%; height: 15px; margin-bottom: 5px;"></div>
Shell Textured Layer*, Patch, Patch Fill Reinforcement	<div style="background-color: black; width: 100%; height: 15px; margin-bottom: 5px;"></div> <div style="background-color: black; width: 100%; height: 15px; margin-bottom: 5px;"></div> <p style="text-align: center;">(OR ALTERNATIVELY)</p> <div style="background-color: black; width: 100%; height: 15px; margin-bottom: 5px;"></div> <div style="background-color: black; width: 100%; height: 15px; margin-bottom: 5px;"></div>	400519-001 400520-001 400624-001 400625-001	<div style="background-color: black; width: 100%; height: 15px; margin-bottom: 5px;"></div> <div style="background-color: black; width: 100%; height: 15px; margin-bottom: 5px;"></div>	<div style="background-color: black; width: 100%; height: 15px; margin-bottom: 5px;"></div> <div style="background-color: black; width: 100%; height: 15px; margin-bottom: 5px;"></div>
Gel	Silicone Gel: <div style="background-color: black; width: 100%; height: 15px; margin-bottom: 5px;"></div>	400595-001 400594-001	<div style="background-color: black; width: 100%; height: 15px; margin-bottom: 5px;"></div>	<div style="background-color: black; width: 100%; height: 15px; margin-bottom: 5px;"></div>
Dip Coat Fill	<div style="background-color: black; width: 100%; height: 15px; margin-bottom: 5px;"></div> <p>(Silicone)</p>	400001-001	<div style="background-color: black; width: 100%; height: 15px; margin-bottom: 5px;"></div>	N/A

* - Component only present on Siltex Gel-filled Mammary Prostheses

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GEL-FILLED MAMMARY PROSTHESES RAW MATERIALS (cont.)

Component	Material Name(s)	Part Number(s)	Manufacturer/ Supplier	Master Access File
Packaging	Inner Thermoform [REDACTED]	104226-002 104226-004 104226-005 104288-001	[REDACTED]	N/A
	Outer Thermoform [REDACTED]	104227-001 104289-001	[REDACTED]	N/A
	Small Inner Lid ([REDACTED])	102739-001	[REDACTED]	[REDACTED]
	Large Inner Lid ([REDACTED])	102738-001	[REDACTED]	[REDACTED]
	Small Outer Lid ([REDACTED])	102746-001	[REDACTED]	[REDACTED]
	Large Outer Lid ([REDACTED])	102746-002	[REDACTED]	[REDACTED]
	Box ([REDACTED])	102800-001 102801-001 102587-001 102587-002	[REDACTED]	N/A

GEL-FILLED MAMMARY PROSTHESES RAW MATERIALS (cont.)

Component	Material Name(s)	Part Number(s)	Manufacturer/ Supplier	Master Access File
Indirect Manufacturing Materials**		400018-001		N/A
		400044-001		N/A
		400034-001		N/A
		400516-001		N/A
		[REDACTED] 400065-001		N/A
		0) 400006-001		N/A
		400447-001 400447-002		N/A
		[REDACTED] 103028-017 through 103028-048 & 103066-001 through 103066-020		N/A
		[REDACTED] 313957-001 to 313957-018 & 313982-001 to 313982-020	[REDACTED]	N/A
		213029-008	Pollock Paper Co.	N/A

** - not present in the finished device, contacts components during manufacturing only

PUBLISHED PRECLINICAL INFORMATION ON MATERIALS TOXICITY

The safety information provided by the preclinical data for Mentor Silicone Gel-Filled Mammary Prostheses presented in this report is further supplemented by extensive information available in the published scientific and medical literature. A full literature review, incorporating human clinical data, epidemiological findings, as well as pertinent animal data – and covering the full range of potential safety issues – will be included in Mentor's upcoming clinical module of this PMA submission. The focus of the literature information presented in this module, however, is specifically on providing a background of current knowledge in preclinical toxicology of silicone materials. The findings of Mentor's own preclinical studies presented in this module are supplemented by this preexisting body of information. Taken together, these preclinical data on silicone gel-filled breast implants and their constituents provide confirmation of the safety of these devices. Based on their review of the preexisting body of scientific and medical literature, a multidisciplinary panel of scientific and medical experts commissioned by the Institute of Medicine concluded that, "In general, there do not appear to be long-term systemic toxic effects from silicone gel implants or from unsuspected compounds in these gels or elastomers detected by these animal experiments."

The Institute of Medicine Committee on the Safety of Silicone Breast Implants is but one of three highly respected panels of scientific and medical experts that have come to essentially the same conclusions regarding the safety of these devices. (The other two panels were the Independent Review Group of the Medical Devices Agency of the U.K. and the National Science Panel commissioned by Judge Pointer in the MDL-926 litigation)

In 1997, the Department of Health and Human Services contracted with the Institute of Medicine (IOM) of the National Academy of Sciences to conduct an independent review of past and ongoing research on silicone breast implants. Sources of funding for this detailed investigation included the Office of Women's Health of the Food and Drug Administration. The 13-member committee included experts in the fields of preventive and internal medicine, nursing, family and women's health, rheumatology, clinical and basic research, epidemiology, immunology, neurology, silicone chemistry, toxicology, breast and other cancer, plastic surgery (the expert's practice was self limited to pediatric surgery), and radiology or mammography. Active steps were taken by IOM to avoid conflicts of interest in constituting the committee. The scope of the resulting IOM report, *Safety of Silicone Breast Implants*, published in 2000 is broad, and includes detailed evaluation of preclinical toxicology studies on silicone fluids, gels and elastomers, as well as other constituents of silicone gel-filled breast implants, which are in most cases identical to or nearly identical to those materials present in Mentor Silicone Gel-Filled Mammary Prostheses. Chapter 4 of the IOM report, entitled *Silicone Toxicology*, provides a detailed peer-reviewed¹ expert evaluation of the available information (primarily preclinical) pertinent to the toxicology of silicone materials. The full content of Chapter 4 is

¹ Among the external reviewers of the report was John Doull, M.D., editor emeritus of the primary textbook in the field of toxicology, *Casarett and Doull's Toxicology: The Basic Science of Poisons*.

presented below, along with Mentor's own annotations (clearly identified) which provide supplemental information, some of which was published very recently:

Silicone Toxicology²

SCOPE AND CRITERIA FOR THE TOXICOLOGY REVIEW

This chapter reviews studies of the toxicology of silicone compounds carried out over the past 50 years. It does not review immunological studies, except occasionally when immune system toxicology is part of a report covering other toxicology. Otherwise, immunological studies are discussed in Chapter 6.³ Silicone compounds include a great many chemical entities; a recent compilation lists toxicological data on 56 different siloxanes (Silicones Environmental Health and Safety Council, 1995). This chapter identifies silicone compounds as they are listed in individual reports, but it is organized by route of exposure not by type of compound. Silicone fluids, gels, and elastomers are covered since they are components of silicone breast implants.

Although the most relevant exposures are reviewed, that is, tissue injections and subcutaneous implants, the committee, unlike other recent reviews (Kerkvliet, 1998) also decided to include other (nonimplantation) exposure routes, such as dermal, oral, and inhalation, since data from such studies may provide some insights into systemic silicone toxicology. The committee included citations on the toxicology of silica in the reference list of this report, because there has been considerable mention of silica as a component of breast implant elastomers. However, the toxicology of silica is not reviewed here because the committee found no valid scientific evidence for the presence of or exposure to silica in tissues of women with breast implants.⁴ Some compounds not found in breast implants (and identified as such) are included briefly, sometimes to complete a survey of silicone species and other times because they have been mentioned in the current debate on the toxic effects of implants. It is important to note that toxicology studies often report silicone dose levels substantially in excess of any doses that could be achieved on a relative weight basis in women with silicone breast implants.

² Chapter 4 from: Bondurant, S., V. Ernster and R. Herdman, Eds. ©2000. *Safety of Silicone Breast Implants*. Committee on the Safety of Silicone Breast Implants, Division of Health Promotion and Disease Prevention, Institute of Medicine. (Washington, D.C., National Academy Press). *The full text of this chapter and the entire IOM report are available online at www.nap.edu.*

³ Mentor Annotation: A detailed evaluation of studies involving potential immunological and neurological effects of silicone, most of which include human data, will be included in the upcoming clinical data module of Mentor's PMA submission.

⁴ Mentor Annotation: Mentor concurs with the view of IOM that there is no evidence of exposure or release of silica from the elastomer shells of silicone mammary prostheses. Nevertheless, the issue of potential biological effects of silica, which involves human data as well as animal data, will be addressed in the full literature review accompanying the upcoming Clinical module of this PMA submission.

Earlier in this report, the committee emphasizes the relevance of published, peer-reviewed scientific reports and assigns secondary importance to technical reports from industry. In this chapter, however, studies done in-house by industry or by commercial testing laboratories have been analyzed. Such reports are often reviewed first in-house, then by the sponsor and panels of outside experts, and eventually by a regulatory agency, which also looks at original data. The conflict of interest inherent in experimentation by an organization with an economic interest in the outcome is recognized. Nevertheless, the committee found many of industry's technical studies informative, useful, and consistent with sound science. The studies cited here consisted of about 50 individual articles from the open scientific literature between 1948 and 1999 and about the same number of industry technical reports. Reviews available to the committee summarized data from some reports not reviewed by or not available to the committee. For example, the Silicones Environmental Health and Safety Council (1995) examined many reports on various organic silicon compounds that are not found in breast implants and reviewed some reports not accessible to the committee. This review was useful in presenting an overall picture of the generally low toxicity of silicones and identifying particular compounds that had toxicity. The report of the Independent Review Group (IRG, 1998) (and earlier versions of the Medical Devices Agency's work), and the report of the National Science Panel (Kerkvliet, 1998) which are described in Appendix C looked at essentially the same body of toxicology information as the committee. The IRG report included proprietary data not available to the committee, and as noted, the committee examined routes of exposure and listed silica references neither of which are included in the IRG or National Science Panel reports. Since the IRG, which had some proprietary data, concluded that silicones were bland substances with little toxicity, such data seem unlikely to have changed the committee's findings in any substantial way. Also, the committee believes that the inclusion of dermal, oral and inhalation toxicology studies in this report provided additional security in conclusions about the biological and toxicological behavior of relevant silicones.

Kerkvliet lists three major reasons why toxicology studies are helpful in assessing the safety of a drug or consumer product such as silicone breast implants. (1) Toxicology studies in animals may identify a hazard—that is, whether a given product can cause adverse health effects. (2) Studies may also clarify dose responses—that is, how much of an entity is necessary to produce effects. (3) Studies may provide mechanistic information—that is, how and under what circumstances an agent produces effects (Kerkvliet, 1998). Such studies, reviewed here, will not "fulfill the manufacturers' responsibility to demonstrate the safety of ... implants"⁵ as Kessler urged in 1992 (Angell, 1995), since unanticipated events cannot be predicted or complete safety proven. Accumulating qualitative and quantitative data on the general toxicity of

⁵ Mentor Annotation: The demonstration of the safety of Mentor's silicone gel-filled mammary prostheses is provided by the full content of the PMA submission of which this module is a part.

silicones, however, allow a reasonable degree of confidence that silicone compounds in breast implants are not hazardous.

BRIEF HISTORY OF SILICONE TOXICOLOGY

The principles of safety evaluation have not changed much over the past 50 years. However, analytical tools, the ability to measure chemicals in the body, and the science of molecular biology, which allows association of complex changes in a few cells or molecules with various disease states, have advanced considerably. These advances affect evaluations of the toxicology of silicones over time and are reflected in more recent studies.

One of the first (if not the first) systematic evaluations of the toxicology of commercial silicones was conducted during World War II at the Dow Chemical Company. Silicone intermediates (chlorosilanes and ethoxysilanes) and selected commercial silicones were tested in rats, rabbits, and guinea pigs. The chlorosilanes and some ethoxysilanes were found to be highly corrosive; they represented significant industrial handling hazards. Methyl- and mixed methyl- and phenylpolysiloxanes, on the other hand, had very low toxicity. For practical purposes, they were divided into three groups: fluids, compounds, and resins. Five methylpolysiloxane and two methylphenylpolysiloxane fluids were tested (hexamethyldisiloxane, 0.35 centistoke [cS]; dodecamethylpentasiloxane, 2 cS; DC 200 fluid, 50 cS; DC 550 fluid, 550 cS; DC 702 fluid, 35 cS; DC 200 fluid, 350 cS; and DC 200 fluid, 12,500 cS). None of these killed rats or guinea pigs when given orally at doses up to 30 ml/kg. Some of the fluids had laxative effects not unlike mineral oil. DC 200 fluid (50 cS) "seemed literally to flow through the animals." The fluid with the lowest viscosity (hexamethyldisiloxane, 0.65 cS) did not have a laxative effect, but produced some mild inebriation and subsequent central nervous system depression. This suggests that there might be some absorption of this compound from the gastrointestinal tract. Repeated administration of DC 200 oil (350 cS) by stomach tube, up to dose levels of 20 g/kg, did not produce gross signs of toxicity such as reduced weight gain, changes in organ weight, or organ pathology.

Intraperitoneal injection was well tolerated, except for hexamethyldisiloxane, which produced extensive adhesions within the peritoneal cavity. This compound also produced inflammation and necrosis at the sites of subcutaneous and intradermal injections and proved lethal on repeated intraperitoneal injections. Other silicone fluids in the peritoneal cavity elicited only reactions "typical ... of an irritating foreign body" with nodules containing the fluid in the omentum and visceral peritoneum. Eye irritation was transitory and no skin irritation was observed with these fluids (Rowe et al., 1948).

Shortly after the report by Rowe et al., Kem et al. (1949) reported their results from feeding rats 0.05%-0.2% silicone-containing diets (a polydimethylsiloxane

[PDMS], G.E. Dri-Film, No. 9977) and injecting silicone suspensions at unknown (but probably low) doses, intraperitoneally and intravenously in mice, and intra- and subcutaneously and in the muscles of rabbits. Hematological and gross and microscopic pathology examinations after 13 weeks were all normal, and the animals had no loss in body weight or other signs of toxicity (Kern et al., 1949).

Two silicone compounds (DC 4 Ignition sealing compound and DC Antifoam A) were examined. Both agents caused transient conjunctival irritation, but no corneal damage when introduced directly into the eyes. No skin irritation was seen. Feeding of Antifoam A at concentrations up to 1% to rats did not produce any untoward effects. In a six-month feeding study in dogs, Antifoam A also exhibited no toxicity (Child et al., 1951). Three types of silicone resins (DC 2102, a methylpolysiloxane, DC 993, a methylphenylpolysiloxane; and DC Pan Glaze, which was similar to DC 993) were evaluated. Acute oral administration of up to 3 g/kg in guinea pigs was not toxic (higher doses could not be administered), and intraperitoneal injection in rats or dermal application in rabbits produced no signs of irritation. Rats fed Pan Glaze at concentrations up to 3% for 50 days gained weight normally, and on microscopic examination, their organs did not show any signs of toxicity (Rowe et al., 1948, 1950).

The studies described by Rowe et al. (1948) reflect state-of-the-art toxicity testing at that time. They were done in a respected laboratory by competent toxicologists. The untoward effects observed with some compounds did not alarm toxicologists. These effects were found only after exposure to high doses of the test agent. According to an old classification, substances with a probable human lethal dose in excess of 15 g/kg were considered practically nontoxic (Casarett, 1975). These investigators commented that "for the past few years, an attempt has been made to keep pace with the rapid development of these products so that toxicological information would be available upon which the health hazards of these materials could be evaluated." Only a few selected samples from each class of compounds were studied, but the experimental toxicology of silicone compounds did not yield data that suggested a need for fundamental, mechanistically oriented experimentation.

When these and some other early studies were reviewed in 1950, silicone fluids with a viscosity of 350 cS were described as having exceedingly low toxicity. Some animal toxicity tests, such as oral and subcutaneous administration and eye irritation, were even performed on one of the authors of this study (Barondes et al., 1950). By then-current standards of toxicology, silicone fluids had to be considered harmless, devoid of any obvious acute toxic potential, and thus presumably safe.

THE CURRENT DATABASE

A recent review of silicone toxicology summarized a substantial database (Silicones Environmental Health and Safety Council, 1995). This document does not list any references which makes it impossible to determine whether the data were published or to discover when the studies were done. It is not possible, therefore, to evaluate adherence to modern good laboratory practice regulations, protocols, and procedural requirements. Carcinogenesis studies done before the mid-1970s had different protocols and procedural requirements than later studies and, by today's standards, must be considered less reliable. This may apply to other test systems as well. The Silicones Council review analyzed a total of 629 studies (see Table 4-1), more than half of them done with PDMS linears (Chemical Abstracts Service [CAS] No. 63148-62-9). Compounds that are of concern because a large number of people are exposed to them and because they are found in breast implants, that is, D₄ and D₅ (where D₄ and D₅ represent cyclic tetramer and pentamer, respectively), comprise 17% of studies. There are few chronic lifetime or carcinogenesis studies (less than 3) and immunological studies (less than 5). Acute and sub-acute toxicity and irritation studies are in the majority (57). Some of the Silicones Council studies summarized briefly in this current database may also be reviewed subsequently in other parts of this chapter. As noted, this material presents an overall picture of silicone toxicity based on a general review of many data sources covering a wide variety of compounds. Specific studies on breast implant compounds are relied on by the committee for conclusions relevant to the safety of silicone breast implants, however.

RESULTS OF STUDIES IN FOUR MAIN GROUPS

Group I

A: Dimethylsiloxanes

A total of 123 reports on cyclic polydimethylsiloxanes (D₃, D₄, D₅, and D₆) were reviewed. These compounds are volatile and potentially of concern in manufacturing; however, they also are used in consumer products, such as hair sprays, and are found in breast implants, although in very low amounts (see Chapter 3). They are practically nontoxic on ingestion, dermal application, or inhalation, although they are mildly irritating when placed directly on the skin or in the eyes. Subacute gavage studies showed that these compounds had no untoward effect other than a reversible increase in liver weight due to increases in both cell number and cell size at doses ranging up to 2,000 mg/kg. Skin application did not cause toxicity; however, some D₅ penetrates the skin. No signs of toxicity were observed in subacute and chronic inhalation studies, except the development of hepatomegaly in some animal species, which was reversible on cessation of exposure. No evidence for carcinogenicity was found. Bacterial and mammalian mutagenicity studies were generally negative.

Developmental and reproductive studies failed to show teratogenic effects or effects on fertility, except when exposure conditions were high enough to cause maternal toxicity in a rabbit study with D₄. Immunotoxicity was studied following intraperitoneal, intramuscular, subcutaneous, and dermal exposure. D₄ had a substantial adjuvant effect for humoral but not cell-mediated immune reactions when injected subcutaneously. Pharmacokinetic studies showed that these compounds are absorbed following oral administration or inhalation, but that skin penetration is very poor. Most of the compounds were excreted in the urine following intravenous administration.

B: Linear Dimethylsiloxanes

Fifty one reports on L₂, L₃ and L₄ (where L = linear polymer) were reviewed. Linear polymers of this size are unlikely to be found in breast implants (Kala et al., 1998; reference not found in the original but added for this report, see Chapter 2). Systemic toxicity after oral, dermal, or inhalation exposure is low. However, linear siloxanes appear to have significant potential for dermal irritation in animals and humans. An in vitro study with human cells suggested that the materials are biocompatible. Evidence for modulation of immune function was obtained in some tests, although the biological significance of these findings was questioned.

Table 4-1 Summary of Toxicity Studies

CAS No.	Group	Acute	Subacute or Subchronic	Chronic	Irritation	Developmental, Reproductive	Pharmaco-Kinetic	Immunological	Cytotoxicity, Mutagenicity	Bio-compatibility
541-05-9 D ₃	IA	3	3	2					3	
556-67-2 D ₄ ^a	IA	8	15		11	5	12	3	9	
541-02-6 D ₅	IA	9	12				2	1	6	1
541-02-6	IA	1			1					
540-97-6 D ₆	IA	2	1				1			
Mixture ^a							1	1	3	
107-46-0	IB	21	6				1		8	3
107-51-7	IB	1						2		1
141-62-8	IB	1	2	2				1		2
Mixture							1			
141-63-9	IC		2		3		2	1	1	1
677-62-90-7	IC	1								
69430-24-6	IC	1								
68037-74-1	IC	1	1							
70131-67-8 ^a	IC	11	1	3		2			5	1
63148-62-9 ^a	IC	52	45	9	88	66	19	19	41	5
2554-06-5	II	2				1			1	
546-56-5	II	1							3	
2374-14-3	II	9	6						7	
68037-59-2	III	2								
67762-94-1	III				1			1		2
680-83-14-7	III	3				1				
Mixture									2	
999-97-3	IV	11							1	
2627-95-4	IV	8	1					1		
Others									3	
Total ^b		149	95	16	116	75	39	30	93	16

^aDenotes human data are available. ^bSum of all studies: 629.

C: Polydimethylsiloxanes

A total of 516 reports on L₅, L₆, L₇, L₉, L₁₃, L₁₆, D₇, D₈, D₉, D₁₅, D_x (cyclosiloxanes, dimethyl(cyclopolydimethylsiloxanes), DMPS (dimethylmonomethylpolysiloxanes, dimethylpolysiloxanes), DMSS (dimethylsilicones and siloxanes, reaction products with silica), SSHS (siloxanes and silicones, dimethyl hydroxy-terminated), PDMS, and L_x (linears) were reviewed. The database on the toxicity of these compounds is extensive. Acute exposure by different routes showed only minimal toxicity. The compounds have minimal potential for skin irritation. Subchronic studies involving oral administration of the agents did not reveal any systemic toxicity. On prolonged dermal application, sometimes under occlusion, some edema and scarring are observed, but no systemic toxicity. Implants of these materials under the skin usually produce granulomatous inflammatory changes and fibrosis. Subcutaneous implantation of PDMS gels in rats produced local sarcomas, such as are commonly seen in rats implanted with inert foreign bodies (solid-state carcinogenesis). An oral carcinogenicity study failed to produce any positive data. Multiple tests found a lack of genotoxicity. Tests for reproductive toxicity following oral or dermal exposure failed to show any clearly positive results. On occasion a small increase in fetal abnormalities was found, although the agents are not considered teratogenic.

In 29 of 35 studies, no effects on the male gonads were found. The summary document, without providing references however, mentions that some PDMS fluids given by gavage at 3.3 ml/kg for six days were associated with reduced seminal vesicle weights, whereas others, given for up to 20 days at similar doses, had no such effects. Spermatogenic depression was found in two of ten rabbits treated with 2 ml/kg PDMS for 20 days. Dermal application of 2 ml/kg for 28 days decreased testicular weight. In the case of one PDMS fluid (not characterized), a no-observable-adverse-effect level (NOAEL) of 50 mg/kg per day for a 28-day exposure was established. All of these dose levels are orders of magnitude greater that could be achieved in women with breast implants on a milliliter- or milligram-per-kilogram body weight basis. No immunotoxic potential was identified, although in some studies, adjuvant activity was noted with an increase in humoral but not cell-mediated immunity. The results were not seen with any consistency, and studies were often of poor quality. The absence of virtually any toxicity following acute exposure by oral and dermal routes was confirmed in human volunteers.

Group II—Non-Dimethyl Siloxanes

Thirty reports were reviewed. The acute oral LD₅₀ (mean lethal dose) of these compounds is influenced by solvent effects. Reproductive studies indicated some adverse effects on the male reproductive tract. In addition, the agents produced severe ulceration and necrosis of rabbit skin during the 21-day treatment. Significant histopathological changes in rabbit liver and kidney were

seen after four days' treatment at 3.3 mg/kg. No genotoxicity was observed. Agents in Group II are polymer precursors, and no exposure is anticipated outside manufacturing sites. The committee found no evidence that these compounds are in breast implants.

Group III—Other Siloxane Polymers and Copolymers, DHPS, DMMVS (siloxanes and silicones, dimethyl, methylvinyl), and DMDS (siloxanes and silicones, diphenyl)⁶

Ten reports were reviewed. The studied compounds are reactive, and they cross-link easily. Use of the toxicity of starting materials is not appropriate in judging the toxicity of cured cross-link products. There appears to be limited industrial exposure and no exposure of the general public. Acute toxicity, irritation, and sensitization are minimal. These compounds are not known to occur in silicone breast implants.

Group IV—Other Materials

Forty-four reports were reviewed. Toxicity following oral exposure is low, and for inhalation a one-hour LC₅₀ (50% lethal concentration) between 23 and 111 mg/ml was measured. The lowest-observable-adverse-effect level (LOAEL) for lung hemorrhage was 5.6 mg/l. Tetramethyldivinylsiloxane was severely irritating to the skin under occlusive conditions. No evidence for genotoxicity or immunotoxicity was reported. These compounds are not known to occur in silicone breast implants.

TOXICOLOGY OF SUBCUTANEOUSLY IMPLANTED OR INJECTED SILICONES

Acute and Subchronic Studies with Silicone Fluids and Gels

Early toxicological experiments were designed to evaluate the effects of silicone liquids and solids implanted under the skin of experimental animals. Such experiments mimic silicone breast implants in many ways, although there are some important differences. Silicone breast implants are more complex. They may have varied surfaces, including coating with polyurethane. They may also contain many different chemical species, including potentially toxic compounds such as platinum. On the other hand, in many of these studies, actual gel and elastomer components of breast implants were tested.

In one early study, medical series 360 Dow Corning PDMS fluid, 350 cS, was injected in massive (up to 540 ml over 27 weeks) doses subcutaneously in rats and guinea pigs. There was very little or no local inflammation. The injected

⁶ Mentor Annotation: As discussed elsewhere in the IOM report, methyl diphenyl copolymers are used in the low-bleed elastomer shells of Mentor silicone gel-filled mammary prostheses (see discussion following this chapter from the IOM report).

fluid became encapsulated by thin, transparent connective tissue in multiloculated cysts. No systemic toxicity was observed. However, it was not clear whether the material was eventually absorbed, redistributed within the body of the animals, or excreted (Ballantyne et al., 1965). To further elucidate this point, mice were injected subcutaneously or intraperitoneally with 1 ml of Dow Corning 360 silicone fluid, 350 cS, followed by intravenous carbon particles to induce reticuloendothelial blockade. Silicone was found in macrophages in regional lymph nodes in all animals and in macrophages in the adrenal in some intraperitoneally injected animals. Unlike the previous high-dose experiment, all other organs were normal (Ben-Hur et al., 1967). A high-dose exposure in man, multiple massive subcutaneous injections of silicone (1 liter at a time), eventually led to diffuse tissue distribution of the material in various organs (primarily the lungs) of this patient who succumbed to adult respiratory distress syndrome (Coulaud et al., 1983).

In mice as in rats, subcutaneous injection of 5 ml of Dow Corning 360 medical fluids did not produce any untoward effects (Andrews, 1966). The same author reported the case of an 18-year-old woman injected subcutaneously twice with 20 ml of 360 fluid. In examining a blood smear, neutrophils and mononuclear cells containing clear vacuoles were seen, which presumably contained silicone. The smear, however, was taken from an incised injection site where leukocytes had direct access to a silicone deposit, and this finding could not be confirmed by Hawthorne et al. (1970), who examined white cells from rats with high silicone exposures (see below). Nedelman (1968) injected various room temperature vulcanized (RTV) medical-grade Silastics mixed with Dow Corning 360 fluid and stannous octoate catalyst subcutaneously in the back of hamsters and supraperiostally in the jaw and palate of rabbits in doses of 0.5-2.0 ml and followed them for one week to three months. He reported that the Silastic was well tolerated and elicited only a mild connective tissue response. In another study in mice, Rees et al. (1967) observed a redistribution of silicone fluid within the body when injected in 1-ml amounts intraperitoneally or in larger amounts subcutaneously (6 ml in a single dose, 1 ml in repeated doses). Deaths occurred when the mice received more than 7 ml of PDMS by subcutaneous injection, an amount corresponding to about 280 ml/kg or about 14 liters in an average woman. Macrophages, presumably containing silicone, accumulated in multiple organs, including adrenal, lymph nodes, liver, kidney, spleen, ovaries, pancreas, and others (Rees et al., 1967). Whether the wider distribution of silicone injected at high doses results from access to, and distribution by, the circulatory system is unknown. The study by Rees et al. prompted Autian (1975a) to warn against the injection of silicone fluid in humans. He was also influenced by the local complications of silicone injection in women, which were well known by that time. Ashley et al. (1971) briefly reported injecting Dow Corning MDX 40411 in amounts ranging from 1 to 500 ml into mice, rats, guinea pigs, rabbits, and monkeys, with the formation of thin capsules, very little tissue reaction, and no systemic effects. This 350-cS fluid was also injected in small (4 ml) amounts into patients for cosmetic effect

without complications. Very few data were reported, and the follow-up of the patients was three months on average (Ashley et al., 1971). Cutler et al. (1974) observed no ill effects on mice of PDMS fluid similar to Antifoam A mixed with 6% amorphous silica injected subcutaneously (0.2 ml) or fed at 0.25 and 2.5% from weaning for 76 weeks. Distribution to liver, spleen, kidneys, and perirenal fat was not detected.

In a more recent study, Dow Corning silicone 360 fluid and gel (1 ml per mouse), and elastomer and polyurethane (0.6-cm-diameter disks) were placed subcutaneously in B6C3 F1 mice (Bradley et al., 1994a,b). Animals were examined first over a 10-day period, then for 180 days. Silicone implantation did not affect any of the selected toxicological endpoints, including survival, weight gain, body and organ weights, hematology, serum chemistry, and bone marrow cytology. No effects on humoral immunity or cell-mediated immunity were found, and host resistance in two bacterial models was not altered.

Although, on occasion, widespread tissue distribution with potentially toxic or even fatal outcomes is seen when very large doses of silicone fluid are deposited subcutaneously or intraperitoneally (1 liter or more in humans, 7 ml in mice), quite substantial amounts are usually well tolerated. A subcutaneous injection in rodents (and most other animal species) is not directly comparable to a subcutaneous injection in humans however, because in most animals a large potential space is provided between mobile skin and underlying muscular fascia that can accommodate a substantial amount of fluid. In humans, silicone, if injected in large amounts, may be forced into the circulation and thus to distant organs, as suggested in the cases mentioned earlier (Andrews, 1966; Coulaud et al., 1983).

Silicones are present in medical devices and instruments (e.g., coatings for tubing and syringes). This has prompted some investigators to inject silicones intravenously, intraperitoneally, or even into the subdural space of the lumbar spinal cord. Intravenous or intracardiac injection of 2 ml of PDMS in dogs did not produce any changes in clotting time, hemoglobin concentration, or plasma surface tension. No changes in electrocardiograms or electroencephalograms were noted (Fitzgerald and Malette, 1961). These authors cited others who had injected larger doses intraarterially or intravenously causing embolisms in various organs. Intraperitoneal injections of Dow Corning MD 44011, a silicone fluid that was actually injected in women for breast augmentation (see Chapter 1), at doses up to 62 ml in 60 rats were tolerated without any apparent adverse effects for up to one year (Hawthorne et al., 1970). Intraperitoneal injections of up to 3 ml of PDMS in mice resulted in a reduction of cell size in abdominal and pericardial fat tissue. In addition, in many abdominal organs such as adrenal, liver, kidney, spleen, pancreas, ovary, and lymph nodes, focal silicone-containing macrophage infiltrates were seen (Rees et al., 1967). Migrating silicone could produce granulomas on the surface of organs (Brody and Frey, 1968). In the course of investigating adjuvant effects, Lake and Radonovich

(1975) reported that intraperitoneally injected low molecular weight silicones (L₃, L₄, D₄, L₅) caused a transient (48 hour) increase in interferon production and a reduction in colloidal carbon clearance by macrophages of the reticuloendothelial system in mice. Higher molecular weight silicones did not have these effects.

PDMS lubricant used in disposable syringes was injected into the lumbar subdural space in rabbits (0.3 ml) and monkeys (0.5 ml) and into the cisterna magna of rats (0.1 ml). No signs of neurotoxicity or histopathological alterations attributable to the silicone injections were observed. All of the radiolabeled silicone injected intracisternally remained in the brain, spinal cord, and vertebral column (Hine et al., 1969). Chantelau et al. (1986) calculated that 0.15-0.25 mg silicone lubricant might be lost from an insulin syringe with each use, or about 200 mg per year, assuming multiple injections per day for diabetes. Others have reported lower estimates of 30-40 ug from an insulin syringe with each use, or up to 30 mg in a year (Collier and Dawson, 1985). An average lifetime human dose would be at most several grams of silicone if the higher estimate was used; Hine's doses in experimental animals, therefore, equal or exceed lifetime human doses on a milligram-per-kilogram body weight basis. In another study, direct injection of silicone gel into peripheral nerve did not result in findings of toxicity of silicone to nerve tissue (Sanger et al., 1992).

Short-Term Studies with Solid Implants

Solid silicone implants also were generally well tolerated by experimental animals. Dogs, examined up to one year after implantation of sponges subcutaneously, intraperiostally, or placed directly onto bone, tolerated the implantation well, and the material was not invaded by bone or periosteum (Marzoni et al., 1959). Actual breast implant materials, such as Dow Corning Q7-2245 elastomer, in a biological safety screen consisting of tissue cell culture, systemic toxicity, rabbit intracutaneous and pyrogen tests, guinea pig sensitization, and rabbit 90-day implants, elicited no local or systemic responses (Munten et al., 1985), nor did Q7-2167/68 gel in a similar screen (Malczewski, 1985a). Subcutaneous implantation of medical-grade polysulfone-based silicone elastomer in rabbits was not carcinogenic up to 18 months. This study was of insufficient duration to be conclusive, however (Lilla and Vistnes, 1976).

In another implant study, nine different Silastic materials were implanted subcutaneously, intramuscularly, and intraperitoneally into 20 young adult purebred beagle dogs for six months to two years. The materials provoked a minimal foreign body reaction and the formation of a fibrous capsule but no general adverse effects (Dow Corning Corporation, 1970). Two years is, nevertheless, a short time compared to a life expectancy in beagles of 12-15 years. Thus, this study does not allow conclusions on such long-term effects as carcinogenesis.

James et al. (1997) recently evaluated one-week and two-month local cellular responses to PDMS, compared with the responses produced by impermeable cellulose acetate Millipore filters. Expression of leukocyte antigens for helper-inducer, T-suppressor-cytotoxic, and macrophage leukocyte antigens, proliferating cell nuclear antigen, and in situ labeling of DNA strand breaks as indicators of DNA damage and apoptosis were measured. The response to silicone did not differ from the response to impermeable cellulose acetate filters. On the other hand, porous cellulose filters, known not to produce local sarcomas, produced more intense inflammatory responses but minimal fibrosis. Within the fibrotic capsule surrounding the tumorigenic implants, cell proliferation and apoptosis were increased and associated with DNA breaks. The authors pointed out that persistent DNA damage and elevated cell proliferation are usually associated with genomic instability and malignant transformation. Similar studies might thus be carried out on human tissue surrounding silicone implants.⁷ Van Kooten et al. (1998) in the course of evaluating human fibroblast proliferative responses to smooth and variously textured Dow Corning Medical Grade Silastic found no influence of toxic leachables that might have been released from the silicone samples using 3-(4,5-dimethylthiazole-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) conversion testing of cellular biochemical activity.

Long-Term (Carcinogenicity) Studies

From the moment silicone compounds became available for implants in humans, long-term effects were of particular concern. It was recognized that subcutaneous implantation of silicone compounds in rodents would produce local tumors at the implantation site. Solid-state carcinogenesis had been discovered in the 1940s and was a well-known phenomenon in plastics toxicology. In addition, the possibility was entertained that implants might release agents capable of producing tumors at distant sites. In a study of carcinogenesis in which animals were observed for up to two years, silicone rubber implanted intraperitoneally did not produce any tumors, but subcutaneous implants caused local sarcomas (Hueper, 1961). An RTV silicone elastomer with a stannous octoate catalyst was also implanted under the skin, intraperitoneally, and subdurally in the brain. No implant-related tumors were found during an observation period of up to 22 months (Agnew et al., 1962). A review of the entire literature on solid-state carcinogenesis induced by silicone compounds was published in 1967. In rats, but not mice, local sarcomas developed at the sites of silicone rubber implants (a 29-40% incidence following placement of single implants). Silicone gel or fluid produced only one sarcoma in 30 rats and no tumors in mice. The authors also pointed out that many of the reported experiments were not lifetime and therefore of too short duration to evaluate carcinogenicity properly (Bryson and Bischoff, 1967).

⁷ Mentor Annotation: Although the findings of such proposed experiments might address a scientific curiosity, the data discussed elsewhere in this report that is suggestive, though not definitive, of a reduced incidence of breast cancer would argue against any potential significance with respect to cancer risk.

In 1972, Bischoff again reviewed silicone toxicity and carcinogenicity. Despite problems with the referencing of this review that interfere with discovery of the original data, the summarized data show a significant trend for tumor development in female, but not male, rats following intraperitoneal injection of silicone fluid. Subcutaneous administration of silicone fluid produced no tumors in rats, but an increased incidence of mesenchymal tumors was observed at the injection site in mice. No such tumors were found with controls (it is not clear how controls were injected). Bischoff (1972) concluded that silicone fluid had a low-grade carcinogenic potential in rodents. In the absence of the original data, it is difficult to evaluate this conclusion. However solid silicone compounds, implanted subcutaneously, clearly produce local tumors of mesenchymal origin at the site of implantation in rats. Silicone shares this property with numerous other agents.

The salient features of solid-state carcinogenesis have been reviewed (Autian, 1975a). The phenomenon is seen in rodents, mainly rats. Implantation of an inert material (e.g., acrylic, cellulose, Teflon, glass, bakelite, silicone, polystyrene, polyurethane, polyethylene) under the skin elicits, after a latent period, the local growth of a mesenchymal malignant tumor. To have such an effect, the implant must have a minimum size. Smooth implants are more effective than rough or perforated disks. Initially, the foreign body will be surrounded by granulomatous tissue that eventually forms a thin capsule. If the foreign body is removed within the first six months after implantation, no tumors develop. Removal of the test material later may or may not be followed by tumor development, but if the tissue pocket is removed, regardless of timing, no tumor will develop. The same amount of material introduced in powdered form under the skin does not produce tumors.

Later studies of the carcinogenicity of silicone implants, gels or solids, confirmed their ability to produce local sarcomas in rodents. In rats, silicone implants produced significantly fewer tumors at the implant site than did polyvinyl chlorides or polyhydroxyethyl methacrylate (Maekawa et al., 1984). Silicone amputation stump implants were placed in dogs, and the animals were observed up to 10 years (Swanson et al., 1984). While there was a benign foreign body giant-cell reaction to local silicone, no silicone particles or giant-cell responses were observed in distant organs, and the implants were well tolerated.

Surgitek breast implant components, silicone gel-SCL, silicone gel-Meme, silicone elastomer SCL, and standard elastomer coated with type A adhesive and polyurethane foam were examined in a two-year rat study with negative (Millipore filters, 0.65-um pore size) and positive (Millipore filters, 0.025-um pore size) controls. Test materials were implanted subcutaneously in the back at four different sites, and the animals were observed for up to 104 weeks. Survival was comparable for the negative control group and the polyurethane

foam group, but significantly decreased in all other groups. However, body weight gains were similar in all groups. Subcutaneous tissue masses at sites of implantation were found in all groups. Tumor incidence ranged from 3% (polyurethane foam) to 53% (positive controls), and the two silicone gels had incidences of 27 and 19%, respectively. Most tumors were malignant, but rarely metastasized, and all were of mesenchymal origin. There was no evidence of systemic toxicity during the conduct of this study. At both interim and final sacrifice, there were no changes in organ weight, clinical chemistry, or hematology that could be attributed to an effect of the test agents. Age-associated inflammatory, degenerative, or neoplastic changes were seen on pathological examination, but the groups did not differ significantly. It was concluded that implantation of silicone gel-SCL or silicone gel-Meme at a higher dose than usual in humans did not produce any signs of systemic toxicity in female rats (Lemen and Wolfe, 1993).

Most recently, a lifetime implant study with Dow Corning Q7-2159A silicone gel, used in breast implants, tested whether a silicone implant would produce tumors at other than the implant site. A group of animals with subcutaneous polyethylene disk implants was also examined. The study, begun in 1990, involved a total of 700 female rats. Seven groups were formed: a control group, three groups receiving silicone gel implants (total surface areas 6.6, 18.0, and 48.8 cm²), and three groups receiving polyethylene disks (total surface areas 0.79, 3.1, and 12.6 cm²). The animals were observed for 104 weeks. Data for survival, body weight gain and food consumption, incidence of neoplastic and nonneoplastic lesions, organ weights, hematology, urinalysis, and clinical chemistry were all analyzed with appropriate statistical methods, designed to show dose-responses, trends, and significance of differences in lesions among treated and control groups (Klykken, 1998). The design, execution, data analysis, and quality control procedures used in this study represent today's state of the art in the conduct of carcinogenesis bioassays. Survival was somewhat shorter in animals that had silicone gel- or polyethylene-induced sarcomas at the implantation sites. In non-tumor-bearing animals, life span was not reduced. Incidence of local tumors increased with implant surface area and was higher in the polyethylene-treated animals. Silicone gel did not produce tumors at a site distant from the implantation site. Similarly, there were no observations of systemic toxic effects in silicone gel-implanted animals.

There was weak statistical evidence of decreased incidence of mammary gland malignant and benign epithelial tumors following gel exposure and of thyroid c-cell carcinomas and adenomas in animals treated with the largest polyethylene disks, compared to controls. In all animals, including the ones with implant site sarcomas, a reduced tumor incidence was also found for brain, mammary gland, pituitary, and all sites combined. Others have suggested that silicone gel implants might be associated with a lower incidence of malignancy in experimental systems. Dreyfuss et al. (1987) noted that a group of 60 rats with experimental silicone gel-filled implants experienced fewer mammary cancers

caused by injection of N-methyl-N-nitrosourea 14 days after implantation than were seen in 60 rat control groups or groups with gel, elastomer, or polyurethane implanted as component sheets rather than fabricated into implants. This was the only positive finding in a group of negatives involving exposure to different silicones and different timing of injections (Dreyfuss et al., 1987). In another study, tumor size was diminished in the presence of tissue expanders in rats injected with mammary cancer cells compared to control and sham-operated rats. In still another study, rats with silicone implants in three locations, including beneath the mammary gland, developed fewer tumors after N-methyl-N-nitrosourea injection compared to sham controls, and mice with implants developed fewer spontaneous carcinomas compared to mice with implants of free gel or silicone sheets or sham operations (Ramasastry et al., 1991; Su et al., 1995). These studies and the epidemiological evidence of lower relative risks of breast cancer in implanted women (cited in Chapter 9) are suggestive, but they are not adequate to provide conclusive evidence for a decreased cancer risk in women with silicone breast implants.

Reproductive Toxicity Following Implantation with Silicones

Most women who receive silicone breast implants are of childbearing age. For this reason, reproductive, developmental, and teratologic effects of exposure to silicones and the effect of silicone implantation on breast feeding are particularly relevant. Many of the human data on exposure and responses to silicone are reviewed in Chapter 11. The reproductive toxicity and teratogenesis of some silicones relevant to those found in breast implants have been addressed directly in a few experimental animal studies.

Dow Corning 360 medical-grade fluid, 350 cS, and two other PDMS fluids were administered in comparatively high doses (20, 200, or 12,000 mg/kg) to male and female rats, mice, and rabbits. Basic guidelines issued by the Food and Drug Administration (FDA) for reproductive toxicity testing were followed. General reproductive performance (exposure of males and females before and during gestation), embryogenesis (exposure of pregnant females during the critical period of gestation), and postnatal performance were evaluated. Altogether, several hundred rats, rabbits, mice, and their offspring were examined, and no adverse teratologic, reproductive, or mutagenic effects were observed (Kennedy et al., 1976).

PDMS fluid, 350 cS, at dose levels of 5, 10, and 20 g/kg body weight was injected over ten days in one group of pregnant rats and all at once in another group of rats one week before mating. The sole effect observed was a significant postimplantation loss in the 5- and 10-g/kg PDMS dose groups of predosed animals. This effect prompted use of the predosing regimen and dose levels of 1, 10, and 20 g/kg PDMS in a definitive assay with 0.85 saline controls. The 20-g/kg dose level was selected to approximate the exposure of a 50-kg woman to sudden and complete rupture of two 500-g silicone gel breast

implants. In this final test, no clinical signs of toxicity were evident in the mothers. No effects were found in the fetuses, and no postimplantation loss was observed. Under the conditions tested, the compound had no teratogenic effect (Bates et al., 1985, 1991).

In a later study, Surgitek silicone gel-SCL, silicone gel-Meme, and polyurethane were implanted under the skin of rabbits at six different locations, 17 rabbits per group. Doses were calculated to represent up to three times the expected human exposure for the gels and up to ten times for the polyurethane. After six weeks the rabbits were mated and then killed on gestation day 29. There were no effects of the treatment on implantation efficiency, pregnancy rates, fetal viability, postimplantation loss, or fetal weights. In animals exposed to polyurethane, some fetal malformations were observed, but the incidence per litter was not significantly different from controls. These findings were considered incidental. Materials implanted under the skin did not appear to produce either maternal toxicity or fetal abnormalities (Lemen, 1991).

More recently, silicone gel Q7-2159A and elastomer Q7-2423/Q7-2551 were evaluated for reproductive toxicity and teratogenesis in rats and rabbits. Altogether, the studies examined three different dose levels for the gel (3, 10, and 30 ml/kg) and two different disk sizes for the elastomer. In the reproductive toxicity studies, 30 male and 30 female rats were used per group, and in the teratology study, 25 pregnant rabbits were used in each group. Test articles were implanted in male rats 61 days, and in female rats 47 days, before mating and in female rabbits 42 days prior to insemination. Implantation of the gel or of the elastomer disks and their continuous presence before or during pregnancy and lactation did not cause observable effects in parents or neonates and had no discernible teratogenic effects. These two studies reflect the current state of the art in reproductive toxicity and teratogenesis testing (Siddiqui et al., 1994a,b). Finally, a two-year gel implant study of Dow Coming Q7-2159A and Dow Coming MDF-0193 in rats has been reviewed (Ruhr, 1991). This report examines the data for evidence that silicone implantation leads to changes in the male or female endocrine system. Fifty male and female rats were implanted with the test materials, and no changes in the endocrine system were found during what amounted to a lifetime study.⁸

Distribution and Migration of Subcutaneously Implanted Material

The fate of subcutaneously implanted silicone has been directly addressed in a few studies. A total of eight male rats received a single subcutaneous injection

⁸ Mentor Annotation: McKim et al. (2001) recently reported results from their investigation of the potential estrogenic and antiestrogenic activity of D₄ and hexamethyldisiloxane (HMDS) in a uterotrophic assay in immature rats. D₄ exhibited weak estrogenic activity, but was approximately 585,000 times less potent than ethinyl estradiol in Sprague-Dawley rats and 3.8 million times less potent than ethinyl estradiol in Fischer F-344 rats. The NOAEL for D₄ identified in this study was 100 mg/kg. HMDS did not reveal any estrogenic activity at doses up to 1200 mg/kg; a small antiestrogenic effect at this high dose level of HMDS was observed when coadministered with ethinyl estradiol.

of PDMS fluid labeled with carbon-14 (^{14}C). More than 94% of the radioactivity remained at the site of injection, and very small percentages (around 0.1%) were detected in expired air, urine, and feces. Less than 0.02% was eventually found to have migrated to different tissues, presumably via the lymphatics (LeBeau and Gorzinski, 1972). The movement of subcutaneously implanted, radiolabeled PDMS gel Q7-2159A was followed over a 20-week period (Isquith et al., 1991). Male and female CD-1 mice received a middorsal 0.5-ml implant of gel synthesized by equilibrating [^{14}C]octamethylcyclotetra-siloxane with dodecamethylpentasiloxane under acidic conditions. Over a period of 20 weeks, only 0.006% in males and 0.009% in females was found to be mobile. A very small amount of radiolabeled silicone was excreted, in large part during the first week postimplantation. What remained in the body beyond the injection site was found primarily in lymph nodes draining the implantation site. The injection sites were collected, but not analyzed. This precludes calculation of the usual mass balance (silicones not specifically measured elsewhere were assumed to have remained in the injection depot), but generally, silicone concentrations (calculated from radioactivity) in different tissues and organs were micrograms per gram of tissue, orders of magnitude lower than the amount injected (500 mg). In a report from the FDA, Young (1991) reanalyzed data from a 1966 Dow Coming study of the movement of [^{14}C]poly(dimethylsiloxane) injected subcutaneously in mice and followed over 90 days. A small fraction of the injected radioactivity appeared in the urine and feces with a half-life of 2 days initially and 56 days for redistributed radioactivity, but 99.97% of the silicone was stable (Young, 1991). These studies appear to show that very little of a gel implant leaves the site of deposition.

Raposo do Amaral et al. (1993) injected rats with 2 ml of silicone gel at two different sites. The animals were killed at intervals of 3, 7, 15, 30, 60, 180, 240, 420, and 450 days. The authors did not detect any silicone gel in lung, heart, spleen, liver, stomach, or gonads, although they could see it in the local tissues surrounding the capsule formed around the injected gel. No silicone was found in the regional lymph nodes draining the implant. However, these tissues were examined for silicone by light microscopy, which is an insensitive detection method. The reaction of local lymph nodes to injected silicone gel (1.5 ml injected subcutaneously into male Wistar rats), was measured with rigorous quantitative morphometric techniques at intervals up to 365 days (Tiziani et al., 1995). There was no evidence of lymph node hyperplasia, giant cells, or silicone droplets.

There was no morphometric difference in lymph nodes from gel-injected or saline-injected animals, and it was concluded that the silicone gel had not migrated. Swanson et al. (1984, 1985) evaluated a patient at autopsy after 12 years' exposure to silicone elastomer joint implants and also evaluated three dogs with elastomer implants after 10 years' exposure. Silicone elastomer particles were found locally around the implants, but a complete organ and

reticuloendothelial system review revealed no particles at distant sites and only a few silicone particles in an axillary node of the autopsied patient (Swanson et al., 1984,1985). Silicone rubber fragments placed in the peritoneal cavities of rats were found in the spleens of these animals, associated with a giant-cell reaction after four days (Guo et al., 1994). Barrett et al. (1991) found silicone particles locally and in regional nodes (when examined) of patients with penile implants. Examinations for particles in more distant sites were not undertaken. These examples are typical of reports of local and some regional node presence of silicone elastomer particles from various kinds of implants, which generally provoke some giant-cell, but no systemic, reaction (Barrett et al., 1991). Inflammatory reactions are limited to joints exposed experimentally to particulate silicone elastomer in rabbits by injection (or in humans from joint implants); unexposed joints are not inflamed (Worsing et al., 1982). More distant migration of small (median diameter, 73 μm) silicone particles to lung and lymph nodes and, less frequently, to kidney and brain was observed in seven female dogs injected with a silicone-polyvinylpyrrolidone paste. There was no tissue reaction around the particles (Henly et al., 1995). Tiziani et al. (1995) concluded from this sort of evidence that regional node reactions were more likely to particulate elastomers than to silicone gel implanted in their drainage areas.

In a recent study, mice received subcutaneous injections of 250 mg of breast implant distillate, a low molecular weight siloxane mixture containing D₃, D₄, D₅, D₆, L₅ and L₆ (Kala et al., 1998). These materials are released by gel fluid diffusion from breast implants in very low concentrations (see Chapter 3). Animal tissues were analyzed at 3, 6, 9, and 52 weeks by gas chromatography-mass spectroscopy. Commercially available D₄, D₅, and D₆ were used as standards. The distribution of individual cyclosiloxanes in brain, heart, liver, kidney, lung, lymph nodes, ovaries, uterus, spleen, and skeletal muscle was measured. Concentrations for the individual cyclosiloxanes were all in the range of less than 1 μg (brain, liver) to a maximum of 7 μg (lymph nodes, ovaries) per gram of tissue. When calculated as total cyclosiloxanes, concentrations were highest in lymph nodes, uterus, and ovaries after six weeks, in the range of 1 to 14 $\mu\text{g/g}$ of tissue. The authors reported that they could detect silicone in all organs examined up to one year later. Linear siloxanes were found at 4 to 5 $\mu\text{g/g}$ of brain and up to 8 $\mu\text{g/g}$ of lung. Large variations in the concentrations of the siloxanes between individual animals were noted. This study shows that in mice a small percentage of low molecular weight siloxanes injected in the suprascapular area can migrate in microgram amounts to different tissues. The experiment gives data on tissue concentrations only.

A mass balance study—that is an analysis of the amount of siloxanes injected, distributed, and excreted—was not carried out in this experiment. Such an analysis, usually a part of tissue distribution studies of chemicals as noted earlier, would have provided information on how much silicone was dislocated from the injection site, retained, or lost from the animal. The data on the total

siloxane concentrations in different organs allow others to estimate a mass balance, however. Average organ concentrations were 7 ug/g wet tissue weight at most. If uniform distribution is assumed for a 25-g mouse, this provides for a total of 175 ug siloxane distributed from the injection site, or about 0.07% of the administered dose (250 mg). By allowing for the fact that the migratory part of the gel (a low molecular weight siloxane fluid distillate), not the gel itself, was injected, these results are consistent with those of Isquith discussed earlier. Kala et al. (1998) reported similar weight gains at one year in control and experimental mice, suggesting that in this study, a large (10 g/kg) dose of low molecular weight linear and cyclic siloxanes appears to have been well tolerated. In a subsequent study, this group injected even larger doses of a distillate containing D₃-D₆ intraperitoneally in mice and observed inflammatory changes in liver and lung. The LD₅₀ for distillate was about 28 g/kg body weight, and for D₄ alone 6-7 g/kg body weight (Lieberman et al., 1999). It is not clear what relevance these studies have for women with silicone breast implants, since test article doses were given that were orders of magnitude greater than possible from breast implants, and LD₅₀s in these ranges have historically been considered indicative of lack of toxicity (Casarett, 1975; Marshall et al., 1981). It was also not clear to the committee why a distillate, instead of an extract or simply reference compounds, was used, since the possibility that some of these compounds were created during distillation once again raises the question of relevance for women with silicone breast implants.⁹

⁹ Mentor Annotation: A state-of-the-art pharmacokinetic study of ¹⁴C-octamethylcyclotetrasiloxane (D₄) in Fischer 344 rats after single and multiple exposures to 7, 70 or 700 ppm was recently published by Plotzke et al. (2000). The investigators reported that: "Retention of inhaled D₄ was relatively low (5-6% of inhaled D₄). Radioactivity derived from ¹⁴C-D₄ inhalation was widely distributed to tissues of the rat. Maximum concentrations of radioactivity in plasma and tissues (except fat) occurred at the end of exposure and up to 3 h postexposure. Maximum concentrations of radioactivity in fat occurred as late as 24 h postexposure. Fat was a depot, elimination of radioactivity from this tissue was much slower than from plasma and other tissues. With minor exceptions, there were no consistent gender effects on the distribution of radioactivity and the concentrations of radioactivity were nearly proportional to exposure concentration over the exposure range. Excretion of radioactivity was via exhaled breath and urine, and, to a much lesser extent, feces. Urinary metabolites included dimethylsilanediol and methylsilanetriol plus five minor metabolites. Relative abundance of these metabolites was the same from every test group. Elimination was rapid during the first 24 h after exposure and was slower thereafter (measured up to 168 h postexposure). In singly-exposed female (but not male) rats, small dose-dependent shifts in elimination pathways were seen. After multiple exposures, the elimination pathways were dose- and gender-independent."

Data from this study formed the basis for development of a physiologically-based pharmacokinetic (PBPK) model for D₄ by Anderson et al. (2001) which concluded that "high pulmonary and hepatic clearance, coupled with induction of metabolizing enzymes at high exposure concentrations, rapidly remove free D₄ from the body and ensure that there is no accumulation on multiple exposures."

Luu and Hutter (2001) published a different PBPK model for D₄ that challenges the Anderson et al. (2001) findings and predicts accumulation following multiple exposures. Numerous apparent flaws in the methodology of Luu and Hutter, however, have been asserted by Meeks (2002) and Anderson et al. (2002). Meeks (2002), in his critique, reported actual measured concentrations of D₄ in blood and fat from rats exposed to 700 ppm D₄ by inhalation (6hr/day, 5 days/week) for 15 days and 6 months, which "confirm that D₄ does not accumulate in the body." Mentor Corporation is currently pursuing an independent evaluation of the competing models by an expert PBPK modeler to assess the validity of the competing claims.

GENERAL TOXICOLOGY OF SILICONE COMPOUNDS, INCLUDING LOW MOLECULAR WEIGHT CYCLIC AND LINEAR POLY(DIMETHYLSILOXANES)

Exposure to silicone compounds is widespread. A comparatively small number of people in industry may experience high exposures by dermal or inhalation routes. A large population may experience low-level exposure through consumer products including food. Toxicity testing has thus had to consider these routes of exposure. The committee has reviewed some of the studies of dermal, oral, and inhalation exposure to silicone in experimental animals for this reason and also because such studies provide some insights into the systemic toxicity of silicones that may be relevant to the toxicology of silicone breast implants.

Dermal Exposure

There are few studies on direct dermal toxicity of silicones, probably because early investigators recognized that silicones had no skin irritating properties and were generally considered nontoxic (Barondes et al., 1950). Nevertheless, a study conducted in rabbits with trifluoropropylmethyl-cyclotrisiloxane revealed some toxicity. In the highest-dose group (400 mg/kg), 40 of the animals died, and there was significant reduction in body weight gain (Siddiqui and Hobbs, 1982). Dermal (and oral) exposure to some organopolysiloxanes, not found in breast implants, resulted in adverse effects on the reproductive systems of male and female rats, rabbits, and dogs. Dermal application for 28 days produced testicular or seminal vesicle atrophy in rabbits (Bennett et al., 1972; Hayden and Barlow, 1972). Maternal weight loss, increased resorption, and decreased viability of young were observed in female rabbits treated dermally with a phenylmethylcyclotrisiloxane. However, the material was not considered teratogenic. Application of the same silicone fluids to human skin did not lead to an increase in silicone blood or urine concentration (Hobbs et al., 1972; Palazzolo et al., 1972). Although some interest in these compounds has been expressed by women with implants or by other investigators, there is no evidence that they are found in silicone breast implants.

Oral Exposure

Oral toxicity for most silicone compounds is very low. For two silicone oils (poly(sec-butylmethylsiloxane) and polydimethylsilicones), the LD₅₀ was greater than 24 g/kg. Agents with such a high LD₅₀ are generally considered nontoxic (Marshall et al., 1981). More recently, the oral toxicity of Dow Corning 200 fluid, 10 cS, a PDMS fluid, was examined in a 28-day and then a 13-week feeding study. Rats received the test material in the diet at concentrations from 1 to 10% in the 28-day study and from 0.5 to 5% in the 13-week study. Corneal opacities, identified as corneal crystals, and other corneal inflammatory changes were noted in the higher-dose groups, presumably due to

direct contact with the fluid on the fur. Changes in clinical chemistry were limited to a significant decrease in mean triglycerides, and in low-density and very low-density lipoproteins. A NOAEL could be set at greater than 100,000 parts per million (ppm) of the test substance, provided the corneal lesions were the result of a topical effect for the 28-day study, and at greater than 50,000 ppm for the 13-week study (Tomkins, 1995). Dow Corning 200 fluid, 350 cS, another PDMS fluid, was evaluated in a similar experiment. The same corneal lesions were noted both in the 28-day and the 13-week studies, and again were attributed to topical contact. No changes in clinical chemistry were noted. In the 13-week study, male and female rats were also given the test substance by gavage (500 and 2,500 mg/kg per day). The NOAEL for this substance could be set at greater than 50,000 ppm, again if the corneal lesions are assumed to be the result of a topical effect (Tomkins, 1995).

Some silicone fluids may be absorbed from the gastrointestinal tract. In one male monkey given ¹⁴C-labeled Dow Corning 360 fluid, very little absorption occurred, and more than 90% of the radioactivity was eventually recovered in the feces (Vogel, 1972). On the other hand, in rats repeatedly given octamethylcyclotetrasiloxane (D₄) approximately 23-33% of the silicone species were detected in urine, and less than 0.3% was found in the feces (possibly resulting from contamination by urine) (Malczewski et al., 1988). Metabolites originating from exposure to D₄ are under investigation (Varaprath et al., 1997)¹⁰, as are studies designed to clarify whether inducers of hepatic drug-metabolizing enzymes alter its metabolism (Plotzke and Salyers, 1997). In commenting on the results of these studies at the Institute of Medicine (IOM) scientific workshop, Meeks noted that these metabolic changes were similar to those induced by common sedatives (McKim 1995, 1996a,b; see R. Meeks, IOM scientific workshop, 1998).

Some early studies examined the carcinogenicity of orally administered silicone compounds. Rowe et al. (1950) fed Dow Corning Antifoam A at a concentration of 0.3% to rats over their lifetime. Survival and growth rate were not affected. However, survival rates, in both controls and exposed animals were not very good by today's standards. No tumors were found, but the low survival rate and the use of only one dose that did not approach a maximum tolerated dose, which is required in current practice, make this negative study inconclusive (Rowe et al., 1950). Carson et al. (1966) fed Dow Corning Antifoam A and Dow Corning 360 fluid, 50 and 350 cS, at 1% of diet to rabbits and rats for 8 months and 1 year, respectively. They observed no differences in body weight, organ weight, hematological, urine, or serum chemistry tests, the microscopic examination of organs, or overall survival between control or experimental groups. Earlier, Kimura et al. (1964) had reviewed studies of methylpolysiloxane.

¹⁰ Mentor Annotation: The full-length, peer-reviewed findings of this investigation were published by Varaprath et al. in 1999.

A silicone antifoam compound consisting of a mixture of 6% finely divided (amorphous) silicon dioxide and 94% PDMS was administered in the diet, at concentrations of 0.25 and 2.5% to male and female outbred mice, respectively (Cutler et al., 1974). This experiment was begun at weaning and terminated 76 weeks later. In the same study, some animals received a single subcutaneous injection of 0.2 ml silicone or 0.2 ml paraffin. All visibly altered tissues as well as lung, heart, stomach, small intestine, spleen, liver, and kidney from about ten male and female mice in each treatment group were examined microscopically. No treatment-related increase in nonneoplastic or neoplastic lesions was found. Cysts and some fibromas were observed at the injection site in mice injected with silicone oil or paraffin, the latter producing fibromas more frequently than the former. Although carcinogenesis was not observed at the dose levels examined, this study performed in 1974 would not fulfill today's criteria for a carcinogenesis bioassay. The study was terminated early, histopathology was incomplete, and no indication was given of how close the higher dose used was to a maximum tolerated dose.

Although the studies of polydimethylsilicone reviewed so far offer little evidence of toxicity, this is not true for all silicone compounds. A series of papers, published in the early 1970s, provides experimental evidence that certain organosiloxanes have estrogenic activity. Several agents were evaluated. The most active of them was *cis*-2,6-diphenylhexamethylcyclotetrasiloxane. This and similar chemicals caused an array of effects in the reproductive systems of male animals and on reproduction in female animals (Bennett et al., 1972; Hayden and Barlow, 1972; Hobbs et al., 1972; LeFevre et al., 1972; LeVier and Boley, 1975; LeVier and Jankowiak, 1975; LeVier et al., 1975; Nicander, 1975). Some human data are available from patients with prostate cancer. The biological half-life varied between 14 and 23 hours (Pilbrandt and Strindberg, 1975). As noted earlier in this chapter, women with breast implants and some recent investigators have expressed an interest in these compounds. However, the toxic effects of these compounds have not been observed in experimental silicone gel implant toxicological studies, and there is no evidence that they are present in silicone breast implants.

Inhalation Exposure¹¹

Because silicone compounds are present in hairspray and shampoo, adverse health effects following inhalation of these compounds have been explored. The toxicity of aerosolized D₄ was evaluated, first in a dose-setting study of four weeks' duration, then in a three-month study (Kolesar, 1995a,b). Exposures were six hours a day, five days a week at concentrations of D₄ ranging from 200 to 1,333 ppm (2.4-15.8 g/m³, grams per cubic meter) eventually reduced in the three-month study to 12 g/m³ (1,000 ppm). The animals were observed for clinical signs of toxicity, and food consumption was monitored. A few animals died during the first week when exposed to 15 g/m³, necessitating reduction of the dose to 12 g/m³. No treatment-related clinical signs were observed at the lower dose levels, but changes in hematology and clinical chemistry were seen. Enlargement of the liver and its cells was dose dependent and more pronounced in females. Changes in the respiratory tract were interpreted as adaptive responses to mild irritation. In females exposed to the highest concentration (12 g/m³), minimal to marked vaginal mucification accompanied by moderate degrees of ovarian atrophy was noted. A separate group of animals was allowed to recover in air for one month following the exposure. Practically all of the abnormalities eventually disappeared, indicating reversibility of the effects of exposure. These exposures are considered quite high.

In a later study, Fischer 344 rats were exposed to D₄ at concentrations ranging from 7 to 540 ppm (80 mg to 6.4 g/m³) for six hours a day, 5 days a week, for 28 days (Klykken et al., 1997). In addition to the usual endpoints measured, immune function was assessed by splenic antibody-forming assay and enzyme-linked immunosorbent assay (ELISA). The only change noted was liver enlargement, which was reversible after a two-week recovery period in male rats exposed to 540 ppm and females exposed to 20-540 ppm (0.24 to 6.4 g/m³). No immune system changes were observed.

¹¹ Mentor Annotation: The results from state-of-the-art inhalation studies of D₄ and D₅ in experimental animals have provided the most sensitive toxicity endpoints as the basis for establishing the no-observable-adverse-effect levels used in Mentor risk assessments (to be included in the upcoming Chemistry module of this PMA submission).

For D₄, the most sensitive toxicity endpoint observed in rodent bioassays has been a dose-related increase in liver weights (reversible following removal of exposure). In the inhalation toxicity study of D₄ reported by Klykken et al. (1999), the lowest-observable-adverse-effect level (LOAEL) was found to be 0.24 mg/L and the no-observable-adverse-effect level (NOAEL) to be 0.085 mg/L for an exposure period of 6h/day, 5d/wk for 28 days in Fischer 344 rats. In a study evaluating the retention, distribution, metabolism and excretion of D₄ in Fischer 344 rats, Plotzke et al. (2000) reported that 5 to 6 percent of an inhaled dose is retained. Assuming a body weight of 350 g, a minute ventilation rate for rats of 240 mL (Hayes 2001), and 5 percent retention (Plotzke et al. 2000), the NOAEL is equivalent to approximately 1.05 mg D₄/kg body weight/day.

For D₅, a similar 28-day inhalation toxicity study in Fischer 344 rats reported by Burns-Naas et al. (1998) identified a NOAEL (also based on reversible increase in liver weight) of 1.14 mg/L. The exposure period was the same as for the D₄ study described above, 6hr/d, 5d/wk for 28 days. Assuming a body weight of 350 g, a minute ventilation rate for rats of 240 mL (Hayes 2001), and 5 percent retention (based on the D₄ data of Plotzke et al. 2000), the NOAEL is equivalent to approximately 14 mg D₅/kg body weight/day.

This protocol was repeated with D₅, except that exposures ranged from 0.4 to 3.5 g/m³ (expressed as milligrams per liter in the original, 27-240 ppm) (Kolesar, 1995c,d). At one month, all animals survived and gained weight normally. Upon termination of the study, only slight interstitial inflammation in the lung and some liver cell enlargement were noted in the highest-dose group. In the three-month study, reduced weight gain was observed in the highest-dose group. Hematology, clinical chemistry, and urinalysis were unremarkable. Histopathological changes were observed in the lungs of animals exposed to the higher concentrations of D₅, both those killed immediately after exposure and those allowed to recover for an additional month in air. More frequent interstitial ovarian and vaginal lesions were also seen in the highest-dose group. Exposures used in all these studies were quite high, perhaps unrealistically so.

The effects of inhaled D₄ and D₅ were also evaluated in reproductive toxicity tests. Male and female rats were exposed to D₄ concentrations ranging from 70 to 700 ppm (0.83-8.3 g/m³) for six hours a day for a minimum of 28 days or for 70 days prior to mating. Exposure continued throughout the gestation and lactation periods (except on day 21 of gestation and days 1-4 of lactation). Offspring were further exposed following weaning on day 21 until day 28. They were thus potentially exposed to the test agent while in utero, throughout suckling, via inhalation or dermal contact during lactation, and via inhalation after weaning. Maternal toxicity consisting of slight reduction in body weight gain and hepatomegaly at autopsy was observed at dose levels of 300, 500, and 700 ppm (3.5, 5.9, and 8.3 g/m³). In the highest-dose group, there was a consistent and reproducible reduction in fetal implantation sites and a decrease in mean live litter size. In the offspring, no exposure-related signs of toxicity were observed (Stump, 1996a). No effect on litter size or pup viability and no signs of maternal toxicity were found in a study with D₅, when maternal animals were exposed to concentrations of 26 and 132 ppm (0.38-1.9 g/m³) (Stump, 1996b).

Decamethylcyclopentasiloxane (D₅) was also evaluated in a different laboratory (Lambing, 1996)¹². Exposures were six hours a day, seven days a week, for a total of 28 exposures, with exposure concentrations ranging from 10 to 160 ppm (0.15-2.4 g/m³). A two-week recovery period was included in the experimental design. There were no test-related effects on survival, clinical condition, body weight gain, food consumption, clinical chemistry, and urinalysis at any exposure level. There were no adverse effects on immunoglobulin M (IgM) antibody response to a T-dependent antigen (sheep red blood cells). Changes noted were a 5% decrease in hematocrit, enlargement of the liver, and increased lung weight, all reversible upon cessation of exposure. Microscopically, increased alveolar macrophage accumulation and some interstitial inflammation in the lungs were observed. Goblet-cell hyperplasia was found in the nasal passages, which was thought to be reversible. If the histopathological changes

¹² Mentor Annotation: The full-length, peer-reviewed findings of this investigation were published by Burns-Naas et al. in 1998.

confined to level one in the nasal passages are taken into account, the no-observed-effect-level (NOEL) would be less than 10 ppm. A NOAEL for systemic toxicity (liver weight increase) was identified at 75 ppm (1.1 g/m³) and for immunosuppression at 160 ppm (Lambing, 1996).

Presumably because some systemic effects such as liver enlargement were observed during inhalation of D₄, a series of pharmacokinetic studies has been initiated. Rats were exposed by nose-only inhalation technique to D₄ labeled with ¹⁴C. Concentrations used ranged from 7.5 to 716 ppm (90 mg to 8.5 g/m³). The animals were killed immediately after exposure and at selected intervals thereafter up to 168 hours. The animals retained approximately 5.5% of the total radioactivity delivered. Radioactivity was found in all tissues and reached maximum levels between zero and three hours after exposures, except in fat, which seemed to serve as a depot for radioactivity. Half-times of retention for combined radioactivity ranged from 68 hours in plasma to 273 hours in various tissues. Radioactivity was mostly excreted by breathing and excretion was most rapid within the first 12 hours. An initial rapid decline followed by a longer terminal elimination phase was also observed in a study where rats were exposed for 14 days, first to unlabeled, and for 1 day to labeled, D₄ vapor (Ferdinandi and Beattie, 1996a,b, 1997). Exposures in these inhalation studies reached very high levels.

Studies have been performed to examine the implications of liver enlargement (McKim, 1995,1996a,b)¹³. Male and female rats were exposed for four weeks to D₄ at airborne concentrations of 70 and 700 ppm. Animals were killed from 3 to 28 days after exposure and after 7 and 14 days of recovery. In females, liver size increased early during exposure. At the end of the study, liver weights were approximately 110% of controls in females and 117% in males. However, following cessation of exposure, there was a rapid decrease in liver weight. Some liver enzymes and proteins were increased. It was concluded that D₄ acted like a "phenobarbital-type" inducer in rat liver. Essentially similar observations were made in studies with inhaled and oral D₅ (McKim, 1997). A metabolic study in rats showed that 75 to 80% of intravenously administered ¹⁴C labeled D₄ appeared in urine as dimethylsilicone diol, methylsiliconetriol and five other minor metabolites within 72 hours (Varaprath et al., 1997).

Because D₄ is found in personal care products such as hairsprays, shampoos, and deodorants and, together with D₅, has been found in indoor atmospheres, a potentially large number of people are exposed daily (Shields et al., 1996). Very small amounts of these compounds are found in breast implants (see Chapter 3), constituting exposures substantially lower than those possible from other, ubiquitous sources. Recent studies have examined the effects of inhaled D₄ on humans. At a concentration of 10 ppm, a one-hour inhalation did not alter human lung function. Deposition of D₄ was calculated to be around 12%.

¹³ Mentor Annotation: The full-length, peer-reviewed findings of this investigation were published by McKim et al. in 2001.

Measurement of plasma concentrations showed a rapid nonlinear blood clearance. Immune function was evaluated by several parameters, such as measurement of serum acute-phase reactants, interleukin-6 (IL-6) levels, establishment of lymphocyte subsets, blast transformation in isolated peripheral mononuclear cells, natural killer (NK) cell cytotoxicity, and in vitro production of cytokines. No signs of an immunotoxic or systemic inflammatory response were found (Looney et al., 1998; Utell et al., 1998).

The authors pointed out that their studies did not preclude possible immunological effects with exposures of longer durations or at higher concentrations. Since the route of exposure was via inhalation, the negative findings should not be relied on when assessing the immunological effects of implanted silicones in humans. Nevertheless, the low order of toxicity observed when D₄ is absorbed and distributed systemically after administration by inhalation or oral routes, tends to support the observations of lack of D₄ toxicology after systemic exposure by implant or injection. The committee did not find data that would allow comparisons between possible systemic exposure to D₄ from common consumer products to large numbers of the general population and estimated exposures from silicone gel-filled breast implants.¹⁴

In Vitro Assays

Few in vitro studies on silicone materials have been published in the open literature. The LC₅₀ of D₄, decamethyltetrasiloxane (L₄), and tetramethyl-tetravinylcyclotetrasiloxane (D'₄) on B-cell lymphoma, plasmacytoma, and macrophage cell lines ranged from 30 to 50 micromolar (8.6-14.4 mg/l, D₄). At lower concentrations, there were biochemical signs of cytotoxicity. Exposed macrophages produced more IL-6 than did untreated cells (Felix et al., 1998). On the other hand, WI-38 human fibroblast, mouse fibroblast, and Chinese hamster ovary cells, when grown in contact with silicone gel used in breast implants, were not adversely affected, even when exposed up to 12 days. Flow cytometry, a sensitive analytical technique, did not reveal any changes in cell-cycle characteristics (Cocke et al., 1987).

Results from in vitro mutagenicity assays are not conclusively negative, although they are suggestively so. Poly-*sec*-butylsilicate ester (Silicate Cluster 102, Olin Corp.) and PDMS (SF-96, G.E. Corp.) were negative in the Ames test (TA-1535, TA-100, TA-1538, and TA-98), with and without metabolic activation (Marshall et al., 1981). In 1988, it was reported that 12 silicone compounds all tested negative for genotoxicity in salmonella (Ames test), *Saccharomyces cerevisiae*, and *Escherichia coli* test systems. Hexamethyldisiloxane (L₂) and D₄ at one dose and several other compounds,

¹⁴ Mentor Annotation: Shipp et al. (2000) provided an estimate of 158 ug/kg/day for the daily intake resulting from exposure to D₄ in a wide variety of personal care products. Owing to currently reduced use of D₄ in roll-on antiperspirants, a current conservative estimate of daily intake from personal care products is 78 ug/kg/day (Meeks 2002).

among them methyltriethoxysiloxane, produced sister chromatid exchange, although often no dose-response relationship was found, and the results were considered inconsistent. Chromosome aberrations were also found with some of the compounds (Isquith et al., 1988). In an evaluation of the mutagenicity of Dow Corning 7-9172 Part A (used to make gel) with several tester strains, with and without metabolic activation systems, no positive responses were found (Isquith, 1992). Six siloxanes were recently examined for mutagenic activity in rat fibroblasts (Felix et al., 1998). Only one compound, tetravinyltetramethylcyclotetrasiloxane was found to give a weak positive response. The study was prompted by the observation that silicones could produce plasmacytomas in highly sensitive mouse strains. Since only one compound was found to be mutagenic, it was concluded that possible nonmutagenic mechanisms might also be responsible for plasmacytoma development.¹⁵

PLATINUM

The potential toxicity of several platinum compounds has received some attention because they have been used as catalysts in the manufacture of silicone gels and solids. Platinum is present in small amounts in implants (see Chapter 3, in which the amount of platinum and the question of its form are discussed). Reports that this platinum is in the form of platinate (Lykissa et al., 1997) are unconfirmed (Lewis and Lewis, 1989; Lewis et al., 1997). Inhalation of platinum compounds is recognized as a problem in the smelting and refining industry. Platinum can produce chemical pneumonitis (Furst and Rading, 1998). Inhalation of complex salts of platinum, but not elemental platinum, can cause progressive allergic and asthmatic reactions. Skin contact with platinum, particularly its chlorides, which are powerful skin sensitizers, can cause contact dermatitis (American Conference of Governmental Industrial Hygienists, 1998). Cisplatin, an agent used in cancer chemotherapy, is highly toxic to the gastrointestinal tract, kidney, bone marrow, and peripheral nervous system. This compound does not occur in silicone breast implants, however.

Early toxicity tests, conducted on a minimum number of animals, showed little if any signs of toxicity for two platinum compounds, Dow Corning Platinum Nos. 1 and 2 (Groh, 1973). Acute oral toxicity was greater than 6.8 g/kg, and upon instillation of the liquids into the eyes of rabbits, only a slight and transient irritation was noted. Moderate to marked skin alterations were seen after repeated application of the undiluted substances. Edema and hyperemia were mentioned, but without any quantitative scores. Studies with Dow Corning X-2-

¹⁵ Mentor Annotation: Vergnes et al. (2000) published a very thorough evaluation of the genotoxicity of octamethylcyclotetrasiloxane (OMCTS or D₄). Their report included the results of both in vitro assays (bacterial mutagenicity, in vitro chromosomal aberration in CHO cells, sister chromatid exchange in CHO cells) and in vivo assays (in vivo chromosomal aberrations in rat bone marrow). The study authors concluded that "the results of these studies indicate that OMCTS does not possess significant in vitro genotoxic potential" and that "no adverse genetic findings were seen in the in vivo screen for chromosome aberrations."

7018 gave essentially similar results (Groh, 1972). The platinum catalysts, when compounded into an elastomer, were nontoxic to human embryonic lung cells in tissue culture. However, in liquid form, the catalysts were toxic, although this effect was abolished for Platinum No. 2 by heating. This seems to indicate that compounding might eliminate toxicity by inactivating reactive sites (Jackson, 1972). The oral toxicity of TX-82-4020-02 (H_2PtCl_6 reacted with tetramethyldivinylsiloxane and then diluted with Dow Corning SFD-119 fluid) was greater than 20 g/kg, and no signs of toxicity were observed during a two-week observation period or upon autopsy of rats (de Vries and Siddiqui, 1982).

BALB/c female mice received injections of ammonium hexachloroplatinate in the left footpad. Comparison of the weight of left popliteal lymph nodes with nodes collected from the right hind leg showed that five, six, and seven days later, the weight of the lymph nodes was increased. This was taken as evidence that platinum in its multi-valent state has immunogenic potential (Galbraith et al., 1993). The skin sensitizing potential of Platinum Nos. 2 and 4 was recently examined in a study with guinea pigs (Findlay and Krueger, 1996a,b). On day 1 of the test, the guinea pigs received six intradermal injections of Dow Corning 2-0707 Intermediate (Platinum No. 4) or Dow Corning 3-8015 (Platinum No. 2) intradermally into the skin of the back over the shoulder region. Negative (phosphate-buffered saline) and positive (1-chloro-2,4-nitrobenzene) controls were similarly injected. On days 7 and 8, the same agents were reapplied, this time topically and under occlusion. A first challenge was applied on day 22 and a second challenge on day 29; 24 and 48 hours after the challenge doses, the skin was examined and scored for signs of irritation with a quantitative procedure (Draize scale). For this experiment, both agents were found to be moderate skin sensitizers in guinea pigs although previous studies were said to be inconsistent with this result (Lane et al., 1998).¹⁶ Available data provide little evidence that the platinum catalysts would have a particular systemic toxicity.

¹⁶ Mentor Annotation: More definitive data regarding the potential for sensitization in humans to the platinum catalyst used in the manufacture of silicone medical devices was provided in an October 2000 submission to the U.S. EPA (OTS0559082-1) by Dow Corning Corporation presenting the results of repeat insult patch testing in human subjects (Galvin 1999a, b): "Repeated human insult patch studies were conducted on both platinum intermediates [DOW CORNING® 3-8015 Int and DOW CORNING® 2-0707 Int] to assess their potential to cause skin sensitization by long repeated topical applications of test substance to the skin of selected subjects. The repeated insult patch test is a predictive patch study that can detect weak human sensitizers which require multiple applications to induce a cell-mediated (Type IV) immune response sufficient to cause an allergic reaction. Each study was comprised of three phases: (1) induction, (2) rest, and (3) challenge. The induction phase consisted of nine consecutive applications of the test substance and subsequent evaluation of patch sites. Semi-occlusive 2cm x 2 cm patches were applied to the infrascapular area of the back to the left or right of the midline. Following the ninth evaluation, the subjects were dismissed for a 14-day rest period. The challenge phase was initiated during the sixth week of the study, with identical patches applied to sites previously unexposed to the study material. These patches were removed after 24 hours and the sites graded after an additional 24 and 48 hours, i.e., 48 and 72 hours after application. One hundred four subjects completed the study. Under the conditions employed in these studies, there was no evidence of skin sensitization in humans to DOW CORNING® 3-8015 Int (Platinum 2) and DOW CORNING® 2-0707 Int (Platinum 4)."

They may have sensitizing potential, but it is not clear whether this is a function of the platinum itself or of the entire molecule.

Harbut and Churchill (1999) reported a small case series of eight women with the onset of asthma at varying intervals after placement of silicone breast implants. These authors speculated that the respiratory signs and symptoms were the result of exposure to hexachloroplatinate in their implants. No evidence for this was reported. Conclusions regarding platinum toxicity in women with breast implants should await evaluations that positively relate platinum to the symptomatology; these might include some or all of elevated serum platinum levels, positive skin prick tests for platinate, positive radioallergosorbent (RAST) or other tests for platinum-specific antibodies, remission of allergic symptoms or reduction of serum platinum levels or skin prick or other allergic tests on explantation in women with no other known exposures to platinum (Biagini et al., 1985; Rosner and Merget, 1990). Absent these tests, diagnoses of platinum toxicity in women with implants are speculative only. Since allergies and asthma are extremely common in the general population, they should be common in women with breast implants, yet epidemiological studies do not report this. These complaints are not prominent in lists of problems with breast implant patients (see Appendix B of this report), and one cohort study of 222 women with breast implants and 80 control women without implants found breathing difficulties to be significantly less frequent ($p < 0.05$) in the women with silicone breast implants (K.E. Wells et al., 1994). It should also be kept in mind that platinum exposure from vehicle exhaust catalysts is increasing and is reflected in serum levels but not in any known health condition (Farago et al., 1998). The committee could not find any such positive platinum-specific evaluations in women with breast implants and thus finds that evidence is lacking for an association between platinum in silicone breast implants and local or systemic health effects in women who have these implants. If the platinum in breast implants is in zero valence form in the final cured state in excess vinyl as reported by Stein et al. (1999), and if it is in microgram quantities as is usually added to gel (Lane et al., 1998), as the current evidence suggests, then a biologically plausible rationale for platinum related health problems in women with silicone breast implants does not presently exist. Many silicone-containing implants other than breast implants (listed in Chapter 2) are found at high frequency in the general population and presumably contain platinum also; the committee is not aware of any evidence that platinum toxicity is present in these persons.

TIN¹⁷

The committee reviewed information bearing on the possible effect of tin on the safety of silicone breast implants. Stannous octoate, stannous oleate or dibutyltin dilaurate catalysts are generally involved in formulation of only part

¹⁷ Mentor Annotation: Tin catalysts are not used in the manufacture of Mentor Silicone Gel-Filled Mammary Prostheses.

of an implant, e.g., the adhesive sealant in the case of Dow Corning and McGhan Medical or the RTV elastomer shells of saline implants in the case of Mentor and McGhan Medical Corporations. HTV gel-filled shells are platinum catalyzed (B. Purkait, personal communication, Mentor Corporation, May 1999; Eschbach and Schuiz, 1994). Tin has been added at low concentrations (e.g., 0.038 stannous oleate to formulate adhesives [about 1.4 ug of tin per Dow Corning implant] or targeted at 70-80 parts per million tin from dibutyltin dilaurate in the case of Mentor saline implant shells and about the same in the case of McGhan shells). Tin has been analyzed at non detectable to 0.73 ppm in saline or dichloromethane extracts of Dow Corning implant silicone gel (J. M. Curtis, Dow Corning, personal communication, May 11, 1999, Lane et al., 1998) or non detectable by inductively coupled plasma atomic emission spectroscopy and cold vapor atomic absorption spectroscopy in saline, ethanol, methylene chloride or hexane extracts in the case of Mentor implant shells (B. Purkait, Mentor Corporation, personal communication, May 1999), or measured within a range of 15 to 100 parts per million in saline shells and non detectable in saline extracts of shell elastomer by inductively coupled plasma atomic emission spectroscopy (R. Duhamel, McGhan Medical Corporation, personal communication, 1999). Normal tissue concentrations of tin can be higher than the levels in implants (0.25-130 ppm, Clayton and Clayton, 1994). Total tin in an average implant¹⁸, therefore, could vary from 1 or 2 ug to 10 mg as an upper limit in Dow Corning, Mentor or McGhan implants.

The toxicology of inorganic and organic tin was reviewed extensively for the U.S. Public Health Service (Agency for Toxic Substances and Disease Registry, 1992) and a few studies of particular tin soap catalysts are available from industry. Human data for organotins are sparse to nonexistent as are experimental animal data on parenteral exposures. The human permissible industrial exposure limit for organotin of 0.1 mg/m³ calculates to a maximum exposure of 14.3 mg/kg per day (American Council of Governmental Industrial Hygienists, 1998). In general, animal data indicate oral toxic levels at more than 10 mg (for the most toxic), although absorption of oral doses is poor, and on inhalation no observable adverse effect levels (NOAELs) over 1 mg/m³. RTV elastomer with stannous octoate was implanted under the skin, intraperitoneally and subdurally in rats. Although no toxic or carcinogenic effects were observed over 22 months, this early study was not designed to examine tin toxicity (Agnew et al., 1962). Other similar implant studies of stannous octoate catalyzed elastomers were also negative but were not designed to evaluate tin (Nedelman, 1968). Likewise, Dow Corning elastomers with 1, 3 and 5 stannous octoate were implanted subcutaneously and intramuscularly in rabbits for 10 or 30 days, and no clear dose response was observed, only the usual foreign body

¹⁸ This depends on saline shell weights which are quite variable, ranging it is said, from a lower limit of 5 g (B. Purkait, Mentor Corporation, personal communication, 1999) to 10 to 30 g (J.M. Curtis, Dow Corning Corporation, personal communication, 1999) to a maximum upper limit as high as 100 g (with a lower average value; R. Duhamel, McGhan Medical Corporation, personal communication, 1999). Also, these weights are dependent on implant model and whether the shell is smooth or textured.

reaction. In another Dow Corning study, the oral LD₅₀ was 3.4g/kg (R. Meeks, Dow Corning, personal communication, 1999). Studies of dibutyltin dilaurate found LD₅₀ levels ranging from 85 mg/kg intraperitoneally to between 175 and 1240 mg/kg body weight orally. In general, these substances were not carcinogenic (Agency for Toxic Substances and Disease Registry, 1992; American Conference of Governmental Industrial Hygienists, 1998; Clayton and Clayton, 1994; Hazardous Substances Data Bank; Mellon Institute of Industrial Research, 1994; National Cancer Institute). These data suggest that toxic effects of even the most toxic (triorganotins—which have not been found in breast implants) tin compounds are seen at doses above those possible from breast implants even in the most unlikely event of complete release of all the tin into the breast. Moreover, the tin in breast implants appears to be of relatively low toxicity among organic tin compounds, and given the difficulty in extracting it, as noted above, and the durability of silicone elastomer, as noted elsewhere in this report, unlikely to be significantly available to surrounding tissues. The committee concluded that there is currently no evidence for toxic effects of retained tin catalysts at the very low exposures likely from silicone breast implants.

CONCLUSIONS

Historically, silicone toxicology has tended to focus on short-term, acute and subacute studies and has suffered from a proportionate dearth of chronic, lifetime, and immunologic studies, as noted earlier in this chapter. Presumably, this reflects early conclusions that silicones were inert. Some silicones have clear biological effects. None can be said to be inert, if this implies an absence of tissue reaction, but the term has perhaps been used as a proxy to indicate that the toxicity of many silicones is of such low order that they comprise a useful class of biomaterials for medical implants.

Older silicone toxicology studies have deficiencies by current standards, but the body of toxicological information is substantial and improving. More chronic studies are being done, although modern regulatory requirements will undoubtedly generate a closer identification of silicones (and other substances) in implants and more specific toxicological studies of appropriate duration. Nevertheless, no significant toxicity has been uncovered by studies of individual compounds found in breast implants. Toxicology studies have examined carcinogenic, reproductive, mutagenic, teratologic, immunotoxic, and local and general toxic and organ effects by exposure routes that are varied and range to very high dose levels. Even challenges by doses that are many orders of magnitude higher than could be achieved on a relative-weight basis in women with silicone breast implants are reassuring. Toxic effects that have been found occur at very high, even extreme, exposure levels (e.g., D₄, D₅). The fact that some organic silicon compounds may have, as one would expect with any large family of chemical compounds, biologic or toxicologic effects is not relevant to

women with breast implants since these compounds are not found in breast implants, as noted here and in Chapter 2.

Studies using whole fluids, gels, elastomers, or experimental implant models injected or implanted in ways that are directly relevant to the human experience with implants are also reassuring. These studies show that depots of gel, whether free or in implants, remain almost entirely where injected or implanted. Even low molecular weight cyclic and linear silicone fluids appear to have low mobility. Half-lives of low molecular weight silicones in body fluids and tissues have been measured infrequently, but known values appear to be on the order of 1 to 10 days. In general, there do not appear to be long-term systemic toxic effects from silicone gel implants or from unsuspected compounds in these gels or elastomers detected by these animal experiments.

Some have speculated that platinum found in silicone gel and elastomer may be responsible for allergic disease in women with silicone breast implants. Very little platinum, microgram quantities, is present in implants, most investigators believe it to be in the zero valence state, and it likely diffuses through the shell at least over a considerable period of time. Evidence for resulting systemic disease at such exposures is lacking.

Toxicological studies of tin compounds used in silicone breast implants are scarce, and generally not of parenterally administered tin. The data on organotins indicate that tin catalysts are among the less toxic, and they have not been extractable from implants shells by saline and some organic solvents. Based on the data available, the committee concluded that evidence is also lacking for tin toxicity at the very low amounts present in saline implants and at the virtually absent levels in gel filled implants.

[NOTE - Bibliography of references cited in the chapter above and by Mentor can be found at the very end of this literature review section.]

Mentor Corporation concurs with the IOM committee's findings that "there do not appear to be long-term systemic toxic effects from silicone gel implants or from unsuspected compounds in these gels or elastomers detected by these animal experiments," and that there is no credible evidence linking platinum in silicone gel and elastomer with systemic disease.

In addition to the extensive IOM review of preclinical data presented above for a wide range of silicone materials (as well as platinum), discussion is provided below of safety information for [REDACTED] materials and xylene, the primary solvent used by Mentor in the manufacture of silicone gel-filled mammary prostheses.

[REDACTED] Materials

As noted in the IOM report, the low-bleed shells of Mentor silicone gel-filled mammary prostheses are constructed of [REDACTED] elastomer. No pertinent literature data on this copolymer and the raw materials used in its manufacture were identified. As part of Mentor's overall safety testing program for this device, both short- and long-term preclinical testing of the [REDACTED] has been performed. The results of this testing, which did not reveal any significant adverse effects, are included elsewhere in this PMA module.

Xylene

1. Introduction

Xylene is a colorless, volatile solvent with a sweet odor. It has a molecular weight of 106.16 and a boiling point between 137 and 140°C. It is practically insoluble in water, but is miscible with absolute alcohol, ether, and other organic liquids (ATSDR 1995; HSDB 2002). Xylene is used in solvents and thinners, in chemical synthesis, as an ingredient in the coating of fabrics and papers, in the manufacture of polymers and pharmaceuticals, and as an ingredient in airplane fuel and gasoline (ATSDR 1995). There are three isomeric forms of xylene: meta-xylene (m-xylene), para-xylene (p-xylene), and ortho-xylene (o-xylene). Mixed xylene, the commercial product, is a mixture of the three isomers.

This review of the toxicity of xylene focuses on data most relevant to the type of exposure associated with the medical device.

2. Pharmacokinetics

Xylene is well absorbed after oral and inhalation exposure (ATSDR 1995). Absorption following ingestion has been shown to occur in humans but has not been quantified. Animal data indicate that xylene is almost completely absorbed (87-92%) following an oral dose of 1.7-1.8 g (ATSDR 1995). Results of studies in human subjects indicated that 50 to 73% of inhaled xylene is retained. Dermal absorption of xylene vapor is considered minor compared to that absorbed through the lungs, although dermal absorption associated with direct skin contact may be significant (Forsyth and Faust 1994).

Xylene is very soluble in blood and is rapidly distributed throughout the body. Due to its lipophilic nature, xylene is primarily distributed to adipose tissue and, at least in animals, to lipid-rich tissues of the brain, fat, and blood and to well-perfused organs such as the liver and kidney (ATSDR 1995).

Regardless of route, isomer, or administered amount, xylene is predominately metabolized by mixed function oxidases in the liver via oxidation of the side-chain methyl group to methylbenzoic acids, which are then excreted in urine free or conjugated with glycerine as methyl hippuric acids (ATSDR 1995; Forsyth and Faust 1994). In humans and animals, about 80-95% of absorbed xylene is excreted as urinary metabolites and approximately 5% is released unchanged through exhaled breath (ATSDR 1995; Forsyth and Faust 1994).

3. Noncarcinogenic Health Effects

a. Acute Toxicity

Data on the acute toxicity of xylene in humans come from case reports of accidental and occupational exposures. Poisoning due to accidental ingestion of a paint thinner containing 90% xylene resulted in hepatitis, which reversed within 20 days (NIOSH 1975). Acute ingestion of unknown quantities of xylene has been reported to produce coma, severe gastrointestinal discomfort and to possibly lead to death due to respiratory failure in humans (Forsyth and Faust 1994). If aspiration into the lungs occurs, chemical pneumonitis, pulmonary edema, and hemorrhage may result (NIOSH 1975). Transient skin irritation, vasodilation, and dryness and scaling of the skin have been associated with acute dermal exposure to m-xylene in hand immersion studies (ATSDR 1995). Eczema and skin irritation have been reported in workers who have come in contact with xylene solvents (Low 1989).

The acute toxicity of xylene is classified as slight, based on the toxicity classification scheme of Casarett and Doull's Toxicology (Doull et al. 1980). Rabbits and guinea pigs treated topically with mixed xylenes showed signs of mild to moderate skin irritation (ATSDR 1995).

Reported acute oral LD50 values in rats for mixed xylenes range from 3,523 mg/kg to 8,600 mg/kg. In mice, the acute oral LD50 for mixed xylene is 5,627 mg/kg for males and 5,251 mg/kg for females (ATSDR 1995). Symptoms of acute oral exposure in experimental animals include impaired visual function, increased liver weights, and central nervous system effects including tremor, prostration, hunched posture, loss of hind leg movement, and labored breathing (ATSDR 1995; NTP 1986).

b. Subchronic and Chronic Toxicity

Repeated dermal exposure of humans to xylene may cause drying and defatting of the skin, leading possibly to dermatitis (Cavender 1994). No other human studies involving subchronic or chronic exposure to xylenes were located.

Rats administered 200 and 800 mg/kg/day m- and p-xylene by gavage for 13 weeks exhibited no treatment related effects other than increased salivation, decreased average body weight, and

decreased heart weight at the high dose (ATSDR 1995). No adverse effects were observed in rats administered up to 1,000 mg/kg/day mixed xylene by gavage for 13 weeks. Mice receiving 2,000 mg/kg/day mixed xylene by gavage developed short and shallow breathing, unsteadiness, tremors and paresis within 5 to 10 minutes of exposure (NTP 1986). Enlarged livers and kidneys have been observed in rats exposed to 150, 750, or 1,500 mg/kg/day mixed xylenes by gavage for 90 days (Forsyth and Faust 1994). Intermediate dermal exposure to undiluted xylene produced dryness, scaling and localized edema of the skin in mice (ATSDR 1995).

The National Toxicology Program (NTP 1986) conducted a two-year gavage study of mixed xylene in rats and mice. Decreased body weight gain and increased mortality were observed in rats exposed to 250 or 500 mg/kg/day. Mice exposed to 1,000 mg/kg/day exhibited hyperactivity. No compound-related histopathological lesions were observed in any of the treated animals (NTP 1986). The NOAEL for male rats in these studies was 250 mg/kg/day. The results of this bioassay were used by the U.S. Environmental Protection Agency to derive an oral reference dose (RfD) for xylene (U.S. EPA 2002).

c. Reproductive and Developmental Effects

No human data useful for assessing the potential reproductive or developmental toxicity of xylene are available (ATSDR 1995).

No reproductive effects were found in rats following inhalation exposure to mixed xylenes during pre-mating, mating, pregnancy, and lactation at xylene vapor concentrations as high as 500 ppm (ATSDR 1995). ATSDR (1995) noted that histopathological examination of reproductive organs following subchronic and chronic exposure to xylene (including the 1986 bioassay conducted by NTP) revealed no effects.

Several studies involving oral, inhalation and dermal exposure to xylene suggest that xylene may be associated with fetotoxic effects, although a number of the available studies are limited and most of the effects may have been secondary to maternal toxicity (ATSDR 1995).

An increased incidence of cleft palate and decreased fetal weight were reported in rats following maternal exposure by oral gavage to 2,060 mg/kg/day mixed xylene in cottonseed oil on gestation days 6-15. Maternal toxicity (31.5% mortality) was evident at a dose of 3,100 mg/kg/day. Doses of 1,030 and 520 mg/kg/day resulted in no apparent maternal or reproductive toxicity (Marks et al. 1982, as cited in ATSDR 1995). Nawrot and Staples (1980, as cited in Forsyth and Faust 1994) evaluated the developmental toxicity of individual isomers of xylene in the mouse. Mice administered 3,000 mg/kg/day of m-xylene on gestation days 6 to 15 exhibited a significantly increased incidence of resorptions and overt maternal toxicity. These effects as well as an increased incidence of cleft palate were observed in offspring of mice receiving 2,250 and 3,000 mg/kg/day o- and p-xylene. No apparent fetal or maternal toxicity was observed at 900 mg/kg/day with any isomer (Nawrot and Staples 1980, as cited in Forsyth and Faust 1994).

Inhalation studies in rats and mice indicate that exposure to vapors of mixed xylene or xylene isomers may induce increased fetal death, decreased fetal weight, delayed skeletal

development, skeletal anomalies, enzymatic changes in fetal organs, and maternal toxicity (ATSDR 1995). These studies showed a large variation in air concentrations of xylene producing developmental effects and those producing no developmental effects, and may be due to such factors as interspecies or interstrain differences in sensitivity, purity of xylene, exposure duration, endpoint measured, concurrent maternal toxicity, and study quality (ATSDR 1995).

No evidence of fetal toxicity was seen in rats exposed dermally to a 1% solution of xylene by the coapplication of a surfactant (ATSDR 1995). Decreased enzyme activity in fetal and maternal brain tissue and impaired motor activity in dams was reported by Russian investigators (Mirkova et al. 1979, as cited in ATSDR 1995) following dermal exposure to 200 mg/kg/day. ATSDR (1995) noted deficiencies (including inadequate information on dosing schedule and xylene composition) in this study.

Based on the developmental toxicity data for xylene following oral administration in experimental animals, it would appear that the oral NOAEL for developmental effects of xylene is 1,030 mg/kg/day, and the LOAEL is 2,060 mg/kg/day.

4. Carcinogenicity

The available data indicate that xylene is not genotoxic in humans and animals. Xylene did not induce sister chromatid exchanges or chromosomal aberrations in two occupational studies (ATSDR 1995). Dominant lethal mutations or chromosomal abnormalities were not induced in rats orally exposed to xylene (Reprotext 1994). Xylene was not mutagenic in bacteria, yeast, mammalian, and other in vitro and in vivo assays (ATSDR 1995).

Data regarding the development of cancer in humans following exposure to mixed xylene or individual isomers are limited to occupational studies. Because of limitations in the available epidemiologic studies, conclusions cannot be reached regarding the carcinogenicity of xylene associated with inhalation exposure (ATSDR 1995). No information was located regarding carcinogenic effects in humans following ingestion or dermal exposure to xylene.

Animal carcinogenicity data are limited to oral studies with mixed xylene and dermal studies in which the isomeric composition of xylene is not known. In a two-year study conducted by NTP (1986), there was no evidence of carcinogenicity in rats or mice at any dose tested. Animals were administered mixed xylene at doses of 250 or 500 mg/kg/day (rats) or 500 or 1,000 mg/kg/day (mice) in corn oil for 103 weeks. Maltoni et al. (1985) administered 500 mg/kg/day by gavage in olive oil to rats for 104 weeks. A higher incidence of malignant tumors in males and females than in controls was reported; however, the results of this study provide insufficient evidence of carcinogenicity because survival rates and specific tumor types were not provided (U.S. EPA 2002). Undiluted xylene applied to the skin of mice for 25 weeks produced tumors in 1 of 40 mice (Forsyth and Faust 1994). Negative results were reported in initiation-promotion experiments with xylene as the initiator and croton oil as the promoter (Forsyth and Faust 1994). U.S. EPA (2002) categorizes xylene as a Group D carcinogen, not classifiable as to human carcinogenicity.

5. Safety Assessment

U.S. EPA (2002) has derived an oral RfD for xylene of 2 mg/kg/day. This RfD is derived from a two-year study conducted by NTP. As stated above, rats were administered 250 or 500 mg/kg/day and mice were administered 500 or 1,000 mg/kg/day mixed xylenes by gavage for 103 weeks. A slight decrease in body weight gain was observed in rats at the high dose. There was a dose-related increased mortality in male rats, which was significantly greater in the high dose group compared to controls. Mice given 1,000 mg/kg/day exhibited hyperactivity. There were no compound-related histopathological lesions in any of the treated animals. Therefore, the high dose in the rat study is considered a frank effect level (FEL) and the low dose a NOAEL. An uncertainty factor of 100 for species-to-species extrapolation and to protect sensitive individuals was applied.

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DESCRIPTION OF STANDARD BIOLOGICAL TEST PROCEDURES USED BY MENTOR

In order to perform many of the raw material qualification tests and some of the final device qualification tests, Mentor contracts with outside laboratories to perform widely accepted basic toxicity/biocompatibility tests usually per USP and/or ISO guidelines. Because these standard tests were performed by a limited number of vendors, and are repeatedly performed for most of the raw materials used in or during the manufacturing process, this section of the submission will generically describe how the tests were performed. The actual results of the tests can be found in the Raw Materials Testing Section and Finished Product Testing Section of this PMA section.

NAmSA is Mentor's primary contract laboratory for performing lot-to-lot biological testing associated with incoming inspections and for some standard finished product tests. Other laboratories have been used on occasion for selected tests.

Material extractions are required for several of the qualification tests. The ratios for extraction have been established by USP and are provided below:

Material Thickness:

< 0.5 mm - ratio of 120 cm²: 20 mL

≥ 0.5 mm - ratio of 60 cm²: 20 mL

Irregularly shaped objects - ratio of 4 grams: 20 mL

Standard Extraction Vehicles:

Sodium Chloride (saline)

Cottonseed oil (CSO)

Temperature and Time:

Extraction in 0.9% sodium chloride and cottonseed oil (distilled water also) are usually performed at one of four standard temperatures and times:

121°C for 1 hour

70°C for 24 hours

50°C for 72 hours

37°C for 72 hours

The temperature selected is based on the heat resistance of the material. Extractions in cell culture media (Minimum Essential Medium - MEM) are performed at 37°C for 24 hours.

The biocompatibility test systems applied here are designed to subject the material to a variety of conditions simulating the environment found within the human body. These tests range from acute toxicity to 90-day implantation. Completion of these tests provides assurance that the

material is biocompatible and may be safely used as part of gel-filled mammary prostheses. A description of the biocompatibility tests performed in the biological qualification of a material or component is shown below.

MEM Elution

Objective: To evaluate the cytotoxicity of a test material extract using an in-vitro mammalian cell culture test.

Procedure: The test article is extracted in Minimum Essential Medium (MEM) for 24 hours at 37°C per USP Guidelines. A portion of the extract is placed in direct contact with a confluent monolayer of L-929 Mouse Fibroblast cells. As a negative control, cells are exposed to a MEM solution that underwent the extraction procedure in the absence of test material or in the presence of a negative control material. Cell exposure to a MEM extract of latex, USP Positive Control material, or positive control material supplied by the testing laboratory served as the test's positive control. The sample and controls are incubated for up to 72 hours at 37°C, after which the cell cultures are examined microscopically and scored for cytotoxicity.

At NAMSA, a nontoxic, intermediate, or toxic result is based upon presence (+) or absence (-) of: a confluent monolayer, vacuolation, cellular swelling, and crenation (abnormal notches on cell surfaces due to shrinkage of cells). The percent of cellular lysis at 24, 48, and 72 hours is also determined. These parameters constitute the cytotoxic effect (CTE).

At Nelson Laboratories (Salt Lake City, UT) the magnitude of cell response is scored on a scale of 0 to 4: (results from three wells are averaged to give a final cytotoxicity score)

- 0 negative; cell destruction not significantly greater than negative control
- 1 $\leq 20\%$ rounding, occasional lysed cells
- 2 >20 to $\leq 50\%$ rounding, extensive cell lysis
- 3 > 50 to $\leq 70\%$ rounding and lysed cells
- 4 nearly complete cell destruction.

The sample passes if the negative controls and the medium controls have a 0 score, three of the positive controls have a score of 3 or higher, and none of the cell cultures exposed to the sample shows greater than grade 2.

ISO MEM Elution Method

Objective: To evaluate the cytotoxicity of a test material extract using an in-vitro mammalian cell culture test.

Procedure: The test article is extracted in Minimum Essential Medium (MEM) for 24 hours at 37°C per USP guidelines. A portion of the extract is placed in direct contact with a confluent monolayer of L-929 Mouse Fibroblast cells. As a reagent control, cells are exposed to a MEM solution that underwent the extraction procedure in the absence of test material. As a negative control, cells are exposed to a MEM solution that underwent the extraction procedure in the presence of a negative control material. Cell exposure to a MEM extract of latex, USP Positive Control material, or positive control material supplied by the testing laboratory served as the test's positive control. The sample and controls are incubated for 48 hours at 37°C, after which the cell cultures are examined microscopically and scored for cytotoxicity using the following system:

Grade	Reactivity	Condition of Culture
0	None	Discrete intracytoplasmic granules; no cell lysis
1	Slight	Not more than 20% of the cells are round, loosely attached, and without intracytoplasmic granules; some lysed cells are present.
2	Mild	Not more than 50% of the cells are round and devoid of intracytoplasmic granules; no extensive cell lysis and empty areas between cells.
3	Moderate	Not more than 70% of the cell monolayer contains rounded cells or is lysed.
4	Severe	Nearly complete destruction of the cell monolayer.

For the suitability of the system to be confirmed, the negative controls must have been a grade of 0 (reactivity none) and the positive controls must have a grade of 3 or 4. The test article passed if all three of the monolayers exposed to the test medium showed no greater than a grade of 2 (mild reactivity). The test would have been repeated if the controls did not perform as anticipated and/or if all three test wells did not yield the same conclusion.

Agarose Overlay

Objective: To evaluate the cytotoxic potential of a test article or extract using an *in vitro* mammalian cell culture.

Procedure: The agarose overlay procedure is a cytotoxicity test used to detect the presence of diffusible toxic substances. A monolayer of L-929 Mouse Fibroblast cells is grown to confluence and overlaid with Minimum Essential Medium (MEM) supplemented with serum, antibiotics, neutral red, and agarose. The test sample or a filter disk saturated with 0.1 ml of the test sample extract is placed on the solidified agarose surface. The same is done for the positive and negative controls. Following an incubation period of at least 24 hours at a temperature of 37°C, the cultures are examined for cell decolorization and lysis.

NAmSA uses a scoring system based on cell decolorization and cell morphology. This results in a CTE (Cytotoxic Effect) score of either toxic or nontoxic using the following criteria.

CTE SCORE	OBSERVATIONS
(N) Nontoxic	Normal cell morphology in proximity to test sample
(T) Toxic	Cellular death and/or degeneration associated with the area beneath the test sample and possibly extended beyond the perimeter of test sample. Where a zone of lysis is observed, the distance from the edge of the sample to the edge of the zone was measured and reported in millimeters (mm).

A sample is non-cytotoxic if the cell lysis is not significantly greater than that of the negative control.

ISO Agarose Overlay

Objective: To evaluate the cytotoxic potential of a test article or extract using an *in vitro* mammalian cell culture.

Procedure: The agarose overlay procedure is a cytotoxicity test used to detect the presence of diffusible toxic substances. A monolayer of L-929 Mouse Fibroblast cells is grown to confluence and overlaid with Minimum Essential Medium (MEM) supplemented with serum, antibiotics, neutral red, and agarose. The test sample or a filter disk saturated with 0.1 ml of the test sample extract is placed on the solidified agarose surface. The same is done for the positive and negative controls. Following an incubation period of at least 24 hours at a temperature of 37°C, the cultures are examined for cell decolorization and lysis.

For this ISO version of the test, NAmSA uses a macroscopic and microscopic scoring system based on cell decolorization and cell morphology. This results in a CTE (Cytotoxic Effect) score of either toxic or nontoxic using the following criteria.

Grade	Reactivity	Conditions of Cultures
0	None	No detectable zone around or under specimen
1	Slight	Some malformed or degenerated cells under specimen
2	Mild	Zone limited to area under specimen
3	Moderate	Zone extends 5-10 mm beyond specimen and up to 4 mm
4	Severe	Zone extends greater than 10 mm beyond specimen

For the suitability of the system to be confirmed, the negative controls must have been a USP grade of 0 (reactivity none) and the positive controls must

have a zone of lysis. The test article passed if all three of the cell cultures exposed to the test article showed no greater than a USP grade 2 (mild reactivity). The test would have been repeated if the controls did not perform as anticipated and/or if all three test wells did not yield the same conclusion.

USP Mouse Systemic Toxicity (and ISO Acute Systemic Toxicity)

Objective: To evaluate acute systemic toxicity of leachables extracted from the test article within seventy-two hours following a single intravenous or intraperitoneal injection in mice.

Procedure: The USP Mouse Systemic procedure evaluates the potential for a single injection of a material extract to cause a systemic toxic effect. The test material is extracted under USP guidelines in any of the following vehicles: 0.9% sodium chloride, USP solution (SC); alcohol in saline 1:20 solution (AS); polyethylene glycol 400 (PEG); and/or cottonseed oil, NF (CSO). The control blanks are the extract vehicles that underwent the extraction procedure in the absence of the test material. The PEG and its control blank are diluted with sodium chloride after extraction to obtain 200 mg of PEG per ml.

Healthy, young albino mice ranging in body weight from 17 to 23 grams are used as test animals. The animals, identified by fur marking or ear punch, are group housed in stock cages and offered food and water ad libitum. Two groups, each consisting of five mice, are used for each extract medium. One group of animals is injected (50 ml/kg SC, AS, and CSO; 10 g/kg PEG) with the test article extract, while the other group is injected with the control solution (blank extract). The sodium chloride and alcohol in saline solution are injected intravenously; the polyethylene glycol and cottonseed oil are injected intraperitoneally. The animals are observed for abnormal behavior at 0, 4, 24, 48, and 72 hours post injection.

Initial and final animal body weights are recorded, as well as abnormal reactions and mortalities. A negative response is one in which, during the observation period, animals treated with the sample extract do not demonstrate a significantly greater reaction than the animals treated with a negative control. If two or more mice died, or if abnormal behavior such as convulsions or prostration occur in two or more mice, or if body weight loss greater than 2 grams occurs in three or more mice, the test sample does not meet the USP test requirement.

USP Rabbit Intracutaneous Toxicity

Objective: To evaluate the local dermal irritant or toxic effects of leachables extracted from the test article following intracutaneous injection in rabbits.

Procedure: Materials are extracted under USP guidelines in any of the following vehicles: 0.9% sodium chloride, USP solution; alcohol in saline 1:20 solution; polyethylene glycol 400 (PEG); and/or cottonseed oil, NF. The control blanks are the extract vehicles that underwent the extraction procedure in the absence of the test material. The PEG extract and its control blank are diluted with sodium chloride after extraction to obtain 120 mg of PEG per ml.

Two to four (depending on the number of extraction vehicles used) healthy New Zealand White rabbits, free of significant dermal blemishes, are used as test animals. The same rabbits may be used for all extract media. Animals are housed individually, fed daily, and allowed water ad libitum. Prior to injection, the hair is closely clipped from the injection site of each rabbit. Exactly 0.2 ml of the test article extract is injected intracutaneously into five separate sites on one side of the back, while 0.2 ml of the negative control solution (blank extract) is introduced into separate sites on the other side of the back. Observations for erythema (redness) and edema (swelling) are made at 24, 48, and 72 hours after injection.

The rabbits are studied for erythema and edema separately using a scale of 0 to 4. A score of 0 indicates a negative response, whereas a grade of 4 reflects a maximal response. The grading scale for erythema and edema is discussed below:

- | | |
|---|--|
| 0 | No erythema; no edema |
| 1 | Very slight erythema; very slight edema (barely perceptible) |
| 2 | Well defined erythema; slight edema |
| 3 | Moderate to severe erythema; moderate edema |
| 4 | Severe erythema with slight eschar (injury in depth); severe edema |

The cumulative average erythema and edema score for each test article extract and corresponding control blank are calculated. For each extract, a difference in average scores (test minus control blank) of 1.0 or less is considered to be acceptable. A difference of 0.6 to 1.0 indicates a slight, but acceptable, reaction. A difference of >1.0 is considered to be unacceptable. Additionally, the average score for each test extract and blank is calculated for each interval. Any adverse reaction noted in the test extract is compared to the corresponding blank. A material passes if the difference in average erythema/edema score of the test extract minus the negative control is 1.0 or less.

ISO Acute Intracutaneous Reactivity

Objective: To evaluate the local dermal irritant or toxic effects of leachables extracted from the test article following intracutaneous injection in rabbits.

Procedure: Materials are extracted under USP guidelines in any of the following vehicles: 0.9% sodium chloride, USP solution; alcohol in saline 1:20 solution;

polyethylene glycol 400 (PEG); and/or cottonseed oil, NF. The control blanks are the extract vehicles that underwent the extraction procedure in the absence of the test material. The PEG extract and its control blank are diluted with sodium chloride after extraction to obtain 120 mg of PEG per ml.

Two to four (depending on the number of extraction vehicles used) healthy New Zealand White rabbits, free of significant dermal blemishes, are used as test animals. The same rabbits may be used for all extract media. Animals are housed individually, fed daily, and allowed water ad libitum. Prior to injection, the hair is closely clipped from the injection site of each rabbit. Exactly 0.2 ml of the test article extract is injected intracutaneously into five separate sites on one side of the back, while 0.2 ml of the negative control solution (blank extract) is introduced into separate sites on the other side of the back. Observations for erythema (redness) and edema (swelling) are made at 24, 48, and 72 hours after injection.

The rabbits are studied for erythema and edema separately using a scale of 0 to 4. A score of 0 indicates a negative response, whereas a grade of 4 reflects a maximal response. The grading scale for erythema and edema is discussed below:

- 0 No erythema; no edema
- 1 Very slight erythema; very slight edema (barely perceptible)
- 2 Well-defined erythema; well-defined edema
- 3 Moderate erythema; moderate edema
- 4 Severe erythema to eschar formation preventing grading of erythema; severe edema

For each animal, the erythema and edema scores obtained at each time interval will be added together and divided by the total number of observations. This calculation will be conducted separately for each test extract and reagent control. The score for the reagent control will be subtracted from the score for the test extract to obtain the Primary Irritation Score. The Primary Irritation Score of each animal will be added together and divided by the total number of animals. The value obtained is the Primary Irritation Index (PII). The primary Irritation Index is characterized by number and description as follows: 0 - 0.4 (negligible), 0.5 - 1.9 (slight), 2.0 - 4.9 (moderate), 5.0 - 8.0 (severe). Any adverse reaction noted in the test extract will be compared to the corresponding reagent control.

Hemolysis (Direct Contact)

Objective: To determine whether the test article or leachables from the test article will cause hemolysis *in vitro*.

NAmSA procedure for pre-1998 testing: A 0.2ml sample of rabbit blood is added to two test tubes each containing test article and 10 ml of 0.9% sodium chloride solution. Positive and negative sample controls are prepared in a similar fashion. The sample preparations are then incubated for 1 hour at 37°C followed by centrifugation for 10 minutes at not less than 1000 x g. Absorbance values are obtained spectrophotometrically (545 nm) for the test sample preparation (SC Test) and compared to that of the negative control. This determines the extent of red blood cell lysis caused by the test article extract. The negative control (SC Negative Control) consists of a sodium chloride blank. USP purified water acts as the positive control (PW Positive Control). Any extractions are performed per USP guidelines.

The percent hemolysis of the sample is determined according to the formula:

$$\% \text{ Hemolysis} = \frac{\text{SC Test} - \text{SC Negative Control}}{\text{PW Positive Control}} \times 100$$

A mean hemolysis value of the duplicate test samples of 5% or less is considered acceptable.

NAmSA procedure for post-1998 testing: Using a USP 60 cm²:20 ml ratio, material samples are covered with an appropriate amount of 0.9% sodium chloride USP solution (SC). Pooled rabbit blood is diluted with SC to a constant hemoglobin concentration. Based on a ratio of 1 ml diluted blood:8 ml vehicle, the appropriate volume of diluted blood is added to the test sample and control (positive and negative) tubes. The tubes are inverted and then left stationary for 4 hours at 37°C. Following the incubation, the supernatants are centrifuged for 15 minutes at 100 - 200 x g, transferred to new centrifuge tubes and centrifuged again for 5 minutes at 700 - 800 x g. A 1 ml aliquot of each test supernatant and positive and negative supernatant is added to individual 3 ml portions of Drabkin's reagent and allowed to stand for 15 minutes at room temperature. The absorbance of each sample is determined spectrophotometrically at 540 nm. A hemoglobin standard curve is prepared using Sigma Hemoglobin Reference Standard and Drabkin's Reagent. The hemoglobin concentration of each test article sample and the positive control are then determined by linear regression using this hemoglobin standard curve. The percent hemolytic index is calculated as follows:

$$\% \text{ Hemolytic Index} = \frac{\text{Hemoglobin Conc. Of SC test}}{\text{Hemoglobin Present}} \times 100$$

The hemoglobin present is confirmed by adding 0.2 ml of diluted blood to 10 ml Drabkin's Reagent and obtaining absorbance readings at 540 nm.

A hemolytic grade is assigned as follows:

Hemolytic Index	Hemolytic Grade
0 - 2%	Non-hemolytic
3 - 10%	Slightly Hemolytic
11 - 20%	Moderately Hemolytic
21 - 40%	Markedly Hemolytic
Above 40%	Severely Hemolytic

A mean hemolytic index of the duplicate SC test samples of 2% or less is considered to be non-hemolytic. For the suitability of the system to be confirmed, the negative control must have an absorbance value ≤ 0.02 and the positive control must have a hemolytic index $\geq 85\%$.

Hemolysis (Extract Method)

Objective: To determine whether leachables extracted from the test article will cause a significant level of hemolysis *in vitro*.

NAmSA procedure for pre-1998 testing: Using a USP ratio of 60 cm²:20 ml (sample surface area to volume of vehicle), the test sample is extracted for 72 hour at 50°C or 24 hours at 37°C. The negative control consists of a sodium chloride blank. The positive control consists of USP purified water (PW). A 0.2ml sample of rabbit blood (collected in EDTA on the day of the test) is added to 10 ml of the test sample extract or the controls, incubated for 1 hour at 37°C, followed by centrifugation for 10 minutes at not less than 1000 x g. Absorbance values are obtained spectrophotometrically (545 nm) for the test sample preparation (SC Test) and compared to that of the negative control. This determines the extent of red blood cell lysis caused by the test article extract. The percent hemolysis of the sample is determined according to the formula:

$$\% \text{ Hemolysis} = \frac{\text{Sample absorbance} - \text{SC negative control}}{\text{PW positive control}} \times 100$$

A mean hemolysis value of the duplicate test samples of 5% or less is considered acceptable.

NAmSA procedure for post-1998 testing: Using a USP 60 cm²:20 ml ratio, material samples are extracted with an appropriate amount of 0.9% sodium chloride USP solution (SC) for 1 hour at 121°C. Pooled rabbit blood is diluted with SC to a constant hemoglobin concentration. Based on a ratio of 1 ml diluted blood:8 ml vehicle, the appropriate volume of diluted blood is added to the test sample and control (positive and negative) tubes. The tubes are inverted and then left stationary for 4 hours at 37°C. Following the incubation, the supernatants are centrifuged for 15 minutes at 100 - 200 x g, transferred to new centrifuge tubes and centrifuged again for 5 minutes at 700 - 800 x g. A 1 ml aliquot of each test supernatant and positive and negative supernatant is added to individual 3 ml portions of Drabkin's reagent and

allowed to stand for 15 minutes at room temperature. The absorbance of each sample is determined spectrophotometrically at 540 nm. A hemoglobin standard curve is prepared using Sigma Hemoglobin Reference Standard and Drabkin's Reagent. The hemoglobin concentration of each test article sample and the positive control are then determined by linear regression using this hemoglobin standard curve. The percent hemolytic index is calculated as follows:

$$\% \text{ Hemolytic Index} = \frac{\text{Hemoglobin Conc. Of SC test}}{\text{Hemoglobin Present}} \times 100$$

The hemoglobin present is confirmed by adding 0.2 ml of diluted blood to 10 ml Drabkin's Reagent and obtaining absorbance readings at 540 nm.

A hemolytic grade is assigned as follows:

Hemolytic Index	Hemolytic Grade
0 - 2%	Non-hemolytic
3 - 10%	Slightly Hemolytic
11 - 20%	Moderately Hemolytic
21 - 40%	Markedly Hemolytic
Above 40%	Severely Hemolytic

A mean hemolytic index of the duplicate SC test samples of 2% or less is considered to be non-hemolytic. For the suitability of the system to be confirmed, the negative control must have an absorbance value ≤ 0.02 and the positive control must have a hemolytic index $\geq 85\%$.

Material Mediated Pyrogenicity

The test article is extracted in 0.9% sodium chloride solution, USP using the USP ratio of 60 cm²:20 ml (surface area to vehicle volume) for 1 hour at 37°C. No more than 30 minutes prior to injection, the temperature of three New Zealand White Rabbits are recorded. The rabbits then receive an intravenous injection of the extract at a dose of 10 ml/kg body weight. Rabbit temperatures are recorded at 30 minute intervals between 1 and 3 hours after injection. The maximum temperature rise for any animal, compared to that rabbit's baseline temperature, must be less than 0.5°C in order to pass the USP test requirement.

ISO Sensitization Study in the Guinea Pig (Maximization Method)

Objective: To identify potential allergens that could cause delayed dermal contact sensitization.

Procedure: Fifteen albino guinea pigs of the Hartley strain per extract solution are used for this procedure; ten test animals and five negative control animals. The dorsoscapular area of the fifteen animals is shaved prior to the first induction phase. The test material is extracted in 0.9% sodium chloride or cottonseed oil following USP Guidelines.

Induction I

Three pairs of intradermal injections are administered to the test and control animals as follows:

- a. 0.1 ml of 50:50 (v/v) mixture of Freund's Complete Adjuvant (FCA) and the chosen vehicle (both test and Control animals)
- b. 0.1 ml of test material extract solution (or the vehicle in the case of the Control animals)
- c. 0.1 ml of a 1:1 mixture of (a) and (b) [or (a) and the vehicle in the case of the Control animals]

Induction II

Six days after the injections, the animals are shaved again and 0.5 to 1 gram of 10% sodium lauryl sulfate (SLS) in petrolatum is applied to induce mild acute inflammation. The area is left uncovered for 24 hours. After removing any remaining SLS, Whatman No. 3MM filter paper saturated with 0.3 ml of freshly prepared test extract is applied to the previously injected sites. Control animals are similarly patched with reagent control material. Each patch is secured with nonreactive tape and the animal wrapped with an elastic bandage. After 48 hours, the patches are removed.

Challenge

Thirteen days after removal of the Induction II patches, the animals are shaved. The next day, a nonwoven cotton disk in a Hill Top Chamber® is saturated with 0.3 mL of the test extract or control vehicle and applied to the appropriate flank of each animal. After 24 hours the patch is removed, at which time the sites are wiped and shaved. Observations are made for any signs of irritation or sensitization at 2 - 4 hours after shaving and at 48 and 72 hours after challenge patch removal. Scores are recorded for both erythema (redness) and edema (swelling) as follows:

- 0 no visible reaction.
- 1 slight erythema/slight edema
- 2 well defined erythema/well defined edema
- 3 moderate erythema/moderate edema
- 4 severe erythema to slight eschar formation/severe edema

The response, pattern, character, and duration of any test animal reactions are compared to the controls. Any dermal inflammatory response at the test sites greater than that seen in any control condition was considered evidence of a potential allergic response.

Salmonella/Mammalian Microsome Mutagenicity Assay (Ames Test)

Purpose: To evaluate the mutagenic potential of the test article by measuring the ability of its saline and/or ethanol extract (or their metabolites) to induce mutations in histidine dependent strains of Salmonella Typhimurium.

Mentor has used two facilities to perform this test. **The following describes NAmSA's procedure.**

Test Article Preparation: The test article is extracted in saline and ethanol following USP extract ratio requirements. When the test article was less than 0.5 mm in thickness with a measurable surface area, the extractions are carried out at 120 cm²/20 ml extraction medium. When the test article is in excess of 1 mm thickness and of variable geometric dimension, no less than 4 grams test article per 20 ml extraction medium ratio is used. The saline mixture is autoclaved at 121⁰C for 1 hour or incubated at 50⁰C for 72 hours. The ethanol mixture is extracted at 37⁰C for 24 hours or 50⁰C for 72 hours.

Test System: The tester strains (TA98, TA100, TA1535, TA1537, and TA1538) used in this assay are derived from Bruce Ames' parental Salmonella typhimurium LT2. 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).

These strains differ both in the gene in which the mutation occurs and the nature of the defect. The strains have been engineered to have characteristics making them particularly suited to be biological "red flags" of genetic damage. These characteristics include: alterations in the bacterial cell wall which allow the relatively unimpeded passage of large or ionically charged molecules, loss of the endogenous ability to repair certain types of DNA damage, and the presence of genetic factors which facilitate the fixation of DNA damage.

Tester strains TA1538 and TA98 detect frameshift mutations, in what are often known as "hotspots" of DNA. These are regions of the genome, which, when analyzed at the DNA sequence level, appear to have higher frequencies of mutagenic events than the rest of the genome. In the case of TA1538 and TA98, the hotspot occurs at an 8 nucleotide region of GC repeats (C-G-C-G-C-G-C-G) near the site of a frameshift mutation in the his D3052 gene. These strains are extremely useful in detecting the ability of test material, especially

aromatic compounds, to induce frameshift mutations. The frameshift mutations subsequently restore the original nucleotide sequence to the his D3052 gene and the bacteria revert to histidine independence.

Tester strain TA1537, which has a mutation in the his C3076 gene, is also a strain which recognizes compounds inducing frameshift mutations. TA1537 is sensitive to some of the substances which revert TA1538 and TA98, but also reverts in the presence of other compounds as well.

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.

Each of these strains also possesses the rfa wall mutation causing the loss of one of the enzymes responsible for the synthesis of part of the lipopolysaccharide layer of the cell wall. This cell wall deficiency increases the permeability of the cells to certain classes of test agents, such as compounds containing large ring systems, which would be excluded by a normal intact cell wall.

A second stable mutation is a deletion in the uvrB gene that results in a deficient DNA excision-repair system (uvrB) and further enhances each tester strains' sensitivity to some mutagens.

Tester strains TA100 and TA98 also harbor an additional genetic element, the pKM101 plasmid (carrying the R-factor), which increases the sensitivity of these two strains to some mutagens.

Metabolic Activation: The metabolic activation system used in this assay is the Aroclor 1254-induced rat liver S-9 microsomal enzyme fraction. The S-9 is 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 is excised and homogenized. The S-9 microsomal enzyme fraction is prepared, aliquoted, and stored at approximately -70°C. The microsomal enzyme mixture (S-9 mix) is prepared immediately before its use in the mutagenesis assay.

The microsomal enzyme mixture introduces an active enzyme fraction into the assay system. These enzymes may generate metabolic mutagens from the test article extracts and expose the test system to mutagens that might not otherwise be present *in vitro*.

Tester Strain Controls: All tester strain cultures are 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 wall mutation is confirmed by demonstrating a sensitivity to crystal violet. The

presence of the uvrB deletion is confirmed by demonstrating a sensitivity to ultraviolet light. The presence of the pKM101 plasmid is confirmed by demonstrating a resistance to ampicillin.

Negative Controls: Vehicle controls consisted of 0.9% saline and 100% ethanol and are 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 are 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:

Tester Strain	S-9 Present in Culture Plate	Positive Controls	Concentration per Culture Plate
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	75.0 ug
TA1538	Yes	2-Aminoanthracene	1.0 ug
TA1538	No	2-Nitrofluorene	1.0 ug

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

Overlaid on plates containing bottom agar is the top agar mixture. The top agar mixture contains either:

- 1) 100 ul of tester strain and 50 ul of vehicle or test article extract (or extract dilution) and 2.5 ml molten selective top agar, or
- 2) 100 ul of tester strain, 50 ul of vehicle or test article, 500 ul of S-9 mix, and 2.0 ml molten top agar.

After the overlay solidifies, the plates are inverted and incubated for approximately 48 hours. After the incubation period, the plates are evaluated and colonies counted. The condition of the bacterial background lawn is evaluated for evidence of test article extract toxicity relative to the vehicle

control bacterial lawn. The presence of test article precipitate is also noted. A dose level is considered toxic if the following criteria are met:

- 1) a 50% reduction in the mean number of revertants per plate as compared to the mean vehicle control value which is accompanied by an abrupt dose-dependent drop in the revertant count and
- 2) a reduction in the background lawn.

Plate Scoring: For each replicate plating, the mean and standard deviation of the number of revertants per plate are 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, TA1537 and TA1538 a data set is judged positive if the increase in mean revertants at the peak of the dose response is equal to or greater than three times the mean vehicle control value. For strains TA98 and TA100 the increase must be equal to or greater than two times the mean vehicle control value.

For a valid test, each tester strain culture 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.

Mentor also uses BioReliance (Microbiological Associates) of Rockville, MD to perform the Ames test. **The following describes BioReliance's test procedures.**

BioReliance uses the same *Salmonella* test strains as NAmSA with the exception of *E. Coli* tester strain WP2 *uvrA* replacing TA 1538. The positive control for this *E. Coli* tester strain without S9 activation is 1000 µg/plate methyl methanesulfonate. During the plate incorporation test, BioReliance scores the background lawn for evidence of test article toxicity using the following codes:

Code	Description	Characteristics
1	Normal	Distinguished by a healthy microcolony lawn.
2	Slightly Reduced	Distinguished by a noticeably thinning of the microcolony lawn and possibly a slight increase in the size of the microcolonies compared to the vehicle control plate.
3	Moderately Reduced	Distinguished by a marked thinning of the microcolony lawn resulting in pronounced increase in the size of the microcolonies compared to the vehicle control plate.
4	Extremely Reduced	Distinguished by an extreme thinning of the microcolony lawn resulting in increase in the size of the microcolonies compared to the vehicle control plate such that the microcolony lawn is visible to the unaided eye as isolated colonies.
5	Absent	Distinguished by a complete lack of any microcolony lawn over greater than or equal to 90% of the plate.
6	Obscured by Precipitate	The background bacterial lawn cannot be accurately evaluated due to microscopic test article precipitate.
NP	Non-Interfering Precipitate	Distinguished by precipitate on the plate that is visible to the naked eye but any precipitate particles detected by the automated colony counter total less than 10% of the revertant colony count (e.g., less than 3 particles on a plate with 30 revertants).
IP	Interfering Precipitate	Distinguished by precipitate on the plate that is visible to the naked eye and any precipitate particles detected by the automated colony counter exceed 10% of the revertant colony count (e.g., more than 3 particles on a plate with 30 revertants).

For the test article to be evaluated positive, its extract must cause an increase in the mean revertants per plate of at least one tester strain. Data sets for tester strains TA1535 and TA1537 are judged positive if the increase in mean revertants is equal to or greater than three times the mean vehicle control value. Data sets for tester strains TA 98, TA100, and WP2_{uvrA} are judged positive if the increase in mean revertants is equal to or greater than two times the mean vehicle control value.

Many of BioReliance's criteria for a valid test are similar to NAMSA's. In addition, a minimum of one non-toxic dose level is required to evaluate assay data. A dose level is considered toxic if one or both of the following criteria are met: (1) A >50% reduction in the mean number of revertants per plate as

compared to the mean vehicle control value. This reduction must be accompanied by an abrupt dose-dependent drop in the revertant count. (2) A reduction in the background lawn.

Four Week Rabbit Subcutaneous Implant with Histopathology (Short Term)

Objective: To evaluate the local tissue effects (irritation and toxicity) of the test article in direct contact with living subcutaneous tissue.

Procedure: Two albino rabbits of the New Zealand White variety are each implanted with six strips of test material each measuring 10 mm x 1 mm (or the equivalent in sections). Four negative controls consist of 10 mm x 1 mm USP plastic strips.

The animals are clipped free of fur over their dorsum and both flanks. The subcutaneous tissue on each side of the vertebral column is infiltrated with lidocaine. Implantations are performed using 16 gauge needles aseptically loaded with the test material or the negative control. The needle is inserted into the tissue and withdrawn over a stylet to leave behind the material in the tissue. Material strips are implanted 2 to 3 cm apart in the subcutaneous tissue next to the vertebral column. The negative control strips are implanted in the subcutaneous tissue on the opposite side of the vertebral column.

The animals are observed daily and their body weights recorded prior to implantation and at termination. At four weeks, the animals are euthanized and the implant sites are examined macroscopically. Capsule formation and other signs of irritation are scored. Representative tissue sites (test and control) from each rabbit are dissected free and fixed in formalin. These sections are embedded, cut, and stained for evaluation by a board certified pathologist.

At NAMSA, capsule formation or other evidence of reaction is scored on a scale of 0-4 as follows:

- 0 no capsule, no adverse reaction (other than minimal hemorrhage).
- 1 up to 0.5mm capsule or reaction area.
- 2 0.6 to 1.0mm capsule or reaction area.
- 3 1.1 to 2.0mm capsule or reaction area.
- 4 >2.0mm capsule or reaction area.

Mean macroscopic scores for test implants will be compared with mean scores of control sites. In general, the requirements of the test are met if the difference between the average test and negative control scores does not exceed 1.0. The requirements of the test are not met if the difference between the test and control score for two or more implant sites exceeds one for any animal implanted.

90 Day Rabbit Subcutaneous Implant with Histopathology (Long Term)

Objective: To evaluate the local tissue effects (irritation and toxicity) of the test article in direct contact with living subcutaneous tissue.

Procedure: Two albino rabbits of the New Zealand White variety are each implanted with six strips of test material each measuring 10 mm x 1 mm (or the equivalent in sections). Four negative controls consist of 10 mm x 1 mm USP plastic strips.

The animals are clipped free of fur over their dorsum and both flanks. The subcutaneous tissue on each side of the vertebral column is infiltrated with lidocaine. Implantations are performed using 16 gauge needles aseptically loaded with the test material or the negative control. The needle is inserted into the tissue and withdrawn over a stylet to leave behind the material in the tissue. Material strips are implanted 2 to 3 cm apart in the subcutaneous tissue next to the vertebral column. The negative control strips are implanted in the subcutaneous tissue on the opposite side of the vertebral column.

The animals are observed daily and their body weights recorded prior to implantation, monthly, and at termination. At 90 days, the animals are euthanized and the implant sites are examined macroscopically. Capsule formation and other signs of irritation are scored. Representative tissue sites (test and control) from each rabbit are dissected free and fixed in formalin. These sections are embedded, cut, and stained for evaluation by a board certified pathologist.

At NAMSA, capsule formation or other evidence of reaction is scored on a scale of 0-4 as follows:

- 0 no capsule, no adverse reaction (other than minimal hemorrhage).
- 1 up to 0.5mm capsule or reaction area.
- 2 0.6 to 1.0mm capsule or reaction area.
- 3 1.1 to 2.0mm capsule or reaction area.
- 5 >2.0mm capsule or reaction area.

Mean macroscopic scores for test implants will be compared with mean scores of control sites. In general, the requirements of the test are met if the difference between the average test and negative control scores does not exceed 1.0. The requirements of the test are not met if the difference between the test and control score for two or more implant sites exceeds one for any animal implanted.

One Week Rabbit Intramuscular Implant with Histopathology (Short Term)

Objective: To evaluate the potential for a local irritant or toxic response to the test article in direct contact with living muscle tissue.

Procedure: At least two albino rabbits of the New Zealand White variety are each implanted with a minimum of six strips of test material each measuring 10 mm x 1 mm (or the equivalent in sections). The sterile test samples are implanted in the paravertebral muscle on one side of the spine while four sterile negative controls consisting of 10 mm x 1 mm USP negative control plastic strips are implanted in the opposite muscle. All test specimens and controls are implanted aseptically using 16 gauge needles while the animal is under sedation and local anesthesia. The needle is inserted into the muscle and withdrawn over a stylet to leave behind the material in the tissue.

The animals are observed daily and their body weights recorded prior to implantation and at termination. At one week, the animals are euthanized, the paravertebral muscle is dissected free, and the implant sites are located and examined macroscopically. Representative tissue sites (test and control) from each rabbit are dissected free and fixed in formalin. These sections are embedded, cut, and stained for evaluation by a board certified pathologist.

At NAMSA, capsule reaction or other evidence of reaction is scored on a scale of 0-4 as follows:

- 0 no capsule, no adverse reaction (other than minimal hemorrhage).
- 1 up to 0.5mm capsule or reaction area.
- 2 0.6 to 1.0mm capsule or reaction area.
- 3 1.1 to 2.0mm capsule or reaction area.
- 4 >2.0mm capsule or reaction area.

Mean macroscopic scores for test implants will be compared with mean scores of control sites. In general, the requirements of the test are met if the difference between the average test and negative control scores does not exceed 1.0.

30 Day Rabbit Intramuscular Implant with Histopathology (Short Term)

Objective: To evaluate the potential for a local irritant or toxic response to the test article in direct contact with living muscle tissue.

Procedure: At least two albino rabbits of the New Zealand White variety are each implanted with a minimum of six strips of test material each measuring 10 mm x 1 mm (or the equivalent in sections). The sterile test samples are implanted in the paravertebral muscle on one side of the spine while four sterile negative controls consisting of 10 mm x 1 mm USP negative control plastic strips are implanted in the opposite muscle. All test specimens and controls are implanted aseptically using 16 gauge needles while the animal is under sedation and local anesthesia. The needle is inserted into the muscle and withdrawn over a stylet to leave behind the material in the tissue.

The animals are observed daily and their body weights recorded prior to implantation and at termination. At 30 days, the animals are euthanized, the paravertebral muscle is dissected free, and the implant sites are located and examined macroscopically. Representative tissue sites (test and control) from each rabbit are dissected free and fixed in formalin. These sections are embedded, cut, and stained for evaluation by a board certified pathologist.

At NAMSA, capsule reaction or other evidence of reaction is scored on a scale of 0-4 as follows:

- 0 no capsule, no adverse reaction (other than minimal hemorrhage).
- 1 up to 0.5mm capsule or reaction area.
- 2 0.6 to 1.0mm capsule or reaction area.
- 3 1.1 to 2.0mm capsule or reaction area.
- 4 >2.0mm capsule or reaction area.

Mean macroscopic scores for test implants will be compared with mean scores of control sites. In general, the requirements of the test are met if the difference between the average test and negative control scores does not exceed 1.0.

90-Day (or Thirteen Week) Rabbit Intramuscular Implant with Histopathology (Long Term)

Objective: To evaluate the potential for a local irritant or toxic response to the test article in direct contact with living muscle tissue.

Procedure: At least three albino rabbits of the New Zealand White variety are each implanted with a minimum of six strips of test material each measuring 10 mm x 1 mm (or the equivalent in sections). The sterile test samples are implanted in the paravertebral muscle on one side of the spine while four sterile negative controls consisting of 10 mm x 1 mm USP negative control plastic strips are implanted in the opposite muscle. All test specimens and controls are implanted aseptically using 16 gauge needles while the animal is under sedation and local anesthesia. The needle is inserted into the muscle and withdrawn over a stylet to leave behind the material in the tissue.

The animals are euthanized after 90 days of implantation and the implant sites are examined macroscopically. The paravertebral muscles are dissected free and the tissue is methodically cut to locate the six implanted test articles and negative controls in each rabbit. Representative tissue sites (test and control) from each rabbit are dissected free and fixed in formalin. These sections are embedded, cut, and stained for evaluation by a board certified pathologist.

At NAMSA, capsule reaction or other evidence of reaction is scored on a scale of 0-4 as follows:

- 0 no capsule, no adverse reaction (other than minimal hemorrhage).
- 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.

Mean macroscopic scores for test implants will be compared with mean scores of control sites. In general, the requirements of the test are met if the difference between the average test and negative control scores does not exceed 1.0.

At American Edwards Laboratory, the implantation sites are evaluated somewhat differently. A positive response includes visible inflammation or necrosis of the tissue adjacent to the test sample. A negative reaction shows no visible evidence of tissue damage. The test material is judged nontoxic by this procedure if four of the five test material implant sites are negative.

Microscopic evaluation is usually performed as well. A minimum of two representative tissue implant sites for each test and control for each rabbit are excised and histologically prepared. The evaluation is conducted by a board certified pathologist. Any resulting response determined from the difference between test and control values will be graded as non-irritant, slight, moderate, or severe irritant (NAmSA) or simply as a nonirritant or irritant (American Edwards Laboratories).

LOW BLEED GEL-FILLED MAMMARY PROSTHESES RAW MATERIALS TESTING

Mentor's raw material testing is meant to insure that the materials used in manufacturing of implantable devices is deemed biocompatible and non-toxic prior to fabrication of finished products. These early tests are used to screen for materials with obvious biological incompatibilities. However, final verification of a materials biological suitability for a device is only made after biological testing has been completed on sterile finished product.

This section describes the raw materials qualification testing for the Smooth and Siltex Gel-filled Mammary Prostheses. Because Mentor uses the same materials in like components and in multiple components for Smooth and Siltex Gel-filled Mammary Prostheses, all of the materials listed below apply to all of the product lines contained in this PMA. Laboratory reports for all material biocompatibility testing presented in this section are located in the Biological Testing Appendices.

The testing requirements for mammary prosthesis raw materials depend upon whether the material will be implanted long-term, implanted short-term, used intra-operatively, is only part of the packaging, comes in contact with the device during manufacturing, or is used only as an aid in the manufacturing process. Based upon its intended use Mentor Texas SOP-HS-136 (Material Characterization and Qualification) identifies chemical, physical and/or biological tests to be performed on the materials.

The remainder of this section details what biological materials qualification tests have been performed for every listed material used in the fabrication of Smooth and Siltex Gel-filled Mammary Prostheses (see the raw materials table in Section III, Device Description and Raw Materials), and the results of the tests. Generic descriptions of how the standard biological tests have been performed can be found in Section V, Description of Standard Biological Test Procedures Used By Mentor of this submission. Laboratory test reports for all biocompatibility tests referred to in this section are located in the Biological Testing Appendices.

MATERIALS IN THE FINAL PRODUCT:

██████████ Elastomer ██████████

██████████ has been biocompatibility tested as part of ██████████
██████████ 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 ██████████
██████████ 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 slabs or extracts from the cured slabs, passed all of the above biocompatibility tests. [See Mentor's PMA #P910037/A49, dated April 9, 1999, Vol 7, pg 1313 for the original copy of the MAF access letter.]

[REDACTED] **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] in the testing information, Sections 30 - 34 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 slabs or extracts from the cured slabs, passed all of the above biocompatibility tests. [See Mentor's PMA #P910037/A49, dated April 9, 1999, Vol 7, pg 1312 for the original copy of the MAF access letter.]

Elastomer [REDACTED] -

Mentor sponsored testing performed by NAmSA around 1994 in accordance with Good Laboratory Practices:

Agar Overlay -

A biocompatibility test was conducted on [REDACTED] elastomer in order to determine the potential for cytotoxicity. A 1 cm² portion of [REDACTED] elastomer, a USP negative control, and a positive control were each placed on an agarose surface directly overlaying a confluent monolayer of L-929 mouse fibroblast cells. After incubation at 37°C for 24 hours, the cell culture was examined macroscopically for cell decolorization to determine the zone of cell lysis and microscopically to determine cell morphology in proximity to the test article.

Under the conditions of this study, the test article showed no evidence of causing cell lysis or toxicity. As anticipated the negative control was nontoxic and the positive control was toxic. The test article, [REDACTED] elastomer, would not be considered toxic to L-929 mouse fibroblast cells in this test.

Hemolysis, *in vitro*, direct contact -

Duplicate tubes of 2 gm of the test article, [REDACTED] elastomer [REDACTED], added to 0.9% sodium chloride USP solution (SC) were prepared. A 0.2 ml sample of whole rabbit blood was then added to the test article in 10 ml of the SC vehicle. A 0.2 ml sample of whole rabbit blood was also mixed with 10 ml of SC (negative control) and 10 ml purified water (positive control). Each tube was inverted gently to mix the contents and incubated at 37° C for one hour. Spectrophotometric readings at 545 nm were taken for each solution. The percent hemolysis for each solution was calculated from the absorbance values. Under the conditions of this study, the combined SC and blood in direct contact with the test article would not be considered hemolytic; the mean hemolysis value of 0% was acceptable.

Hemolysis, *in vitro*, saline extract -

Hemolysis testing of the test article extract was performed to determine whether leachables from the test article would cause hemolysis *in vitro*. The test article, [REDACTED] elastomer [REDACTED], was extracted (90 cm² of the test article to 30 ml volume of the extract vehicle) in 0.9% sodium chloride USP solution (SC) at 121⁰C. for 1 hour. The test material was not considered hemolytic since the mean hemolysis value of 0% was acceptable.

Sensitization (Maximization), Guinea Pig, Saline Extract -

Sodium chloride extracts, 4 gm [REDACTED] 20 ml 0.9% sodium chloride (SC) of the test article, were evaluated for delayed contact sensitization. The test article was extracted in SC for 72 hours at 50° C. The vehicle, SC, was similarly prepared to serve as the control. The challenge sites were scored over a 96 hour period for erythema and edema. Test article sites were compared to the negative control challenge (control vehicle) and the positive control (0.1% 1-chloro-2,4-dinitrobenzene (DNCB) solution. Under the conditions of this study, the SC test article extracts and the test article [REDACTED] elastomer, showed no evidence of causing delayed dermal contact sensitization in the guinea pig.

[REDACTED] sponsored [REDACTED] elastomer biocompatibility testing as part of its [REDACTED] for this material. See Group IV, Biological Testing in the [REDACTED] testing information, Section 26-30 for copies of the reports. [See Mentor's PMA #P910037/A33 dated November 9, 1995, Vol. 1, pg 13 for the [REDACTED].] The testing consisted of the following:

In Vitro Cytotoxicity Study (USP Elution Method)

[REDACTED] elastomer test article (designated by the vendor for testing as [REDACTED]) was extracted in serum-supplemented Minimum Essential Medium at a ratio of 60 cm² surface area of test article to 20 ml extraction vehicle. The test article extract was placed onto duplicate confluent monolayers (80% or greater by 48 hours) of L-929 mouse fibroblast cells. Separate confluent monolayers (80% or greater) of L-929 mouse fibroblast cells were prepared for negative and positive controls. The test and negative monolayers were examined microscopically at 48 hours to determine any change in cell morphology. The monolayer in the positive control plates was examined at 24 hours.

Scoring for cytotoxicity was based on a grading scale from 0 (no toxicity) to 4 (severe toxicity) The test article meets the requirements of the test if neither of the monolayers exposed to the test medium showed greater than a grade 2 (mild).

Under the conditions of this study the MEM test extract showed no evidence of causing cell lysis or toxicity greater than a grade 2 (mild). The negative control and the positive control performed as anticipated. The MEM test extract meets the requirements of the USP.

In Vitro Cytotoxicity Study (MEM Elution Method)

██████████ elastomer test article (designated by ██████████) was extracted for 37°C for 24 hours in serum-supplemented Minimum Essential Medium at a ratio of 60 cm² surface area of test article to 20 ml extraction vehicle. The test article extract was placed onto duplicate confluent monolayers (80% or greater by 48 hours) of L-929 mouse fibroblast cells. Separate confluent monolayers (80% or greater) of L-929 mouse fibroblast cells were prepared for negative and positive controls. Microscopic scoring was based on monolayer confluence, vacuolization, swelling, crenation, and % lysis.

Test and negative control monolayers were incubated at 37° C and examined microscopically to score for cytotoxicity at 24, 48, and 72 hours of incubation. The positive control monolayer titer was observed at 24 hours and the result compared to the NAMSA historic value. The positive control must be toxic within plus or minus one dilution of the NAMSA historical value. Under the conditions of this study, the MEM test extract would not be considered toxic to L-929 mouse fibroblast cells.

USP Mouse Systemic Toxicity -

The purpose of this study was to determine whether leachables extracted from the test article would cause acute systemic toxicity following injection into mice. The ██████████ elastomer test article (identified by ██████████) was extracted for 1 hour at 121° C in 0.9% sodium chloride (SC) and cottonseed oil (CSO) at a ratio of 60 cm²:20 ml (test article surface area to volume of extraction vehicle). These extracts were injected into five mice (per abstract) by either the intravenous or intraperitoneal route at a dose of 50 ml/kg. Similarly, 5 mice were dosed with corresponding blank SC and CSO vehicles. The animals were observed immediately and at 4, 24, 48, and 72 hours after systemic injection for adverse reactions. Under the conditions of this study, the test article extracts would not be considered systemically toxic to the mouse at the prescribed USP dosage tested.