Preface:

Animals placed in Column "E" in this report were enrolled in studies undertaken for product registration purposes based on regulatory guidelines of the FDA 21 CFR 312.23 for pharmacology and toxicology studies and the Red Book. Guidance for study design and conduct also conformed with recommendations by the International Conference on Harmonization Guidelines. This guidance includes Part VII, DHHS, FDA, International Conference on Harmonization; Guidance on non-clinical safety studies for the conduct of human clinical trial for pharmaceuticals, Federal Register, Vol. 62, #227, November 25, 1997.

As per the ICH Guideline M3(R1) regulatory citation, *" The goals of the non-clinical safety evaluation include a characterization of toxic effects with respect to target organs, dose dependence, relationship to exposure, and potential reversibility. This information is important for estimation of an initial safe starting dose for the human clinical trials and the identification of parameters for clinical monitoring for potential adverse effects. The non-clinical safety studies...should be adequate to characterize potential toxic effects under the conditions of the supported clinical trial."*

During the conduct of an animal toxicology study that is required by regulatory agencies, it is possible that some of the clinical signs of toxicity may result in more than momentary pain and/or distress. However, if one does not allow these signs of toxicity to develop, then the primary scientific goal of characterizing the toxic effects of the test article will not be achieved (and the study would be considered invalid by the regulatory authorities). Results of toxicology studies become part of the safety assessment of the potential new human drug that will result in the determination of an initial exposure of human subjects and the identification of parameters for clinical monitoring for potential adverse effects of the drug on people. During the conduct of an animal toxicology study, each drug-related effect is evaluated by the attending veterinary staff and the study director to determine if treatment to alleviate more than momentary distress/pain could interfere with the regulatory purpose/scientific goal (conduct) of the study. Treatments that could interfere with the purpose of conduct of the study are prohibited by FDA Good Laboratory Practice regulations [§ 58.90 (c)] and are withheld to assure that toxic effects can be evaluated.

Depending upon the nature of the compound, certain other regulations and guidelines promulgated by the FDA, EPA, TSCA, FIFRA and the OECD also apply and are listed in the Applicable Guidelines/Regulations section below.

Animals are placed in Category "E" following retrospective analysis. Retrospective categorization of pain or distress was made by the Attending Veterinarian (or their designee, also a laboratory animal veterinarian) in conjunction with the Study Director. Professional judgment calls, particularly with regard to the diagnosis of distress, were purposely conservative with a default of category E if there was any doubt.

The following are applicable guidelines and regulations covering the conduct of studies at all Charles River Laboratory Preclinical Services facilities (listed below).

- Redbook 2000 Toxicological Principles for the Safety Assessment of Food Ingredients November 2003.
- PART VII, DHHS, FDA, International Conference on Harmonization; Guidance on Non-Clinical Safety Studies for the Conduct of Human Clinical Trial For Pharmaceuticals, Federal Register, Vol. 62, No. 227, Nov 25, 1997
- EPA Health Effects Test Guidelines OPPTS 870.3050, 28-Day Oral Toxicity in Rodents, July 2000
- EPA Health Effects Test Guidelines OPPTS 870.3150, 90-Day Oral Toxicity in Non-Rodents, August 1998
- EPA Health Effects Test Guidelines OPPTS 870.3100, 90-Day Oral Toxicity in Rodents, August 1998
- EPA Health Effects Test Guidelines OPPTS 870.4100, Chronic Toxicity, August 1998
- EPA Health Effects Test Guidelines OPPTS 870.3500, Preliminary Developmental Toxicology Screen, March 1994

- EPA Health Effects Test Guidelines OPPTS 870.3600, Inhalational Developmental Toxicity Study March 1994
- EPA Health Effects Test Guidelines OPPTS 870.3700, Prenatal Developmental Toxicity Study, August 1995
- EPA Health Effects Test Guidelines OPPTS 870.3800, Reproduction and Fertility Effects, August 1995
- OECD Guideline for the Testing Of Chemicals, Repeated Dose 90-day Oral Toxicity Studies in Non-Rodents, 409, September 1998
- OECD Guideline for the Testing Of Chemicals, Repeated Dose 90-day Oral Toxicity Studies in Rodents, 408, September 1998
- OECD Guideline for the Testing Of Chemicals, Repeated Dose 28-day Oral Toxicity Studies in Rodents, 407, July 1995
- U.S. Food and Drug Administration (1994). International Conference on Harmonization; Guideline on detection of toxicity to reproduction for medicinal products. *Federal Register*, September 22, 1994, Vol. 59, No. 183.
- U.S. Food and Drug Administration. Good Laboratory Practice Regulations; Final Rule. 21 CFR Part 58; last revised April 1, 2002; U.S. Federal Government Archives.
- Japanese Ministry of Health, Labour and Welfare (1997). Good Laboratory Practice Standard for Safety Studies on Drugs, MHW Ordinance Number 21, March 26, 1997.
- Organisation for Economic Co-operation and Development (1998). The Revised OECD Principles of Good Laboratory Practices [C(97) 186/Final].
- U.S. Food and Drug Administration (2003). *Guidance for Industry - Photosafety Testing,* Center for Drug Evaluation and Research (DCER), May 2003
- Organisation for Economic Co-operation and Development (1987). *Guidelines for Testing of Chemicals*. Section 4, No. 402: Acute Dermal Toxicity, pp. 1-7
- Organisation for Economic Co-operation and Development (1992). *Guidelines for Testing of Chemicals*. Section 4, No. 406: Skin Sensitization, pp. 1-9.
- *Drug Registration Requirements in Japan,* 4th Edition (19910. Yakuji Nippo, Ltd., Tokyo, pp. 61-64.
- U.S. Food and Drug Administration (2005) Investigational New Drug Application, Title 21, Part 321.23, 8.ii.a
- Organisation for Economic Co-operation and Development (1998). The Revised OECD Principles of Good Laboratory Practices [ENV/MC/CHEM(98)17]
- U.S. Dept of Health and Human Services Food and Drug Administration. Guideline for Industry; detection of toxicity to reproduction for medicinal products, (ICH) S5A; September, 1994. Rockville.
- Pharmaceutical Affairs Bureau, Ministry of Health, Labour and Welfare, GLP standard ordinance for non-clinical laboratory studies on safety of drugs, MHW Ordinance No. 21; March 26, 1997. Japan.
- OECD Environment Directorate. OECD Principles of Good Laboratory Practices, [C(97) 186/Final] (1998); Environmental Health and Safety Division
- U.S. Food and Drug Administration (1993). Points to consider in the characterization of cell lines used to produce biologicals.
- European Pharmacopoeia Monograph 5.2.3, Cell substrates for production of vaccines for human use. 01/2005:50203
- U.S. Dept of Health and Human Services Food and Drug Administration. Guideline for Industry; toxicokinetics: the assessment of systemic exposure in toxicity studies, ICHS3A; March, 1995, Rockville (MD).
- International Conference on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use: Guidance for Industry, MD(R1) Nonclinical Safety Studies for the Conduct of Human Clinical Trials for Pharmaceuticals.
- FDA Guidance for Industry characterization and qualification of cell substrates and other biological materials used in the production of viral vaccines for infectious disease indications Feb 2010.
- European Pharmacopea monograph 5.2.3 Cell substrates for production of vaccines for human use 01/2005;50203

REVIEW OF CATETORY "E" STUDIES

The following studies have been listed in Category "E" based upon the guidelines stated in the preface at the beginning of this report. The study designs that resulted in certain animals being placed retrospectively into Category "E" were required by federal regulations and guidelines listed in the applicable regulations/guidelines section below. For the purpose of this report studies have been given a unique number that corresponds to the actual study number. For reasons of confidentiality, actual study numbers are not presented but are available to the USDA for on-site inspection or report follow-up. Category "E" explanations/details are listed separately for each study.

Study: #1

Animals: 3 Rabbits

Type of Study: Embryo-Fetal Development Study

Guidelines/Regulations:

- U.S. Food and Drug Administration. Good Laboratory Practice Regulations; Final Rule. 21 CFR Part 58; last revised April 1, 2002; U.S. Federal Government Archives.
- ICH Harmonized Tripartite Guideline. Detection of Toxicity to Reproduction for Medicinal Products, and Toxicity to Male Fertility S5 (R2). Parent Guideline dated 24 June 1993. Adopted by CPMP, September 93, issued as CPMP/ICH/386/95. This study evaluates ICH Harmonised Tripartite Guideline stages C and D of the reproductive process.
- U.S. Dept of Health and Human Services Food and Drug Administration. Guideline for Industry: detection of toxicity to reproduction for medicinal products, (ICH) S5A; September, 1994. Rockville (MD) Federal Register, September 22, 1994, Vol. 59, No. 183.
- Japanese Ministry of Health, Labour and Welfare (1997). Good Laboratory Practice Standard for Safety Studies on Drugs, MHW Ordinance Number 21, March 26, 1997.
- Organisation for Economic Co-operation and Development (1998). The Revised OECD Principles of Good Laboratory Practices [ENV/MC/CHEM(98)17]

Diagnosis:

Three rabbits from this embryo-fetal development study.

#9107 experienced body weight loss (21% over approximately twenty-one days) and a reduction in feed consumption during that time period. Supplemental feed items and a secondary water source were provided. While no other clinical signs were observed prior to euthanasia it is possible that the inappetance and weight loss may have been consistent with more than momentary distress prior to euthanasia and therefore this rabbit was conservatively categorized as E.

#9134 experienced body weight loss (24% over approximately twelve days) and a reduction in feed consumption during that time period. Supplemental feed items and a secondary water source were provided. The animal was euthanized when its clinical condition declined. While the animal was euthanized when its clinical condition declined, it is possible that the inappetance and weight loss prior to euthanasia may have been consistent with more than momentary distress so this rabbit was conservatively categorized as E.

#9175 experienced body weight loss (20% over approximately eight days) and a reduction in feed consumption during that time period. Supplemental feed items and a secondary water source were provided. The animal was euthanized when it was determined that it was no longer pregnant. While no other clinical signs were observed prior to euthanasia it is possible that the inappetance and weight loss may have been consistent with more than momentary distress prior to euthanasia and therefore this rabbit was conservatively categorized as E.

Study: #2

Animals: 2 Rabbits

Type of Study: Dosage Range-Finding Embryo-Fetal Development Study

Guidelines/Regulations:

- This study was conducted to support subsequent required regulatory studies, and by that requirement, a maximum tolerated dose (MTD) is required to be included in the full study to assess the safety of the test material. This dosage-range study was done to determine dosage selection for future studies that will be based on U.S. Department of Health and Human Services Food and Drug Administration: Guidelines for Industry: detection of toxicity to reproduction for medicinal products, (ICH) S5A; September 1994, Rockville, MD. These guidelines require that dosages produce some maternal toxicity. Conducting the dosage range study prior to a full study, allows the determination of a toxic dose acceptable while only using a few animals. Without conduct of this dosage-range study prior to the required full developmental toxicity studies, larger numbers of animals may be needed for the full study.

Diagnosis:

Two rabbits from this dosage range-finding embryo-fetal development study.

#3913 experienced body weight loss (23% over approximately nine days) and a reduction in feed consumption during that time period. Supplemental feed items and a secondary water source were provided. The animal was euthanized when it was determined that it was no longer pregnant. While no other clinical signs were observed prior to euthanasia it is possible that the inappetance and weight loss may have been consistent with more than momentary distress prior to euthanasia and therefore this rabbit was conservatively categorized as E.

#3915 died acutely. Prior to death, the animal experienced body weight loss (23% over approximately sixteen days) and a reduction in feed consumption during some of that time period. Supplemental feed items and a secondary water source were provided. While no other clinical signs were observed prior to acute death it is possible that the prior inappetance and weight loss may have been consistent with more than momentary distress and therefore this rabbit was conservatively categorized as E.

Study: #3

Animals: 2 Rabbits

Type of Study: Embryo-Fetal Development Study

Guidelines/Regulations:

- U.S. Food and Drug Administration. Good Laboratory Practice Regulations; Final Rule. 21 CFR Part 58; last revised April 1, 2002; U.S. Federal Government Archives.
- ICH Harmonized Tripartite Guideline. Detection of Toxicity to Reproduction for Medicinal Products, and Toxicity to Male Fertility S5 (R2). Parent Guideline dated 24 June 1993. Adopted by CPMP, September 93, issued as CPMP/ICH/386/95. This study evaluates ICH Harmonised Tripartite Guideline stages C and D of the reproductive process.
- U.S. Dept of Health and Human Services Food and Drug Administration. Guideline for Industry: detection of toxicity to reproduction for medicinal products, (ICH) S5A; September, 1994. Rockville (MD) Federal Register, September 22, 1994, Vol. 59, No. 183.
- Japanese Ministry of Health, Labour and Welfare (1997). Good Laboratory Practice Standard for Safety Studies on Drugs, MHW Ordinance Number 21, March 26, 1997.
- Organisation for Economic Co-operation and Development (1998). The Revised OECD Principles of Good Laboratory Practices [ENV/MC/CHEM(98)17]

Diagnosis:

Two rabbits from this embryo-fetal development study.

#2508 experienced body weight loss (22% over approximately twenty three days) and a reduction in feed consumption during that time period. Supplemental feed items and a secondary water source were provided. While no other clinical signs were observed prior to euthanasia it is possible that the inappetance and weight loss may have been consistent with more than momentary distress prior to euthanasia and therefore this rabbit was conservatively categorized as E.

#2518 experienced a reduction in feed consumption for approximately 9 days. Supplemental feed items and a secondary water source were provided. Clinical signs including cool to touch, decreased activity and ataxia were noted. The animal was euthanized when its clinical condition declined. While the animal was euthanized when these clinical signs were observed, it is possible that the inappetance and weight loss prior to euthanasia may have been consistent with more than momentary distress so this rabbit was conservatively categorized as E.

Study: #4

Animals: 1 Hamster

Type of Study: Fertility and Early Embryonic Development Study

Guidelines/Regulations:

- U.S. Food and Drug Administration. Good Laboratory Practice Regulations; Final Rule. 21 CFR Part 58; last revised April 1, 2002; U.S. Federal Government Archives.
- ICH Harmonized Tripartite Guideline. Detection of Toxicity to Reproduction for Medicinal Products, and Toxicity to Male Fertility S5 (R2). Parent Guideline dated 24 June 1993. Adopted by CPMP, September 93, issued as CPMP/ICH/386/95. This study evaluates ICH Harmonised Tripartite Guideline stages C and D of the reproductive process.
- U.S. Dept of Health and Human Services Food and Drug Administration. Guideline for Industry: detection of toxicity to reproduction for medicinal products, (ICH) S5A; September, 1994. Rockville (MD) Federal Register, September 22, 1994, Vol. 59, No. 183.
- Japanese Ministry of Health, Labour and Welfare (1997). Good Laboratory Practice Standard for Safety Studies on Drugs, MHW Ordinance Number 21, March 26, 1997.
- Organisation for Economic Co-operation and Development (1998). The Revised OECD Principles of Good Laboratory Practices [ENV/MC/CHEM(98)17]

Diagnosis:

One hamster from this fertility and early embryonic development study.

#1922 experienced a reduction in feed consumption and associated body weight loss for approximately 6 days. Supplemental feed items, administration of supplemental fluids and a secondary water source were provided. The animal was euthanized when it did not respond to supportive care. While the animal was euthanized when its clinical condition declined, it is possible that the inappetance and weight loss prior to euthanasia may have been consistent with more than momentary distress so this hamster was conservatively categorized as E.

Study: #5

Animals: 1 Rabbit

Type of Study: Embryo-Fetal Development Study

Guidelines/Regulations:

- U.S. Food and Drug Administration. Good Laboratory Practice Regulations; Final Rule. 21 CFR Part 58; last revised April 1, 2002; U.S. Federal Government Archives.
- ICH Harmonized Tripartite Guideline. Detection of Toxicity to Reproduction for Medicinal Products, and Toxicity to Male Fertility S5 (R2). Parent Guideline dated 24 June 1993. Adopted by CPMP, September 93, issued as CPMP/ICH/386/95. This study evaluates ICH Harmonised Tripartite Guideline stages C and D of the reproductive process.
- U.S. Dept of Health and Human Services Food and Drug Administration. Guideline for Industry: detection of toxicity to reproduction for medicinal products, (ICH) S5A; September, 1994. Rockville (MD) Federal Register, September 22, 1994, Vol. 59, No. 183.
- Japanese Ministry of Health, Labour and Welfare (1997). Good Laboratory Practice Standard for Safety Studies on Drugs, MHW Ordinance Number 21, March 26, 1997.
- Organisation for Economic Co-operation and Development (1998). The Revised OECD Principles of Good Laboratory Practices [ENV/MC/CHEM(98)17]

Diagnosis: One rabbit from this embryo-fetal development study.

#8927 experienced body weight loss (19-26% over approximately fourteen days) and a reduction in feed consumption during some of that time period. Supplemental feed items and a secondary water source were provided. While no other clinical signs were observed prior to euthanasia it is possible that the inappetance and weight loss may have been consistent with more than momentary distress prior to euthanasia and therefore this rabbit was conservatively categorized as E.

Study: #6

Animals: 3 Rabbits

Type of Study: Embryo-Fetal Development Study

Guidelines/Regulations:

- U.S. Food and Drug Administration. Good Laboratory Practice Regulations; Final Rule. 21 CFR Part 58; last revised April 1, 2002; U.S. Federal Government Archives.
- ICH Harmonized Tripartite Guideline. Detection of Toxicity to Reproduction for Medicinal Products,

and Toxicity to Male Fertility S5 (R2). Parent Guideline dated 24 June 1993. Adopted by CPMP, September 93, issued as CPMP/ICH/386/95. This study evaluates ICH Harmonised Tripartite Guideline stages C and D of the reproductive process.

- U.S. Dept of Health and Human Services Food and Drug Administration. Guideline for Industry: detection of toxicity to reproduction for medicinal products, (ICH) S5A; September, 1994. Rockville (MD) Federal Register, September 22, 1994, Vol. 59, No. 183.
- Japanese Ministry of Health, Labour and Welfare (1997). Good Laboratory Practice Standard for Safety Studies on Drugs, MHW Ordinance Number 21, March 26, 1997.
- Organisation for Economic Co-operation and Development (1998). The Revised OECD Principles of Good Laboratory Practices [ENV/MC/CHEM(98)17]

Diagnosis: Three rabbits from this embryo-fetal development study.
#1137 experienced a reduction in feed consumption for approximately 4 days. Supplemental feed items and a secondary water source were provided. Clinical signs including abdominal distension and dehydration were noted. The animal was euthanized when its clinical condition declined. While the animal was euthanized when its clinical condition declined, it is possible that the inappetance and weight loss prior to euthanasia may have been consistent with more than momentary distress so this rabbit was conservatively categorized as E.
#1163 died acutely. Prior to death, the animal experienced body weight loss (23% over approximately nine days) and a reduction in feed consumption during that time period. Supplemental feed items and a secondary water source were provided. While no other clinical signs were observed prior to acute death it is possible that the prior inappetance and weight loss may have been consistent with more than momentary distress and therefore this rabbit was conservatively categorized as E.
#1178 experienced body weight loss (24% over approximately sixteen days) and a reduction in feed consumption during that time period. Supplemental feed items and a secondary water source were provided. The animal was euthanized when it was determined that it was no longer pregnant. While no other clinical signs were observed prior to euthanasia it is possible that the inappetance and weight loss may have been consistent with more than momentary distress prior to euthanasia and therefore this rabbit was conservatively categorized as E.

Study: #7
Animals: 2 Hamsters
Type of Study: Fertility and Early Embryonic Development Study
Guidelines/Regulations:

- U.S. Food and Drug Administration. Good Laboratory Practice Regulations; Final Rule. 21 CFR Part 58; last revised April 1, 2002; U.S. Federal Government Archives.
- ICH Harmonized Tripartite Guideline. Detection of Toxicity to Reproduction for Medicinal Products, and Toxicity to Male Fertility S5 (R2). Parent Guideline dated 24 June 1993. Adopted by CPMP, September 93, issued as CPMP/ICH/386/95. This study evaluates ICH Harmonised Tripartite Guideline stages C and D of the reproductive process.
- U.S. Dept of Health and Human Services Food and Drug Administration. Guideline for Industry: detection of toxicity to reproduction for medicinal products, (ICH) S5A; September, 1994. Rockville (MD) Federal Register, September 22, 1994, Vol. 59, No. 183.
- Japanese Ministry of Health, Labour and Welfare (1997). Good Laboratory Practice Standard for Safety Studies on Drugs, MHW Ordinance Number 21, March 26, 1997.
- Organisation for Economic Co-operation and Development (1998). The Revised OECD Principles of Good Laboratory Practices [ENV/MC/CHEM(98)17]

Diagnosis:
Two hamsters from this fertility and early embryonic development study.
#1221 and #1305 experienced body weight loss (31-38% over approximately seven to eighteen days) and a reduction in feed consumption during that time period. Supplemental feed items, supplemental heat source, subcutaneous fluid administration and a secondary water source were provided. Intermittent clinical signs including dehydration, unkempt fur and abnormal posture were noted. The animals were euthanized when their clinical condition declined. While the animals were euthanized their clinical condition declined, it is possible that some of these clinical signs or the inappetance and weight loss prior to euthanasia may have been consistent with more than momentary distress so these hamsters were conservatively categorized as E.

Study: #8

Animals: 1 Rabbit

Type of Study: Dosage Range-Finding Study

Guidelines/Regulations:

- This study was conducted to support subsequent required regulatory studies, and by that requirement, a maximum tolerated dose (MTD) is required to be included in the full study to assess the safety of the test material. This dosage-range study was done to determine dosage selection for future studies that will be based on U.S. Department of Health and Human Services Food and Drug Administration: Guidelines for Industry: detection of toxicity to reproduction for medicinal products, (ICH) S5A; September 1994, Rockville, MD. These guidelines require that dosages produce some maternal toxicity. Conducting the dosage range study prior to a full study, allows the determination of a toxic dose acceptable while only using a few animals. Without conduct of this dosage-range study prior to the required full developmental toxicity studies, larger numbers of animals may be needed for the full study.

Diagnosis:

One rabbit from this dosage range-finding study.

#6130 experienced body weight loss (25% over approximately four days) and a reduction in feed consumption during that time period. Supplemental feed items, subcutaneous fluid administration, oral electrolytes and a secondary water source were provided. Intermittent clinical signs including dehydration, decreased activity, abdominal distention and abnormal fecal output were noted. The animal was euthanized when it didn't respond to supportive care. While the animal was euthanized when its clinical condition declined, it is possible that some of these clinical signs or the inappetance and weight loss prior to euthanasia may have been consistent with more than momentary distress so this rabbit was conservatively categorized as E.