4.3.1 Developmental toxicity

OP-1 had no maternal or developmental toxicity when administered by IV injection to pregnant Sprague-Dawley rats in either of 2 rat studies [Stryker Biotech Studies 98-004 and 00-005, (P060021, Appendix 2, pages 6436 and 6531)] using up to 3.5 mg/kg/day administered on gestation day (GD) 6 to 17 (up to 35 times the human dose, administered daily for 12 days) or by daily IV injection to pregnant New Zealand white rabbits using up to 0.4 mg/kg/day (GD 6 to 18; 4 times the human dose, administered daily for 13 days) (Stryker Biotech Study 00-006; P060021, Appendix 2, page 6740). In a placental transfer study (Stryker Biotech Study 99-004; P060021, Appendix 2, page 7025), rats were given 3.8 mg/kg (38 times the human dose) of OP-1 and less than 1% was transferred to the fetus. When rabbits were intentionally immunized with OP-1 using an adjuvant, no maternal or developmental toxicity was observed, despite the presence of significant IgG and IgM in the dams and kits. Relevant studies are summarized below.

Effect on Embryo-Fetal Development

Rat Studies

A GLP study (Stryker Biotech Study 98-004; P060021, Appendix 2, page 6436) was conducted using OP-1 formulated in 20 mM acetate buffer, pH 4.1. The purpose of the study was to provide information concerning the potential maternal and developmental toxicity of OP-1 in the pregnant rat and to serve as a dose range finding study for a radiolabeled OP-1 placental transfer study (Stryker Biotech Study 99-004; P060021, Appendix 2, page 7025). In Stryker Biotech Study 98-004, the test article was administered via IV injection to 24 time-mated female Sprague-Dawley rats on GD 6 to 17 at daily dose levels of 0, 0.035, 0.35, and 3.5 mg/kg/day using 6 rats per group. Study animals were observed twice daily for mortality and for obvious toxic effects. Each animal was given a detailed physical examination on GD 4, 6, 9, 12, 15, 18, and 20. During the treatment interval, these latter examinations were conducted one-half to 2 hours post-dosing. Body weights and food consumption were recorded at regular intervals throughout gestation. All females were euthanized on GD 20, and given a macroscopic postmortem evaluation. Gravid uterine weights and corpora lutea/uterine implantation data were recorded. Fetuses were weighed, given a gross external examination and necropsied. Maternal necropsies were also performed. There was no evidence of maternal or developmental toxicity among the animals treated with OP-1. The maternal body weight and observation data and the fetal body weight, sex distribution, and external examination data were similar among the animals treated with OP-1 and controls. IV administration of OP-1 at doses up to 3.5 mg/kg/day
4.3.1 Developmental toxicity
(Content from P060021/A011 Section IV, Preclinical: section 4.9, page 60-65)

on GD 6 to 17 (35 times the human dose for 12 consecutive days) showed no evidence of maternal or developmental toxicity.

Another GLP study was conducted using OP-1 formulated as a 5% lactose solution (Stryker Biotech Study 00-005; P060021; Appendix 2, page 6531). The test article was administered via IV injection to 96 mated female Sprague-Dawley rats on GD 6 to 17 at dose levels of 0, 0.004, 0.04, and 0.4 mg/kg/day using 24 rats per group. Study animals were observed twice daily for mortality/morbidity and for obvious pharmacologic and/or toxicologic effects. On GD 6 to 20 each animal was removed from the cage and given a detailed physical examination. During the treatment interval, these physical examinations were conducted approximately 1 hour post-dosing. Body weights and food consumption were recorded at regular intervals throughout gestation. All females were sacrificed on GD 20, given a macroscopic postmortem evaluation, and a complete necropsy was performed. During these evaluations, gravid uterine weights and corpora lutea/uterine implantation data were recorded. Fetuses were given a gross external examination and weighed. Approximately half of the fetuses in each litter were fixed in Bouin’s solution and those from control and high-dose animals were examined for soft tissue defects. The remaining fetuses were examined for skeletal malformations and ossification variations. There was no evidence of test article attributed maternal or developmental toxicity. The maternal body weight and observation data and the fetal body weight, sex distribution, and external examination data were similar between OP-1 and control groups. There also were no adverse effects related to OP-1 in fetal soft tissue or skeletal tissue. IV administration of OP-1 at doses up to 0.4 mg/kg/day on GD 6 to 17 (4 times the human dose at the highest dose used, given daily for 12 days) showed no maternal or developmental toxicity in rats.

Rabbit Studies

A study was conducted using OP-1 formulated as a 5% lactose solution (Stryker Biotech Study 00-006; P060021, Appendix 2, page 6740). The purpose of this study was to evaluate the potential maternal and developmental (including potential teratogenic) toxicity of OP-1 when administered by IV injection to pregnant rabbits. The test article was administered via IV injection to 92 time-mated female New Zealand white rabbits on GD 6 to 18 at dose levels of 0, 0.004, 0.04, and 0.4 mg/kg/day using 23 rabbits per group. Study animals were observed twice daily for mortality/morbidity and for obvious pharmacological and/or toxicological effects. On GD 6 to 18, each animal was removed from the cage and given a detailed physical examination. During the treatment interval, physical examinations were conducted approximately 1 hour post-dosing. Body weights and food consumption were recorded at regular intervals throughout
4.3.1 Developmental toxicity
(Content from P060021/A011 Section IV, Preclinical: section 4.9, page 60-65)

gestation. All females were sacrificed on GD 29, given a macroscopic postmortem evaluation, and subjected to a complete necropsy. During these evaluations, gravid uterine weights and corpora lutea/uterine implantation data were recorded. Fetuses were given a gross external examination and weighed. Following anesthesia, each fetus was subjected to a visceral exam after which it was fixed in alcohol, and the eyes and brain evaluated. The fetuses were then examined for skeletal malformations and ossification variations. There was no evidence of test article attributed maternal or developmental toxicity. In instances where statistical differences were noted, mean values were within historical control ranges and the effects were not attributed to test-article administration. The maternal body weight and observation data and the fetal body weight, sex distribution, and external examination data were similar among the animals treated with OP-1 and controls. There were no adverse effects associated with OP-1 observed in fetal soft tissue or skeletal tissue. IV administration of OP-1 at doses up to 0.4 mg/kg/day (4 times the human dose, given daily for 13 days) on GD 6 to 18 of gestation showed no evidence of maternal or developmental toxicity in rabbits.

**Placental Transfer**

In a placental transfer study (Stryker Biotech Study 99-004; P060021, Appendix 2, page 7025), rats were given 3.8 mg/kg of OP-1 IV (38 times the human dose). The amount of OP-1 was estimated in the tissues by measuring radioactivity levels and autoradiography. It was determined that less than 1% of the dose was transferred to the fetus. Considering that no more that 2 to 3% of the dose is found in the bloodstream after implantation (Refer to P060021/A011 Section 3.2.2, page 39), the expected potential transfer of OP-1 may be negligible.

**Immunogenicity**

Studies were performed to evaluate the potential adverse effects of an immune response to OP-1 on offspring from rabbits intentionally immunized with human recombinant OP-1 and adjuvant. Results of these studies are summarized below.

**Phase 1–Immunization Pilot Study**

The objective of this study was to establish an immunization schedule that could be utilized in subsequent studies (Stryker Biotech Study 04-002; P060021, Appendix 2, page 7101). Serum samples from immunized rabbits were tested and analyzed for IgG and IgM isotype immunoreactivity. It was determined that complete Freund’s adjuvant and multiple boosts were required to elicit a robust antibody response. Each immunized rabbit received 350 µg of OP-1 for
the primary immunization and subsequent boosts. This immunization schedule was used in the ensuing study phases.

**Phase 2–Maternal Transfer of Antibody/Prenatal Study**

The objectives of this study (Stryker Biotech Study 04-010; P060021, Appendix 2, page 7158) were to evaluate the maternal transfer of anti-human OP-1 antibodies to offspring and potential developmental toxicity in offspring. Female New Zealand white rabbits were immunized with either 350 µg OP-1 in 0.5 mL complete Freund’s adjuvant or adjuvant alone (control) on Day 1. Adjuvant was used to enhance the immune response to OP-1. All animals were subsequently boosted on Days 14, 28, and on GD 12 with either 350 µg OP-1 in 0.5 mL incomplete Freund’s adjuvant (OP-1) or adjuvant alone (control). Immunizations were given SC during a period that included the mating period (with unimmunized males) and subsequent gestation period. All animals were observed twice daily for signs of morbidity, mortality, injury, changes in body weight, and access to food and water.

Blood samples were collected from the dams for measurement of serum anti-OP-1 antibodies on Day 1 (prior to dosing), Day 28 (prior to mating), and on GD 8, 14, 20, and 29. Samples were analyzed by ELISA in the Bioassay Development Laboratory at Stryker Biotech for titers of IgG and IgM antibodies to OP-1. Antibody titers were measured in the serum of the dams, fetuses, and umbilical cord blood. Nearly all of the serum samples from the dams and the fetuses had significant (and similar) titers of IgG and IgM immunoreactivity. IgM and IgG were also present in the umbilical cord blood (GD 29) of the dams at about the same level found in the maternal and fetal (pooled) serum samples. Little or no immunoreactivity was evident in the control groups.

The rabbits were euthanized on GD 29, and each dam was subjected to a complete necropsy, including a uterine examination, and selected tissues were removed, weighed, and preserved in fixative. The total number of implantations, early and late resorptions, number of corpora lutea, fetal mortality, sexes, and body weights were recorded. All fetuses were given an external visceral examination. Selected organs were dissected from one fetus/sex/litter, weighed, and preserved. All fetuses were processed for skeletal staining. Malformations and developmental variations were recorded. During this process, fetal and umbilical cord blood samples were collected and pooled by litter for serum anti-OP-1 antibody determination.

All animals survived to scheduled termination. Injection-site irritation attributed to Freund’s adjuvant was noted in both groups. There was no mortality or differences in pregnancy status between groups. There were no differences in body weight, food consumption, and uterine or
4.3.1 Developmental toxicity
(Content from P060021/A011 Section IV, Preclinical: section 4.9, page 60-65)

ovarian parameters. Likewise, no effect of treatment was evident from macroscopic observations. The only hints of activity were small and statistically insignificant reductions in thymus and spleen weights in the OP-1 dams. In a similar subsequent study, no differences were noted in either thymus or spleen weight in the dams at the time of weaning (see below). All other maternal organ weight data for the OP-1 group were comparable to controls and unaffected by treatment. Pregnancy rates in the control and OP-1 groups were 87% (20/23) and 96% (22/23), respectively, yielding 19 litters from controls and 22 litters from OP-1 groups with viable fetuses for evaluation on GD 29. No animals aborted or delivered early. No effect of treatment with OP-1 was evident from gravid uterine weights, adjusted body weight gain GD 0 to 29, or uterine implantation data. Likewise, no effect of treatment was evident in fetal body weights, sex ratio, fetal organ weights, or fetal external and visceral examinations. Sporadic skeletal malformations were noted in the OP-1 group among the fetuses involving sternebrae (fused), thoracic centra (fused, misshapen), and/or neural arch (misshapen). The total number of malformations was not statistically significant nor outside the reference ranges for historical controls.

Phase 3–Postnatal Study

This study (Stryker Biotech Study 04-011; P060021, Appendix 2, page 7429) was conducted to evaluate the maternal transfer of anti-OP-1 antibodies to offspring and to perform a postnatal evaluation of dams and litters. A summary of the study and its results is provided below. Female New Zealand white rabbits were immunized with either 350 µg OP-1 in 0.5 mL complete Freund’s adjuvant (OP-1) or adjuvant alone (control) on Day 1. The rabbits were subsequently boosted with either 350 µg OP-1 in 0.5 mL incomplete Freund’s adjuvant or adjuvant alone on Days 14, 28, and GD 12 and 26. The immunization process was done over approximately 6 weeks, and included the mating period (with untreated males) and the subsequent gestation and lactation period. All animals were observed twice daily for signs of morbidity, mortality, injury, changes in body weight, and the availability of food and water during the entire period. Females were allowed to deliver, and the dams and kits were observed over a 28-day lactation period. Litter size was recorded at birth lactation day (LD) 0, 7, 14, 21, and 28. At these same time points, the kits were weighed and given an external examination.

Maternal blood samples were collected for measurement of serum anti-OP1 antibodies on Day 1 (prior to dosing), Day 28 (prior to mating), on GD 8, 14, 20, 26, and on LD 0, 7, 14, 21, and 28. On LD 7, 14, 21, and 28, blood samples for serum anti-OP-1 antibody determination were collected and pooled from 4 kits in each litter. Milk samples for anti-OP-1 antibody
determination were collected from all dams with surviving kits on LD 4, 7, 14, 21, and 28. Antibody titers in milk and serum of dams and kits were determined. In general, nearly all dams and kits in the OP-1 groups had significant titers of IgG during the full course of the experiment. Little or no immunoreactivity was evident in the control groups. As in the previous study, IgM was also present in dam and kit sera, although levels waned in the kits by the end of the study. In milk samples from the OP-1 group, IgG titers were significant throughout the postnatal phase including the final measurement on LD 28.

On LD 28, each dam was subjected to a complete necropsy. These examinations were also conducted on all surviving kits on LD 28. The sex of each kit was recorded at necropsy. Protocol-designated organs for the dams and one randomly selected kit/sex/litter were collected and weighed. One OP-1 immunized dam was euthanized moribund in extremis on LD 19. This female was maintaining a litter of 8 kits at euthanasia. The deterioration in health of this animal in the OP-1-treated group was considered spurious and unrelated to treatment. All other OP-1-treated animals survived to scheduled termination.

During the clinical examinations, several animals in the control and OP-1-treated group had localized swellings and ulcerations in the region of the injections (shoulder, dorsal thorax). The occurrence of these findings along with scabbing in this area in a few animals was considered related to irritation at the injection site, possibly from the adjuvants, and not indicative of OP-1 toxicity. No effect of treatment was evident from maternal body weights, body weight gain, or food consumption during the premating, gestation, or lactation periods. Likewise, no effect of treatment was evident from pregnancy rates, parturition parameters (gestation length, litter size, stillborn indices), organ weights, or macroscopic observations. Pregnancy rates in each group were consistent with 23 control females and 24 OP-1 females delivering litters for evaluation. No effect of treatment was evident from litter size, kit clinical examinations at birth and during lactation, kit survival indices until weaning, kit sex ratios, or macroscopic examinations.

Mean OP-1 kit body weights were comparable to controls at birth (Day 0), but were slightly lower than controls for the remainder of lactation. From LD 7 to weaning (LD 28), kit weights in the OP-1 group were about 10 to 15% lower than controls. Generally these differences in weights were not statistically significant. The one exception occurred on LD 21; male kit weight (254.5 g) at this time point was about 14% less than controls (296.2 g). A mitigating factor was the large variation in kit weights based on litter size (which is expected). There were 21% fewer kits (63 versus 79) in the adjuvant control group than in OP-1 group. Although the difference between the two treatment groups was within expectations for variation, smaller litters tend to
have larger kits, a characteristic that is enhanced when the kits started to consume more of the maternal diet. Based on this variability and the general lack of statistical significance on the other lactation days, the lower kit weights do not appear to be indicative of a treatment-related response. Kit organ weights in litters from the OP-1 immunized dams were also generally similar to the control group. As expected, the slight difference in body weight between the groups created situations in which a few organ weight/body weight ratios were elevated in the OP-1 kits, including the brain and kidney. However, the absolute mean weights of the two organs in the OP-1 versus control groups were not different. Also the kidney weight/brain weight ratio of the 2 groups was similar. The only organ weight that changed in absolute terms compared to brain weight was the spleen in female kits. The organ weight was reduced by about 19.6% (similar to the difference in body weight).

In conclusion, in this series of rabbit perinatal and postnatal studies, OP-1 immunization with adjuvants was not maternally toxic, despite the fact that the animals were hyperimmunized with OP-1 and all dams had demonstrable IgG and IgM immunoreactivity in serum and breast milk. Immunization did not affect mating performance, fertility, or parturition parameters. Moreover, the IgG and IgM responses were not associated with toxicity in neonates through the time of weaning. Swelling and ulcerations at the injection site noted at clinical examination and confirmed macroscopically as thickened skin, were seen with similar frequency in control and treated animals and considered related to the injection procedure and/or adjuvants and unrelated to treatment.

**Ectopic Bone Studies**

Studies done by Reddi et al. have shown that collagen combined with BMP induces bone in a variety of anatomical environments (including the SC space). [Reddi, 2003] Consequently, it is not surprising that in many of the studies described throughout Section 2, Pharmacology, and Section 4.3, Toxicology, (e.g. Stryker Biotech Studies 04 008, 00 003, 96 001, and 97 002), ossified tissue and heterotopic bone (at the site of implantation or injection) are found after implantation or IV injection of preparations containing OP-1. Indeed, bone formation is the basis of the proposed therapeutic use of OP-1® Putty. It should be noted, however, that ectopic bone (bone that forms away from the site of implantation or injection) does not form at sites distant from the site of injection or implantation of OP-1; even when the molecule is given systemically at large repeated doses. Also, when implanted for the purpose of spinal fusion, OP-1® Putty does not migrate or cause islands of unwanted ossification in the surgical site. Even when intentionally applied under the dura during a spinal fusion, the new subdural bone did not
cause any noticeable adverse neurologic effects (Stryker Biotech Study 97-038; P060021, Appendix 2, page 1025). The results from these studies suggest that OP-1 is unlikely to form islands of unwanted ectopic bone when implanted in accordance with its intended use.

**Toxicology Conclusions**

OP-1® Putty appears to be safe at doses that are large in comparison with doses used in experimental models of spinal fusion and in clinical practice. It remains confined to the site of administration and does not circulate at high levels in the blood or into distant tissues. As OP-1 diffuses from the implantation site, it is cleared quickly by the kidneys. The lack of systemic distribution may be partially responsible for the large safety multiples observed in the use of OP-1. However, even when given by repeat IV administration, the molecule is nontoxic at high multiples except for implantation-site irritation which is expected given the pharmacology of the molecule. When large amounts of the molecule were given in the form of OP-1® Implant, pleomorphic sarcomas were observed at the site of implantation. This response is attributable to a solid state carcinogenicity phenomenon that is unique to rodents. It might be expected, based on the role of OP-1 in development that its use might cause developmental toxicity or teratogenicity. However, when administered at high repeated IV doses, OP-1 had no adverse maternal or perinatal effects. Even when rabbits were intentionally immunized with xenogeneic OP-1 using an adjuvant, there were no significant developmental abnormalities. In conclusion, there were no observations in the toxicology studies that would preclude the use of OP-1 at its intended dose for spinal fusion.

**Reference**