Endotoxicity and Cytotoxicity Studies

1) Aug 2005	**In Vitro Chromosomal Aberration Study – Cytotoxicity (File: TS39)**
Investigator	**NAMSA, Northwood, Ohio**
Summary	Chinese Hamster Ovary (CHO) cells were grown in monolayer with and without metabolic activation. Growth medium was replaced by the product with various concentrations and incubated. Cytotoxicity was monitored by means of microscopically observing inner-cell structures and chromosomes and scanning for damage.
Conclusion	No cytotoxicity can be detected at concentrations up to 100%.

Genotoxicity Studies

1) Aug 2005	**Genotoxicity: Chromosomal Aberration Study Dose Range finding (File: TS39)**
Investigator	**NAMSA, Northwood, Ohio**
Summary	Chinese Hamster Ovary (CHO) cells were grown in monolayer with and without metabolic activation. Various concentrations of the product were placed in the growth medium and incubated for 20 hours. Cells were stained and visually examined microscopically for any visual signs of toxicity or chromosome aberration.
Conclusion	The product does not cause any visually detectable damage to the chromosomes up to very large doses (50% solutions).

2) Dec 2005	**Genotoxicity: Chromosomal Aberration Study in Mammalian Cells (File: TS35)**
Investigator	**NAMSA, Northwood, Ohio**
Summary	Chinese Hamster Ovary (CHO) cells were grown in monolayer with and without metabolic activation. Concentrations of the product up to 50% were placed in the growth medium and incubated for 20 hours. Cells were stained and visually examined microscopically for chromatid gaps or breaks, chromosome breaks or fragments of several different types, or chromosome interchanges. Percentages of chromosomal aberrations were determined and statistically analyzed for each aberration class.

Conclusion	The product does not cause damage to the chromosomes. There was often less chromosomal aberration in the cultures with the product than in cultures with the salt water control. No evidence of increased chromosome damage of any sort was found.

3) Jan 2006	**Genotoxicity: Reverse Mutation Study (File: TS39)**
Investigator	**NAMSA, Northwood, Ohio**
Summary	Reverse genetic mutations were counted in 5 different strains of specially prepared mutant bacteria, sensitive to genetic reverse mutation due to toxicity. Cultures were exposed to various high concentrations of the product and compared to cultures with negative and positive controls. The number of reverse mutations indicates how strong the expected mutagenic effect of the product has on DNA. All tests were run in triplicate.
Conclusion	The product is not a potential mutagen. No significant changes in reverse mutation were observed in any of the bacterial strains at any dose.

4) 2005	**Gene Expression Analysis for Toxicity in Rabbits (File: TS22)**
Investigator	**Affimetrix, Functional Genomics Center, University of Albany**
Summary	4 Groups of New Zealand white rabbits received daily injections of various concentrations of MDI-P or saline for 3 days. Full genetic activity for over 10,000 different genes was analyzed from samples taken before and after exposure and from the control groups. From this analysis the genetic expressions of inflammatory cytokines, immune activation messengers and other genetic markers of toxic response were measured.
Conclusion	No genes were expressed that indicated a toxic response to MDI-P. There was a significant 2X down regulation of the SOCS2 gene families, indicating a reduction of inflammation and a significant 7X up regulation in the TIA1 genes calling for programmed cell death of damaged cells.

Accute Toxicology Studies

1) Jun 1994	**Fourteen Day Acute Intravenous Toxicity Study of the Product in Mice (File: TS6)**
Investigator	**Biological Test Center, Irvine, California**

Summary	The product was injected into 4 groups of 5 mice, 4 times on the first day, and once a day thereafter for 14 days. The 4 groups received various doses up to 8 ml/kg body weight (over 50 times the equivalent amount expected to be in the blood of a typical human adult). The mice were monitored carefully for any signs of toxicity including death, signs of irritation or illness, and behavioral abnormalities.
Conclusion	Large amounts of the product in the blood do not cause signs of illness or behavioral changes in mice.

2) Jan 2002	**Rising Dose Tolerance Study of the Product Administered to Dogs (File: TS10)**
Investigator	**WIL Research, Ashland, Ohio**
Summary	The product was injected into 2 male and 2 female Beagle dogs every 2 days with rising doses 10, 20, 40 ml/Kg for the first 3 days and 20 ml/Kg (over 300 times the maximum amount expected to be in the blood of a typical human adult) for the remaining 4 days. Blood Pressure, body weight, and heart ECG's were recorded before and after each dosing. Comprehensive blood tests were also done. Careful monitoring for any signs of toxicity or behavioral abnormalities was done daily. Nothing out of the normal was observed for any animal. Blood pressure, ECG scans and blood work were all normal across the board.
Conclusion	Extremely large daily amounts of the product in the blood of dogs do not cause any abnormal vital signs, weight loss, blood chemistry or signs of illness or adverse behavioral changes over a period of 7 days.

3) Feb 2002	**A 4-Week Toxicity Study of the Product in Dogs (File: TS11)**
Investigator	**WIL Research, Ashland, Ohio**
Summary	The product was injected into four groups of 3 male and 3 female Beagle dogs, with doses from 5 to 20 ml/Kg every day for 28 consecutive days (many hundreds of times the maximum amount expected to be in the blood of a typical human adult). Blood pressure, body weight, and heart ECG's were recorded before and after each dosing. Comprehensive blood and urine tests were also done. Careful monitoring for any signs of toxicity or behavioral abnormalities was done daily. Nothing out of the normal was observed for any animal. Blood pressure, ECG scans and blood/urine work were all normal across the board. Literally volumes of data were collected on each animal.

Conclusion	Extremely large daily amounts of the product in the blood of dogs do not cause any abnormal vital signs, weight loss, blood/urine chemistry or signs of illness or adverse behavioral changes over a period of 28 days.

4) Jan 2006	**Mouse Peripheral Blood Micronucleus Study: Dose Range Finding (File: TS40)**
Investigator	**NAMSA, Northwood, Ohio**
Summary	The product was injected into 4 groups of 10 mice at various doses up to 20 ml/Kg (50 times the maximum equivalent amount expected in the blood of a typical adult) once a day for 3 days. Body weight and careful behavioral observations were made. All animals had normal body weight and behavior during testing.
Conclusion	Extremely large concentrations of the product in the blood of mice do not cause any abnormal or adverse behavioral effects or body weight changes.

5) 2005	**Toxicological Study – 3 Day Acute Toxicity Study of the Product in Rabbits (File: TS27)**
Investigator	**James Clagett, Ph.D., Snohomish, Washington**
Summary	Various concentrations of the produc t or saline were injected into 4 groups of New Zealand white rabbits for 3 days. Comprehensive blood analysis, including white blood cell counts, ALT, GLU, BUN, Albumin and hematocrit levels were done. Histopathologies of the colon, esophagus, eye, heart, kidney, intestines, liver, lung , reproductive organs, muscle, spleen, stomach and tongue were microscopically performed to check for any abnormalities. After MDI-P exposure, the AST:ALT ratio was lower by 60%, serum protein and albumin levels were reduced 48%. No signs of toxicity were observed.
Conclusion	Extremely large concentrations of the product in the blood of rabbits do not cause any adverse effects or tissue damage to major organs. Changes in blood composition were potentially beneficial.

6) Jan 2006	**Central Nervous and Behavioral Effects of Mice Exposed Nasally to the Product (File: TS41)**
Investigator	**James Clagett, Ph.D., Snohomish, Washington**

Summary	4 groups of 5 mice were exposed to nebulized vapor of the product through a nose cone over a 28 day dosing period. Breathing volumes, respiratory rates were monitored 3 times a week. Telemetric body movements and body temperature to measure normal function of the central nervous system was sampled every 3 minutes over a 15 to 17 hour period every other day. Blood/urine samples and microscopic observations of select organs were made. There was no irritation to the soft tissues of the nose, mouth, throat and eyes due to long-term contact of the product vapor. No change was observed in respiratory rates, breathing volumes, blood/urine markers or major organs. There was no evidence of central nervous system damage.
Conclusion	Nebulized product does not cause irritation to soft tissues of the nose, mouth, throat, eyes or lungs in mice over long-term exposures. There are no signs of toxicity, inflammation or damage to any of the major organs due to breathing the product.

7) Mar 2006	**Cardiovascular, Pulmonary and Residual Toxic Effects on Dogs Exposed Nasally to the Product (File: TS21,TS32)**
Investigator	**MPI Research, Mattawan, Michigan**
Summary	Nebulized product, through a mask, was administered to 1 control group and 3 treatments groups of 4 male and 4 female Beagle dogs about 5 hours every day for 28 days. Cardiovasular (ECG) and pulmonary activity was monitored before, during and after dosing. Blood and urine panels were taken every week, with full blood-gas analysis. Full histopathological analysis was done on all major organs/systems. Dogs often slept comfortably through the procedure.
Conclusion	Nebulized product vapor does not cause irritation to the soft tissues of the nose, mouth, throat, eyes or lungs in dogs over long-term exposures. No abnormal macroscopic or microscopic damage, blood or urine markers, blood gas findings, are observed.

Long-Term Toxicology Studies

1) Jan 2005	**A Chronic Six-Month Toxicology Study of the Product in Mice (File: TS30)**
Investigator	**James Clagett, Ph.D., Snohomish, Washington**

Summary	The product was injected into 4 groups of 4 mice at various concentrations (up to 50 times the maximum equivalent amount expected in the blood of a typical adult) once a week for 28 weeks. Body weight and careful behavioral observations were made. All animals had normal body weight and behavior during testing. Heart, liver, kidney, spleen, intestine and lung histopathologies were performed and all normal for every animal.
Conclusion	Long-term, high concentrations of the product in the blood of mice do not cause any abnormal or adverse behavioral effects, signs of toxicity or tissue damage.