CENTER FOR DRUG EVALUATION AND RESEARCH

APPLICATION NUMBER:

022472Orig1s000

PHARMACOLOGY REVIEW(S)

DEPARTMENT OF HEALTH & HUMAN SERVICES
Food and Drug Administration

Memorandum

Date: March 4, 2014
From: Miyun Tsai-Turton PhD, MS; Pharmacologist; DMEP
Subject: Pharmacology/Toxicology Memo
To: NDA 22-472 Afrezza/Mannkind
Reference: Pharmacology/Toxicology Review & Evaluation of NDA 22-472 (eCTD SN 0074)

Background

Afrezza or Technosphere insulin (TI) is inhaled as a dry powder with the aid of a Gen2 inhaler. Technosphere insulin is comprised of recombinant human insulin, a novel excipient fumaryl diketopiperazine (FDKP), the MannKind proprietary excipient and primary component of Technosphere, and (b) (4) polysorbate 80 (PS80). FDKP undergoes acid-induced intermolecular self-assembly in aqueous solution resulting in adsorption of insulin onto the Technosphere particles to form Technosphere insulin.

This NDA 22472 (originally submitted under IND 61729) has gone through two review cycles since 2008 and both cycles resulted in Completed Response letters being issued. All correspondents between FDA and the sponsor to address deficiencies were documented in DARRTS. The NDA was resubmitted on Oct 2013.

Drug Product

Technosphere Insulin is provided as a 0.35 mg (10 U) insulin cartridge (3.33 mg TI powder) and a 0.7 mg (20 U) insulin cartridge (6.67 mg TI powder), based on cartridge fill weights.
The highest anticipated clinical dose for TI is 4 U/kg/day or 300 U/day (assuming 75 kg body weight). It will be administered during meal time 3 to 4 times daily. Appropriate doses for each patient will be established by titration. **Note: The sponsor's designation of U refers to Units of Afrezza not International Units of insulin. For example 300 U Afrezza=99 mg TI= (b) (4) mg insulin + (b) (4) mg Technosphere (FDKP).**

Regulatory History

- March 2009 – original NDA submission with the **MedTone** device
- **Dec 2009 - Nonclinical reviews are in DARRTS. Pharm/tox recommended approval. Labeling recommendation was also included in the review.**
- March 2010 – FDA issued a CR letter containing clinical, clinical pharmacology, labeling, and device deficiencies
- June 2010 – EOR meeting was held. The sponsor resubmitted this NDA application with the **Gen2C** device later that month.
- Jan 2011 – FDA issued another CR letter containing clinical, clinical pharmacology, product quality, device, and labeling deficiencies.
- May 2011 – EOR meeting was held.
- Oct 2011 – Type C meeting: written response was granted.
- Nov 2011 – The briefing document for the Written Response was submitted (eCTD SN 0064).
- **Jan 2012 - Nonclinical memo is in DARRTS.** The sponsor submitted one 28 day repeat-dose toxicity study to support their impurity limits (MKC-PC-2010-0042; 37 out of 813 pages was provided). This study focused on TI-related impurities/degradants.

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- August 2012 – Meeting package was submitted re: Labeling and Human Factors Usability. Written Responses Letter was issued in Nov 2012.
- Feb 2013 – Meeting package was submitted re: Clinical and Labeling. Written Responses was issued in March 2013.
- Oct 2013 – Resubmission of NDA

There are two pivotal Phase III clinical trials conducted in T1DM (Study No. MKC-TI-171) and T2DM (Study No. MKC-TI-175). These studies intend to demonstrate that the efficacy of the TI-Gen2 group (Technosphere Insulin Inhalation Powder administered using the Gen2C inhaler in combination with a basal insulin) will be non-inferior to the insulin aspart group (insulin aspart in combination with a basal insulin) by measuring HbA1c levels in T1DM or T2DM patients.

Nonclinical Aspect of Afrezza

During 1st cycle NDA submission, Pharm/tox reviewed all nonclinical studies with Technosphere Insulin (TI) and/or Technosphere particles alone (FDKP was the novel excipient). Pharm/tox concluded that there might be lung irritation, based on 39 week inhalation dog study, where minimal to mild alveolar/bronchial interstitial neutrophil infiltration was seen in lungs for all TI-treated groups. However, these findings regressed after 8 weeks recovery.

During 2nd cycle NDA (re)submission, pharm/tox did not provide any additional comments since there were no new nonclinical studies submitted. Even though the delivery device was changed from the MedTone system in 1st review cycle to Gen2C system in 2nd review cycle, such a switch in device did not affect the outcome of Afrezza's nonclinical development program.

With this resubmission, the sponsor includes 1) one study (MKC-PC-2010-0042) to qualify insulin impurities, 2) assessment of PCNA (proliferating cell nuclear antigen) evaluation of lung tissues from the prior 26 week rat inhalation toxicity study and the 39 week dog inhalation toxicology study, and 3) updated safety margins based on FDKP exposure data obtained from a new clinical study (MKC-TI-176) with Gen2 inhaler. *MKC-TI-176 is a Phase I study in healthy volunteers using Gen2 inhaler, with doses of 10U (10U cartridgex1), 30U (10U + 20U cartridge), 60U (20U cartridgex3) and 80U (20U cartridgex4).*

The MKC-PC-2010-0042 was originally submitted under IND 61729 annual report period: Jan 2010-Jan 2011. This was a 28-day toxicity rat study with 14-day recovery for insulin impurities qualification. The study was previously reviewed (see memo in DARRTS dated Jan 2012. At that time, only 37 out of 813 pages were submitted for review. Based on this study, impurities were appropriately quantified and there were no impurity-related safety concerns. See Appendix for study details.

Reassessment of PCNA Analysis

26 Week Rat Toxicity Study

In the 26 week rat toxicity study (MKC-PC-2003-2002), exposure to Technosphere particles at 10 mg/kg/day in males and 12.8 mg/kg/day in females caused an increase in cell replication in the epithelium of large bronchioles in female rats and terminal bronchioles in male and female rats when compared to air exposed rats. Such increase in cell replication in the large and terminal bronchioles, but not the alveoli, of rats may be due to the load of Technosphere particles impacting the conducting airway epithelium. Due to their size, inhaled particles would be expected to deposit primarily in the conducting airway. On the contrary, exposure to TI (particles + insulin) at both of the exposure levels did not increase cell replication in the epithelium of large bronchioles or terminal bronchioles of male or female rats. There was also no increase of cell replication observed in the alveolar epithelium of male or female rats in this study.

Group Identification, Exposure, and Number of Male Rats

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Group ID	Theoretical Achieved Dose	Number of Rats
1	Air Control	10
2	Technosphere particles 10.5 mg/kg/d	10
3	Technosphere Insulin 1.05 mg/kg/d, 2.94 IU/kg/d, respectively	10
4	Technosphere Insulin 1.91 mg/kg/d, 5.18 IU/kg/d, respectively	10

Group Identification, Exposure, and Number of Female Rats

Group ID	Theoretical Achieved Dose	Number of Rats
1	Air Control	10
2	Technosphere particles 12.8 mg/kg/d	10
3	Technosphere Insulin 0.404 mg/kg/d, 1.14 IU/kg/d, respectively	10
4	Technosphere Insulin 1.28 mg/kg/d, 3.46 IU/kg/d, respectively	10

Mean Percent PCNA-Labeled Epithelial Cells in Male Rats at 26 Weeks

Group ID	Exposure	Percent Labeled Large Bronchiolar Epithelial Cells Mean (SD)	Percent Labeled Terminal Bronchiolar Epithelial Cells Mean (SD)	Percent Labeled Alveolar Epithelial Cells Mean (SD)
1	Air Control	0.67 (0.41)	0.78 (0.49)	0.64 (0.30)
2	Technosphere particles 10.5 mg/kg/d	1.77 (1.34)	2.04 (1.04)	0.57 (0.30)
3	Technosphere Insulin 1.05 mg/kg/d, 2.94 IU/kg/d, respectively	0.92 (0.51)	1.23 (0.44)	0.79 (0.35)
4	Technosphere Insulin 1.91 mg/kg/d, 5.18 IU/kg/d, respectively	1.04 (0.50)	1.06 (0.47)	0.74 (0.33)

Mean Percent PCNA-Labeled Epithelial Cells in Female Rats at 26 Weeks

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Group ID	Exposure	Percent Labeled Large Bronchiolar Epithelial Cells Mean (SD)	Percent Labeled Terminal Bronchiolar Epithelial Cells Mean (SD)	Percent Labeled Alveolar Epithelial Cells Mean (SD)
1	Air Control	0.65 (0.42)	0.79 (0.39)	0.55 (0.36)
2	Technosphere particles 12.8 mg/kg/d	1.25 (0.57)	1.46 (0.52)	0.73 (0.40)
3	Technosphere Insulin 0.404 mg/kg/d, 1.14 IU/kg/d, respectively	0.63 (0.35)	0.79 (0.39)	0.46 (0.23)
4	Technosphere Insulin 1.28 mg/kg/d, 3.46 IU/kg/d, respectively	0.78 (0.56)	0.93 (0.34)	0.52 (0.20)

39 Week Dog Toxicity Study

In the 39 week dog toxicity study (MKC-PC-2003-0004), PCNA stained cells within alveolar and bronchiolar epithelia were qualified. There were no differences observed in the cell proliferating activity of control and treated groups (TI at 0.39 mg/kg/day in males and 1.92 mg/kg/day in females) of the sacrifice occasions between sexes. Since cell proliferation activity was observed in the lungs of animals across all treatment groups and sacrifice occasions, the labeling was attributed to a response to the inhalation procedure and unrelated to treatment with test or control article.

Grp	Treatment	Dose (mg/kg/day)[b]	Sacrificed Week 26		Sacrificed Week 39		Recovery Animals	
			M	F	M	F	M	F
1	Air Control		4	4	4	4	2	2
2	Technosphere particles (Low dose):	2.4			4	4		
3	Technosphere particles (High dose)	10.9			4	4		
4	TI (Low dose)	0.39/0.04[a]			4	4		
5	TI (High dose)	1.92/0.22[a]	4	4	4	4	2	2

[a] Shown are doses for TI in (mg/kg/day) / Insulin (mg/kg/day)

[b] Mean of males and females

Abbreviations: F = Female; Grp = group; M = Male; TI = Technosphere® Insulin

There was no indication that insulin caused or exacerbated epithelial cell replication or proliferation. No hyperplastic or metaplastic signals indicative of cell proliferation were observed. The NOAEL was considered 1.91 mg/kg/day in males and 1.28 mg/kg/day in females for TI and 10.5 mg/kg/day for males and 12.8 mg/kg/day for females for Technosphere particles.

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Summary of PCNA analysis (generated by the reviewer based on individual data provided by the sponsor)

Group	Day 183				Day 274				Recovery			
	M		F		M		F		M		F	
	Alveoli	Others	Alveoli	Others	Alveoli	Others	Alveoli	Others	Alveoli	Others	Alveoli	Others
1 - Air	0	7	3	85	1	5	14	14	0	0	1	2
2 – T low					1	8	18	14				
3 – T high					1	9	11	20				
4 – TI low					2	11	2	2				
5 – TI high	1	3	1	5	0	5	1	1	0	0	3	8

Individual data of PCNA analysis (provided by the sponsor in the original NDA submission)

(b) (4)

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Impurities

The original NDA submission included studies to address FDKP impurities. This resubmission includes a new study to qualify insulin related compounds present at (b) (4) % (MKC-PC-2010-0042) and presumably adjust specification limits for these impurities. The primary degradation products of insulin in TI were A21 desamido insulin and high molecular weight proteins (HMWP) which are primarily covalent insulin dimers. Insulin impurities containing modification of a single amino acid residue were present at much lower levels are grouped together by the sponsor as total other impurities (TOI). The results of this 28-day rat toxicity study administered product containing these impurities qualify acceptance criteria of (b) (4) % for A21 (b) (4) for HMWP and (b) (4) for TOI. See Appendix for study details.

Safety Margin Readjustment

In original NDA submission, human FDKP exposure used to calculating safety margins were determined using data from clinical studies with the MedTone® Inhaler. Subsequent to the completion of this section of the NDA, clinical trials were conducted with the Gen2 Inhaler. The Gen2 Inhaler produced human FDKP exposure values that were higher than when using the MedTone Inhaler. The sponsor provided their calculations in the tables below.

Drug product of 10U = 3.3 mg TI = (b) (4) mg insulin + (b) (4) mg T (FDKP)
Maximum daily dose of 300 U/day = 99 mg TI = (b) (4) mg insulin + (b) (4) mg T

The sponsor indicates that the maximum human dose is changed from 315 U Afrezza/day with the prior MedTone device to 4 U/kg/day or 300 U/day (assuming a 75kg human) with the Gen 2 device. A human dose range study with the Gen2 device was performed for 10-80 U doses, which shows dose proportionality. Higher doses were not tested. Human PK data indicates that 15 U delivered by the MedTone device is comparable to 10U delivered by the Gen2 device. Based on the comparability across devices and adjustment for maximum human dose it seems reasonable the safety margins calculated across devices should be similar. Therefore the initial labeling comments from the Dec 18, 2009 Pharm/Tox Supervisory Memo should still be valid. The sponsor has decided to readjust safety margins for nonclinical studies based on technosphere alone. This is rationale based on the focus of the toxicology program being the FDKP in the Technosphere and not rh-insulin. However the label needs to reflect the drug product being administered. These cross-species calculations are only estimates because of the nature of the route of drug administration in the animals (nose, nose-mouth passive inhalation) compared to humans with an inhaler device.

Safety Margin: Repeated Dose Toxicity Studies

In 26 week inhalation **rat** study, there were increases in pulmonary organ weights, which correlated with T administration, in a dose-related manner at Week 26. Histopathological findings, such as eosinophilic globules and epithelial degeneration in the nasal cavities, were observed in M and F given T and TI in non dose-dependent fashion, suggesting a local effect of T inhalation. These changes were diminished in females, but not in males, during the recovery period. In addition, the PCNA (proliferating cell nuclear antigen) analysis showed a slight change in epithelial cell activity in the upper airway bronchial cells in both T and TI groups at Week 26. However, no histopathological changes of epithelial hyperplasia were noted in any of the respiratory tree tissues. Carcinogenicity studies confirmed an absence of preneoplastic/neoplastic findings. Furthermore, there was minimal to slight myocardial degeneration/necrosis seen in 50% T-treated males at 11.7 mg/kg/day. Three deaths post-dosing were also found in T-treated group.

In 39 week inhalation **dog** study, there were minimal to mild alveolar and/or bronchial-interstitial neutrophil infiltrate of the lungs in both TI groups after 39 weeks of treatment. These findings regressed after 8 weeks of recovery. This lung irritation appeared to be in a T-dose dependent

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manner. Other findings, such as minimal to mild thymic atrophy (high-dose T), minimal to moderate hypocellularity of the seminiferous epithelium and minimal to mild germ cell degeneration (low-dose T and high-dose TI) were noted.

Species	***Duration***	***Route***	***Doses (mg/kg/day) NOAEL/NOEL***	***Findings***
Spraque-Dawley	26 wk w/ 30 d recovery, daily for 35-180 min	Inhalation Pivotal (nasal)	T: 11.7 TI: 1.05/0.04 (m/f), 1.91/1.28 (m/f) **$NOAEL_T$: < 11.7** **$NOAEL_{TI}$: 1.91(m)/1.28(f)**	▪ ↓ body wt in T control and HD TI groups in T dose-dependent manner ▪ ↓ glucose at HD TI groups ▪ ↑ pulmonary wt in T dose-related fashion ▪ Eosinophilic globules and epithelial degeneration were noted in the nasal cavities of both T and TI groups ▪ ↑ PCNA (bronchial cells) in TI groups, diminished during 4 wk recovery. ▪ 50% T-treated males: myocardial degeneration/necrosis, no functional effects seen.
Beagle	26/39 wk	Inhalation Pivotal (nose & mouth)	T: 2.4, 10.9 TI: 0.39, 1.92 **$NOAEL_T$: 2.4** **$NOAEL_{TI}$: 1.92**	▪ ↓ Serum glucose ▪ Neutrophil infiltrate of the lung in TI groups after 39 wk, regressed during 8 wk recovery ▪ Thymic atrophy in 6/8 high-dose T group at Week 29 ▪ Hypocellularity of the seminiferous epithelium and germ cell degeneration in ¼ low-dose T and 2/4 high-dose TI groups at Week 39

In all species tested, safety margins for Technosphere® particles alone were considered underestimated since Technosphere NOAELs were tested the at the maximum dose. Safety margins for Technosphere® insulin were modest because doses were limited by hypoglycemia, which was an expected pharmacological activity of insulin.

Safety margin: **The NOAELs for T in both rats and dogs are approx. 1.5x in rats and <1x in dogs, based AUCs for FDKP.**

According to the sponsor re: human exposure:
Maximum daily dose of 99 mg TI
Approx. 89 mg Technosphere, with AUC = 398,155 ng•min/mL

In 26 week rat study:
NOAEL of T = 11.7 mg/kg/day in rats
AUC (FDKP) on Day 182 = 514362 ng•min/mL (male) and 703445 ng•min/mL (female)
Combined M and F AUC = 608904 ng•min/mL

In 39 week dog study:
NOAEL of T = 2.4 mg/kg/day in dogs
AUC (FDKP) on Day 182 = 102,604 ng•min/mL (male) and 112,946 ng•min/mL (female)
Combined M and F AUC = 107775 ng•min/mL

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Safety Margin: Reproductive Toxicity Studies

In rats, Technosphere® particles did not have effects on male fertility, however pre- and post-natal implantation loss was observed at 100 mg/kg/day (NOAEL=30 mg/kg/day). There is an effect on pre/postnatal development (i.e. learning impairment at higher doses of T). In rabbits, Technosphere® particles seemed to produce maternal toxicity (i.e. weight loss at 2 mg/kg/day) and some malformations in fetuses (i.e. absent lung lobe and gallbladder in survived fetuses and cleft palate in dead fetus).

Species	***Duration***	***Route***	***Doses (mg/kg/day) NOAEL/NOEL***	***Findings***
Spraque-Dawley rats	M: 4 wk prior to mating F: 2 wk prior to mating - GDs - 7, twice daily	SC injection	T : 10, 30, 100 Fertility NOAEL for F0 (M/F): 100 NOAEL for F1 (litters): 100	▪ No maternal toxicity ▪ No effect upon fertility ▪ No early embryonic developmental toxicity
Spraque-Dawley rats	GDs 6-17 (f) 21 d (m)	SC injection	T: 10, 30, 100 Pivotal Seg II NOAEL for F0 (F): 100 NOAEL for F1 (litters): 100	▪ No maternal toxicity ▪ Only minor anomalies (e.g. dilated ureters), but no difference between the control and treated groups
New Zealand White rabbits	GDs 7-19, once daily	SC injection	T: 2, 10, 100 Pivotal Seg II NOAEL for F0 (F): < 2 NOAEL for F1 (litters): < 2 *Sponsor: NOAEL for F0: 10* *Sponsor: NOAEL for F1 (litters): 100*	▪ Maternal toxicity: One mortality at 100 mg/kg/day, Scant and soft/liquid feces, and Reduced body weigh at 2 mg/kg/day ▪ Malformations (i.e. cleft palate in one dead fetus, absence lung lobe and gallbladder in survived fetuses) at 2 mg/kg/day
Spraque-Dawley rats	GDs 7 to LDs 20, twice daily	SC injection	T: 10, 30, 100 Pre/postnatal NOAEL for F0 (F): 100 NOAEL for F1 (M): 10 NOAEL for F2 (F): 100 *Sponsor: NOAEL for F1: 100*	▪ F0: No maternal toxicity ▪ F1: Some pups failed to learn from at 30 mg/kg/day groups ▪ F1: Decreased male reproductive organ weights (testes and epididymides absolute weights) at 100 mg/kg/day ▪ F2: No T-treated gross external alternations observed in F2 generation.

***Safety margin*:** **FDPK safety margin is approx. 21x in rats and 1.3x in rabbits, based on AUC.**

According to the sponsor re: human exposure:
Maximum daily dose of 99 mg TI
Approx. 89mg Technosphere, with AUC = 398,155 ng•min/mL

Seg II rat study:
For NOAEL of T = 100 mg/kg/day in rats
AUC on GD 7 = 8,335,854 ng•min/mL

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Seg II rabbit study:
For NOAEL of T < 2 mg/kg/day in rabbits
AUC on GD 19 (at 2 mg/kg/day) = 506,098 ng•min/mL

Safety Margin: Carcinogenicity Studies

In rats, no pre-neoplasia and/or neoplasia were seen in the lung with both Technosphere® particles (46 mg/kg/day) and Technosphere® Insulin (up to 1.23 mg/kg/day). In mice, there was no evidence of increased oncogenicity associated with Technosphere® particles (25 or 75 mg/kg/day) or with Technosphere® Insulin (2.5 and 5 mg/kg/day in males or 0.6 and 1.25 mg/kg/day in females).

Species	***Duration***	***Route***	***Doses (mg/kg/day)***	***Findings***
Spraque-Dawley rats	104 wk	Inhalation Pivotal	T:1.1041/0.6784 (m/f), 41/46 (m/f) TI: 0.6572/0.4413 (m/f), 1.2313/0.7608 (m/f) Insulin: 0.0787/0.0529 (m/f), 0.1457/0.0900 (m/f)	▪ Adrenal cortical carcinoma (significant in high-dose TI females) ▪ Malignant astrocytoma (in low- and high-dose T males) ▪ Malignant schwannoma in nasal cavity (of low-dose T males) ▪ Fibroma in the skin/subcutis (in low-dose TI males and females) ▪ Pituitary adenoma/carcinoma (in low-dose TI males) *Lacks of dose-response and low incidences, within historical (background) rates, indicate not drug-related.*
rasH2 mice	26 wk	SC injection Pivotal	T: 25/75 TI: 2.5/0.6 (m/f), 5/1.25 (m/f)	▪ Bronchiolar-alveolar adenoma/carcinoma in the lung ▪ Hemangioma/hemangiosarcoma, primarily spleen ▪ Harderian gland adenoma/carcinoma ▪ Squamous cell neoplasms at multiple sites – stomach and skin/subcutis ▪ Myeloproliferative neoplasia *Found in the sham, vehicle control, high T, and high TI groups, not drug related.*

Safety margin: **FDPK safety margin is approx. 5x in rats, based on relative body surface area across species.**

According to the sponsor re: human exposure:
Maximum daily dose of 99 mg TI
Approx. 89 mg/day Technosphere = 55 mg/m2 (based on 60 kg adult)

104 week inhalation rat study:
For NOAEL of T = 41/46 mg/kg/day in M/F rats (246/276 mg/m2)

Updated Safety Margin for FDKP Provided by the Sponsor

Safety margins for FDKP, administered as Technosphere particles, from

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toxicology studies

Species	Duration	NOAEL[1] (mg/kg)	NOAEL[2] (mg/m^2)	AUC* (ng·min/mL)	Multiples based on: mg/kg	mg/m^2	AUC**
Mouse	13-week, subcutaneous	75	240	4,080,875	57.7	4.7x	10.2x
Rat	4-week, inhalation	61.7	432	391,500 (783,000)	47.5x	8.5x	1.0x (2.0x)
Rat	26-week, inhalation	11.7	82	608,904 (1,217,808)	9x	1.6x	1.5x (3.1x)
Dog	4-week, inhalation	40.3	806	2,931,000	31x	15.8x	7.4x
Dog	39 week, inhalation	10.9	218	461,584	8.4x	4.3x	1.2x
Human	Daily inhalation	1.3	51	398,155^	-	-	-

* Average of male and female AUC

1) NOAEL represents highest dose of Technosphere particles tested in each study

2) Conversion factors from CDER Guidance Document: Estimating the Maximum Safe Starting Dose in Initial Clinical Trials for Therapeutics in Adult Healthy Volunteers; July 2005

Mouse (body weight 24 g) conversion factor mg/kg to mg/m^2 = 3.2
Rat (body weight 250 g); conversion factor mg/kg to mg/m^2 = 7
Dog (body weight 10kg); conversion factor mg/kg to mg/m^2 = 20
Human (body weight 70kg); conversion factor mg/kg to mg/m^2 = 39

** Note: Rat AUC values in parenthesis represent conservative estimates of FDKP exposure including the 3-hour period that animals were in the chamber (See PK report MKC-PC-2006-0024). Concentrations of FDKP appear to reach steady state (~80% of C_{max}) at about 40 minutes into a 180 minute exposure and are assumed to have a linear increase from t=0 to 40 minutes. Unaccounted FDKP exposure is estimated as Cmax x 0.8 (1 40 minutes) + Cmax x 0.8 (40 minutes x 0.5) ≈ 2x AUC at end of exposure. Sources for data found in study reports: Mouse (MKC-PC-2005-0011), Rat (PDC-PC-1996-0002 and MKC-PC-2003-0002), Dog (PDC-PC-1997-0001 and MKC-PC-2003-0004) and , for maximum daily human exposure, see Clinical Summary (Study MKC-TI-176)

^Human FDKP AUC at maximum daily dose of 315U with earlier MedTone Inhaler was 224,245 ng·min/mL. Revised FDKP AUC at maximum daily dose (4U/kg or 300 Units) with Gen2 Inhaler was 398,155 ng·min/mL.

Summary of Safety Margin Update

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Species	Duration	Original NDA			Resubmission Update		
		Multiples based on^:			Multiples based on^^:		
		mg/kg	mg/m^2	AUC	mg/kg	mg/m^2	AUC
Mouse	13-week, subcutaneous	57.7	4.7x	18.1x	57.7	4.7x	10.2x
Rat	4-week, inhalation	47.5x	8.5x	1.7x (3.5x)	47.5x	8.5x	1.0x (2.0x)
Rat	26-week, inhalation	9x	1.6x	2.7x (5.4x)	9x	1.6x	1.5x (3.1x)
Dog	4-week, inhalation	31x	15.8x	13.1x	31x	15.8x	7.4x
Dog	39- week, inhalation	8.4x	4.3x	2.1x	8.4x	4.3x	1.2x

^ Human FDKP AUC at maximum daily TI dose of 315U with the MedTone inhaler was 224,245 ng·min/mL (MKC-TI 141)

^^ Revised human FDKP AUC at the maximum daily TI dose (4U/kg or 300 Units) with the Gen-2 inhaler was 398,155 ng·min/mL (MKC-TI-176)

Safety margins for FDKP, administered as Technosphere particles, from carcinogenicity studies

Species	Duration	NOAEL[1] (mg/kg)	NOAEL[2] (mg/m^2)	AUC*/C_{max}** (ng·min/mL)	Multiples based on: mg/kg	mg/m^2	AUC
Mouse	26-weeks subcutaneous	75	240	2,860,975*	57.7	4.7	**7.2**
Rat	104-weeks inhalation	43.5	305	5374 ng/mL**	33.5	6.0	**9.5**^
Human^^	Daily inhalation	1.3	51	**398,155/563**	-	-	-

* Average of male and female AUC

**Average of male and female C_{max} (ng/mL FDKP)

^Safety Margin multiple based on C_{max} (C_{max} in humans is ≈ **563 ng/mL**)

1) NOAEL represents highest dose of Technosphere particles tested in each study

2) Conversion factors from CDER Guidance Document: Estimating the Maximum Safe Starting Dose in Initial Clinical Trials for Therapeutics in Adult Healthy Volunteers; July 2005

Mouse (body weight 24 g) conversion factor mg/kg to mg/m^2 = 3.2

Rat (body weight 250 g); conversion factor mg/kg to mg/m^2 = 7

Human (body weight 70kg); conversion factor mg/kg to mg/m^2 = 39

Sources for data found in study reports: Mouse (MKC-PC-2007-0003), Rat (MKC-PC-2004-0001) and, for maximum daily human exposure, see Clinical Summary (Study MKC-TI-176)

^^Human FDKP AUC at maximum daily dose (315 Units) with earlier Medtone Inhaler was 224,245 ng·min/mL. Revised FDKP AUC at maximum daily dose (4U/kg assuming 75 kg body weight or 300 Units) with Gen2 Inhaler was 398,155 ng·min/mL; Cmax ≈ 563 ng/mL.

Overall Conclusion: The results of these additional data, changes, and subsequent evaluations do not change the original overall non-clinical conclusions.

Labeling

Sponsor proposed Labeling

8	**Use in Specific Populations**
8.1	**Pregnancy**

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FDA recommendations

8	**Use in Specific Populations**

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8.1	Pregnancy Teratogenic Effects: Pregnancy Category C AFREZZA has not been studies in pregnant women. AFREZZA should not be used during pregnancy unless the potential benefit justifies the potential risk to the fetus. In pregnant rats given SC doses of 10, 30, and 100 mg/kg/day of carrier particles (vehicle without insulin) from gestation day 6 through 17 (organogenesis), no major malformations were observed at up to 100 mg/kg/day (a systemic exposure 21 times the human systemic exposure, resulting from the maximum recommended daily dose of 99 mg Afrezza based on AUC. In pregnant rabbits given SC doses of 2, 10, and 100 mg/kg/day of carrier particles (vehicle without insulin) from gestation day 7 through 19 (organogenesis), adverse maternal effects were observed at all dose groups (at human systemic exposure following a 99 mg Afrezza dose, based on AUC. Fetal mortality and malformations observed included cleft palate, non-patent nares, absent intermediate lung lobs, absent gallbladder, as well as vertebral/rib/scapular malformations and irregular ossified skull bones. In pregnant rats given SC doses of 10, 30, and 100 mg/kg/day of carrier particles (vehicle without insulin) from gestation day 7 through lactation day 20 (weaning), decreased epididymis and testes weights and impaired learning were observed in pups at ≥ 30 mg/kg/day (a systemic exposures less than the human systemic exposure at the maximum daily AFREZZA dose of 99 mg based on AUC).
8.2	**Nursing Mothers** Many drugs are excreted in human milk. A study in rats indicates that the carrier is excreted in milk at approximately 10% of maternal exposure levels. It is therefore highly likely that AFREZZA is excreted in human milk. A decision should be made whether to discontinue nursing or suspend use of the drug since AFREZZA has not been studied in lactating women.
8.3	**Pediatric Use** AFREZZA has not been studied in patients younger than 18 years of age.

13	**Nonclinical Toxicology**
13.1	**Carcinogenesis, Mutagenesis, Impairment of Fertility** In a 104 week carcinogenicity study, rats were given doses up to 46 mg/kg/day of the carrier and up to 1.23 mg/kg/day of insulin, by nose-only inhalation. No increased incidence of tumors was observed at systemic exposures equivalent to the insulin (5 times the systemic exposure to Technosphere at a maximum daily AFREZZA dose of 99 mg based on a comparison of relative body surface areas across species). In a 26 week carcinogenicity study, transgenic mice (TgrasH2) given doses up to 75 mg/kg/day of Technosphere and up to 5 mg/kg/ day of AFREZZA. No increased incidence of tumors was observed. AFREZZA was not genotoxic in Ames bacterial mutagenicity assay and in the chromosome aberration assay, using human peripheral lymphocytes with or without metabolic activation. The carrier alone was not genotoxic in the in vivo

	mouse micronucleus assay. In female rats given SC doses of 10, 30, and 100 mg/kg/day of carrier particles (vehicle without insulin) beginning 2 weeks prior to mating until gestation day 7, there were no adverse effects on male fertility at doses up to 100 mg/kg/day (a systemic exposure 21 times that following the maximum daily AFREZZA dose of 99 mg based on AUC). In female rats there was increased pre- and post-implantation loss at 100 mg/kg/day (21 times higher systemic exposure than the maximum daily AFREZZA dose of 99 mg based on AUC) but not at 30 mg/kg/day (less than the systemic exposure from an AFREZZA daily dose of 99 mg).

Reference ID: 3464275

Appendix 1

STUDY No. MKC-PC-2010-0042

Study title: Insulin Impurities: A 28-day Subcutaneous Toxicity Study with a 14-Day Recovery Period in Spraque-Dawley Rats

Key study findings:

- Spraque-Dawley rats were treated with a mixture of insulin-related compounds (0, 0.0244, 0.1435, or 0.3021 mg/kg/day) twice daily for 28 days via sc injection and with 14 day recovery period. ***Since the sponsor submitted incomplete final study report, most data was not reviewed in details.***
- There was one death (attributed to hypoglycemia) in HD group.
- No test article-related adverse effects on clinical signs, body weight, food consumption, ocular toxicity, coagulation, and organ weights.
- There were several changes in hematological parameters (i.e. white blood cell populations in treated groups and neutrophils, monocytes, eosinophils, basophils, and large unstained cells in HD group). These changes were recoverable.
- There were dose-dependent increases in AST and TRIG levels on Day 29. These changes were recoverable.
- There were also increased urine volume and decreased specific gravity in HD males. These changes were recoverable.
- Dark areas were seen at injection sites of both control and treated animals. This correlated with microscopic findings (i.e. sc haemorrhage and mixed/mononuclear cell infiltrates).
- One test-article related microscopic finding was noted. Dark areas of the glandular stomach were seen in 2/20 MD animals and 8/19 HD animals. This correlated with microscopic findings (i.e. focal/multifocal erosion in 1/20 MD animals and 13/19 HD animals, focal/multifocal mucosal congestion/haemorrhage in 4/20 MD animals). These were not present during the recovery period.
- The NOAEL for this study was a total mean daily dose of 0.024 mg/kg/day (A21: 0.0097 mg/kg/day, HMWP: 0.0034 mg/kg/day, and combined other insulin related impurities (b) (4) 0.0113 mg/kg/day) due to stomach findings (i.e. hemorrhage and erosion in the glandular stomach) in MD and/or HD groups.

Study no.: MKC-PC-2010-0042
Volume #, and page #: 813 pages
Conducting laboratory and location: (b) (4)
Date of study initiation: Nov 2nd 2010
GLP compliance: Yes
QA report: yes (x) no ( )
Drug, lot #, and % purity: Lot No. FD10294 with 7.52% purity

- Test article is a mixture of 9 insulin impurities

Reporting group	Name of group	Components (Molecular Weight)
1		(b) (4)
2		
3		
4		
5		
6		
7		

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Description of insulin-related compounds tested

Compound	Description
A21	Deamidation of asparagine at A21 to aspartic acid
HMWP	Covalently linked insulin dimers
Total Others	(b) (4)

Methods

- *Doses:* 0, 0.0244, 0.1435, or 0.3021 mg/kg/day. The test article was prepared as a mixture of impurities without insulin to avoid exaggerated insulin pharmacology, hypoglycemia.

Group Numbers	Group Designation	Nominal dose Level per day / per injection (mg/kg)	Nominal dose concentration (µg/mL)#	Toxicology Animals				Toxicokinetics Animals	
				Main		Recovery			
				Male	Female	Male	Female	Male	Female
1	Control*	0	0	10	10	5	5	3	3
2	Low Dose	0.034 / 0.017	4.26	10	10	-	-	6	6
3	Mid Dose	0.168 / 0.084	21.00	10	10	-	-	6	6
4	High Dose	0.335 / 0.167	41.85	10	10	5	5	6	6

* Control animals received the Control/Vehicle [1X PBS (phosphate buffered saline), pH 7.4]

Mean achieved daily dose levels for insulin degradation products

Group Number	Group Designation	Mean daily dose for insulin-related compounds (mg/kg/day)				
		Calculated from mean of dosing solution assays				Nominal
		A21	HMWP	*Combined (b) (4) (b) (4)	TOTAL	
1	Control	0	0	0	0	0
2	Low Dose	(b) (4)				
3	Mid Dose					
4	High Dose					

* Individual results of the insulin impurities are included in the formulation analysis report

Amendment No 1

Group Number	Group Designation	Mean daily dose for insulin-related compounds (mg/kg/day)				
		Calculated from mean of dosing solution assays				Nominal
		A21	HMWP	*Combined (b) (4) (b) (4)	TOTAL	
1	Control	0	0	0	0	0
2	Low Dose	(b) (4)				
3	Mid Dose					
4	High Dose					

* Individual results of the insulin impurities are included in the formulation analysis report.

Mean achieved doses of individual insulin-related compounds

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Component	Mean Daily Dose (mg/kg/day) Group 2	Group 3	Group 4
A21			(b) (4)
HMWP			
Other Impurities* (b) (4)			(b) (4)
TOTAL			

(b) (4)

- *Species/strain:* Spraque-Dawley rats
- *Number/sex/group or time point (main study):* 10/sex/group
- Route, formulation, volume, and infusion rate:
- *Satellite groups:* Recovery: n=5 for control and HD groups and TK: n=3 for control and 6 for LD, MD, HD groups.
- *Age:* 8-10 weeks
- *Weight:* 266-385g for males and 216-258 for females
- *Sampling times:* Blood samples were collected from each TK phase rat on Days 1 and 28: predose, 5, 15, 30 min, 1, 2, 4, and 6 hrs.
- *Unique study design or methodology (if any):* None.

Obervations and Results

Mortality: Once a day)
One HD animal was found dead on Day 28 due to hypoglycaemia.

Clinical signs: Once a day
No test-article related clinical signs were observed in these animals.

Body weights: Days 1, 7, 14, 21, 28 (main and recovery animals), 35 and 42 (recovery animals)
No test-article related adverse effects on the mean body weight in these animals in this study.

Food consumption: Weekly
No test-article related adverse effects on food consumption in these animals during dosing period. However, the mean food consumption for the HD males was lower compared to the concurrent control during the recovery period. This might be due to individual variation.

Ophthalmoscopy: Once pre-treatment, once during Week 4, and at the end of recovery period
No test-article related adverse ocular effects were observed in these animals.

EKG: N/A

Hematology: Day 29 (main) and Day 43 (recovery)

Red blood cell count	Mean corpuscular haemoglobin (calculated)
Haematocrit (calculated)	Mean corpuscular volume
Haemoglobin	Morphology of cells
White blood cell count	Platelet count
WBC differential (absolute)	Reticulocyte (absolute and percentage)
Mean corpuscular haemoglobin concentration (calculated)	

The WBC in the treated groups was differed from the controls in a dose-dependent manner.

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Others, including neutrophils (NEUT), monocytes (MONO), eosinophils (EOS), basophils (BASO), and large unstained cells (LUC), were also elevated in the HD group. These changes were somewhat reversed by the end of recovery period (Day 43). These findings were within normal reference ranges.

Relative difference from control in white blood cell populations at the end of treatment

Parameter	Percentage change by Group and sex					
	Group 2		Group 3		Group 4	
	Male	Female	Male	Female	Male	Female
WBC	-1.7	-12.6	8.9	19.9	14.1	18.9
NEUT	23.1	-29.8	19.2	-19.3	57.6	80.7*
MONO	-6.2	-30.8	62.5*	0.0	100.0**	46.5*
EOS	11.1	0.0	22.2	12.5	22.2	25.0
BASO	-20.0	0.0	0.0	33.3	20.0	33.3
LUC	0.0	0.0	0.0	25.0	66.7	50.0

Statistical significance is noted above as follows: * = p<0.05 and ** = p<0.001 (Dunnett's).

Relative difference from control in white blood cell populations on Day 43 following Recovery period

Parameter	Percentage change by Group and sex			
	Group 4 Day 29		Group 4 Day 43	
	Male	Female	Male	Female
WBC	14.1	18.9	0.2	10.0
NEUT	57.6	80.7*	6.8	-22.0
MONO	100.0**	46.5*	-33.3	-40.0
EOS	22.2	25.0	0.0	16.7
BASO	20.0	33.3	0.0	0.0
LUC	66.7	50.0	0.0	0.0

Statistical significance is noted above as follows: * = p<0.05 and ** = p<0.001 (Dunnett's).

Clinical chemistry: Day 29 (main) and Day 43 (recovery)

Batch 1:	Batch 2:
Alanine aminotransferase	Albumin
Aspartate aminotransferase	Total Protein
Creatinine	A/G ratio (calculated)
Urea	Globulin (calculated)
Batch 3:	Batch 4:
Chloride	Bilirubin (Total)
Potassium	Alkaline phosphatase
Sodium	Glucose
Calcium	Cholesterol (total)
Phosphorous (inorganic)	Triglycerides
	Gamma glutamyl transpeptidase (GGT)

The serum AST and TRIG were higher on Day 29 in the treated groups than the controls in a dose-dependent manner. Both AST and TRIG are linked to the pharmacology of insulin. These changes were reversed by the end of recovery period (Day 43). These findings were within normal historical ranges.

Relative difference from control in AST and triglyceride levels

Reference ID: 3464275

Group Number	Day	Percent difference from control			
		AST		Triglycerides	
		Males	Females	Males	Females
2	29	4.2	3.9	32.3	17.2
3	29	7.7	10.2	71***	31
4	29	13.3	50.8**	87.1***	38
	43	-2.3	-19.8	-18.8	18.2

** = p<0.01 (Dunnett's)
*** = p < 0.001 (Dunnett's)

Urinalysis: Day 29 (main) and Day 43 (recovery)

Bilirubin
Blood
Color and appearance
Glucose
Ketones
pH
Protein
Sediment microscopy
Specific gravity
Urobilinogen
Volume

There were no effects on urinalysis parameters except the HD males had increased urine volumes, corresponding decreased specific gravity. This was comparable to the control group. The toxicological significance of this finding was unknown. NOTE: No data was provided for review.

Gross pathology: Day 29 (main) and Day 43 (recovery)
Dark areas around injection sites were observed but there were no comparable between the control and treated groups. This is probably due to the administration procedure. There changes were still observed at the end of recovery period. They correlated with microscopic findings (i.e. haemorrhage and inflammation). In addition, depressed areas of the glandular stomach were noted in 2/20 MD animals and 9/19 HD animals. These findings correlated with microscopic findings (i.e. erosion).

Incidence of treatment-related macroscopic finding in main study animals

Sex	Males				Females			
Group	1	2	3	4	1	2	3	4
Number of animals examined	10	10	10	9	10	10	10	10
Stomach								
Depressed area	0	0	1	4	0	0	1	4

Organ weights: Day 29 (main) and Day 43 (recovery)
No test article related changes in mean absolute or relative organ weights were seen. There were some significant differences but were not consistent across dosing groups or between sexes. These changes were not associated with any macroscopic or microscopic findings.

Histopathology: Adequate Battery: yes (x), no ( )
Peer review: yes ( ), no (x)

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ORGANS/TISSUES	Retain (•)	Weigh (✓)	Examine (€)	ORGANS/TISSUES	Retain (•)	Weigh (✓)	Examine (€)
Adrenals	•	✓	€	Duodenum	•		€
Animal identification	•			Jejunum	•		€
Aorta (thoracic)	•		€	Ileum	•		€
Blood	•			SC, cervical	•		€
Bone marrow smears (3)	•			Spleen	•	✓	€
Brain	•	✓	€	Sternum & marrow	•		€
Cecum	•		€	Stomach	•		€
Colon	•		€	Testes	•d	✓	€
Epididymides	•d		€	Thymus	•	✓	€
Esophagus	•		€	Thyroid gland/parathyroids	•	✓	€
Eyes	•a		€	Tongue	•		€
Femur & marrow	•		€	Trachea	•c		€
Heart	•	✓	€	Urinary bladder	•		€
Kidneys	•	✓	€	Uterus	•	✓	€
Liver (2 lobes)	•	✓	€	Vagina	•		€
Lungs (2 lobes)	•bc	✓	€				
LN, mandibular	•		€	Abnormal findings	•		€
LN, mesenteric	•		€				
Mammary gland (inguinal)	•		€				
Optic nerves	•a		€	**Additional Tissues presented below**			
Ovaries	•	✓	€	Last dosing site*	•		€
Pancreas	•		€	ID microchip	•		
Pituitary	•	✓	€				
Prostate	•	✓	€				
Rectum	•		€				
SG, mandibular	•		€				
Sciatic nerve	•		€				
Seminal vesicles	•		€				
Skeletal muscle	•		€				
Skin & subcutis (inguinal)	•		€				

a	Davidson's fluid (euthanized animal only)
b	Lungs were infused with 10% neutral buffered formalin (euthanized animal only)
c	Lungs were weighed with trachea and recorded in Provantis as lungs in organ weight record
d	Bouin's fluid (euthanized animal only)
LN	Lymph node
SG	Salivary gland
SC	Spinal cord
€	Examined microscopically
*	All dosing sites were collected but only the last site was examined histopathologically

Notes:

Paired organs weighed together
Parathyroids, mammary gland and optic nerves will only be examined histologically if present in routine sections

Incidence of subcutaneous haemorrhage and subcutaneous mixed or mononuclear cell infiltrates was observed at dosing/injection sites in both control and treated animals. In addition, there was test article related focal or multifocal erosion of the glandular stomach seen in 1/20 MD animals and 13/19 HD animals. Focal or multifocal mucosal congestion/haemorrhage of the glandular stomach was also seen in 4/20 MD animals. These findings were consistent with studies in rats exposed to insulin alone at high doses by sc injection or animals under chronic stress. All stomach microscopic findings were no longer present, suggesting complete recovery.

Incidence and severity of treatment-related microscopic findings

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Study Phase	Main								Recovery			
Sex	Males				Females				Males		Females	
Group	1	2	3	4	1	2	3	4	1	4	1	4
Number of animals examined	10	10	10	9	10	10	10	10	5	4	5	5
Stomach, glandular												
Erosion	0	0	0	5	0	0	1	8	0	0	0	0
minimal	-	-	-	5	-	-	1	8	-	-	-	-
Congestion/haemorrhage	0	0	2	0	0	0	2	0	0	0	0	0
minimal	-	-	2	-	-	-	2	-	-	-	-	-

Toxicokinetics: Days 1 and 28 (predose, 5, 15, 30 min, 1, 2, 4, 6 hours)
TK data was used to confirm systemic exposure of the test article in animals. Twice daily sc injections of the test article in rats for 28 days resulted in consistent insulin detection (with dose-related increases in exposure).

Other:
Formulation analysis: The results of LD, MD, and HD samples collected for analysis were lower than expected: (b) (4) % for Group 2 (b) (4) % for Group 3, and (b) (4) % for Group 4. These measured concentrations did not meet the acceptance criterion of (b) (4) % of the nominal concentration, so the measured concentrations were used to calculate dosage. These low results may be due to loss of protein to the glass and the filter and such loss had more effect on lower dose groups (i.e. more diluted solutions).

Mean achieved daily dose levels for insulin degradation products

Group Number	Group Designation	Mean daily dose for insulin-related compounds (mg/kg/day)				
		Calculated from mean of dosing solution assays				Nominal
		A21	HMWP	*Combined (b) (4) (b) (4)	TOTAL	
1	Control	0	0	0	0	0
2	Low Dose	(b) (4)				
3	Mid Dose					
4	High Dose					

* Individual results of the insulin impurities are included in the formulation analysis report

Overall Conclusion:
This 28 day toxicity study with a 14 day recovery was to qualify a mixture of insulin related compounds (impurities and degradation products) in Technosphere Insulin Inhalation Power (TI). The sponsor identified 7 impurities which were then subdivided into three groups: A21 (A21 desamido insulin), HMWP (high molecular weight proteins, primarily covalent insulin dimers), and Total Others (mixtures of (b) (4) Due to their structural similarity to insulin, all compounds were expected to have biological activity of insulin.

This study showed one mortality in HD group (hypoglycemia), no adverse effects on clinical sign/body weight/food consumption/organ weights, some changes (but recoverable) in hematological parameters (i.e. ↑ WBC/NEUT/MONO), clinical chemistry (i.e. ↑ AST and TRIG), and urinalysis (i.e. ↑ urine volume and specific gravity). The study also found macroscopic changes (dark areas at injection sites in control/treated groups and dark areas in glandular stomach in MD/HD groups). These gross findings further correlated with microscopic findings (i.e. injection site inflammations in both control/treated groups and stomach erosion and inflammation in MD and/or HD groups). Based on all these study findings, NOAEL was established at the LD (total dose of 0.0244 mg/kg/day).

Based on these study findings, the NOAEL was the low dose. However, the sponsor argued the mid dose should be the NOAEL based on the fact that such stomach findings were known

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species-specific pharmacologic effects of insulin doses > 0.087 mg/kg (2.5 U/kg) or chronic. Even though the sponsor provided references to establish the probable association between chronic hyperinsulemia and increased incidence of ulcer, the NOAEL for this study should remain at the low dose, where no toxicity was observed.

All in all, based on this 28 day toxicity rat study, insulin impurities and degradation products in the Technosphere Insulin Inhalation Powder were qualified and had 1 to 3 fold safety margins at the proposed acceptance criteria.

Estimate safety margins based on NOAEL (low dose):

Impurites	Mean Achieved Dose (mg/kg/day)	Level Qualified	Acceptance Criterion	Max. Anticipated Clinical Dose* (mg/kg/day)	Dose Multiple
A21	(b) (4)				
HMWP					
Total other impuritie (b) (4)					
(b) (4)					

This is a representation of an electronic record that was signed electronically and this page is the manifestation of the electronic signature.

/s/

MIYUN M TSAI-TURTON
03/04/2014

KAREN L DAVIS BRUNO
03/04/2014
P/T Review Resub #3 w/labeling comments

Pharmacology/Toxicology Mid-Cycle Deliverables

Since this NDA 22472 is a resubmission. Goal date time line is no longer applied. This resubmission has a 6 month review cycle. The resubmission includes 1) one new study (MKC-PC-2010-0042) to qualify insulin impurities, 2) assessment of Proliferating Cell Nuclear Antigen (PCNA) evaluation of lung tissues from the 26 week rat inhalation toxicology study and the 39 week dog inhalation toxicology study, 3) updated safety margin based on FDKP exposure data obtained from a new clinical study (MKC-TI-176) with the Gen2 Inhaler.

NDA 22472 is for Afrezza (inhaled insulin). The NDA was first submitted in 2009. Original nonclinical review can be found in DARRTS. In original review, there were no nonclinical safety concerns relevant to clinical use. Only possible lung irritation was noted based on 39 week inhalation dog study, where minimal to mild alveolar/bronchial interstitial neutrophil infiltration was seen in lungs of all TI (insulin+FDKP) treated groups. However, these findings regressed following 8 weeks recovery. Hence pharm/tox recommended approval during the last review cycle.

Mid-Cycle Deliverable	Goal Date	Status
Filing Review Meeting • **Deliverable: Regulatory history, summary of pharmacology and toxicology findings from preliminary review of existing data, adequacy of NDA submission**	**45- day of NDA submission**	N/A
Genetox Study Review (impurities and drug substance) • **Deliverable: Draft review, label recommendation/interact with genetox committee**	**2-3 months of NDA submission**	N/A ▪ Negative findings by chromosome aberration assays (T and TI) and by mouse micronucleus test (T only)
Carcinogenicity Study Review • **Deliverable: Identify statistical reviewer**	**1-month of NDA submission**	N/A
• **Deliverable: Schedule ECAC meeting**	**1-month of NDA submission**	N/A
• **Deliverable: Draft review of Carcinogenicity study with statistical input, *Interact with statistician***	**6-months of NDA submission**	N/A.
• **Deliverable: ECAC review of carcinogenicity study, incorporation of eCAC comments in the review, Identification of issues from Carcinogenicity study & related post marketing commitments, labeling recommendation**	**7- month of NDA submission**	N/A ▪ Negative for carcinogenicity
ReproTox Study Review (Seg III and I) • **Deliverable: Draft review, Identify issues (special studies for post marketing commitments), labeling recommendation, *Interact with Reprotox committee***	**5- month of NDA submission**	N/A ▪ Category C labeling is proposed.
Impurity/Extractable Qualification • **Deliverable: Begin review; *interact with CMC***	**3-month of NDA submission-will depend on CMC also**	Currently under review ▪ A new study was conducted to qualify insulin-related impurities (MKC-PC-2010-0042)
• **Deliverable: Identify whether the**	**5-6 month of**	N/A

impurities are qualified, Identify approvability/post marketing commitment issues	**NDA submission-if sponsor has final formulation**	
<u>Toxicokinetic Study Review</u> • **Deliverable: Draft review; *Interact with Clinical Pharmacology* for dose and ADME, *Interact with Medical Reviewer* for adverse event comparison and dose multiples**	**6- months of NDA submission**	N/A
<u>Chronic Toxicity Study Review</u> • **Deliverable: Draft review**	**6- months of NDA submission**	Currently under review. ▪ An assessment of cellular response based on new data from a GLP Proliferating Cell Nuclear Antigen (PCNA) evaluation of lung tissues from the 26 week rat inhalation tox study (MKC-PC-2003-0002; Report No 09-RS-006) was conducted. ▪ A more detailed summary of GLP PCNA analysis from the 39 week dog inhalation tox study (MKC-PC-2003-0004) was conducted. ▪ Safety margins have also been updated based on FDKP exposure data (AUC margins only) obtained in a new clinical study with Gen2 Inhaler (MKC-TI-176)
<u>Pharmacology Study Review</u> • **Deliverable: Draft review, identify PD issues**	**6- months of NDA submission**	N/A
<u>Tox findings compared in animal chronic studies and human phase 3 studies</u> • **Deliverable: Input in ODS database**	**8- months of NDA submission**	Pending completion of comprehensive review

Study MKC-PC-2010-0042

Study design: To qualify insulin impurities administered to rats by sc injection as a solution twice daily for 28 days followed by 14 day recovery.

Dose groups in study MKC-PC-2010-0042

Group	Treatment	Mean Achieved Daily Doses of Insulin Impurities (mg/kg/day)* A21	HMWP	TOI	Main Study (N/sex)	Recovery (N/sex)	Toxicokinetic Groups (N/sex)
1	Control	0	0	0	10	5	3
2	Low Dose	(b) (4)			10	0	6
3	Mid Dose				10	0	6
4	High Dose				10	5	6

Note: Total daily dose was administered as twice daily (6-8 hours apart) subcutaneous injections.
* The primary impurities of TI have been identified as:
1) A21 desamido insulin (A21)
2) High Molecular Weight Proteins (HMWP, primarily covalent insulin dimer), and
3) Total Other Impurities (TOI) identified as (b) (4)

Findings: Hypoglycemia caused mortality in the high dose group (0.3021 mg/kg/day). The other treatment-related change was erosion and congestion/hemorrhage of the glandular stomach (limited to surface of the mucosa), which is consistent with studies in rats exposed to high dose of insulin by sc injection or animals under stress. Such glandular stomach erosion was not noted in the recovery animals. The NOAEL was the low dose of 0.0244 mg/kg/day. The NOAEL can be further broken down by component: (b) (4) mg/kg/day for A21 and (b) (4) mg/kg/day for HMWP, and (b) (4) mg/kg/day for other insulin-related compounds combined (b) (4)

Report Title Insulin Impurities: A 28-Day Subcutaneous Toxicity Study with a 14-Day Recovery Period in Sprague-Dawley Rats						
Species/Strain:	Rat, Sprague-Dawley	**Duration of Dosing:**	28 days	**Study No. MKC-PC-2010-0042**		
Initial Age:	8 to 10 weeks	**Duration of Postdose:**	14 days	**Location in CTD**	**Vol.**	**Page**
Date of First Dose:	November 10, 2010	**Method of Administration:**	Subcutaneous			
		Vehicle/Formulation:	1X PBS (phosphate buffered saline), pH 7.4.	**GLP Compliance: Yes**		
Special Features:		BID dosing				
No Observed Adverse Effect Level:		Total dose = 0.0244 mg/kg/day, Component A21: (b) (4) mg/kg/day, Component HMWP: (b) (4) mg/kg/day and (b) (4) mg/kg/day for other insulin-related compounds combined (b) (4)				

Daily Achieved Dose (mg/kg/day)	0 (Control)		0.0244 (Low Dose)		0.1435 (Mid Dose)		0.3021 (High Dose)	
Number of Animals	**M: 15**	**F: 15**	**M: 10**	**F: 10**	**M: 10**	**F: 10**	**M: 15**	**F: 15**
Insulin Equivalent Toxicokinetics:								
Day 1;								
AUC last (µU equiv•h/mL)	BQL	BQL	29.6	27.3	270	228	701	387
C_{max} (µU equiv/mL)	BQL	BQL	24.9	18.4	156	135	596	279
T_{max} (h)	BQL	BQL	0.250	0.500	0.500	0.250	0.083	0.500
Day 28;								
AUC last (µU equiv•h/mL)	BQL	BQL	61.6	48.6	378	362	816	1446
C_{max} (µU equiv/mL)	BQL	BQL	34.6	24.8	200	218	533	571
T_{max} (h)	BQL	BQL	0.083	0.250	0.500	0.250	0.500	0.500
Died or Euthanized Moribund	0	0	0	0	0	0	1	0

Daily Achieved Dose (mg/kg/day)	0 (Control)		0.0244 (Low Dose)		0.1435 (Mid Dose)		0.3021 (High Dose)	
Number of Animals	M: 15	F: 15	M: 10	F: 10	M: 10	F: 10	M: 15	F: 15
Body Weight (%*) - Day 28	490 (g)	303 (g)	1.2	-5.0	5.5	4.0	0.2	2.0
- Day 42	578 (g)	331 (g)	-	-	-	-	-5.0	2.1
Food Consumption (%*) - Day 28	234 (g)	169 (g)	1.7	-5.9	4.3	6.5	-3.4	3.6
- Day 42	298 (g)	174 (g)	-	-	-	-	-10.7	1.7

- No reportable results.

*At end of dosing period. For controls, group means are shown. For treated groups, percent differences from controls are shown.

-BQL (Below Level of Quantitation)

Noteworthy Findings

Daily Achieved Dose (mg/kg)	0 (Control)		0.0244 (Low Dose)		0.1435 (Mid Dose)		0.3021 (High Dose)	
Number of Animals	M: 15	F: 15	M: 10	F: 10	M: 10	F: 10	M: 15	F: 15
Hematology - Day 29								
WBC (10^9/L)	9.06	6.02	8.91	5.26	9.87	7.22	10.34	7.16
Neutrophils (10^9/L)	0.78	0.57	0.96	0.40	0.93	0.46	1.23	1.03*
Monocytes (10^9/L)	0.16	0.13	0.15	0.09	0.26*	0.13	0.32***	0.19*
Eosinophils (10^9/L)	0.09	0.08	0.10	0.08	0.11	0.09	0.11	0.10
Basophils (10^9/L)	0.05	0.03	0.04	0.03	0.05	0.04	0.06	0.04
Large unstained cells (10^9/L)	0.06	0.04	0.06	0.04	0.06	0.05	0.10	0.06
- Day 43								
WBC (10^9/L)	8.85	4.87	-	-	-	-	8.83	5.36
Neutrophils (10^9/L)	0.88	0.68	-	-	-	-	0.94	0.53
Monocytes (10^9/L)	0.27	0.15	-	-	-	-	0.18	0.09
Eosinophils (10^9/L)	0.10	0.06	-	-	-	-	0.10	0.05
Basophils (10^9/L)	0.03	0.01	-	-	-	-	0.03	0.01
Large unstained cells (10^9/L)	0.06	0.03	-	-	-	-	0.06	0.03

Daily Achieved Dose (mg/kg)	0 (Control)		0.0244 (Low Dose)		0.1435 (Mid Dose)		0.3021 (High Dose)	
Number of Animals	M: 15	F: 15	M: 10	F: 10	M: 10	F: 10	M: 15	F: 15
Serum Chemistry - Day 29								
Aspartate aminotransferase (U/L)	143	128	149	133	154	141	162	193**
Tryglycerides (mmol/L)	0.31	0.29	0.41	0.34	0.53***	0.38	0.58***	0.40
- Day 43								
Aspartate aminotransferase (U/L)	130	162	-	-	-	-	127	130
Tryglycerides (mmol/L)	0.44	0.33	-	-	-	-	0.36	0.39
* p<0.05 ; ** p<0.01;*** p<0.001 (Dunnett's)								
- No Reportable Results								
Urinalysis - Day 29								
Volume (mL)	9	81.032	13	7	13	8	17**	7
Specific gravity	1.035		1.026	1.028	1.025	1.026	1.018***	1.028
- Day 43								
Volume (mL)	10.0	4 1.038	-	-	-	-	4	6
Specific gravity	1.027		-	-	-	-	1.049	1.033
Gross Pathology - Day 29								
Stomach - Depressed area	0/10	0/10	0/10	0/10	1/10	1/10	4/9	4/10
- Day 43								
Stomach - Depressed area	- -	- -	-	-	-	-	- -	- -
Histopathology - Day 29								
Stomach, glandular - erosion Minimal	0/10	0/10	0/10	0/10	0/10	1/10	5/9	8/10
Congestion/hemorrhage Minimal	0/10	0/10	0/10	0/10	2/10	2/10	0/9	0/10
- Day 43								
Stomach, glandular - erosion Minimal	0/5	0/5	0/5	0/5	0/5	0/5	0/4	0/5
Congestion/hemorrhage								

Daily Achieved Dose (mg/kg)	0 (Control)		0.0244 (Low Dose)		0.1435 (Mid Dose)		0.3021 (High Dose)	
Number of Animals	M: 15	F: 15	M: 10	F: 10	M: 10	F: 10	M: 15	F: 15
Minimal - No Reportable Results - - No Noteworthy Findings * p<0.05; ** - p<0.01;*** - p<0.001 (Dunnett's)	0/5	0/5	0/5	0/5	0/5	0/5	0/4	0/5
Pre-terminal animal (4005A) – Clinical Signs - Day 28 Decreased activity Body cold to touch Loss of coordination Difficulty using the hind legs Gross Pathology Dark red area at dosing site Discoloration of the mandibular lymph node Histopathology Heart: Minimal focal myocardial degeneration Liver: Minimal microgranuloma/mononuclear cell infiltrate Epidiymis: Minimal focal interstitial mononuclear cell infiltrate							 √ √ √ √ √ √ √ √ √	
√ = present for pre-terminal animal 4005A								

PCNA analysis: 26 week rat tox study

Findings: Exposure to Technosphere particles at 10.5 and 12.8 mg/kg/day caused an increase in cell replication in the epithelium of large bronchioles in female rats and terminal bronchioles in male and female rats.

Mean Percent PCNA-Labeled Epithelial Cells in Male Rats at 26 Weeks

Males				
Group	Exposure	Percent Labeled Large Bronchiolar Epithelial Cells Mean (SD)	Percent Labeled Terminal Bronchiolar Epithelial Cells Mean (SD)	Percent Labeled Alveolar Epithelial Cells Mean (SD)
1	Air Control	0.67 (0.41)	0.78 (0.49)	0.64 (0.30)
2	Technosphere Particles	1.77 (1.34)	2.04 (1.04)	0.57 (0.30)
3	Low Dose TI	0.92 (0.51)	1.23 (0.44)[a]	0.79 (0.35)
4	High Dose TI	1.04 (0.50)	1.06 (0.47)[b]	0.74 0.33)

[a]p<0.01 compared to Group ; [b]p<0.05 compared to Group 2

Mean Percent PCNA-Labeled Epithelial Cells in Female Rats at 26

Weeks

Females				
Group	Exposure	Percent Labeled Large Bronchiolar Epithelial Cells Mean (SD)	Percent Labeled Terminal Bronchiolar Epithelial Cells Mean (SD)	Percent Labeled Alveolar Epithelial Cells Mean (SD)
1	Air Control	0.65 (0.42)	0.79 (0.39)	0.55 (0.36)
2	Technosphere Particles	1.25 (0.57)[a]	1.46 (0.52)[c]	0.73 (0.40)
3	Low Dose TI	0.63 (0.35)[b]	0.79 (0.39)[d]	0.46 (0.23)
4	High Dose TI	0.78 (0.56)	0.93 (0.34)[b]	0.52 (0.20)

[a] $p<0.05$ compared to Group 1; [b] $p<0.05$ compared to Group 2; [c] $p<0.01$ compared to Group 1; [d] $p<0.01$ compared to Group 2

PCNA analysis: 39 week dog tox study

Findings: PCNA stained cells within alveolar and bronchiolar epithelia were quantified. There were no differences observed in the cell proliferation activity of control and treated groups at any of the sacrifice occasions of between sexes.

Updated safety margin

In original NDA, human FDKP exposures used to calculating safety margins were determined using data from clinical studies with the MedTone® Inhaler. Subsequent to the completion of this section of the NDA, human trials were conducted with the Gen2 Inhaler. The Gen2 Inhaler produced human FDKP exposure values that were higher than when using the MedTone Inhaler since the Gen2 Inhaler delivers powder more efficiently than the MedTone Inhaler.

Summary of Safety Margin Update

Species	Duration	Original NDA Multiples based on^:			Resubmission Update Multiples based on^^:		
		mg/kg	mg/m²	AUC	mg/kg	mg/m²	AUC
Mouse	13-week, subcutaneous	57.7	4.7x	18.1x	57.7	4.7x	10.2x
Rat	4-week, inhalation	47.5x	8.5x	1.7x (3.5x)	47.5x	8.5x	1.0x (2.0x)
Rat	26-week, inhalation	9x	1.6x	2.7x (5.4x)	9x	1.6x	1.5x (3.1x)
Dog	4-week, inhalation	31x	15.8x	13.1x	31x	15.8x	7.4x
Dog	39- week, inhalation	8.4x	4.3x	2.1x	8.4x	4.3x	1.2x

^ Human FDKP AUC at maximum daily TI dose of 315U with the MedTone inhaler was 224,245 ng·min/mL (MKC-TI 141)

^^ Revised human FDKP AUC at the maximum daily TI dose (4U/kg or 300 Units) with the Gen-2 inhaler was 398,155 ng·min/mL (MKC-TI-176)

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/s/

MIYUN M TSAI-TURTON
01/13/2014
Mid-Cycle Review

KAREN L DAVIS BRUNO
01/13/2014

DEPARTMENT OF HEALTH & HUMAN SERVICES
Food and Drug Administration

Memorandum

Date: January 5th 2012
From: Miyun Tsai-Turton PhD, MS; Pharmacology; DMEP
Subject: Pharmacology/Toxicology Memo
To: NDA 22-472 Afrezza/Mannkind

Reference: Pharmacology/Toxicology Review & Evaluation of NDA 22-472 (eCTD SN 0064)

Background

Afrezza or Technosphere insulin (TI) is inhaled as a dry powder with the aid of a Gen2 inhaler. Technosphere insulin is comprised of recombinant human insulin, a novel excipient fumaryl diketopiperazine (FDKP), the MannKind proprietary excipient and primary component of Technosphere, and (b) (4) polysorbate 80 (PS80). FDKP undergoes acid-induced intermolecular self-assembly in aqueous solution resulting in adsorption of insulin onto the Technosphere particles to form Technosphere insulin.

This NDA 22472 (originally submitted under IND 61729) has gone through two review cycles since 2008 and both cycles resulted in Completed Response letters being issued. All correspondents between FDA and the sponsor to address deficiencies were documented in DARRTS.

Regulatory History:

- March 2009 – original NDA submission with the **MedTone** device
- March 2010 – FDA issued a CR letter containing clinical, clinical pharmacology, libeling, and device deficiencies
  NOTE: In original NDA submission, the sponsor did one 28 day repeat-dose toxicity study to support their impurity limits. This study focused on FDKP-related impurities. The pharm/tox was OK with that study.
- June 2010 – EOR meeting was held. The sponsor resubmitted this NDA application with the **Gen2C** device later that month.
- Jan 2011 – FDA issued another CR letter containing clinical, clinical pharmacology, product quality, device, and labeling deficiencies.
- May 2011 – EOR meeting was held.
- Oct 2011 – Type C meeting: written response granted
- Nov 2012 – The briefing document for the Written Response was submitted (eCTD SN 0064).
  NOTE: The pharm/tox is assisting CMC to address comment 7 under Product Quality. The sponsor submitted one 28 day repeat-dose toxicity study (which is being reviewed here) to support their impurity limits. This study focused on TI-related impurities/degradants.

For clinical development thus far, there are two proposed Phase III studies which will be conducted in T1DM (Study No. MKC-TI-171) and T2DM (Study No. MKC-TI-172). There studies intend to demonstrate that the efficacy of the TI-Gen2 group (Technosphere Insulin Inhalation Powder administered using the Gen2C inhaler in combination with a basal insulin) will be non-inferior to the insulin aspart group (insulin aspart in combination with a basal insulin) by measuring HbA1c levels in T1DM or T2DM patients.

Reference ID: 3067377

Study Title: A Phase 3, Multicenter, Open-label, Randomized, Forced-titration Clinical Trial Evaluating the Efficacy and Safety of Technosphere® Insulin Inhalation Powder Administered Using Two Different Inhalation Devices in Combination with Insulin Glargine Versus Insulin Aspart in Combination with Insulin Glargine in Subjects with Type 1 Diabetes Mellitus Over a 24-week Treatment

Study Title: A Phase 3, multicenter, open-label, randomized, forced-titration clinical trial to evaluate the efficacy and safety of Technosphere® Insulin Inhalation Powder administered using two different inhalation devices (Gen2C and MedTone C) in combination with insulin glargine versus insulin aspart in combination with insulin glargine in subjects with Type 2 diabetes mellitus over a 24-week treatment period.

Nonclinical Aspect of Afrezza

During 1st cycle NDA submission, Pharm/tox reviewed all nonclinical studies with Technosphere Insulin (TI) and/or Technosphere particles alone (FDKP was the novel excipient). Pharm/tox concluded that there might be lung irritation, based on 39 week inhalation dog study, where minimal to mild alveolar/bronchial interstitial neutrophil infiltration was seen in lungs for all TI-treated groups. However, these findings regressed after 8 weeks recovery.

During 2nd cycle NDA (re)submission, pharm/tox did not provide any additional comments since there were no new nonclinical studies submitted. Even though the delivery device was changed from the MedTone system in 1st review cycle to Gen2C system in 2nd review cycle, such switch in device did not affect the outcome of Afrezza's nonclinical development program.

According to the most current annual report (report period: Jan 2010-Jan 2011) for IND 61729, there were two ongoing nonclinical studies: MKC-PC-2010-0043 (HPLC analytical method validation and **MKC-PC-2010-0042** *(insulin impurities qualification with a 28 day toxicity study with 14 day recovery in rats)*. There were no nonclinical studies planned. **NOTE: The 28 day toxicity study is reviewed in this memo.**

STUDY No. MKC-PC-2010-0042

Study title: Insulin Impurities: A 28-day Subcutaneous Toxicity Study with a 14-Day Recovery Period in Spraque-Dawley Rats

Key study findings:

- Spraque-Dawley rats were treated with a mixture of insulin-related compounds (0, 0.0244, 0.1435, or 0.3021 mg/kg/day) twice daily for 28 days via sc injection and with 14 day recovery period. ***Since the sponsor submitted incomplete final study report, most data was not reviewed in details.***
- There was one death (attributed to hypoglycemia) in HD group.
- No test article-related adverse effects on clinical signs, body weight, food consumption, ocular toxicity, coagulation, and organ weights.
- There were several changes in hematological parameters (i.e. white blood cell populations in treated groups and neutrophils, monocytes, eosinophils, basophils, and large unstained cells in HD group). These changes were recoverable.
- There were dose-dependent increases in AST and TRIG levels on Day 29. These changes were recoverable.
- There were also increased urine volume and decreased specific gravity in HD males. These changes were recoverable.
- Dark areas were seen at injection sites of both control and treated animals. This correlated with microscopic findings (i.e. sc haemorrhage and mixed/mononuclear cell infiltrates).

Reference ID: 3067377

- One test-article related microscopic finding was noted. Dark areas of the glandular stomach were seen in 2/20 MD animals and 8/19 HD animals. This correlated with microscopic findings (i.e. focal/multifocal erosion in 1/20 MD animals and 13/19 HD animals, focal/multifocal mucosal congestion/haemorrhage in 4/20 MD animals). These were not present during the recovery period.
- The NOAEL for this study was a total mean daily dose of 0.024 mg/kg/day (A21: (b) (4) mg/kg/day, HMWP: (b) (4) mg/kg/day, and combined other insulin related impurities (b) (4) mg/kg/day) due to stomach findings (i.e. hemorrhage and erosion in the glandular stomach) in MD and/or HD groups.

Study no.: MKC-PC-2010-0042
Volume #, and page #: 813 pages (Note: Only 37 out of 813 pages were provided)
Conducting laboratory and location: (b) (4)
Date of study initiation: Nov 2nd 2010
GLP compliance: Yes
QA report: yes (x) no ( )
Drug, lot #, and % purity: Lot No. FD10294 with 7.52% purity

- Test article is a mixture of 9 insulin impurities

Reporting group	Name of group	Components (Molecular Weight)
1		(b) (4)
2		
3		
4		
5		
6		
7		

Description of insulin-related compounds tested

Compound	Description
	(b) (4)

Methods

- *Doses:* 0, 0.0244, 0.1435, or 0.3021 mg/kg/day. The test article was prepared as a mixture of impurities without insulin to avoid exaggerated insulin pharmacology, hypoglycemia.

Reference ID: 3067377

Group Numbers	Group Designation	Nominal dose Level per day / per injection (mg/kg)	Nominal dose concentration (µg/mL)#	Toxicology Animals				Toxicokinetics Animals	
				Main		Recovery			
				Male	Female	Male	Female	Male	Female
1	Control*	0	0	10	10	5	5	3	3
2	Low Dose	0.034 / 0.017	4.26	10	10	-	-	6	6
3	Mid Dose	0.168 / 0.084	21.00	10	10	-	-	6	6
4	High Dose	0.335 / 0.167	41.85	10	10	5	5	6	6

* Control animals received the Control/Vehicle [1X PBS (phosphate buffered saline), pH 7.4]

Mean achieved daily dose levels for insulin degradation products

Group Number	Group Designation	Mean daily dose for insulin-related compounds (mg/kg/day)				
		Calculated from mean of dosing solution assays				Nominal
		A21	HMWP	*Combined (b) (4) (b) (4)	TOTAL	
1	Control	0	0	0	0	0
2	Low Dose	(b) (4)				
3	Mid Dose					
4	High Dose					

* Individual results of the insulin impurities are included in the formulation analysis report

Mean achieved doses of individual insulin-related compounds

Component	Mean Daily Dose (mg/kg/day)		
	Group 2	Group 3	Group 4
A21	(b) (4)		
HMWP			
Other Impurities* (b) (4)			
TOTAL			

*Total combined dose of Impuritie (b) (4) and the individual results of these impurities are included in the formulation analysis report.

- *Species/strain:* Spraque-Dawley rats
- *Number/sex/group or time point (main study):* 10/sex/group
- Route, formulation, volume, and infusion rate:
- *Satellite groups:* Recovery: n=5 for control and HD groups and TK: n=3 for control and 6 for LD, MD, HD groups.
- *Age:* 8-10 weeks
- *Weight:* 266-385g for males and 216-258 for females
- *Sampling times:* Blood samples were collected from each TK phase rat on Days 1 and 28: predose, 5, 15, 30 min, 1, 2, 4, and 6 hrs.
- *Unique study design or methodology (if any):* None.

Obervations and Results

NOTE: Since the sponsor only submitted partial final study report (37 out of 813 pages), most data were not available for review.

Mortality:

- Once a day

One HD animal was found dead on Day 28 due to hypoglycaemia.

Clinical signs:
- Once a day

No test-article related clinical signs were observed in these animals. NOTE: No data was provided for review.

Body weights:
- Days 1, 7, 14, 21, 28 (main and recovery animals), 35 and 42 (recovery animals)

No test-article related adverse effects on the mean body weight in these animals in this study. NOTE: No data was provided for review.

Food consumption:
- Weekly

No test-article related adverse effects on food consumption in these animals during dosing period. However, the mean food consumption for the HD males was lower compared to the concurrent control during the recovery period. This might be due to individual variation. NOTE: No data was provided for review.

Ophthalmoscopy:
- Once pre-treatment, once during Week 4, and at the end of recovery period

No test-article related adverse ocular effects were observed in these animals.

EKG:
- This was not examined in these animals.

Hematology:
- Day 29 (main) and Day 43 (recovery)

Red blood cell count	Mean corpuscular haemoglobin (calculated)
Haematocrit (calculated)	Mean corpuscular volume
Haemoglobin	Morphology of cells
White blood cell count	Platelet count
WBC differential (absolute)	Reticulocyte (absolute and percentage)
Mean corpuscular haemoglobin concentration (calculated)	

The WBC in the treated groups was differed from the controls in a dose-dependent manner. Others, including neutrophils (NEUT), monocytes (MONO), eosinophils (EOS), basophils (BASO), and large unstained cells (LUC), were also elevated in the HD group. These changes were somewhat reversed by the end of recovery period (Day 43). These findings were within normal reference ranges. NOTE: Only summary tables were provided.

Relative difference from control in white blood cell populations at the end of treatment

Parameter	Percentage change by Group and sex					
	Group 2		Group 3		Group 4	
	Male	Female	Male	Female	Male	Female
WBC	-1.7	-12.6	8.9	19.9	14.1	18.9
NEUT	23.1	-29.8	19.2	-19.3	57.6	80.7*
MONO	-6.2	-30.8	62.5*	0.0	100.0**	46.5*
EOS	11.1	0.0	22.2	12.5	22.2	25.0
BASO	-20.0	0.0	0.0	33.3	20.0	33.3
LUC	0.0	0.0	0.0	25.0	66.7	50.0

Statistical significance is noted above as follows: * = p<0.05 and ** = p<0.001 (Dunnett's).

Relative difference from control in white blood cell populations on Day 43 following Recovery period

Parameter	Percentage change by Group and sex			
	Group 4 Day 29		Group 4 Day 43	
	Male	Female	Male	Female
WBC	14.1	18.9	0.2	10.0
NEUT	57.6	80.7*	6.8	-22.0
MONO	100.0**	46.5*	-33.3	-40.0
EOS	22.2	25.0	0.0	16.7
BASO	20.0	33.3	0.0	0.0
LUC	66.7	50.0	0.0	0.0

Statistical significance is noted above as follows: * = p<0.05 and ** = p<0.001 (Dunnett's).

Clinical chemistry:

- Day 29 (main) and Day 43 (recovery)

Batch 1:	Batch 2:
Alanine aminotransferase	Albumin
Aspartate aminotransferase	Total Protein
Creatinine	A/G ratio (calculated)
Urea	Globulin (calculated)
Batch 3:	**Batch 4:**
Chloride	Bilirubin (Total)
Potassium	Alkaline phosphatase
Sodium	Glucose
Calcium	Cholesterol (total)
Phosphorous (inorganic)	Triglycerides
	Gamma glutamyl transpeptidase (GGT)

The serum AST and TRIG were higher on Day 29 in the treated groups than the controls in a dose-dependent manner. Both AST and TRIG are linked to the pharmacology of insulin. These changes were reversed by the end of recovery period (Day 43). These findings were within normal historical ranges. NOTE: Only summary table was provided.

Relative difference from control in AST and triglyceride levels

		Percent difference from control			
Group		AST		Triglycerides	
Number	Day	Males	Females	Males	Females
2	29	4.2	3.9	32.3	17.2
3	29	7.7	10.2	71***	31
4	29	13.3	50.8**	87.1***	38
	43	-2.3	-19.8	-18.8	18.2

** = p<0.01 (Dunnett's)
*** = p < 0.001 (Dunnett's)

Urinalysis:

- Day 29 (main) and Day 43 (recovery)

Bilirubin	Protein
Blood	Sediment microscopy
Color and appearance	Specific gravity
Glucose	Urobilinogen
Ketones	Volume
pH	

There were no effects on urinalysis parameters except the HD males had increased urine volumes, corresponding decreased specific gravity. This was comparable to the control group. The toxicological significance of this finding was unknown. NOTE: No data was provided for review.

Gross pathology:

- Day 29 (main) and Day 43 (recovery)

Dark areas around injection sites were observed but there were no comparable between the control and treated groups. This is probably due to the administration procedure. There changes were still observed at the end of recovery period. They correlated with microscopic findings (i.e. haemorrhage and inflammation). In addition, depressed areas of the glandular stomach were noted in 2/20 MD animals and 9/19 HD animals. These findings correlated with microscopic findings (i.e. erosion). NOTE: Only summary table was provided.

Incidence of treatment-related macroscopic finding in main study animals

Sex	Males				Females			
Group	1	2	3	4	1	2	3	4
Number of animals examined	10	10	10	9	10	10	10	10
Stomach								
Depressed area	0	0	1	4	0	0	1	4

Organ weights (specify organs weighed if not in histopath table):

- Day 29 (main) and Day 43 (recovery)

No test article related changes in mean absolute or relative organ weights were seen. There were some significant differences but were not consistent across dosing groups or between sexes. These changes were not associated with any macroscopic or microscopic findings.
NOTE: No data was provided for review.

Histopathology: Adequate Battery: yes (x), no ( )
Peer review: yes ( ), no (x)

ORGANS/TISSUES	Retain (•)	Weigh (✓)	Examine (€)	ORGANS/TISSUES	Retain (•)	Weigh (✓)	Examine (€)
Adrenals	•	✓	€	Duodenum	•		€
Animal identification	•			Jejunum	•		€
Aorta (thoracic)	•		€	Ileum	•		€
Blood	•			SC, cervical	•		€
Bone marrow smears (3)	•			Spleen	•	✓	€
Brain	•	✓	€	Sternum & marrow	•		€
Cecum	•		€	Stomach	•		€
Colon	•		€	Testes	•d	✓	€
Epididymides	•d		€	Thymus	•	✓	€
Esophagus	•		€	Thyroid gland/parathyroids	•	✓	€
Eyes	•a		€	Tongue	•		€
Femur & marrow	•		€	Trachea	•c		€
Heart	•	✓	€	Urinary bladder	•		€
Kidneys	•	✓	€	Uterus	•	✓	€
Liver (2 lobes)	•	✓	€	Vagina	•		€
Lungs (2 lobes)	•bc	✓	€				
LN, mandibular	•		€	Abnormal findings	•		€
LN, mesenteric	•		€				
Mammary gland (inguinal)	•		€				
Optic nerves	•a		€	**Additional Tissues presented below**			
Ovaries	•	✓	€	Last dosing site*	•		€
Pancreas	•		€	ID microchip	•		
Pituitary	•	✓	€				
Prostate	•	✓	€				
Rectum	•		€				
SG, mandibular	•		€				
Sciatic nerve	•		€				
Seminal vesicles	•		€				
Skeletal muscle	•		€				
Skin & subcutis (inguinal)	•		€				

a	Davidson's fluid (euthanized animal only)
b	Lungs were infused with 10% neutral buffered formalin (euthanized animal only)
c	Lungs were weighed with trachea and recorded in Provantis as lungs in organ weight record
d	Bouin's fluid (euthanized animal only)
LN	Lymph node
SG	Salivary gland
SC	Spinal cord
€	Examined microscopically
*	All dosing sites were collected but only the last site was examined histopathologically

Notes:

Paired organs weighed together
Parathyroids, mammary gland and optic nerves will only be examined histologically if present in routine sections

Incidence of subcutaneous haemorrhage and subcutaneous mixed or mononuclear cell infiltrates was observed at dosing/injection sites in both control and treated animals. In addition, there was test article related focal or multifocal erosion of the glandular stomach seen in 1/20 MD animals and 13/19 HD animals. Focal or multifocal mucosal congestion/haemorrhage of the glandular stomach was also seen in 4/20 MD animals. These findings were consistent with studies in rats exposed to insulin alone at high doses by sc injection or animals under chronic stress. All stomach microscopic findings were no longer present, suggesting complete recovery. NOTE: Only summary table was provided.

Incidence and severity of treatment-related microscopic findings

Study Phase	Main								Recovery			
Sex	Males				Females				Males		Females	
Group	1	2	3	4	1	2	3	4	1	4	1	4
Number of animals examined	10	10	10	9	10	10	10	10	5	4	5	5
Stomach, glandular												
Erosion	0	0	0	5	0	0	1	8	0	0	0	0
minimal	-	-	-	5	-	-	1	8	-	-	-	-
Congestion/haemorrhage	0	0	2	0	0	0	2	0	0	0	0	0
minimal	-	-	2	-	-	-	2	-	-	-	-	-

Toxicokinetics:

- Days 1 and 28 (predose, 5, 15, 30 min, 1, 2, 4, 6 hours)

TK data was used to confirm systemic exposure of the test article in animals. Twice daily sc injections of the test article in rats for 28 days resulted in consistent insulin detection (with dose-related increases in exposure). NOTE: No data was provided for review.

Other:

- Formulation analysis

The results of LD, MD, and HD samples collected for analysis were lower than expected: (b) (4) % for Group 2 (b) (4) % for Group 3, and (b) (4) % for Group 4. These measured concentrations did not meet the acceptance criterion of (b) (4) % of the nominal concentration, so the measured concentrations were used to calculate dosage. These low results may be due to loss of protein to the glass and the filter and such loss had more effect on lower dose groups (i.e. more diluted solutions). NOTE: Only summary data was provided.

Mean achieved daily dose levels for insulin degradation products

Group Number	Group Designation	Mean daily dose for insulin-related compounds (mg/kg/day)				
		Calculated from mean of dosing solution assays				Nominal
		A21	HMWP	*Combined (b) (4) (b) (4)	TOTAL	
1	Control	0	0	0	0	0
2	Low Dose	(b) (4)				
3	Mid Dose					
4	High Dose					

* Individual results of the insulin impurities are included in the formulation analysis report

Overall Conclusion:

This 28 day toxicity study with a 14 day recovery was to qualify a mixture of insulin related compounds (impurities and degradation products) in Technosphere Insulin Inhalation Power (TI). The sponsor identified 7 impurities which were then subdivided into three groups: A21 (A21 desamido insulin), HMWP (high molecular weight proteins, primarily covalent insulin dimers), and Total Others (mixtures of (b) (4) Due to their structural similarity to insulin, all compounds were expected to have biological activity of insulin.

This study showed one mortality in HD group (hypoglycemia), no adverse effects on clinical sign/body weight/food consumption/organ weights, some changes (but recoverable) in hematological parameters (i.e. ↑ WBC/NEUT/MONO), clinical chemistry (i.e. ↑ AST and TRIG), and urinalysis (i.e. ↑ urine volume and specific gravity). The study also found macroscopic changes (dark areas at injection sites in control/treated groups and dark areas in glandular stomach in MD/HD groups). These gross findings further correlated with microscopic findings

Reference ID: 3067377

(i.e. injection site inflammations in both control/treated groups and stomach erosion and inflammation in MD and/or HD groups). Based on all these study findings, NOAEL was established at the LD (total dose of 0.0244 mg/kg/day).

Based on *incomplete* final study report prepared by the CRO ([redacted] (b) (4)), the NOAEL was the low dose. However, the sponsor argued the mid dose should be the NOAEL based on the fact that such stomach findings were known species-specific pharmacologic effects of insulin doses > 0.087 mg/kg (2.5 U/kg) or chronic. Even though the sponsor provided references to establish the probable association between chronic hyperinsulemia and increased incidence of ulcer, the NOAEL for this study should remain at the low dose, where no toxicity was observed.

All in all, based on this 28 day toxicity rat study, insulin impurities and degradation products in the Technosphere Insulin Inhalation Powder were qualified and had 1 to 3 fold safety margins at the proposed acceptance criteria.

Estimate safety margins based on NOAEL (low dose):

Impurites	Mean Achieved Dose (mg/kg/day)	Level Qualified	Acceptance Criterion	Max. Anticipated Clinical Dose* (mg/kg/day)	Dose Multiple
A21	[redacted] (b) (4)				
HMWP					
Total other impurities (b) (4)					
[redacted] (b) (4)					

Pharm/tox-related CMC Issue

- In FDA's Jan 2011 2nd CR letter

  7. *Conduct a study of the insulin adduct impurities and insulin-related degradants in support of the limits on these compounds as proposed in the November 24, 2010 amendment to the NDA. Submit adequate supporting data, including a validated method for monitoring insulin-FDKP adducts on stability.*

- Sponsor's question

• Comment 7: MannKind believes that the completed GLP 28-day rat toxicology study supports the acceptance criteria for A21, total others, and HMWP. Does the Agency agree?

- Sponsor's rationale

Reference ID: 3067377

A 28-day subcutaneous toxicity study with a 14-day recovery period (MKC-PC-2010-0042) was conducted in Sprague-Dawley rats to qualify a mixture of insulin-related compounds (impurities and degradation products) in AFREZZA [Technosphere® Insulin Inhalation Powder] that could exceed (b)(4)% during the intended shelf life. Subcutaneous administration of the insulin-related degradation products twice daily for 28 consecutive days in rats was well tolerated with no clinical signs and no adverse effects on body weight/food consumption, or ophthalmic evaluations. A summary of this toxicology study is provided below and additional details are provided in Appendix 1, MannKind Evaluation and Appendix 2, (b)(4) Study Report.

On-going registration stability studies indicate that the acceptance criteria submitted in the November 24, 2010 amendment, sequence number 0053, 3.2.P.5.1 Specification (Technosphere® Insulin Inhalation Powder) will require modification and support modifying the acceptance criteria for A21 from NMT (b)(4)% to NMT (b)(4)%, HMWP from NMT (b)(4)% to NMT (b)(4)%, and Total Others from NMT (b)(4)% to NMT (b)(4)%. A summary of the on-going registration stability data for A21, HMWP, and Total Others is provided below

Based upon ICH guidelines, insulin impurities and degradation products in the Technosphere Insulin Inhalation Powder are qualified and demonstrate greater than a 10-fold safety margin at the proposed acceptance criteria.

Pharm/tox Response to Comment 7:

Based on your 28 day toxicity study provided in your briefing document, insulin impurities and degradation products seemed appropriately qualified. The impurities were considered qualified at the following levels: A21 - (b)(4)%; HMWP-(b)(4)%; total other impurities -(b)(4)% and are now considered safe.

Other pharm/tox comments:

For the 28 day rat toxicity study report, the (b)(4) Study Report in Appendix 2 was incomplete; there were only 37 out of 813 pages submitted in your briefing document for review. We also do not agree that there was a 10-fold safety margin at the proposed acceptance criteria, using the mid dose as your NOAEL. The NOAEL of this study should be based on the dose which there was no observable toxicity, which would be the low dose used in this study.

Reference ID: 3067377

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/s/

MIYUN M TSAI-TURTON
01/05/2012

KAREN L DAVIS BRUNO
01/05/2012

DEPARTMENT OF HEALTH & HUMAN SERVICES
Food and Drug Administration

Memorandum

Date: December 18, 2009
From: Karen Davis-Bruno PhD; Pharmacology Supervisor; DMEP
Subject: Pharmacology/Toxicology Supervisor's Memo
To: NDA 22-472 Afrezza/Mannkind

Reference: Pharmacology/Toxicology Review & Evaluation of NDA 22-472

Afrezza or Technosphere insulin (TI) is inhaled as a dry powder with the aid of a MedTone inhaler. Technosphere insulin is comprised of recombinant human insulin, a novel excipient fumaryl diketopiperazine (FDKP) and primary component of Technosphere, and (b) (4) polysorbate 80 (PS80). FDKP undergoes acid-induced intermolecular self-assembly in aqueous solution resulting in adsorption of insulin onto the Technosphere particles to form Technosphere insulin. A 28-day rat inhalation toxicity study with Technosphere particles containing (b) (4) PS80 was conducted which was unremarkable in its outcome. This exposure to PS80 represents a 25X higher rat PS80 dose than with the maximum human TI dose on a mg/kg basis.

The proposed clinical indication is Type 1 and Type 2 diabetics, administered at mealtime 3-4X daily. The maximum recommended human daily dose (MRHD) is 315 U of insulin in 105 mg TI powder ((b) (4) mg insulin + (b) (4) mg T). TI is intended as rapid acting insulin with a faster onset of action and shorter duration than other marketed insulins. This pharmacokinetic profile is proposed to result from the efficient delivery of TI powder into the deep lung where it dissolves rapidly. This is in part a function of the Technosphere particle diameter being 2-2.5 µm. (b) (4) Larger particles may be deposited in the upper airways/bronchial tree. The insulin from these larger particles may also be absorbed. A variation in the particle size pulmonary distribution (because of differing inhalation flow rates) may affect the amount of insulin absorbed. The mechanism involved in the rapid systemic absorption of insulin from Technospheres has not been established. It may be related to some unidentified action of FDKP on the pulmonary cells. Cell-based studies were performed to demonstrate that FDKP and TI do not disrupt cellular tight junctions and FDKP does not increase the permeability of cell membranes. Technospheres administered alone. did not result in a reduction in blood glucose, confirming that FDKP has no effect on blood glucose concentrations.

Nonclinical Development Program The nonclinical development program for TI focused on the effects of local toxicity of inhaled insulin and on characterization of FDKP/Technospheres. FDKP is not metabolized and undergoes renal excretion, unchanged. The pharmacology/toxicology of inhaled human insulin has been well established in humans and animals over the last 90 years.

***Afrezza Carcinogenicity*:** A 2-year carcinogenicity study in rat and a subcutaneous transgenic mouse study (Tg.rasH2) were performed. The results of the carcinogenicity assessment with Technosphere and TI indicate an absence of any drug-induced neoplastic findings with administration either via inhalation (2-year rat) or subcutaneous (Tg.rasH2 mouse) routes at systemic exposures of Technosphere (FDKP) 13X and 1X respectively, compared to the AUC at the MRHD (105 mg). Cell proliferation activity (proliferating cell nuclear antigen; PCNA) was confirmatory of the absence of neoplasias/pre-neoplastic signals as assessed in alveolar and bronchiolar cells across treatment and control groups from the rat carcinogenicity study. The genotoxicity of Technosphere and insulin was assessed in an Ames bacterial mutagenicity assay, chromosome aberration assay in human peripheral blood lymphocytes with and without metabolic activation as well as a mouse micronucleus assay with Technosphere alone. All genotoxicity studies were negative.

Carcinogenicity of Related Insulin Products

Exubera (NDA 21-868; 2006) was the first marketed recombinant human insulin in powdered form, delivered via a pulmonary inhaler. Marketing ceased in 2007 by Pfizer because the product failed to gain acceptance among patients and physicians. In April 2008, Pfizer announced that Exubera may have been associated with lung cancer (6/4740 patients vs. 1/4292 placebo patients from clinical trials). This incidence was not statistically significant. Carcinogenicity studies were not performed with Exubera because recombinant human insulin is identical to the endogenous hormone and treatment is considered replacement therapy. Chronic toxicity studies indicated there was no effect on cell proliferation indices in alveolar or bronchiolar areas of the lung in either species. Compared to control animals, there were no drug-related adverse effects in either rats or Cynomolgus monkeys following 6-month duration repeat-dose chronic toxicity studies with regard to pulmonary function, gross or microscopic morphology of the respiratory tract or bronchial lymph nodes.

Lantus (NDA 21-081) is a recombinant human insulin analogue (insulin glargine) which is modified to slow the release of microprecipitated insulin glargine from the subcutaneous injection site. Two year life-time carcinogenicity bioassays with insulin glargine were performed in mice and rats at doses up to 0.455 mg/kg at exposures 10-times in the rat and 5-times in the mouse compared to human exposures at the starting dose of 10 IU (0.008 mg/kg/d) based on body surface area comparisons across species (mg/M^2). Female mice had survival issues during the study and the findings were inconclusive as a result of the excessive mortality in all dose groups attributable to hypoglycemia. Malignant histiocytomas were observed at the injection sites in male rats and male mice. The histiocytomas reached statistical significance in the male rats (2-fold human exposure at 10 IU) but was not statistically significant in the male mice. Histiocytomas were not observed in female rats, saline control, or in the insulin comparator group using a different (non-acidic) vehicle. Indices of mitogenicity and relative IGF-1 receptor binding were reported as slightly higher for insulin glargine compared to human insulin.

Afrezza General Toxicology: The chronic inhalational toxicology studies included 6-month rat and 9-month dog studies. Toxicology data with Technospheres suggest some potential for respiratory irritation with therapeutic use. The chronic toxicity studies (rat,

dog) indicate minimal to mild respiratory irritation at 2X human systemic exposure. This did not correlate to any preneoplastic or neoplastic findings as discussed previously.

In the 26-week chronic rat study with dose groups: 11.7 mg/kg/d for T, low dose (LD) TI (1.05 M, 0.404 F mg/kg/d) and high dose (HD) TI (1.91 M, 1.28 F mg/kg/d) administered by nose only inhalation, respiratory findings including increased lung weights, nasal cavity eosinophilic globules and epithelial degeneration were observed. These findings were recoverable in females but remained in the males following a 4-week drug withdrawal period. A slight increase in bronchiolar cell PCNA (but not alveolar cells) in the upper respiratory tract in T and in LD, HD TI groups may be associated with particulate impaction. This is in contrast to the results from the 2-year rat carcinogenicity study showing an absence of bronchiolar and alveolar cell PCNA. Insulin is known to have an adaptive effect on late phase G1 in conjunction with IGF-1, therefore an increased proliferation rate of exposed, regenerating cells such as the bronchial cells is not surprising. However since these cells did not show neoplastic findings and PCNA analysis was negative after lifetime exposure in rat, the slight increase in bronchiolar cell PCNA in the chronic rat study may be considered an adaptive response. These results showing a slight increase in the upper airway (bronchial) but not in the deep alveolar tissues suggest that rats were exposed to larger Technosphere particles than proposed for human use with the MedTone (DPI) inhaler. In addition to pulmonary effects, approximately half the males treated with T (11.7 mg/kg/d) had evidence of myocardial degeneration/necrosis which was not observed in any TI treated groups nor was this observed in female rats or in the dog. The 2-year rat carcinogenicity study with lifetime exposure did not show any increase in treatment related cardiac findings above concurrent controls. The NOAEL for the chronic rat toxicity study was 1.91 mg/kg/d in males and 1.28 mg/kg/d in females. Based on FDKP exposure, the NOAEL was 2-fold greater than the maximum anticipated daily human therapeutic exposure (105 mg Afrezza) based on AUC.

In the 2-year rat carcinogenicity study, with nose only inhalation, the nasal cavity of the HD Technosphere (41, 46 mg/kg/d) treated group had goblet cell hyperplasia of the respiratory epithelium, accumulation of eosinophilic droplets in the olfactory and respiratory epithelium. These findings were considered an adaptive response to chronic inhalation of Technospheres because they were seen at comparable severity/frequency in the control and TI treated groups. Consistent with this observation, is the minimal to slight mixed macrophage and neutrophils infiltrates and degenerative skeletal muscle myopathy seen in the subcutis in the SC transgenic mouse carcinogenicity study in all groups, including controls.

In the 39-week chronic dog study with administered doses of: LD T (2.4 mg/kg/d), HD T (10.9 mg/kg/d and LD TI (0.39 mg/kg/d) and HD TI (1.92 mg/kg/d) were tested using oronasal exposure. Respiratory findings included increased minimal to mild neutrophils infiltration of the lungs in the HD TI group which recovered following an 8-week drug withdrawal. Thymic atrophy and hypocellularity of the seminiferous epithelium and germ layer degeneration were observed in the T and TI groups. These findings are commonly associated with immature dogs, consistent with age 7-8 month old dogs at

study initiation. These male reproductive effects were not observed in the subsequent reproductive toxicity test battery. The NOAEL for the chronic dog toxicity study was 1.92 mg/kg/d. Based on FDKP exposure this NOAEL provides an exposure margin 2-fold greater than the maximum anticipated daily human therapeutic exposure (105 mg Afrezza) based on AUC comparisons across species.

Afrezza Reproductive Toxicity: A reproductive and developmental toxicology study battery was performed in rats with assessment from preconception through lactation and during organogenesis in rabbits via subcutaneous exposure (SC) to Technospheres. There were no adverse effects on fertility in male rats administered a dose of 100 mg/kg/d which is 180X the projected maximum human exposure (105 mg Afrezza) based on AUC comparison. This suggests that the male seminiferous epithelium and germ cell degeneration in seen in the chronic dog toxicity study could be attributable to the immaturity of the dogs used instead of drug-associated findings. In assessing female rats fertility an increased incidence of pre- and post-implantation loss was observed at 100 mg/kg/d (180X human exposure) but not at 30 mg/kg/dwhich is 50X the maximum human exposure (105 mg Afrezza) based on AUC comparison. FDKP was detected in rat fetal circulation at concentrations comparable to maternal plasma concentrations on gestation day 18. FDKP was present in rat milk at ~10% of the maternal systemic exposure.

The rabbit is more sensitive to Technosphere (FDKP) than the rat during organogenesis. Maternal toxicity is seen in pregnant rabbits as evidenced by significantly decreased body weight at all doses. Mortality is seen in pregnant female rabbits at 100 mg/kg/d. In pregnant rabbits given subcutaneous doses of Technosphere (2, 10, 100 mg/kg/d), resulted in malformations in 2-3 fetuses (1 dead fetus with many of these findings) which included: cleft palate, non-patent nares, absent intermediate lung lobes, gall bladder as well as vertebral/rib/scapular malformations and irregular ossified skull bones at 2 mg/kg/d which is 10X the human systemic exposure at the 105 mg Afrezza dose based on AUC. However since there is clear evidence of maternal toxicity in rabbits at the 2 mg/kg/d dose these fetal findings may be attributed to this confounder. Pregnant women would not be dosed to toxicity. The rabbit appears to have greater systemic exposure (AUC=10X higher) to Technospheres than the rat at the same mg/kg dose and greater exposure may account for the increased toxicity observed in the rabbit.

Pregnant rats given subcutaneous doses of 10, 30, 100 mg/kg/d Technosphere during organogenesis resulted in no major treatment related findings at 180X the projected human systemic exposure at the 105 mg TI dose based on AUC comparison. In pregnant rats given the same doses from organogenesis through weaning (lactation day 20), decreased absolute epididymis and testes weights (without histopathological correlates) and impaired learning were observed in pups at ≥30 mg/kg/day. These developmental delays at 50X the human systemic exposure at the 105 mg Afrezza dose based on AUC comparison across species occur at exceedingly high systemic exposures.

Afrezza Immunogenicity: The immunogenicity assessment of TI and Technosphere particles following inhalation or SC administration to rats for 28 days followed by a 28

day recovery period indicate no adverse effects on lymphoid tissue, lymphocyte subpopulations or T-cell dependent antibody responses. There was a decrease in absolute and relative percentages of NK lymphocytes observed in animals given 75 mg/kg/d SC Technospheres but the absence of an SC control group makes the interpretation of this observation unclear. Anti-drug antibody responses were not evaluated in chronic toxicity studies for this application.

Potential Impurities/Extractables/Leachables

Additional studies evaluating the toxicity of FDKP manufacturing process impurities as well as qualification of extractables, leachables and foreign particles were provided by the sponsor.

Impurities: (b) (4) FDKP process impurities (b) (4) were identified and qualified. The major process impurities (b) (4) were qualified in a 28-day rat SC toxicology study where each of these impurities were tested at (b) (4) % respectively. The results of this study support the sponsor's proposed release specifications. This 28-day rat impurity qualification study tested impurities levels that were >10X the maximum human TI dose of 105 mg based on mg/m^2 body surface area comparison across species. Impurities: (b) (4) were not qualified in this study because they were previously qualified in other studies at >8X the maximum human TI dose based on mg/m^2 body surface area comparison across species. FDKP impurity (b) (4) was not qualified because it was removed during reformulation to TI. Bacterial mutagenicity studies with FDKP impurities alone or FDKP containing impurities were not conducted due to solubility limitations in vitro. Genotoxicity testing of FDKP were negative.

Impurity	NOAEL (mg/m^2)	Impurity @ MRHD (mg/m^2)	Multiple of human impurity dose based on specification*
(b) (4)			

Reference is made to the ONDQA chemistry review of Dr. Alan Schoeder regarding concerns for the safety of leachables/extractables/foreign particles in the insulin/device combination of Afrezza.

Leachables: Drug product was stored for 12 months at 5°C plus 4 months at 25 ^{o}C/60% relative humidity. The headspace of the cartridge was evaluated for potential leachables consisting of the five largest extractable peaks (b) (4) The five largest extractable peaks, which make up the list of potential leachables, are listed below along with their quantitation limits. Since the five largest potential leachables were not present, the likelihood of finding other, smaller extractables peaks as leachables in the drug

product is low.

(b) (4)

The Division of Pulmonary and Allergy Products (DPAP) accept the extractable/leachable qualification limits in the Product Quality Research Institute (PQRI) proposal for orally inhaled and nasal drug products. The PQRI qualification threshold of 0.15 µg/d for leachables having a structural alert for inhalational products is used. (b) (4)

Therefore there potential leachables listed at or below their proposed product specification does not elicit a safety concern.

Extractables: Analysis for potential extractables found using extreme testing conditions, for the inhaler mouthpiece and chamber (b) (4) have been performed.

(b) (4)

(b) (4) The risk seems to be quite small here, for a dry powder inhaler.

Foreign particulates: The mass of foreign particles (b) (4) µm in size) obtained from the emitted dose of the drug product observed in multiple batches is provided in section 3.2.P.5.6.8 of the original NDA (Technosphere Insulin Inhalation System). Estimation of quantification uses the following assumptions: (b) (4) The following is the applicant's summary of the characterization of foreign particulates in the drug product:

(b) (4)

At FDA's request, the applicant has (subsequent to the original NDA) also proposed acceptance criteria for particle sizes larger than (b) (4) microns. The revised foreign particulate acceptance criteria are listed below:

Table 2. Proposed Acceptance Criterion for Foreign Particulates between (b) (4) µm

	Total µg	Total Count	Total Count (b) (4)
Proposed Acceptance Criterion			

Since the sponsor is setting the specification for foreign particles as NMT (b) (4) µg/d limit (particles (b) (4) µm) based on an assumption that (b) (4) a worse case scenario and this specification

clearly meets the EPA limits and in accordance with an IPAC-RS "International Pharmaceutical Aerosol Consortium on Regulation and Science", this is acceptable.

Afrezza Product Labeling

Sponsor's proposed label:

(b) (4)

1 Page(s) of Draft Labeling have been Withheld in Full as B4 (CCI/TS) immediately following this page

(b) (4)

Conclusions:
The pharmacology and toxicology of insulin has been established over the last 90 years. Therefore the supporting nonclinical data for Afrezza have focused on the novel components of the inhalational Technosphere delivery system. A complete nonclinical development program of repeat-dose, genetic, reproductive/developmental, local tolerance, sensitization, immune toxicology and carcinogenicity studies have been performed. The results of these studies have suggested some potential for pulmonary irritation with Afrezza at maximum clinical exposures (105 mg Afrezza). This is based on minimal to mild lung irritation observed in rats and dogs following chronic exposure to Technospheres by inhalation at 2-fold higher exposures in animals relative to therapeutic exposure at the maximum clinical dose (105 mg Afrezza). These findings in test species did not have any functional significance on respiratory function. The respiratory irritation appeared to recover with discontinuation of Technosphere inhalation in animals. Evidence of pulmonary inflammation was not observed following chronic inhalational administration in rats and dogs, including lifetime exposure in rat. Based on the limited nature of these nonclinical findings, this application is recommended for approval with the enclosed labeling comments.

Application Type/Number	Submission Type/Number	Submitter Name	Product Name
NDA-22472	ORIG-1	MANNKIND CORP	INSULIN HUMAN (RDNA ORIG)INH POWDER

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KAREN L DAVIS BRUNO
12/18/2009

DEPARTMENT OF HEALTH AND HUMAN SERVICES
PUBLIC HEALTH SERVICE
FOOD AND DRUG ADMINISTRATION
CENTER FOR DRUG EVALUATION AND RESEARCH

PHARMACOLOGY/TOXICOLOGY REVIEW AND EVALUATION

NDA NUMBER:	**22-472**
SERIAL NUMBER:	**000**
DATE RECEIVED BY CENTER:	**March 16th 2009**
PRODUCT:	**Afrezza (Inhaled Insulin)**
INTENDED CLINICAL POPULATION:	**T1DM and T2DM**
SPONSOR:	**MannKind Corporation**
DOCUMENTS REVIEWED:	**EDR**
REVIEW DIVISION:	**Division of DMEP Drug Products (HFD-510)**
PHARM/TOX REVIEWER:	**Miyun Tsai-Turton, Ph.D., M.P.H**
PHARM/TOX SUPERVISOR:	**Karen Davis-Bruno, Ph.D.**
DIVISION DIRECTOR:	**Mary Parks, M.D.**
PROJECT MANAGER:	**Haley Seymour/Rachel Hartford**

Date of review submission to Division File System (DFS): December 7th 2009

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EXECUTIVE SUMMARY

I. Recommendations

A. Recommendation on approvability

Pharmacology recommends approval of this application.

B. Recommendation for nonclinical studies

No further nonclinical studies will be needed.

C. Recommendations on labeling

See draft labeling changes on pages 456 – 458.

II. Summary of nonclinical findings

A. Brief overview of nonclinical findings

In nonclinical studies, both Technosphere® Insulin (TI), insulin absorbed to Technosphere® particles) and Technosphere® particles (T) alone were tested. Technosphere® particles are comprised of a novel excipient, fumaryl diketopiperazine (FDKP). TI doses were limited by the pharmacology of insulin, which resulted in hypoglycemia, so higher doses of Technosphere® particles were administered alone to maximize FDKP exposure.

The **toxicology** of TI and T was characterized in **repeated-dose inhalation** studies in rats and dogs. TI and T were well-tolerated in general. The primary toxicity was related to expected exaggerated insulin pharmacology (hypoglycemia) at high doses. In 26 week rat study, 11.7 mg/kg/day for T and 1.05/0.404 (male/female, low-dose TI) and 1.91/1.28 (male/female, high-dose TI) mg/kg/day TI were tested by nose only inhalation. There were some findings: **1)** increased lung weights in rats with no histological correlation, **2)** eosinophilic globules and epithelial degeneration observed in nasal cavities of T group, which regressed in females but remained in males after a 4-week recovery, **3)** slightly increased bronchial proliferating cell nuclear antigen activity in the upper respiratory tract, probably related to exposure duration and upper airway particulate impaction in T alone (in males) and low/high-dose TI (in males and females) groups, and **4)** degeneration and necrosis of the myocardium and infiltration of mononuclear cells in approx. 50% males treated with T, which remained in males after 4 week recovery. Such cardiac finding, associated with T, was not observed in dog histopathological finding. The airway findings were consistent with minimal to mild respiratory irritation at 2x human systemic exposure and did not correlate to any preneoplastic or neoplastic findings. This suggests the possibility of some mild airway irritation with clinical use. The $NOAEL_T$ was < 11.7 mg/kg/day and the $NOAEL_{TI}$ was 1.91/1.28 (male/female) mg/kg/day. The safety margin based on FDKP exposure was approx 1.5-fold greater than maximum anticipated daily exposure.

In 39 week dog study, 2.4 (low-dose T) and 10.9 (high-dose T) mg/kg/day T and 0.39 (low-dose TI) and 1.92 (high-dose TI) mg/kg/day TI were tested. There were some findings: **1)** increased minimal to mild neutrophil infiltrate of lungs in high-dose TI group, which regressed after an 8-week recovery and **2)** thymic atrophy, hypocellularity of the seminiferous epithelium, and germ cell degeneration seen in both T and/or TI groups. The $NOAEL_T$ was 10.9 mg/kg/day and the $NOAEL_{TI}$ was 1.92 mg/kg/day. The safety margin based on FDKP exposure was approx 2-fold greater than maximum anticipated daily exposure.

Genotoxicity studies were conducted in vitro with Technosphere (T) and Technosphere + insulin (TI) and in a mouse micronucleus assay (T only). T and TI showed no apparent genotoxic potential.

Carcinogenicity studies in rats (inhalation) and transgenic mice (subcutaneous) were conducted. There was no evidence of increased oncogenicity, as well as microscopic pathological findings, related to TI or T. NOAELs for T in the mouse (75 mg/kg/day) and rat (46 mg/kg/day) carcinogenicity studies were at the maximum doses tested, and represented approx. 1.2- and 0.2-fold of the projected maximum human exposure (or max blood levels) respectively.

Technosphere® particles were evaluated in **reproductive and developmental toxicity** studies in rats and/or rabbits (subcutaneous). In rats, there was no T-induced impairment of fertility and no teratogenic findings at 100 mg/kg/day, and some effects (learning impairment and decreased male reproductive organ weights) on offspring at 30 and 100 mg/kg/day. Exposure was confirmed in pregnant animals, and a separate rat fetal TK study confirmed in utero. FDKP was also present in the mother's milk in rats at about 10% of the maximum concentration detected in the systemic circulation. FDKP was also detected in rat fetal circulation at concentrations comparable to the maternal plasma concentrations of GD (gestation day) 18. In rabbits, there was maternal weight loss and malformations observed at 2 mg/kg/day T. In addition, T had safety margins for tolerability and embryo/fetal development, ranging from approx. 3 to 8-fold human exposure multiples in rats and rabbits) during pregnancy.

Additional studies to **toxicity of process impurities** were also performed. (b) (4) impurities (b) (4)) were identified and qualified in toxicology studies. Major impurities (b) (4) were further studied in 28-day SC rat study and there was no additional systemic toxicity seen in these animals, compared to the parent drug.

B. Pharmacologic activity

In vitro study results, from an evaluation of FDKP binding to 63 diverse receptors or enzyme of neurotransmitter-related receptors, steroids, ion channels, secondary messenger, prostaglandins, growth factors or hormones, and brain or gut peptides showed that only 1 of 63 receptors (γ-Bungarotoxin insensitive nicotinic receptor was inhibited at > 50%. In follow-up experiments, the K_i values ranged from 95.9 to 333 μM, suggesting weak inhibition. The anticipated

maximum concentration of FDKP in humans is approx. (b) (4) μM, so the safety margin is 96-fold the maximum anticipated clinical dose for this receptor.

In vitro study results, from inhalation studies in rats and dogs, showed that blood glucose reductions were observed in rats and dogs, in a dose-dependent manner,. In dogs, following TI inhalation, the nadir in glucose levels happened approx. 20 to 60 min postdose. This was correlated to mean peak serum insulin levels that occurred approx.10 min postdose. On the other hand, following T inhalation, no reduction in blood glucose was observed, suggesting that FDKP had no effect on blood glucose levels.

C. Nonclinical safety issues relevant to clinical use

There might be lung irritation, based on 39 week inhalation dog study, where minimal to mild alveolar/bronchial interstitial neutrophil infiltration was seen in lungs of all TI-treated groups. These findings regressed following 8 weeks recovery.

2.6 PHARMACOLOGY/TOXICOLOGY REVIEW

2.6.1 INTRODUCTION AND DRUG HISTORY

NDA number: 22-472
Review number: 001
Sequence number/date/type of submission: 0000 (original application as a 505(b)(1) submission), March 16th 2009, e-CTD submission.
Information to sponsor: Yes ( ) No (x)
Sponsor and/or agent: MannKind Corporation (Paramus, NJ)
Manufacturer for drug substance: MannKind Corporation (Danbury, CT)
Reviewer name: Miyun Tsai-Turton, Ph.D., M.P.H.
Division name: DMEP
HFD #: 510
Review completion date: December 1st, 2009

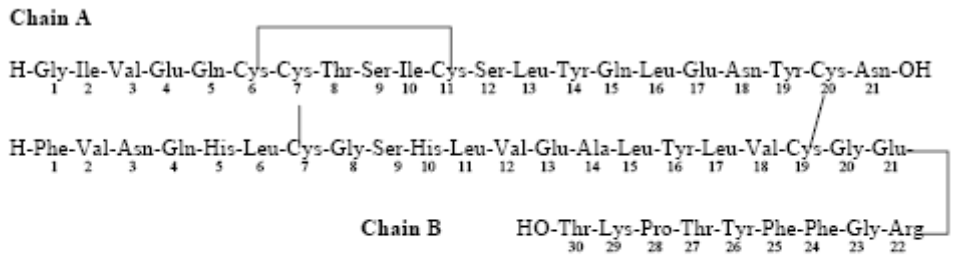
Drug:

Trade name: Afrezza
Generic name: Insulin monomer human [rDNA origin]
Code name: FDKP/Fumaryl Diketopiperazine/224
Chemical name: FDKP as:

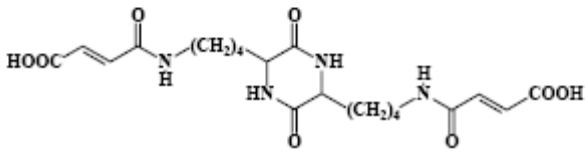
(*E*)-3,6-bis[4-(*N*-carboxy-2-propenyl)amidobutyl]-2,5-diketopiperazine
3,6-bis[*N*-fumaryl-*N*-(n-butyl)amino]2,5-diketopiperazine

CAS registry number: 11061-68-0 (insulin); 294659-59-9 (FDKP)
Molecular formula/molecular weight: $C_{257}H_{383}N_{65}O_{77}S_6$ / 5808 (insulin)
$C_{20}H_{28}N_{65}N_4O_8$ / 452.46 (FDKP)
Structure: See below.

Recombinant Human Insulin



FDKP



Technosphere + insulin (TI) drug product is prepared by absorption of recombinant-human insulin onto Technosphere particles which are comprised of FDKP. FDKP undergoes acid-induced intermolecular self assembly in water.

Relevant INDs/NDAs/DMFs: IND 61,729, DMF (b) (4)

Drug class: Hyperglycemic agent

Intended clinical population: T1DM (b) (4) and T2DM (b) (4)

Clinical formulation: 15 U insulin (5 mg TI powder) and 30 U insulin (10 mg TI powder), Technosphere® Insulin (TI) Inhalation Powder/MedTone® Inhaler combination: human insulin, fumaryl diketopiperazine (FDKP), (b) (4) polysorbate 80.

Note from the reviewer: MRHD = 315 U insulin (105 mg TI powder in divided doses over 3 meals (90 U or 30 mg/meal) and 1 snack (45 U or 15 mg).

Component	Grade	Quantity (per mg of formulation)	Function
Insulin Human Recombinant	USP	3.0 U[a]	Drug Substance
Fumaryl Diketopiperazine (FDKP)	In-house	(b) (4)	Raw material to form carrier particle
Polysorbate 80	NF	(b) (4)	
(b) (4)			
(b) (4)			

Route of administration: Oral inhalation using a novel device; MedTone® Inhaler

Disclaimer: Tabular and graphical information are constructed by the reviewer unless cited otherwise.

Studies reviewed within this submission: PD, safety pharm, PK, and tox

Pharmacodynamics (3 studies)

- MKC-2002-2009: IC50/Ki in vitro study (non-GLP)
- MKC-2006-0023: PD/PK study in dogs
- MKC-2006-0024: PD/PK study in rats

Safety Pharmacology (8 studies)
- PDC-PC-2002-0007: CNS study in rats
- PDC-PC-2002-0005: CV in vivo study in dogs
- MKC-PC-2005-17: CV in vitro study
- MKC-PC-2006-38: Renal study in rats
- MKC-PC-2007-21: Pulmonary study of T in rats - fxn
- MKC-PC-2007-22: Pulmonary study of TI in rats - fxn
- MKC-PC-2007-23: Pulmonary study of T in rats - RR and TV
- MKC-PC-2007-24: Pulmonary study of TI in rats – RR and TV

Pharmacokinetics (23 studies)
- *Absorption*
  - PDC-PC-2000-2011: T in rat lung
  - MKC-PC-2005-0003: 14-day sc PK study in CD-1 mice (T)
  - MKC-PC-2006-0001: PK sc study in male dogs (TI)
  - MKC-PC-2006-0002: PK sc study in minipigs (TI)
  - MKC-PC-2006-0034: AME after a single iv in rats (T)
  - MKC-PC-2006-0035: AME after a single iv in rats (TI)
  - MKC-PC-2006-0036: AME after a single iv in dogs (T)
  - MKC-PC-2007-0005: AME after a single IT, sc, or po in rats (T)
  - MKC-PC-2007-0009: AME after a single IT, sc, or po in dogs (T)
  - MKC-PC-2007-0010: AME after a single IT or po in rats (TI)
  - MKC-PC-2007-0011: AME after a single iv in dogs (T)
  - MKC-PC-2007-0012: AME after a single iv in rats (TI)
  - MKC-PC-2007-0013: AME after a single iv in rats (T)
- *Distribution*
  - PDC-PC-2002-0008: PK/Distribution after a single IT in rats (TI)
- *Metabolism*
  - PDC-PC-2000-0004: Cytochrome P450 activity (T)
  - MKC-PC-2003-0007: Metabolite stability and profiling using hepatocytes (T)
  - MKC-PC-2004-0009: In vitro metabolism of FDKP
  - MKC-PC-2006-0014: Cytochrome P450 (T and TI)
- *Elimination*
  - PDC-PC-2000-0007: Excretion in urine after iv in rats (T)
  - MKC-PC-2004-0020: Metabolite profile in urine and recovery in feces after a single IT or sc in rats (T)
- *PK Drug Interaction and Other PK*
  - MKC-PC-2003-0018: Protein binding in plasma (T)
  - MKC-PC-2006-2008: PK of TI after sc, iv, po in rats (TI)
  - MKC-PC-2006-0009: PK of TI after crossover sc and po in dogs (TI)

Single dose toxicity (3 studies)
- PDC-PD-1993-0001: acute oral tox rat study
- PDC-PC-1993-0002: acute oral tox rat study
- PDC-PD-1004-0002: acute oral tox dog study

Repeat dose toxicity (11 studies)
- PDC-PC-1994-0001: 4-week study in dogs
- PDC-PC-1996-0001: 5-day study in dogs

- PDC-PC-1996-0002: 4-week study in rats
- PDC-PC-1997-0001: 4-week study in dogs
- PDC-PC-1999-0002: 26-week study in rats
- PDC-PC-2000-0003: 13-, 26-, 39-week study in dogs
- *MKC-PC-2003-0002: 26-week study in rats*
- *MKC-PC-2003-0004: 39-week study in dogs*
- MKC-PC-2003-0022: 5-day study in dogs
- MKC-PC-2005-0011: 13-week study in CD-1 mice
- MKC-PC-2006-0011: 28-day study in rasH2 Hybrid mice

Carcinogenicity (2 studies)
- *MKC-PC-2004-0001: 104-Weekinhalation study in rats*
- *MKC-PC-2007-0003: 26-Week sc study in transgenic mice*

Reproductive Toxicity (7 studies)
- Fertility and early embryonic development (T only)
  - *PDC-PC-2001-0006: fertility study in rats*
  - MKC-PC-2004-0003: development study in rabbits
- Embryofetal development (T only)
  - PDC-PC-2002-0012: embryo-fetal dev study in rats
  - *PDC-PC-2001-0003: teratology study in rats*
  - *MKC-PC-2004-2004: dev tox study in rabbits*
  - MKC-PC-2006-0010: fetal TK study in rats
- Prenatal and postnatal development (T only)
  - *PDC-PC-2004-0008: peri/post-natal repro tox study in rats*

Local tolerance & special toxicity (14 studies)
- MKC-PC-2007-0019: skin irritation in rabbits
- PDC-PC-2001-0001: antigen
- MKC-PC-2007-0004: phototox
- MKC-PC-2008-0001: ecotox, Daphnia magna
- MKC-PC-2008-0002:: ecotox, green alga
- MKC-PC-2008-0005: PS80 tox/TK profile
- *MKC-PC-2008-2006: impurity*
- MKC-PC-2008-0016: skin irritation w/ 3 FDKP intermediates
- MKC-PC-2008-0018: delayed contact hypersensitivity w/ 3 FDKP intermediates
- MKC-PC-2008-0019: cute oral tox w/ 3 FDKP intermediates
- *MKC-PC-2008-0020: immunotox*
- MKC-PC-2008-0021: partition coefficient of FDKP
- MKC-PC-2008-0023: eye irritation w/ 3 FDKP intermediates
- MKC-PC-2008-0024:biodegradability of FDKP

Studies not reviewed within this submission: None.

Note: For NDA reviews, all section headings should be included.

Introduction and drug history:

Afresa™ is an ultra rapid acting prandial insulin for treatment of T1DM and T2DM in adults. It consists of Technosphere® Insulin Inhalation Powder pre-metered into unit dose cartridges and

the MedTone® inhaler. Technosphere® Insulin (TI) Inhalation Powder is a dry powder formulation of insulin. It is administered by the reusable, high resistance, breath-powered MedTone® inhaler. TI is comprised primarily of insulin and a novel excipient, fumaryl diketopiperazine (FDKP).

TI is formed by adsorbing insulin into the surface of Technosphere® particles. Technosphere particles are formed prior to insulin adsorption by the acid-induced crystallization of FDKP. Subsequently, those crystals were self-assembled into particles with a compact, approximately spherical shape. TI particles have a median diameter of approx. 2.5 µm and are sized appropriately for inhalation into the deep lung. Following inhalation, TI particles dissolve immediately at physiological pH and insulin and FDKP are absorbed systemically. Peak circulating insulin concentrations occur within 12-14 min after administration. This closely mimics the natural mealtime insulin release characteristics of healthy individuals.

Regulatory history of Technosphere® Insulin Inhalation System is listed below, provided by the sponsor.

Type of Interaction	Event	Date	Location of Documentation in NDA
IND Serial Submission 0000	Acceptance of IND 61,729 for Technosphere® Insulin Inhalation System	2001-01-03	N/A
Special Protocol Assessment – Nonclinical	CAC Review: 2 Year Rat Carcinogenicity Protocol & Dose Selection	2004-06-03	1.6.3 Correspondence Regarding Meetings
Type B Meeting	End of Phase 2 Meeting	2004-10-12	1.6.3 Correspondence Regarding Meetings
Special Protocol Assessment - Clinical	Response to Special Protocol Assessment: MKC-TI-030	2005-04-21	1.6.3 Correspondence Regarding Meetings
Special Protocol Assessment – Clinical	Response to Special Protocol Assessment: MKC-TI-102	2005-10-14	1.6.3 Correspondence Regarding Meetings
Special Protocol Assessment - Clinical	Agency Comments on MKC-TI-105 Protocol	2006-04-10	1.6.3 Correspondence Regarding Meetings
Type B Meeting Request	Response to CMC Meeting Request	2006-11-09	1.6.3 Correspondence Regarding Meetings
Type B Meeting Request	Response to CMC Device Question	2007-01-12	1.6.3 Correspondence Regarding Meetings
Special Protocol Assessment – Nonclinical	CAC Review: 6 Month Transgenic Mouse Carcinogenicity Protocol & Dose Selection	2007-05-02	1.6.3 Correspondence Regarding Meetings
Letter Correspondence	FDA Response to MannKind CMC Batch Related Questions	2007-07-30	1.6.3 Correspondence Regarding Meetings
Letter Correspondence	Proposed tradename AFRESA	2007-09-29	N/A (IND 61,729 Serial No. 0222)

Type of Interaction	Event	Date	Location of Documentation in NDA
Letter Correspondence	Proposed tradename AFRESA; Follow-up	2007-11-12	N/A (IND 61,729 Serial No. 0229)
Letter Correspondence	Proposed tradename AFRESA, Follow-up	2007-11-26	N/A (IND 61,729 Serial No. 0231)
Information Amendment	Nov 2007 DSMB Letter - Neoplasia	2008-01-16	N/A (IND 61,729 Serial No. 0239)
Response to Request for Information	MannKind Response to FDA request for information regarding neoplasm and cardiovascular events	2008-02-27	N/A (IND 61,729 Serial No. 0245)
Response to Request for Information	MannKind Response to FDA request for information regarding reports of lung cancer in Technosphere Insulin clinical trials	2008-03-21	N/A (IND 61,729 Serial No. 0250)
Type B Meeting	Pre-NDA Meeting	2008-07-14	1.6.3 Correspondence Regarding Meetings
Response to Request for Information	MannKind Response to FDA request for information regarding cardiovascular event rates	2008-10-06	N/A (IND 61,729 Serial No. 0303)

2.6.2 PHARMACOLOGY

2.6.2.1 Brief summary

The pharmacology program was conducted with both T and TI. Pharmacodynamic studies were done to evaluate the glucose lowering capability of insulin, administered as TI. Safety pharmacology studies included both *in vitro* assays (hERG channel assay) and *in vivo* studies (CV studies in dogs, respiratory function studies in rats, a CNS study in rats, and a renal study in rats) with T and TI.

Primary pharmacodynamics: *In vitro* study results, from an evaluation of FDKP binding to 63 diverse receptors or enzyme of neurotransmitter-related receptors, steroids, ion channels, secondary messenger, prostaglandins, growth factors or hormones, and brain or gut peptides showed that only 1 of 63 receptors (γ-Bungarotoxin insensitive nicotinic receptor) inhibited at > 62%. In follow-up experiments, the K_i values ranged from 95.9 to 333 μM, suggesting weak inhibition. The anticipated maximum concentration of FDKP in humans is approx. (b) (4) μM, so the safety margin is 96-fold the maximum anticipated clinical dose with TI for this receptor. In addition, *in vitro* study results, from inhalation studies in rats and dogs, showed that blood glucose reductions were observed in rats and dogs, in a dose-dependent manner,. In dogs, following TI inhalation, the nadir in glucose levels happened approx. 20 to 60 min postdose. This was correlated to mean peak serum insulin levels that occurred approx.10 min postdose. On the other hand, following T inhalation, no reduction in blood glucose was observed, suggesting that FDKP had no effect on blood glucose levels.

Safety pharmacology: These studies demonstrated that neither T nor TI affected CV, respiratory, CNS, or renal functions.

- CNS safety pharmacology study used a Functional Observational Battery (FOB) test, including grip strength, hind limb splay, body temperature, and motor activity, to detect potential undesirable CNS effect of T and TI, in albino male rats. When compared to predose values, there was a decrease in motor activity in the 0.125 mg/kg TI group. When compared

to high-dose T (12.5 mg/kg) group, there was an increase in motor activity in the 0.125 mg/kg TI group. This might suggest that T could lead to decreased motor activity if compared to untreated control group. This finding was considered, by the sponsor, biologically irrelevant because predose motor activity in the TI group was higher than postdose motor activity. However, with no untreated control group was included in this study, it would be hard to determine the relevance of this finding.

- *In vivo* CV safety pharmacology study demonstrated that there was no evidence of cardiac adverse effects in telemeterized, conscious dogs in a single-dose, as well as in a 39-week dog inhalation toxicity study. There were no effects on ECG or QTc intervals. Hemodynamic changes were seen in dogs received T containing PS80, a component of TI powder. However, these changes were not seen with PS80-free T and were considered to be species-specific. *In vitro* CV safety pharmacology study also showed that T and TI did not significantly block the hERG channel at levels up to 100 μM, which were 65- and > 96-fold over the maximum human serum FDKP levels, respectively.

- Respiratory safety pharmacology studies indicated that T up to 50 mg/kg and TI up to 0.32 mg/kg, via the IV and IT routes, did not alter tidal volume, respiratory rate, airway resistance, mean arterial pressure, dynamic lung compliance, or heart rate in conscious and anesthetized rats. Safety margins for Technosphere particles (T) were 15X and 2X for Technosphere + insulin (TI). Maximum FDKP levels attained in these studies were approx 90-fold greater than the max anticipated human exposure. On a mg/kg basis, for the tidal volume and respiratory rate, safety margins were 33-fold for T and 2-fold for TI. For airway resistance, mean arterial pressure, dynamic lung compliance, and heart rate, safety margins were 33-fold (IV injection) and 15-fold (IT delivery) for T and 2-fold for TI.

- Renal safety pharmacology study revealed that T up to 50 mg/kg and TI up to 0.32 mg/kg had no effects on electrolyte concentration, urine output, and creatinine clearance, in rats via the IV route, suggesting no renal toxicity and normal renal function.

- Drug-drug interaction studies showed that FDKP did not bind to proteins in mouse, rat, rabbit, dog, monkey, and human plasma. Up to 200 μM FDKP did not inhibit CYP1A2, CYP2C8, CYP2C9, CYP2C19, CYP2D6, and CYP3A4 *in vitro*, indicating that FDKP was not an inhibitor of cytochrome P450 enzymes. Therefore, it was unlikely to cause clinically relevant drug-drug interactions when administered with cytochrome P450 substrates, including warfarin, simvastatin, and dextromethorphan. In addition, FDKP+insulin and up to 10 μM FDKP did not induce CYP3A4 activity in cultured human hapatocytes. Thus, neither TI nor T was likely to cause decreased exposure of CYP3A4 substrates.

2.6.2.2 Primary pharmacodynamics

Mechanism of action: To lower blood glucose levels by stimulating peripheral glucose uptake by skeletal muscle and fat, and by inhibiting hepatic glucose production.

Drug activity related to proposed indication:

2 studies were provided by the sponsor.

- MKC-2006-0023: PD/PK study in dogs
- MKC-2006-0024: PD/PK study in rats

MKC-PC-2006-0023: Single Dose Inhalation Pharmacokinetics and Pharmacodynamic Study of Technosphere® and Technosphere®/Insulin in Beagle Dogs [By (b) (4) Study No. 65016]

This study was to determine the PD and PK profile of T and TI following a single oronasal inhalation administration to dogs. Twenty-four animals were allocated to 4 groups (3/sex/group). Groups 1, 3, and 4 animals were exposed once for 15 min where as Group 2 animals were exposed for 65 min. Blood samples were collected predose, during dose (10 min after the start of dosing for all groups, and 20 and 40 min after the start of dosing for Group 2), immediately after dosing (within 5 min), 10, 20, 40, 60 min, 2, 4, 8, 24 hrs after the end of dosing.

Group No.	Group Designation	Exposure time (mins)	Number of animals	
			♂	♀
1	Technosphere® particles - Low	15	3	3
2	Technosphere® particles - High	65	3	3
3	Technosphere® Insulin - Low	15	3	3
4	Technosphere® Insulin - High	15	3	3

Group	Duration of dosing	Bleeding timepoints		
		Pre-dose	During dosing (minutes from the start of dosing)	After dosing (time from the end of dosing)
1	15	Within 1 hour prior to the start of dosing	10	Immediately (within 5 minutes), 10, 20, 40, 60 minutes, 2, 4, 8 and 24 hours
2	65		10, 20, 40	
3	15		10	
4	15		10	

Group Designation	Achieved Dose Level (mg/kg) [A]									Achieved Aerosol Concentration (mg/L) [C]		
	Technosphere® particles [C, D]			Insulin (IU) [C, D]			Total			Techno-sphere® particles	Insulin	Total [B]
	♂	♀	♂&♀	♂	♀	♂&♀	♂	♀	♂&♀			
Technosphere® particles - Low	10.5	11.1	10.8	0	0	0	11.0	11.6	11.3	2.093	0	2.193
Technosphere® particles - High	37.7	40.1	38.9	0	0	0	39.5	42.0	40.8	1.729	0	1.812
Technosphere® Insulin - Low	0.333	0.351	0.342	0.041 (1.08)	0.043 (1.13)	0.042 (1.11)	0.383	0.404	0.394	0.066	0.0081	0.076
Technosphere® Insulin - High	1.07	1.14	1.11	0.150 (3.95)	0.160 (4.21)	0.155 (4.08)	1.34	1.43	1.39	0.213	0.0300	0

[A] Achieved dose levels were estimated using group mean body weights
[B] Achieved aerosol concentrations were measured gravimetrically.
[C] The achieved dose and aerosol concentration values quoted for Technosphere® particles and insulin were measured analytically,
[D] The values for insulin doses included in the table in parentheses are international units (IU) – the conversion factor from mg/kg to IU is 26.315.

Findings: T and TI were well tolerated by the dogs. There were no significant treatment related mortalities or clinical observations noted during or immediately after exposure. For insulin, dose related increases in insulin C_{max} and $AUC_{0\text{-}4h}$ as a function of dose were seen in both males and females. These increases were greater than dose-proportional. No sex differences in insulin t½ and t_{max} were seen for both insulin dose levels. For FDKP, it was detected in all samples collected from all groups, following the initiation of T and TI treatments. Dose-related increases in C_{max} and $AUC_{0\text{-}last}$ were observed in this study. These increases were approx. dose-

proportional. No sex differences in the PK of FDKP were noted. For glucose, a dose-dependent decrease in glucose levels was seen 10 min after the end of dosing in both sexes of Groups 3 and 4, ranging between 19 and 47%, when compared to their respective pre-treatment levels. The lowest glucose levels were seen 20 to 40 min after treatment, down to 44 and 33% of pre-treatment levels for Group 4 males and females, respectively. These glucose levels returned to normal by the 2 hr timepoint in males and by the 4 hr timepoint in females. The decrease in the glucose levels was more pronounced in Group 4 (high TI) than in Group 3 (low TI) animals. As the decrease in glucose levels was expected pharmacology of treatment with insulin, they were not considered to be of any toxicological significance.

MKC-PC-2006-0024: Single Dose Inhalation Pharmacokinetics and Pharmacodynamic Study of Technosphere® and Technosphere®/Insulin in Spraque Dawley Rats [By (b) (4) Study no. 75022]

This study was to determine the PD and PK profile of T and TI following a single nasal inhalation administration to rats. Seventy-two animals were allocated to 4 groups (9/sex/group). All groups, except females from Groups 3 and 4, were exposed once for 60 min. Females from Groups 3 and 4 were exposed for 20 and 36 min respectively. Blood samples were collected during dose (20 and 40 min after the start of dosing), immediately after dosing (within 5 min), 10, 20, 40, 60 min, 2, 4, 8, 24 hrs after the end of dosing.

Group No.	Group Designation	Achieved Dose Level (mg/kg/day) [1]						Achieved Aerosol Concentration (mg/L) [2]		
		Technosphere® particles		Insulin (IU) [4]		Total		Technosphere® particles	Insulin	Total
		♂	♀	♂	♀	♂	♀			
1	Technosphere® particles - Low	11.5	11.7	0	0	11.5	11.7	0.299	0	0.299
2	Technosphere® particles - High	45.8	46.8	0	0	45.8	46.8	1.191	0	1.191
3	Technosphere® Insulin - Low	0.843 [3]	0.288 [3]	0.095 (2.5)	0.033 (0.9)	0.938 [3]	0.321 [3]	0.0221 [3]	0.0025	0.0246 [3]
4	Technosphere® Insulin - High	1.462 [3]	0.898 [3]	0.165 (4.3)	0.101 (2.7)	1.627 [3]	0.999 [3]	0.0381 [3]	0.0043	0.0424 [3]

[1] Achieved dose levels were estimated based on group mean body weights and achieved levels of Technosphere® particles and insulin (measured analytically).
[2] Achieved aerosol concentrations were measured analytically.
[3] The achieved dose and aerosol concentration values quoted for Technosphere® particles were estimated assuming the proportion of insulin was 11.3% in the measured atmosphere, as indicated in the certificate of analysis, as only insulin levels were analyzed for Groups 3 and 4.
[4] The values for insulin doses included in the table in parentheses are international units (IU) – the conversion factor from mg/kg to IU is 26.315.

Male Rats				
Group No.	Duration of Dosing	Pre-Dose	During Dosing (minutes from the start of dosing)	After Dosing (time from the end of dosing)
1	60	Within 1 hour prior to the start of dosing	20, 40	Immediately (within 5 minutes), 10, 20, 40, 60 minutes, 2, 4, 8 and 24 hours
2				
3				
4				

Findings: T and TI were well tolerated by the rats at all doses administered and no treatment related mortalities or clinical observations occurred during or immediately after exposure. For insulin, quantifiable levels of insulin were observed in Groups 3 and 4 after a single nasal inhalation TI administration. The increases in C_{max} and AUC_{0-24h} were dose-dependent. Female

rats seemed to have higher systemic exposures of insulin after adjusting for the administered dose. The elimination half-life of human insulin in rats was short and approx. 1 hr where determinable. For FDKP, it was detectable in the serum of male and female rats after a single nasal T or TI administration for at least 9 hrs after the initiation of administration. Peak serum levels (C_{max}) of FDKP in rats were achieved with 1.17 hrs after the start of dosing at all dose levels. Serum FDKP levels declined bi-exponentially with half-life values ranging from 1.2 to 3.8 hrs, when able to calculate. In general, C_{max} values were higher in female rats than in male rats after adjusting for the administered dose, with the exception of Group 3 in which the males had a higher C_{max}. There was no clear relationship between $AUC_{0\text{-last}}$ values and sex. Increases in dose resulted in increases in FDKP C_{max} and $AUC_{0\text{-last}}$ in both males and females. These increases were nearly dose-proportional. For glucose, dose-dependent decreases in glucose levels were observed after completion of treatment, attaining 19% in Group 4 males and 23% in Groups 4 females, when compared to pre-dose levels. Glucose levels were back to normal, or higher, 60 min after treatment in Group 4 (high TI).

2.6.2.3 Secondary pharmacodynamics

MKC-PC-2002-0009: Receptor Binding: IC50/Ki Determination [By (b) (4) – Non-GLP]

No study summary was provided. The results from this non-GLP study were described in the Module 2.4 Nonclinical Overview. Only 1 of 63 receptors (γ-Bungarotoxin insensitive nicotinic receptor) from the Ames Assay was inhibited at > 50%. The K_i values for this receptor ranged from 95.9 to 333 μM, suggesting weak inhibition.

2.6.2.4 Safety pharmacology

8 studies were provided by the sponsor.

- PDC-PC-2002-0007: CNS study in rats
- PDC-PC-2002-0005: CV in vivo study in dogs
- MKC-PC-2005-17: CV in vitro study
- MKC-PC-2006-38: Renal study in rats
- MKC-PC-2007-21: Pulmonary study of T in rats - fxn
- MKC-PC-2007-22: Pulmonary study of TI in rats - fxn
- MKC-PC-2007-23: Pulmonary study of T in rats - RR and TV
- MKC-PC-2007-24: Pulmonary study of TI in rats – RR and TV

Neurological effects:

PDC-PC-2002-20007: Safety Pharmacological Assessment: Effects of Technosphere® and Technosphere®/Insulin on the Central Nervous System of the Albino Rat [By (b) (4) Study No. 93556]

The study was to detect undesirable effects of T (vehicle) or TI (test article) on the CNS following IV administration to male albino rats. All animals were observed twice daily for signs of reaction to treatment. A FOB (Functional Observational Battery) assessments of grip strength, hind limb splay, and body temperature, and motor activity were performed for all animals once predose and at approx. 15 min, 1, and 24 hrs postdose.

Group/Identification	Dose Level of FDKP1 (mg/kg)	No. of Males
1 Technosphere®	0.125	8
2 Technosphere®	12.5	8
3 Technosphere®/Insulin	0.125	8

Findings: There were no deaths and no clinical signs observed in these rats. No biological significant effects on FOB parameters were observed. However, at the 15 min post-dose timepoint animals given TI at 0.125 mg/kg showed increased total activity counts when compared to the groups given T at 0.125 and 12.5 mg/kg. This trend continued at the 1 hr and 24 hr post-dose timepoints. The significance of this increase motor activity in TI group was uncertain since when compared the animals' predose values, motor activity was decreased. In addition, this finding might suggest that T could lead to decreased motor activities if compared to untreated controls. However, there was no untreated control group except pre-dose values from each dose group.

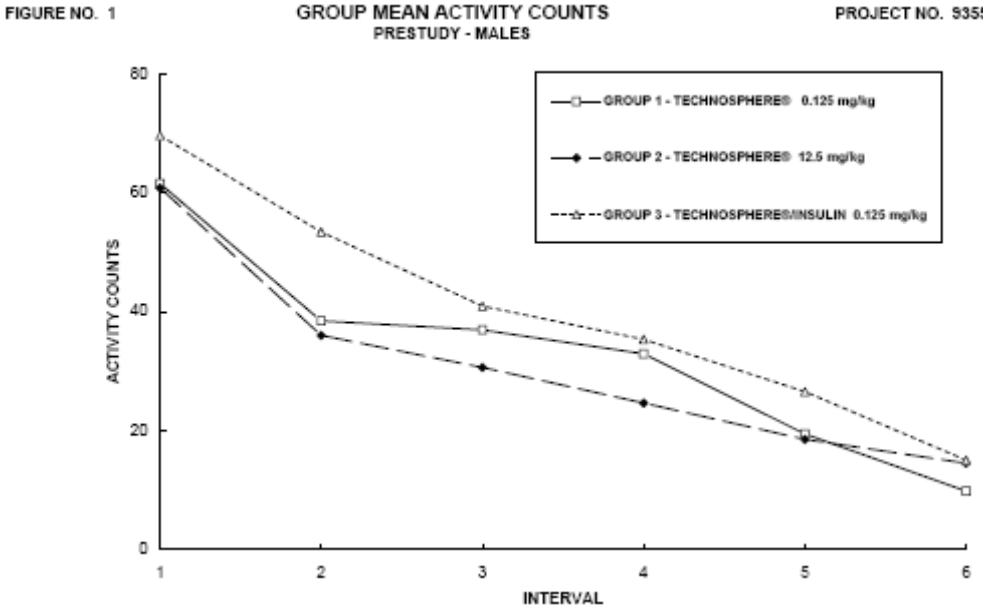


FIGURE NO. 2 GROUP MEAN ACTIVITY COUNTS PROJECT NO. 9355

15 MIN POST DOSE - MALES

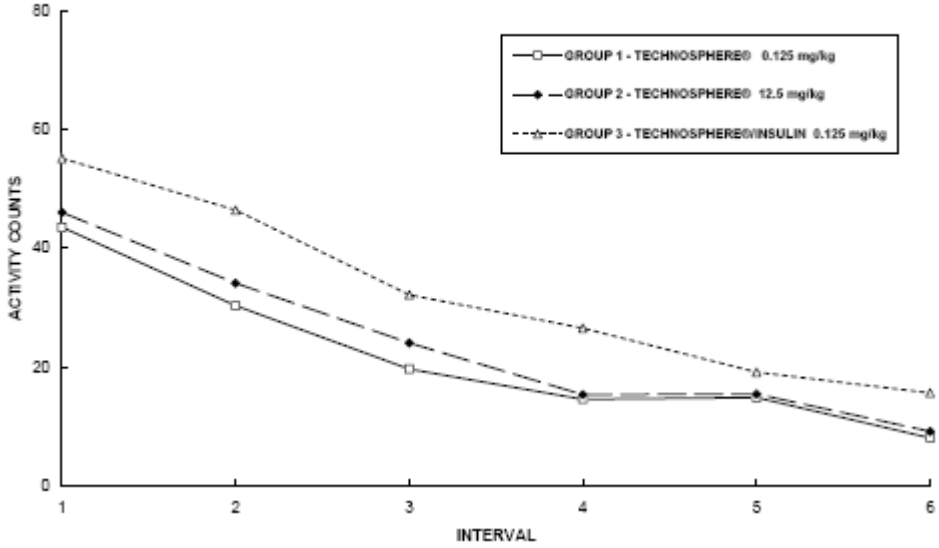
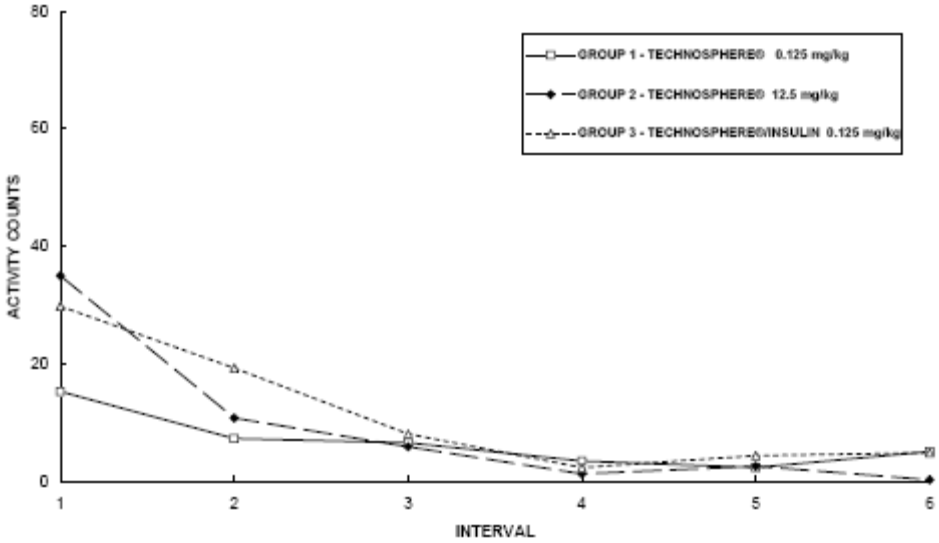
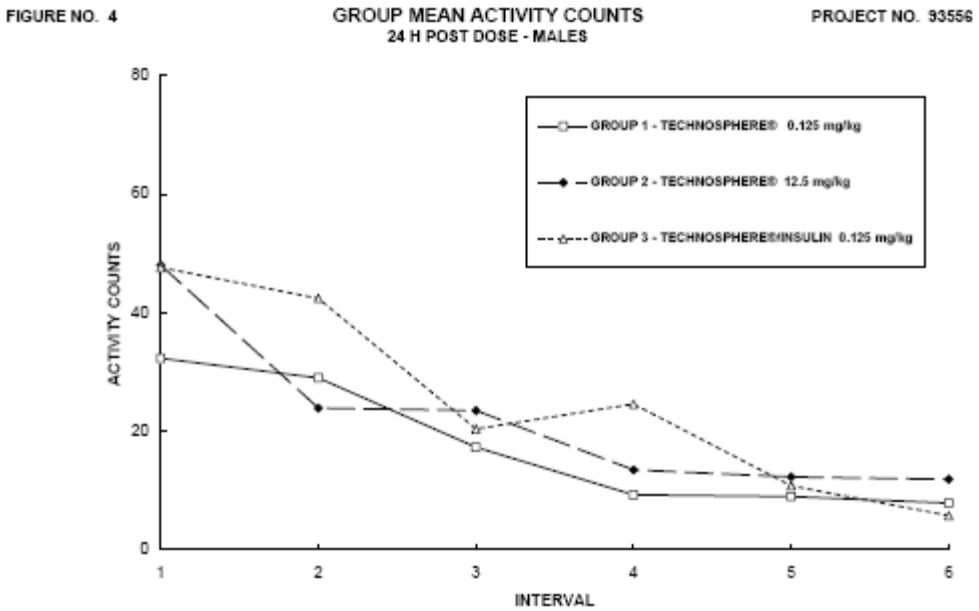


FIGURE NO. 3 GROUP MEAN ACTIVITY COUNTS PROJECT NO. 9355

1 H POST DOSE - MALES



Group Mean Activity Counts - 24 hour post dose - Males



Cardiovascular effects:

PDC-PC-2002-20005: A Pharmacological Assessment of Intravenous Administration of Technosphere®/Insulin Formulations on the Cardiovascular System of the Conscious Telemetered Beagle Dog [By (b) (4) Study No. 93554]

This study was to evaluate the pharmacological effects of T (control article) and TI (test article) with and without Tween, on the cardiovascular system following IV bolus administration to the conscious beagle dog. The vehicle article was PBS in this study. These dogs were instrumented with radiotransmitters for the recording of systemic blood pressure (mean, systolic, and diastolic), heart rat, and modified lead II ECG. The test articles were administered by IV bolus injection to a peripheral vein. These animals were observed for mortality and signs of reaction to treatment twice daily. In addition, details examinations were performed once prior to treatment initiation and after each dosing occasion. Blood samples were collected for plasma histamine levels, approx. 10-14 min postdose, commencing on Dose 4 through Dose 7. Blood samples were also collected for immunoglobulin levels, prior to treatment for Doses 5 through 7, and at approx. 24 hrs post treatment for Doses 4 through 7.

Dose No. Identification	Dose Level* Technosphere® (mg/kg)	Dose Volume (ml/kg)	Number of Animals* Males
1 Vehicle	0	1	6
2 Technosphere®	0.125	1	6
3 Technosphere®	12.5	1	6
4 Tween–free Technosphere®	12.5	1	6
5 Technosphere®/Insulin	0.125	1	6
6 Technosphere®	1.25	1	6
7 Technosphere®**	12.5	1	6

* All six dogs received each of the seven escalating doses administered with at least a 2-day washout period between the beginning of one administration and the start of the next administration.
** The seventh dose administration was from a batch of Technosphere® used previously on PCS-MTL PN 91739.

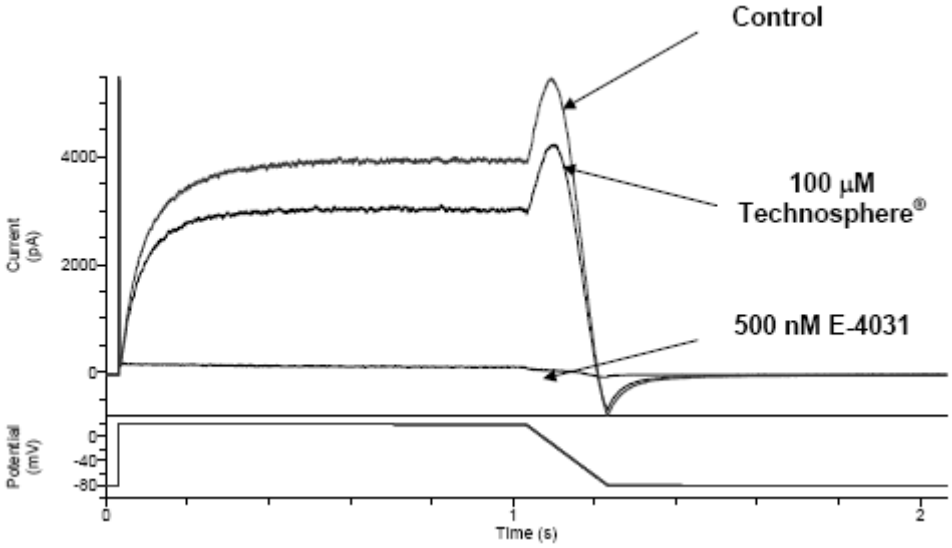
Findings: Treatment-related clinical sings were seen immediately following Dose 3 (125 mg/kg T) on Day 8 in 4/6 dogs. Clinical signs included labored breathing, elevated respiratory rate, defecation, moderate to hyper salivation, pale skin, and reduced activity. There reactions to treatment were consistent with the systemic release of histamine. However, no reaction treatment was observed on Days 1 to 4 or subsequent (Days 53, 57, 64, and 71) dose occasions.

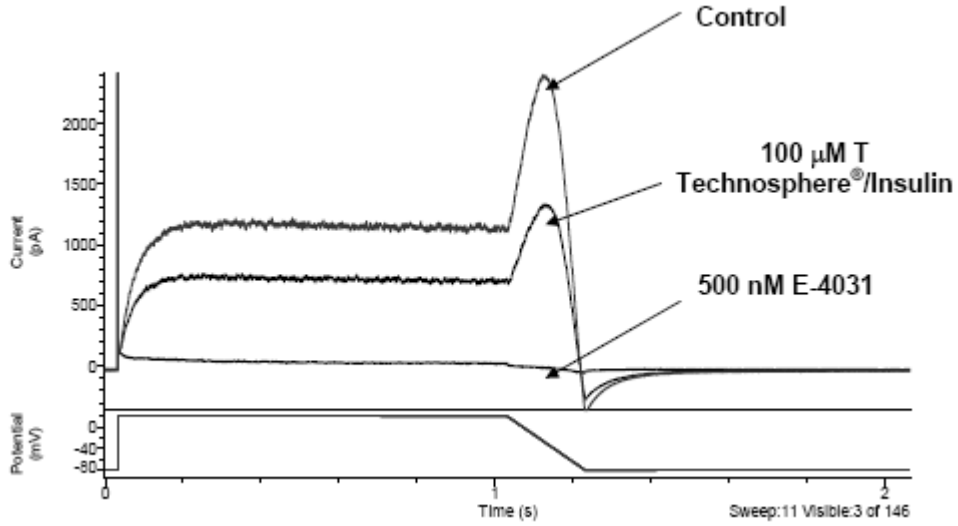
The CV studies showed that on Dose 2, Day 4, there were no hemodynamic effects associated with 0.125 mg/kg T. On Dose 3, Day 8, 2 animals (1001 and 1002) had slight but transient elevations in heart rate post-dosing at 12.5 mg/kg T. However, it was not determined if the blood pressure was reduced or maintained between 0 and 30 min postdose. One animal (1004) had the largest but transient heart rate increase postdose and a slight reduction in blood pressure (approx. 25 mm Hg), which resolved between 2 and 3 hrs postdose. One animal (1005) had a transient reduction in blood pressure (approx. 20 mm Hg), which resolved by approx. 3 hrs postdose. These observations were suspected to be related to the histamine reactions observed in most animals at this dose level. Two other animals (1003 and 1006) were unaffected. On Dose 4, Day 52, there were no hemodynamic effects associated with Tween-free T at 12.5 mg/kg. On Dose 5, Day 57, 3 out of 6 animals (1002, 1003, and 1005) showed slight but transient reduction in systolic blood pressure (approx. 15-20 mm Hg) following dosing with TI at 0.125 mg/kg. These changes were considered to be related to animals' activity and/or the experimental procedure. On Day 6, Day 64, mild but transient blood pressure effects were noted in 50% of the dogs receiving T at 1.25 mg/kg. These were considered likely related to Tween formulation and subsequent histamine release in some animals. On Dose 7, Day 71, 1 animal (1003) had a transient reduction in blood pressure and heart rate postdose at 12.5 mg/kg T. It was considered due to individual variation rather than a direct effect of T.

The ECG results showed that there were no test article related effects following any of the 7 treatments. The histamine levels were also measured in these dogs. At Doses 4-7, very low levels of histamine (< 1.5 ng/ml) were seen in 2/6 to 5/6 animals. In the TI group (Dose 5), 2/6 animals had detectable histamine levels, compared to all other dose groups. At Doses 3 and 7 (given 12.5 mg/kg T), the highest number of animals with detectable histamine levels were observed. However, there was no correlation with the presence of histamine or histamine levels and hemodynamic changes.

MKC-PC-2005-0017: Effect of Technosphere® and Technosphere® Insulin on Cloned hERG Potassium Channels Expressed in Mammalian Cells [By (b) (4), Study No. 050726.NBD]

The *in vitro* effect of T and TI (test articles) on hERG currents were evaluated in HEK293 cells stably expressing hERG channel. E-4031 was a reference substance, which was prepared in DMSO (test article carrier), to selectively inhibits hERG current with IC_{50} of 12 nM. Findings: T inhibited hERG current by 14% (n=3) at 100 µM. Since a mean current inhibition at this concentration did not exceed 20% the lower concentrations selected for this study, IC50 was not determined. In addition, TI inhibited the hERG currents by 1.3%, 12.9%, 27.4%, and 61.2% at 1, 10, 30, and 100 µM. The IC50 of TI for hERG currents was calculated to be 67.64 µM. By contrast, the positive control, 60 nM Terfenadine (positive control article), inhibited the hERG current by 82%.





Pulmonary effects:

MKC-PC-2007-0021: Influence of Technosphere® Powder on Pulmonary Function in the Anaesthetized, Ventilated Spraque-Dawley Rat [By (b) (4) Study no. (b) (4) 07043-RPT01988]

This study was to assess the effects of T (test article) administered 1) at doses of 5 mg via the IT (intratracheal) route, and 2) at a dose of 11 and 50 mg/kg via the IV (intravenous) route, on airways resistance (R_L), dynamic lung compliance (C_{dyn}), heart rate, and mean arterial blood pressure (MAP) in anaesthetized, ventilated male rats, and to determine the FDKP levels in rat serum. There were 5 treatment groups with 8 rats per group. Each rat received a single IT or IV dose. Rats were then anaesthetized, paralyzed, and ventilated via a tracheal cannula with a mixture of air and oxygen. PBS was used as the vehicle article and acetylcholine chloride was used as the reference article in this study.

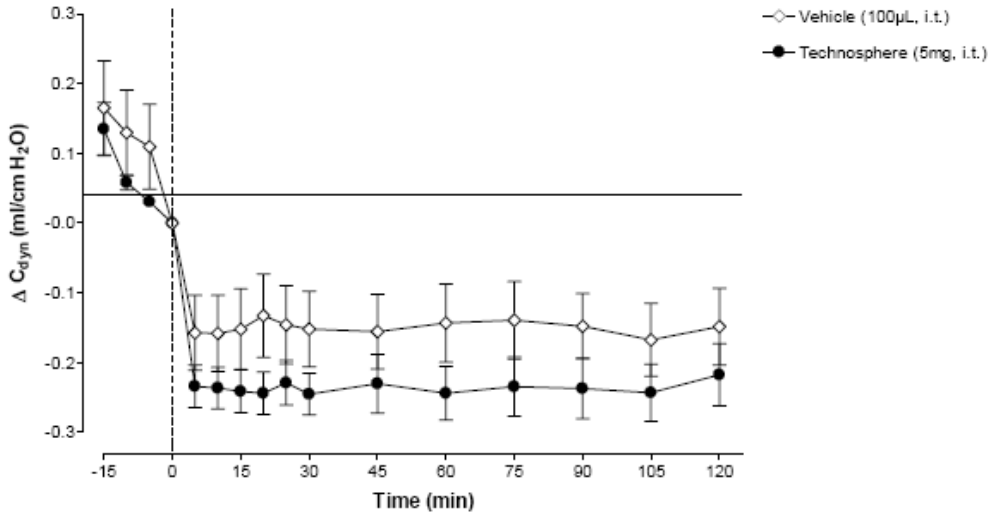
Group 1	Vehicle (100 µL i.t.; n = 8)
Group 2	Technosphere® Powder (5 mg i.t.; n = 8); Concentration 50 mg/mL
Group 3	Vehicle (1 mL/kg, i.v.; n = 8)
Group 4	Technosphere® Powder (11 mg/kg i.v.; n = 8); Concentration 11 mg/mL
Group 5	Technosphere® Powder (50 mg/kg i.v.; n = 8); Concentration 50 mg/mL

Findings: One animal from Group 5 died under anesthesia approx. 45 min after dosing. The death was related to problems in cannulation of the carotid artery.

- For dosing solution analysis, all dosing solution samples contained ≤ 90% of the projected concentration. Actual concentrations ranged from 64.0 to 84.0%. Therefore, the actual doses of the test substance were approx. 3.54 mg (Group 2), 8.14 mg/kg (Group 4), and 33.9 mg/kg (Group 5).

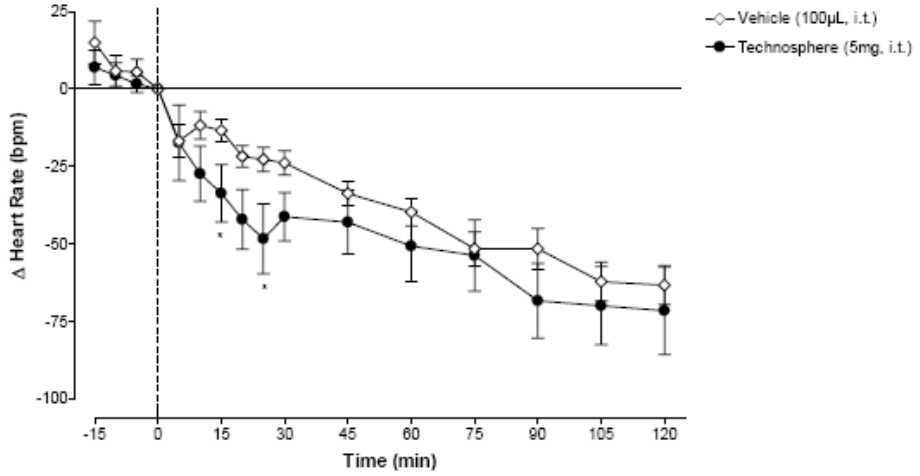
- For respiratory function via the IT route, T (5 mg) had no effect on R_L at any time. A small decrease in C_{dyn} was observed (see figure below). However, these changes were not significantly different when compared to time-matched VH (PBS) treated animals. A small but significant decrease in heart rate was seen 15 and 25 min after dosing, when compared to time-matched VH treated animals (see figure below). No significant decrease was observed at any other time point and it was likely that such effect was not biologically significant. Administration of ACh (100 µg/kg) at 120 min caused a substantial increase in R_L, and a decrease in C_{dyn}, heart rate, and MAP. T at 5 mg did not significantly affect the magnitude of the response to ACh.

Figure 5 **Effect of vehicle and Technosphere® Powder administered intratracheally on change in dynamic lung compliance in anaesthetised, ventilated rats**



Change in Cdyn (mL/cm H_2O) from value at t = 0 (intratracheal dose) in anaesthetised, ventilated Sprague-Dawley rats. Vehicle (100 µL) or Technosphere (5 mg) were given intratracheally at t = 0.
Data show mean + s.e.m. of n = 8 animals / group.

Figure 7 **Effect of vehicle and Technosphere® Powder administered intratracheally on change in heart rate in anaesthetised, ventilated rats**



Change in heart rate (bpm) from value at t = 0 (intratracheal dose) in anaesthetised, ventilated Sprague-Dawley rats. Vehicle (100 µL) or Technosphere (5 mg) were given intratracheally at t = 0.
Data show mean + s.e.m. of n = 8 animals / group except Technosphere (5mg, i.t.) at 15 min where n=7.
* $P < 0.05$ vs time-matched vehicle response; unpaired Student's t-test.

- For <u>respiratory function via the IV route</u>, <u>T (11 and 50 mg/kg)</u> had no effect on R_L and C_{dyn} at any time. Transient decreases in heart rate and MAP were observed right after IV dosing with either VH or T. T had no effect on either heart rate or MAP at any time point in the study. Administration of ACh (100 µg/kg) at 120 min caused a substantial increase in R_L, and a decrease in C_{dyn}, heart rate, and MAP. T at 11 and 50 mg/kg did not significantly affect the magnitude of the response to ACh.

All in all, T had no significant effect at the doses used in this study on either R_L, C_{dyn}, heart rate, and MAP when administered by either the IT and IV routes.

MKC-PC-2007-0022: Influence of Technosphere®/Insulin on Pulmonary Function in the Anaesthetized, Ventilated Spraque-Dawley Rat [By (b) (4) Study no. (b) (4) 07044-RPT01989]

This study was to assess the effects of TI (test article) administered 1) at doses of 1 mg via the IT route, and 2) at a dose of 0.32 mg/kg via the IV route, on airways resistance (R_L), dynamic lung compliance (C_{dyn}), heart rate, and mean arterial blood pressure (MAP) in anaesthetized, ventilated male rats, and to determine the FDKP levels in rat serum. There were 4 treatment groups with 8 rats per group. Each rat received a single IT or IV dose. Rats were then anaesthetized, paralyzed, and ventilated via a tracheal cannula with a mixture of air and oxygen. PBS was used as the vehicle article and acetylcholine chloride was used as the reference article in this study.

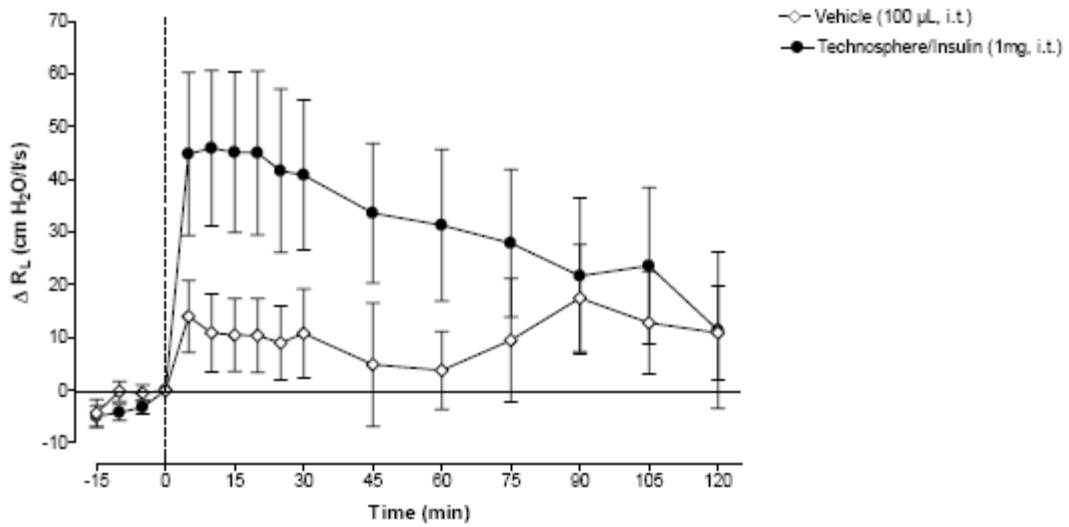
Group 1	Vehicle (100 µL i.t.; n = 8)
Group 2	Technosphere®/Insulin (1 mg i.t.; n = 8); concentration 10 mg/mL
Group 3	Vehicle (1 mL/kg i.v.; n = 8)
Group 4	Technosphere®/Insulin (Dose 0.32 mg/kg i.v.; n = 8); concentration 0.32 mg/mL

Findings:

- For dosing solution analysis, all dosing solution samples contained ≤ 90% of the projected concentration. Actual concentrations ranged from 40.17 to 57.3%. Therefore, the actual doses of the test substance were approx. 0.51-0.57 mg by the IT route and 0.13 mg/kg by the IV route.

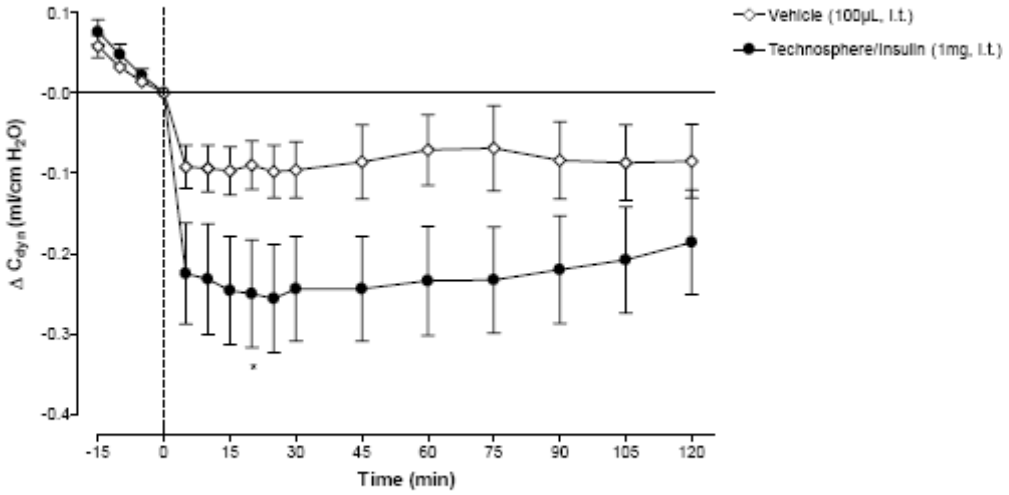
- For respiratory function via the IT route, TI (1 mg) or VH (100 µL PBS) caused an increase in R_L and a reduction of C_{dyn} (see figures below). However, the magnitude of the changes in response to TI was not significantly different from VH except for C_{dyn} at 20 min after dosing (small decrease when compared to time-matched VH treated animals). Since no significant decrease was seen at any other time point, it was likely that this effect was not biologically significant. In addition, TI had no effect on heart rate and MAP at any time after IT administration. Administration of ACh (100 µg/kg) at 120 min caused a substantial increase in R_L, and a decrease in C_{dyn}, heart rate, and MAP. TI at 1 mg did not significantly affect the magnitude of the response to ACh.

Figure 3 **Effect of vehicle and Technosphere®/Insulin administered intratracheally on change in airways resistance in anaesthetised, ventilated rats**



Change in R_L (cm H_2O/L/s) from value at t = 0 (intratracheal dose) in anaesthetised, ventilated Sprague-Dawley rats. Vehicle (100 µL) or Technosphere/Insulin (1 mg) were given intratracheally at t = 0.
Data show mean + s.e.m. of n = 8 animals / group.

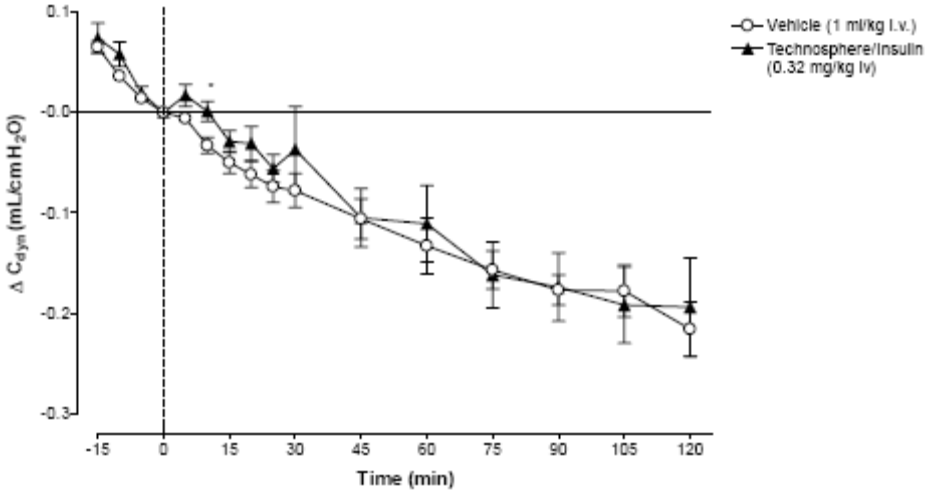
Figure 5 **Effect of vehicle and Technosphere®/Insulin administered intratracheally on change in dynamic lung compliance in anaesthetised, ventilated rats**



Change in Cdyn (mL/cm H_2O) from value at t = 0 (intratracheal dose) in anaesthetised, ventilated Sprague-Dawley rats. Vehicle (100 µL) or Technosphere/Insulin (1 mg) were given intratracheally at t = 0.
Data show mean + s.e.m. of n = 8 animals / group.
* P < 0.05 vs time-matched vehicle; unpaired Student's t-test.

- For respiratory function via the IV route, TI (0.32 mg/kg) had no effect on R_L, heart rate, and MAP at any time. A small but significant increase in C_{dyn} was seen only at 10 min after dosing, when compared to time-matched VH treated (1 ml/kg) animals (see figure below). Since no significant decrease was observed at any other time point, it was likely that the effect was not biologically significant. Administration of ACh (100 µg/kg) at 120 min caused a substantial increase in R_L, and a decrease in C_{dyn}, heart rate, and MAP. TI at 0.32 mg/kg did not significantly affect the magnitude of the response to ACh.

Figure 13 Effects of vehicle and Technosphere®/Insulin administered intravenously on changes in dynamic lung compliance in anaesthetised, ventilated rats



Change in Cdyn (mL/cm H_2O) from value at t = 0 (intravenous dose) in anaesthetised, ventilated Sprague-Dawley rats. Vehicle (1 mL/kg) or Technosphere/Insulin (0.32 mg/kg) were given intravenously at t = 0.
Data show mean + s.e.m. of n = 8 animals / group, except vehicle from 90 min where n = 7.
* P <0.05 vs time-matched vehicle; Student's t-test.

Overall, TI had no biologically significant effect at the doses administered in this study on R_L, C_{dyn}, heart rate, and MAP, when administered by either the intratracheal or intravenous routes.

MKC-PC-2007-0023: Effect of Technosphere® Powder on Respiration Rate and Tidal Volume in Conscious Rats [By (b) (4) Study No. (b) (4) 07044 – RPT01989]

This study was to assess the effect of Technosphere® Powder (test article), at 11 or 50 mg/kg by bolus IV injection, on respiration rate and tidal volume in conscious rats. There were 4 treatment groups with 8 rats per group. Because of the concentration for the high dose group formulation was found to be out of specification, the actual concentration was 64.4% (32.2 mg/kg) of the projected 50 mg/kg. PBS was used as the vehicle article and morphine was used as the reference article in this study.

D	Vehicle for Technosphere® Powder	1 mL/kg
A	Technosphere® Powder	11 mg/kg
B	Technosphere® Powder	50 mg/kg
C	Morphine	10 mg/kg

Findings: For respiration rate, IV administration of T at 11 and 32.2 mg/kg did not significantly affect the respiration rate of rats at any of the time points tested, when compared to VH group data. On the contrary, morphine caused a significant decrease in respiration rate at 5, 10, and 60 min post-dose, when compared to pre-dose values, which was consistent with its known pharmacological activity. For tidal volume, IV administration of T at 11 and 32.2 mg/kg did not significantly affect the tidal of rats at any of the time points tested, when compared to VH group data. However, morphine caused a significant decrease in tidal volume at 5, 10, and 60 min post-dose, when compared to pre-dose values. All in all, bolus IV administration of T at 11 and 32.2

mg/kg did not affect the respiration rate or tidal volume of male rats at any of the time points tested.

MKC-PC-2007-0024: Effect of Technosphere® Insulin on Respiration Rate and Tidal Volume in Conscious Rats [By (b) (4) Study No. ZNA14662.002]

This study was to assess the effect of TI (test article) at 0.32 mg/kg administered by IV injection on respiration rate and tidal volume in conscious Spraque-Dawley male rats. There were 3 treatment groups with 8 rats per group. PBS was used as the vehicle article and morphine was used as the reference article in this study.

B	Vehicle for Technosphere® Insulin	1mL/kg
A	Technosphere® Insulin	0.32 mg/kg
C	Morphine	10 mg/kg

Findings: For respiration rate, IV administration of TI at 0.32 mg/kg did not significantly affect the respiration rate of rats at any of the time points tested, when compared to VH group data. On the other hand, morphine caused a significant decrease in respiration rate at 5, 10, and 60 min postdose, when compared to VH group values, which was consistent with its known pharmacological activity. For tidal volume, IV administration of TI at 0.32 mg/kg did not significantly affect the tidal of rats at any of the time points tested, when compared to VH group data. However, morphine caused a significant decrease in tidal volume at 5, 10, and 60 min post-dose, when compared to VH group values. All in all, bolus IV administration of TI at 0.32 mg/kg did not affect the respiration rate or tidal volume of male rats at any of the time points tested.

Renal effects:

MKC-PC-2006-0038: Effect of Technosphere® Particles and Technosphere® Insulin: Effects on Renal Function in the Rat Following Intravenous Administration [By (b) (4) Study no. 2778-001]

The study was to determine if treatment with T or TI (test articles) would modify renal function in the rats by examining the potential effects on creatinine clearance, urine output, and electrolyte excretion. The vehicle article for the T and TI was 29.6% ammonia in water for injection, whereas the diluent for T and TI was PBS. Groups of 5M and 5F Spraque-Dawley rats were given a single bolus IV administration of VH, test article, or reference compound, via a tail vein. After dosing, the animals were placed into individual metabolic cages and the urine volume measured at 1, 2, 3, 4, 5, 6, and 24 hrs postdose. After the 6 and 24 hr observation, the urine was collected for electrolyte and creatinine analysis. To determine creatinine clearance, a blood sample was also taken following the urine collection at 6 and 24 hr post-dose.

Group	Treatment	Dose level (mg/kg)	Formulation concentration (mg/mL)	Animal Numbers	
				Males	Females
1	Vehicle	-	-	1-5	6-10
2	Technosphere® particles	11	11	11-15	16-20
3	Technosphere® particles	50	50	21-25	26-30
4	Technosphere®/insulin*	0.32	0.32	31-35	36-40
5	Furosemide	10	10	41-45	46-50
6	Untreated	-	-	151-155	156-160

* Insulin dose was approximately 1U/kg.

Findings:

For female rats given T, IV administration at dose levels of 11 or 50 mg/kg (Groups 2 and 3) had no effect on urine output, urinary electrolyte excretion (Na+, Cl-, K+) or creatinine clearance, when compared to the VH-treated animals (Group 1). For male rats given T, IV administration at dose levels of 11 or 50 mg/kg had no effect on urinary electrolyte excretion during 0-6 hrs post-dose, when compared to VH-treated animals. However, for 6-24 hrs post-dose urine sample, there was a significant increase in K+ in both dose groups, Na+ in 11 mg/kg group, and Cl- in 50 mg/kg group. The findings were not thought to be of any biological significance since these electrolyte values were comparable to the levels seen in untreated group. In addition, there was no effect on creatinine clearance in male rats given 11 mg/kg T, when compared to the VH-treated group. On the other hand, there was a significant increase in creatinine clearance in male rats given 50 mg/kg T 6 hr post-dose. The significant was attributable to the relatively low group mean of the VH control group, which was likely to have resulted from n=3 (rather than n=5), due to insufficient plasma samples for analysis from 2 animals in this group. This finding was not biologically significant as the clearance value was comparable to male rats in the untreated group (Group 6). For female and male rats given TI, IV administration at a dose level of 0.32 mg/kg (Group 4) had no effect ton urine output, urinary electrolyte excretion or creatinine clearance, when compared to the VH-treated group (Group 1). For female and male rats given furosemide (Group 5) at 10 mg/kg, it produced a significant increase in urine output, when compared to the VH group. A significant decrease in CI- (males), Na+ (females) and K+ (males and females) was observed at 0-6 hrs post-dose. In addition, a significant decrease in Na+ (males) and increase in K+ (males) were observed at 6-24 hrs post-dose. Moreover, furosemide had no effect on creatinine clearance in male rats, but did have an effect (significant decrease) in female rats at 6 hrs post-dose. Overall, IV administration of T (11 or 50 mg/kg) and TI (0.32 mg/kg) to male and female rats had no effect on creatinine clearance, electrolyte concentration or urine output.

Effect of Technosphere® particles and Technosphere®/insulin on group mean urine output in the rat following intravenous administration

Group	Intravenous treatment and dose level (mg/kg)	Sex	Group mean accumulative urine volume (mL ±sd) at time (hour) post-dose						
			1	2	3	4	5	6	24
1	Vehicle -	M	0.7 ±0.72	2.1 ±0.63	3.2 ±0.96	3.8 ±0.91	3.9 ±0.98	4.1 ±1.01	10.0 ±2.60
		F	1.0 ±0.91	2.0 ±1.37	3.1 ±1.39	3.3 ±1.32	3.6 ±1.26	4.0 ±1.36	8.6 ±3.16
2	Technosphere® particles 11	M	0.7 ±0.60	2.0 ±0.50	3.1 ±0.22	3.6 ±0.67	3.9 ±0.74	4.1 ±0.74	8.9 ±1.29
		F	1.5 ±1.27	3.2 ±1.30	4.0 ±1.72	4.7 ±1.65	4.9 ±1.68	5.0 ±1.58	9.6 ±2.97
3	Technosphere® particles 50	M	1.1 ±0.68	2.5 ±1.15	3.7 ±1.33	4.1 ±1.51	5.1 ±1.39	5.4 ±1.58	10.2 ±2.41
		F	1.2 ±1.11	2.4 ±1.73	2.8 ±1.47	3.5 ±1.44	4.0 ±1.55	4.1 ±1.58	7.5 ±1.54
4	Technosphere® / insulin[a] 0.32	M	0.9 ±0.42	2.3 ±0.64	4.0 ±0.67	4.6 ±0.88	5.2 ±0.82	5.3 ±0.76	10.2 ±2.11
		F	0.5 ±0.21	1.9 ±0.72	2.7 ±1.02	3.1 ±0.98	3.4 ±1.08	3.4 ±1.01	7.7 ±1.95
5	Furosemide 10	M	11.2*** ±1.32	12.9*** ±1.53	13.1*** ±1.35	13.1*** ±1.35	13.3*** ±1.51	13.3*** ±1.45	17.2** ±3.08
		F	10.1*** ±1.04	10.7*** ±1.02	10.8*** ±1.08	10.8*** ±1.08	11.1*** ±1.22	11.5*** ±0.91	14.9** ±1.65
6	Untreated -	M	1.0 ±0.65	2.6 ±1.14	3.6 ±1.25	3.9 ±1.65	4.9 ±1.67	5.4 ±1.39	10.2 ±2.86
		F	1.1 ±0.45	2.6 ±0.45	3.7 ±1.02	4.2 ±0.96	4.8 ±0.90	5.3 ±0.87	10.7 ±1.36

sd standard deviation

M = male, F = female

[a] Insulin dose was approximately 1U/kg.

Statistical significance of difference from vehicle-treated group: **P<0.01, ***P<0.001

Effect of Technosphere® particles and Technosphere®/insulin on group mean electrolyte output and creatinine clearance in the rat at 6 hours following intravenous administration

Group	Intravenous treatment and dose level (mg/kg)	Sex	Group mean electrolyte concentration (mmol/L ±sd) at 6 hours post-dose			Group mean creatinine clearance (mL/min ±sd)
			Cl^-	K^+	Na^+	
1	Vehicle -	M	183 ±16.9	126 ±17.7	129 ±28.4	1.1[a] ±0.15
		F	209 ±63.1	106 ±45.6	175 ±19.0	1.4 ±0.29
2	Technosphere® particles 11	M	189 ±26.1	124 ±9.7	130 ±10.3	1.4 ±0.22
		F	162 ±30.9	80 ±16.8	150 ±15.1	1.3 ±0.22
3	Technosphere® particles 50	M	170 ±25.5	114 ±28.9	138 ±20.1	1.8* ±0.33
		F	193 ±95.1	103 ±49.2	168 ±65.3	1.1 ±0.40
4	Technosphere® / insulin[a] 0.32	M	172 ±13.8	116 ±20.1	121 ±10.2	1.7 ±0.41
		F	216 ±58.6	134 ±40.8	147 ±34.4	1.2 ±0.36
5	Furosemide 10	M	148** ±5.9	35*** ±7.1	134 ±4.0	1.3 ±0.25
		F	145 ±3.7	44* ±5.4	127*** ±3.1	1.0* ±0.11
6	Untreated -	M	177 ±49.7	108 ±24.6	132 ±28.5	2.0** ±0.34
		F	150 ±26.9	73 ±20.4	144 ±10.8	1.4 ±0.43

sd standard deviation
M = male, F = female
n = 5, except for [a] where n=3
[a] Insulin dose was approximately 1U/kg.
Statistical significance of difference from vehicle treated group: *P<0.05, **P<0.01, ***P<0.001

Effect of Technosphere® particles and Technosphere®/insulin on group mean electrolyte output and creatinine clearance in the rat at 24 hours following intravenous administration

Group	Intravenous Treatment and Dose Level (mg/kg)	Sex	Group mean electrolyte concentration (mmol/L ±sd) at 24 hours post-dose			Group mean creatinine clearance (mL/min ±sd)
			Cl^-	K^+	Na^+	
1	Vehicle -	M	252 ±111.8	312 ±132.2	163 ±55.3	1.4 ±0.50
		F	296 ±151.0	450 ±203.5	202 ±101.8	1.2 ±0.22
2	Technosphere® particles 11	M	394 ±24.4	518* ±42.4	268* ±33.6	1.3 ±0.19
		F	273 ±121.3	411 ±194.1	189 ±68.7	1.3 ±0.11
3	Technosphere® particles 50	M	421* ±56.2	491* ±29.9	252 ±64.3	1.5 ±0.28
		F	310 ±68.9	418 ±107.8	213 ±25.8	0.9 ±0.11
4	Technosphere® / insulin* 0.32	M	338 ±134.3	414 ±144.7	243 ±81.0	1.3 ±0.38
		F	378 ±45.3	398 ±28.5	257 ±39.8	1.2 ±0.32
5	Furosemide 10	M	175 ±60.1	619* ±205.5	83* ±34.2	1.5 ±0.26
		F	151 ±36.2	485 ±75.3	106 ±47.0	1.2 ±0.23
6	Untreated -	M	410 ±84.5	482 ±103.3	261 ±61.0	1.4 ±0.26
		F	261 ±48.2	318 ±48.5	162 ±18.2	1.3 ±0.08

sd standard deviation
M = male, F = female
n = 5
* Insulin dose was approximately 1U/kg.
Statistical significance of difference from vehicle-treated group: *P<0.05

Gastrointestinal effects: Not provided.

Abuse liability: Not provided.

Other: None.

2.6.2.5 Pharmacodynamic drug interactions

Not provided by the sponsor.

2.6.3 PHARMACOLOGY TABULATED SUMMARY

2.6.3.1. Pharmacodynamics: Effect on Blood Glucose Levels after a Single Dose Inhalation to Rats

Test Article: Technosphere® and Technosphere Insulin Inhalation Powder

Study Number	**MKC-PC-2006-0024 (b) (4) 75022)**							
Species	**Rats**		**Rats**		**Rats**		**Rats**	
Test article	**Technosphere**		**Technosphere**		**Technosphere Insulin**		**Technosphere Insulin**	
Sex (M/F)/n	9M/9F (Group 1; low)		9M/9F (Group 2; high)		9M/9F (Group 3, low)		9M/9F (Group 4; high)	
Vehicle/formulation	Powder (aerosol)		Powder (aerosol)		Powder (aerosol)		Powder (aerosol)	
Method of administration	Oronasal inhalation		Oronasal inhalation		Oronasal inhalation		Oronasal inhalation	
Analyte	Glucose, Technosphere				Glucose, Technosphere, Insulin			
Assay	Glucose – Accu-Soft Advantage®							
Technosphere (Groups 1,2) or TI (Groups 3,4) (mg/kg)	11.5	11.7	45.8	46.8	0.938	0.321	1.627	0.999
Insulin (mg/kg)	NA	NA	NA	NA	0.095	0.033	0.165	0.101
Insulin (U/kg)	NA	NA	NA	NA	2.5	0.9	4.3	2.7
Pharmacokinetics	**Male**	**Female**	**Male**	**Female**	**Male**	**Female**	**Male**	**Female**
Glucose (Mean ± SD) mmol/L								
Predose	5.8 ± 0.36	5.4 ± 0.32	5.7 ± 0.31	5.4 ± 0.40	6.1 ± 0.29	5.6 ± 0.20	5.9 ± 0.40	5.6 ± 0.31
10 min (during inhalation)	NS	NA	NS	NA	NS	7.8 ± 1.35	NS	7.5 ± 1.04
20 min (during inhalation)	7.5 ± 1.08	7.6 ± 0.55	8.0 ± 0.91	9.3 ± 0.15	7.0 ± 0.23	NA	6.8 ± 0.40	6.3 ± 0.36
40 min (during inhalation)	7.3 ± 0.50	6.7 ± 0.46	6.4 ± 1.30	6.9 ± 0.52	6.6 ± 0.71	NA	6.4 ± 0.25	NA
Immediate (1 min postdose)	6.5 ± 0.30	6.5 ± 0.56	6.8 ± 0.55	7.1 ± 0.35	6.6 ± 0.71	7.3 ± 1.18	5.7 ± 0.66	5.5 ± 0.82
10 min	6.6 ± 0.40	6.7 ± 0.93	6.7 ± 0.53	6.7 ± 0.55	5.8 ± 0.46	7.1 ± 2.54	4.8 ± 0.78	5.3 ± 1.46
20 min	6.8 ± 0.55	6.1 ± 0.21	6.4 ± 1.29	6.1 ± 0.40	6.1 ± 0.49	5.3 ± 0.36	5.2 ± 0.89	4.8 ± 0.62
40 min	6.7 ± 0.49	5.4 ± 0.50	6.6 ± 0.06	6.1 ± 0.44	6.7 ± 0.38	6.0 ± 0.42	5.8 ± 0.90	4.3 ± 1.06
60 min	6.7 ± 1.23	5.7 ± 0.17	6.9 ± 0.50	6.5 ± 0.75	6.6 ± 0.61	6.7 ± 0.76	5.9 ± 0.35	6.7 ± 1.29
2 h	6.8 ± 0.66	5.9 ± 0.67	6.1 ± 1.16	6.0 ± 0.26	6.7 ± 0.38	5.9 ± 1.44	6.4 ± 0.40	5.4 ± 0.28
4 h	6.6 ± 0.21	5.5 ± 0.32	6.3 ± 0.67	5.8 ± 0.50	6.7 ± 1.43	5.9 ± 0.72	6.3 ± 0.15	6.3 ± 0.25
8 h	6.6 ± 0.29	5.8 ± 0.45	6.5 ± 0.71	5.9 ± 0.70	6.6 ± 0.17	6.2 ± 0.90	6.9 ± 0.21	6.1 ± 0.68

Study Number	**MKC-PC-2006-0024 (b) (4) 75022)**							
Species	**Rats**		**Rats**		**Rats**		**Rats**	
Test article	**Technosphere**		**Technosphere**		**Technosphere Insulin**		**Technosphere Insulin**	
24 h	6.2 ± 0.60	5.9 ± 0.46	5.8 ± 1.02	6.3 ± 0.15	6.4 ± 0.70	5.6 ± 0.29	6.1 ± 0.35	5.9 ± 0.25
Location in CTD	**Module 4.2.1.1**							

Additional Information: Slight to moderate reductions in blood glucose levels were observed in groups 3 and 4 animals from 20 or 40 min post dose.

Abbreviations: CTD, common technical document; F, female; M, male; NA, not applicable; No., number; SD, standard deviation.

2.6.3.2. Pharmacodynamics: Effect on Blood Glucose Levels after a Single Dose Inhalation to Dogs

Test Article: Technosphere and Technosphere Insulin Inhalation Powder

Study Number	MKC-PC-2006-0023 ([(b) (4)]65016)							
Species	**Dogs**		**Dogs**		**Dogs**		**Dogs**	
Test article	**Technosphere**		**Technosphere**		**Technosphere Insulin**		**Technosphere Insulin**	
Sex (M/F)/N of animals	3M/3F (Group 1; low)		3M/3F (Group 2; high)		3M/3F (Group 3, low)		3M/3F (Group 4; high)	
Feeding condition	Fasted		Fasted		Fasted		Fasted	
Vehicle/formulation	Powder (aerosol)		Powder (aerosol)		Powder (aerosol)		Powder (aerosol)	
Method of administration	Oronasal inhalation		Oronasal inhalation		Oronasal inhalation		Oronasal inhalation	
Analyte	Glucose, Technosphere				Glucose, Technosphere, Insulin			
Assay	Glucose – Accu-Soft Advantage®							
Technosphere (Groups 1,2) or TI (Groups 3,4) (mg/kg)	11.0	11.6	39.5	42.0	0.38	0.40	1.34	1.43
Insulin (mg/kg)	NA	NA	NA	NA	0.04	0.04	0.15	0.16
Insulin (U/kg)	NA	NA	NA	NA	1.1	1.1	4.0	4.2
Pharmacokinetics	**Male**	**Female**	**Male**	**Female**	**Male**	**Female**	**Male**	**Female**
Glucose (Mean ± SD) mmol/L								
Predose	4.2 ± 0.26	4.4 ± 0.32	4.3 ± 0.15	4.0 ± 0.36	4.1 ± 0.36	4.2 ± 0.57	4.5 ± 0.21	4.5 ± 0.12
10 min (during inhalation)	3.7 ± 0.30	4.1 ± 0.21	4.3 ± 0.84	3.8 ± 0.21	3.6 ± 0.44	3.7 ± 0.55	4.2 ± 0.15	4.9 ± 1.50
20 min (during inhalation)	NA	NA	4.5 ± 0.67	3.8 ± 0.36	NA	NA	NA	NA
40 min (during inhalation)	NA	NA	4.2 ± 1.68	4.1 ± 0.42	NA	NA	NA	NA
End of dosing or 1 min after dosing	4.2 ± 0.30	4.4 ± 0.21	5.0 ± 0.70	4.5 ± 0.57	4.1 ± 0.30	4.2 ± 0.50	3.9 ± 0.40	5.5 ± 3.12
10 min	4.2 ± 0.10	4.7 ± 0.17	4.8 ± 0.74	3.7 ± 0.79	2.6 ± 0.38	3.4 ± 1.12	2.4 ± 1.19	3.0 ± 2.02
20 min	4.2 ± 0.15	4.1 ± 0.31	5.0 ± 0.76	4.2 ± 0.61	2.3 ± 0.50	2.7 ± 1.19	2.0 ± 0.85	1.7 ± 0.35
40 min	4.3 ± 0.25	4.6 ± 0.15	4.6 ± 0.36	4.4 ± 0.20	2.7 ± 0.30	3.1 ± 0.85	2.2 ± 1.23	1.5 ± 0.32
60 min	4.3 ± 0.29	4.4 ± 0.35	4.8 ± 0.70	4.3 ± 0.31	3.0 ± 0.32	3.3 ± 0.93	2.7 ± 0.68	1.5 ± 0.17
2 h	3.4 ± 0.76	3.4 ± 1.04	4.2 ± 0.20	3.9 ± 0.42	3.9 ± 0.26	3.1 ± 1.20	4.1 ± 0.26	2.5 ± 1.04
4 h	4.1 ± 0.17	3.7 ± 0.17	3.7 ± 0.36	3.7 ± 0.40	3.5 ± 0.53	3.8 ± 0.70	4.2 ± 0.15	4.4 ± 0.44
8 h	4.4 ± 0.47	4.6 ± 0.57	4.1 ± 0.20	3.8 ± 0.90	4.4 ± 0.87	3.5 ± 0.40	4.3 ± 0.69	4.5 ± 0.46

Study Number	MKC-PC-2006-0023 ([(b) (4)]65016)							
Species	**Dogs**		**Dogs**		**Dogs**		**Dogs**	
Test article	**Technosphere**		**Technosphere**		**Technosphere Insulin**		**Technosphere Insulin**	
24 h	4.3 ± 0.06	4.5 ± 0.38	4.7 ± 0.49	4.5 ± 0.49	4.2 ± 0.29	4.3 ± 0.58	4.5 ± 0.12	4.6 ± 0.71
Location in CTD	**Module 4.2.1.1**							

Additional Information: Dose dependent reductions in glucose levels were observed following treatment with Technosphere Insulin up to 1 or 2 hours post exposure.

NOTE: The conversion factor from mg/kg to U/kg insulin is 26.315.

2.6.3.3. Secondary Pharmacodynamics: Diversity of Receptor Binding

Test Article: Technosphere Inhalation Powder

Study Number PDC-PC-2002-0009 ([(b) (4)]04-0357)				Location in CTD: Module 4.2.1.1		
Assay	**Radioligand**	**K_d (M)**	**Reference Compound**	**K_i (M) Ref Cpd**	**Activity**	**% Inhibition by FDKP at 1.0E-4**
Neurotransmitter Related						
Adenosine, nonselective	[^{3}H]NECA	7.7E-9	Neca	7.57E-9	No	13.15%
Adrenergic, alpha 1, nonselective	[^{3}H]7-MeOxy-Prazosin	2.0E-10	Phentolamine	8.90E-9	No	13.90%
Adrenergic, alpha 2, nonselective	[^{3}H]RX 821002	1.5E-9	Phentolamine	3.16E-9	No	-1.99%
Adrenergic, beta, nonselective	[^{3}H]DHA	1.74E-9	Alprenolo HCl	4.41E-9	No	1.13%
Dopamine transporter	[^{3}H]WIN 35,428	28.0E-9	GBR12909	1.00E-8	No	0.68%
Dopamine, nonselective	[^{3}H]Spiperone	0.7E-9	Spiperone HCL	4.29E-10	No	3.95%
GABA A, agonist site	[^{3}H]GABA	3.7E-7	GABA	1.06E-8	No	-6.33%
GABA A, BDZ, alpha 1 site	[^{3}H]Flunitrazepam	1.4E-9	Ro15-1788	9.93E-10	No	15.98%
GABA-B	[^{3}H]CGP 54626A	1.2E-9	(±) Baclofen	8.85E-7	No	3.97%
Glutamate, AMPA site	[^{3}H]AMPA	2.8E-8	(±)AMPA HBr	2.15E-8	No	-8.43%
Glutamate, kainate site	[^{3}H]Kainic acid	16E-9	Kainic acid	8.76E-9	No	17.61%
Glutamate, NMDA agonist site	[^{3}H]CGP 39653	7E-9	NMDA	1.28E-5	No	-1.69%

Glutamate, NMDA, glycine (stry-insens site)	[^{3}H]-MDL-105,519	2E-8	MDL-105,519	3.44E-8	No	-15.49%
Glycine, strychnine-sensitive	[^{3}H]Strychnine	28.0E-9	Strychnine nitrate	2.45E-8	No	-6.59%
Histamine, H1	[^{3}H]Pyrllamine	1.3E-9	Triprolidine	5.41E-9	No	10.02%
Histamine, H2	[1251]-Ammopotentidine	7E-10	Tlotidine	9.28E-9	No	-18.64%
Histamine, H3	[^{3}H]N-a-MeHistamine	0.37E-9	N-a-Methylhistamine	6.71E-10	No	20.78%
Melatonin, nonselective	[1251]-2-Iodomelatonin	66E-12	2-Iodomelatonin	1.53E-10	No	-3.55%
Muscarinic, M1 (h)	[^{3}H]Scopolamine, N-Methyl	5E-11	(-)Scopolamine, MeBr	5.48E-11	No	1.99%
Muscarinic, M2 (h)	[^{3}H]Scopolamine, N-Methyl	2.9E-10	(-)Scopolamine, MeBr	2.47E-10	No	18.49%
Muscarinic, nonselective, central	[^{3}H]QNB	1.0E-10	Atropine sulfate	2.18E-10	No	4.83%
Muscarinic, nonselective peripheral	[^{3}H]QNB	0.3E-9	Atropine sulfate	5.27E-10	No	11.61%
Nicotinic (a-bungarotoxin insensitive)	[^{3}H]Epibatidine	63E-12	(±) epibatidine	6.72E-11	Yes	61.89%
Norepinephrine transporter	[^{3}H]Nisoxetine	0.9E-9	Desimipramine HCl	1.80E-9	No	6.13%
Opiod, nonselective	[^{3}H]Naloxone	2.0E-9	Naloxone HCl	1.31E-9	No	3.02%
Orphanin (h)	[^{3}H]Nociceptin	5E-10	Nociceptin	9.67E-10	No	16.42%
Serotonin transporter	[^{3}H]Citalopram, N-Methyl	1.7E-9	Imipramine HCl	2.90E-8	No	11.17%
Serotonin, nonselective	[^{3}H]LSD	7.2E-9	Methysergide maleate	4.60E-9	No	10.50%
Sigma, nonselective	[^{3}H]DTG	3.92E-8	Haloperidol	8.90E-9	No	-6.43%
Steroids						
Estrogen	[1251]3,17B-Estradiol, 16a	5.0E-10	17-B-Estradiol	1.19E-10	No	7.25%
Testosterone (cytosolic)	[3H]Methyltrienolone	4.0E-9	Methyltrienolone	9.79E-10	No	-7.11%
Ion Channels						
Calcium channel, type L (Dihydropyridine site)	[3H]Nitrendipine	0.20E-9	Nifedipine	6.25E-10	No	6.61%
Calcium channel, type N	[1251]-Conotoxin GVIA	1.0E-11	w-Conotoxin GVIA	3.00E-11	No	-0.31%
Potassium channel, ATP-sensitive	[^{3}H]Glibenclamide	0.25E-9	Glibenclamide	3.24E-10	No	12.56%
Potassium channel, Ca2+ Act., VI	[1251]Apamin	7.0E-11	Apamin	1.13E-10	No	-17.59%
Potassium channel, 1[Kr] (hERG) (h)	[^{3}H]Astemizole	100E-9	Terfenadine	6.15E-6	No	5.10%
Sodium, site 2	[^{3}H]Batrachotoxin A 20-a-Benzo	32.0E-9	Aconitine	7.42E-7	No	16.59%
Second Messengers						
Nitric Oxide, NOS (Neuronal-Binding)	[^{3}H]NOARG	25.0E-9	NOARG	2.15E-8	No	-13.92%
Prostoglandins						
Leukotriene, LTB4 (N/A)	No data available	1E-9			No data available	No data available
Leukotriene LTD4	[^{3}H]LTD4	5.0E-9	LTD4	2.64E-9	No	2.90%
Thromboxane A2 (h)	[^{3}H]SQ 29,548	2.0E-9	Pinane-thromboxane	1.00E-7	No	-2.29%
Growth Factors/Hormones						

Corticotropin releasing factor, nonselective	[1251]Tyro-oCRF	4.45E-9	Tyro-o-CRF	2.86E-9	No	-1.62%
Oxytocin	[^{3}H]Oxytocin	1.2E-9	Oxytocin	1.15E-9	No	-5.35%
Platelet activating factor (PAF)	Hexadecyl-[^{3}H]-acetyl-PAF	1.7E-9	C16-PAF	1.47E-8	No	20.22%
Thyrotropin releasing hormone (TRH)	[^{3}H]-(3MeHis2)TRH	2.3E-9	TRH	6.71E-8	No	-2.09%
Brain/Gut Peptides						
Angiotensin ll, AT1 (h)	[1251]-(Sar1-lle8)Angiotensin	8.5E-10	Angiotensin ll (Human)	1.33E-8	No	-1.65%
Angiotensin ll, AT2	[1251]Tyr4-Angiotnsin ll	0.4E-9	Angiotnsin ll (Human)	4.25E-10	No	-16.71%
Bradykinin, BK2	[^{3}H]Bradykinin	0.4E-9	Bradykinin TFA Salt	2.35E-10	No	-4.49%
Cholecystokinin, CCK1 (CCKA)	[1251]CCK-8	3.17E-11	CCK-8 (sulfated)	6.38E-11	No	-3.15%
Cholecystokinin, CCK2 (CCKB)	[1251]CCK-8	0.2E-9	CCK-8 (sulfated)	1.30E-9	No	-1.75%
Endothelin, ET-A (h)	[1251]Endothelin-1	1.6E-10	Endothelin-1	5.96E-10	No	-19.37%
Endothelin, ET-B (h)	[1251]Endothelin-1	2.1E-10	Endothelin-1	1.68E-10	No	-12.02%
Galanin, nonselective	[1251]Galanin	1.0E-10	Galanin (Porcine)	2.83E-10	No	-3.63%
Neurokinin, NK-1	[^{3}H]Substance P	1.4E-9	Substance P	3.15E-9	No	8.98%
Neurokinin, NK2 (NKA) (h)	[1251]-NKA	5E-10	Neurokinin A	6.41E-10	No	20.12%
Neurokinin, NK3 (NKB)	[1251]Eledoisin	1.5E-9	Eledoisin	4.08E-9	No	28.79%
Vasoactive intestinal peptide, nonselective	[1251]VIP	1.0E-9	VIP	2.40E-9	No	17.91%
Vasopressin 1	[^{3}H]Vasopressin-1 Antagonist	0.3E-9	Arg8-Vasopressin	5.09E-9	No	9.19%
Enzymes						
Choline acetyltransferase	[^{14}C]Acetyl Coenzyme	1.5E-6	Bromoacetylcholine	7.33E-7	No	12.33%
Esterase, acetylcholine	Acetylthiocholine	1.4E-4	Eserine	3.70E-7	No	13.71%
Glutamic acid decarboxylase	[^{14}C]Glutamic acid	1.3E-6	AminoOxy acetic acid	2.01E-10	No	4.53%
Oxidase, MAO-A, peripheral	[^{14}C]-5HT (Serotonin)	8.7E-9	Ro 41-1049	6.81E-9	No	19.69%
Oxidase, MAO-B, peripheral	[^{14}C]Phenylethylamine	4.0E-8	Ro 16-6491 HCl	1.70E-8	No	12.02%

IC_{50} determination for Nicotinic (a-Bungarotoxin insensitive)						
Experiment 1			Experiment 2			
FDKP Concentration	% Inhibition	% Specific binding	FDKP Concentration	% Inhibition	% Specific binding	
1.0E-7	-0.47%	100.47%	1.0E-7	-9.68%	109.68	
1.0E-6	-3.31%	103.31%	1.0E-6	-1.63%	101.63	
3.0E-6	3.88%	96.12%	3.0E-6	-9.76%	109.76	
1.0E-5	5.39%	94.61%	1.0E-5	-4.82%	104.82	
3.0E-5	10.46%	89.54%	3.0E-5	3.60	96.40	
1.0E-4	34.35%	65.65%	1.0E-4	14.70	85.30	
3.0E-4	62.14%	37.86%	3.0E-4	27.81	72.19	
1.0E-3	74.96%	25.04%	1.0E-3	55.94	44.06	
(±) epibatidine	IC_{50}	7.79E-11	(± epibatidine	IC_{50}	1.90E-10	
	K_i	3.14E-11		K_i	7.90E-11	
	Slope	-0.82		Slope	-1.03	
FDKP	IC_{50}	2.38E-4	FDKP	IC_{50}	8.01E-4	
	K_i	9.59E-5		K_i	3.33E-4	
	Slope	-0.94		Slope	-0.87	

Abbreviations: AMPA, alpha-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid; Arg, arginine; AT, Angiotensin; ATP, adenosine triphosphate; BK, Bradykinin; C, carbon; Ca, calcium; CCK, cholecystokinin; CRF, corticotropin releasing factor; DHA, docosahexaenoic acid; DTG, 1,3-di-(2-tolyl)guanidine; ET, endothelin; GABA, gamma-aminobutyric acid; H, hydrogen; HBr, hydrogen bromide; hERG, human Ether-a-go-go Related Gene; HCl, hydrochloric acid; IC_{50}, concentration of 50% inhibition; K_d, dissociation constant; K_i, dissociation constant for inhibitor binding; LTB, lymphotoxin beta; LTD, lymphotoxin delta; NK, neurokinin; MeHis, methyl histone; MOA, monoamine oxidase; NMDA, N-methyl-D-aspartic acid; NOARG, NG-nitro arginine; PAF, Platelet activating factor; Ref Cpd, reference compound; TRH, thyrotropin releasing hormone; VIP, vasoactive intestinal peptide.

2.6.3.4. Safety Pharmacology Studies

Test Article: Technosphere (FDKP), Technosphere Insulin (FDKP Insulin)

Organ System Evaluated	Species /Strain	Method of Administration	Dosage (mg/kg)	Sex; Number per group	Findings	GLP Compliance	Study Number
CNS	Rat/ Sprague Dawley	iv	Single dose; 0.125 mg/kg FDKP Insulin 0.125, 12.5 mg/kg FDKP	Male; 8/group	No effect on Functional Observational Battery, assessments of grip strength, hind limb splay, body temperature, or motor activity	Yes	PDC-PC-2002-0007
Cardiovascular	In vitro	In vitro hERG assay	Up to 100 uM FDKP; Up to 100 uM FDKP Insulin	NA	IC50 of FDKP >100 uM; IC50 of FDKP Insulin =67.4 uM	Yes	MKC-PC-2005-0017
Cardiovascular	Dog/ Beagle	iv	Single dose; 0.125 FDKP Insulin; 0.125, 1.25 and 12.5 mg/kg FDKP	Male; 6/group	No QTc prolongation of ECG effects. Hemodynamic changes with Tween formulations (species-specific). No effect on blood pressure with Tween-free formulations	Yes	PDC-PC-2002-0005
Respiratory (Anesthetized)	Rat/ Sprague Dawley	Intratracheal and iv	Single dose; 12.5 mg/kg FDKP intratracheal; 11 and 50 mg/kg FDKP intravenous	Male; 8/group	FDKP had no biologically significant effect at the doses used in this study on either airway resistance, dynamic lung compliance, heart rate, or mean arterial blood pressure.	Yes	MKC-PC-2007-0021
Respiratory (Anesthetized)	Rat/ Sprague Dawley	Intratracheal and iv	Single dose; 2.5 mg/kg intratracheal; 0.32 mg/kg FDKP Insulin intravenous	Male; 8/group	FDKP Insulin had no biologically significant effect at the doses used in this study on either airway resistance, dynamic lung compliance, heart rate, or mean arterial blood pressure.	Yes	MKC-PC-2007-0022

Organ System Evaluated	Species /Strain	Method of Administration	Dosage (mg/kg)	Sex; Number per group	Findings	GLP Compliance	Study Number
Respiratory (Conscious)	Rat/ Sprague Dawley	iv	Single dose; 11 and 32.2 mg/kg FDKP	Male; 8/group	FDKP had no biologically significant effect at the doses used in this study on either respiratory rate or tidal volume.	Yes	MKC-PC-2007-0023
Respiratory (Conscious)	Rat/ Sprague Dawley	iv	Single dose; 0.32 mg/kg FDKP Insulin	Male; 8/group	FDKP Insulin had no biologically significant effect at the doses used in this study on either respiratory rate or tidal volume.	Yes	MKC-PC-2007-0024
Renal	Rat/ Sprague Dawley	iv	Single dose; 0.32 mg/kg FDKP Insulin; 11 and 50 mg/kg FDKP	Male and Female; 5/sex/group	No effect on creatinine clearance, urine output, and electrolyte concentration with FDKP Insulin at 0.32 mg/kg or FDKP up to 50 mg/kg.	Yes	MKC-PC-2006-0038

Abbreviations: CNS, central nervous system; ECG, electrocardiogram; GLP, Good Laboratory Practice; iv, intravenous; QTc, QT interval corrected for rate.

2.6.4 PHARMACOKINETICS/TOXICOKINETICS

2.6.4.1 Brief summary

The PK profiles of insulin (Technosphere® Insulin) and FDKP (Technosphere® particles) were characterized. The ADME of TI and T were also characterized. There studies were done in rats and dogs via various routs including inhalation, PO, SC, and IV. Technosphere particles have a median diameter of 2-2.5 µm; a size designed for deep lung delivery.

The HPLC and LC-MS methods were used to validate assays for FDKP in serum and plasma, test solutions, and air sampling substrates, such as filters. The assays for insulin included ELISA, electrochemiluminescence (ECL) immunoassay, and radioimmunoassay methods.

These PK studies showed that TI was absorbed rapidly in all nonclinical species tested, and the PK profile was consistent with the human PK profile. The PK profiles for both insulin and FDKP have remained the same throughout the clinical and nonclinical TI programs. The mean t_{max} for insulin and FDKP were about 15 min after dosing across all studies. Insulin levels returned to near baseline between 180 and 240 min postdose, and the mean t ½ ranged between 135 and 170 min. Insulin and FDKP exposure following TI inhalation were approx. dose linear.

Clinical Exposure:
1 mg Technosphere Insulin = (b) (4) mg insulin (or 3 U) + (b) (4) mg FDKP
1 U insulin = 0.0347 mg insulin
MRHD = 105 mg Technosphere Insulin

FDKP AUC following 40 mg Technosphere without insulin: 1631.16 (ng*h/mL) (CV% 27.32)
FDKP AUC following 30 U Technosphere with insulin at Day 7 following 7 days administration: 20658 (min*ng/mL) (CV% 36.2)

PK and absorption:

- In rats, following the IV administration, FDKP showed moderate systemic clearance (approx. 2-fold GFR), moderate volume of distribution (1.5-4-fold of body water volume), and a short terminal ½ life. There were no apparent PK differences between T and TI. Theses data showed that insulin did not influence the PK disposition of FDKP. Following the SC administration, systemic exposure was equivalent to the exposure following IV administration. However, following the PO and IT administrations, minimal absorption occurred. There were no notable sex-related differences in the PK of FDKP in rats.

- In dogs, following the inhalation administration, the PK of FDKP and insulin were evaluated with doses up to 46.8 mg/kg T and 1.27 mg/kg TI. After dosing, serum insulin levels rose rapidly in a dose linear manner and peaked at 18 min, with apparently elimination half-lives ranging from 10 to 30 min. Dose-dependent reductions in glucose levels were seen only in the TI dose group up to 1 hr in males or 2 hrs in females. Higher insulin C_{max} and $AUC_{0\text{-}4h}$ were observed in females compared with males. Increased FDKP C_{max} and $AUC_{0\text{-}4h}$ were observed in a dose-dependent manner. In addition, the dose-normalized FDKP systemic exposure was about 4-time higher than that observed following IV administration, indicating that approx. 25% bioavailability by the oronasal route. No sex differences in FDKP C_{max} and $AUC_{0\text{-}last}$, t_{max}, or $t_{1/2}$ were observed. Following the IV administration, FDKP was highly bioavailable and systemic clearance approximated the GFR. Following the SC administration, FDKP was also highly bioavailable. On the other hand, following the PO administration, the relative bioavailability of FDKP was about 3% compared to the SC administration. There was no evidence of any sex-related differences in the PK of FDKP in dogs.

- In both rats and dogs, FDKP bioavailability was high by the IV route, moderate (20 to 30%) by the inhalation route, and poor following the PO administration. Regardless of route of exposure, the primary route of excretion was through the kidney.

Distribution:

- FDKP did not bind to proteins in human, mouse, rat, dog, monkey, and rabbit plasma. Following IT instillation (as a solution) or insufflation (as a dry powder) of ^{14}C-FDKP, radioactivity levels were highest and $AUC_{0\text{-}last}$ was largest in the lungs and trachea (site of

administration) and kidneys (site of excretion). Notable radioactivity was also observed in the thyroid/parathyroid glands, in the walls of the GI tracts, and the wall of the urinary bladder. The lowest radioactivity was found in the eyes, brain, spinal cord, seminal vesicle, and testis.

- In general, the tissue to plasma ratios remained constant over the study interval, indicating that radioactivity was cleared at approx. the same rate from plasma and tissues. The FDKP levels in milk of lactating rats were approx. 1/10 of peak plasma levels, indicating that FDKP distributed into milk to a modest extent. FDKP did cross blood-placental barrier in a facile manner in rats, with comparable levels attained in maternal and fetal blood.

Metabolism:

- *In vitro* studies showed that FDKP was not metabolized in the presence of cryopreserved hepatocytes from mice, rats, rabbits, dogs, and humans, suggesting that FDKP was not a substrate for Phase I or II metabolism. FDKP was also not metabolized when incubated with human liver microsomes in the presence of NADPH. Moreover, *in vivo* studies in rats and dogs showed that FDKP was not metabolized. Based on these findings, metabolism was not likely to contribute to the clearance of FDKP in either animals or humans. FDKP was not metabolized in humans.

Excretion:

- ^{14}C-FDKP excretion was evaluated in rats and dogs following IV, SC, and PO, and IT administrations. Following a single IV dose of ^{14}C-FDKP or ^{14}C-FDKP+insulin to rats, 85-95% of the administered ^{14}C-FDKP was excreted, unchanged, in the urine within 18 hr postdose. The remainder of the administered dose was eliminated by the hepato-biliary route. In addition, following the SC administration to rats, approx. 83% of the administered ^{14}C-FDKP was excreted in the urine, with a minor amount (13-16%) excreted in feces. Following the PO administration to rats, 87-96% of the dose was recovered in feces with 2-5% in the urine, and <1% in bile. All these findings indicated that limited absorption of ^{14}C-FDKP from the GI tract. Following the IT administration, 72-85% of the administrated dose was recovered in feces with 6-12% in the urine, and <1% in bile. Moreover, following a single IV dose of ^{14}C-FDKP to dogs, approx. 95% of the administered dose was recovered in urine and <1% by biliary and fecal elimination.

2.6.4.2 Methods of Analysis

[See under individual study reviews]

12 studies were provided by the sponsor.
Note from the reviewer: study conclusions were cut and pasted from each study report.

MKC-PC-2003-2027: Validation of a HPLC method for the determination of Technosphere on Filers

MKC-PC-2003-2028: Method validation of an LC-MS/MS assay for the determination of fumaryl technosphere in rat serum

The method presented here for the determination of Fumaryl Technosphere in rat serum samples shows an acceptable linearity within the low calibration range of 0.25 ng/mL to 70 ng/mL and the high calibration range of 0.07 µg/mL to 20 µg/mL, with good precision and accuracy. In addition, the method is specific and selective for Fumaryl Technosphere and its internal standard, (b) (4) Fumaryl Technosphere was found to be stable under the storage conditions investigated.

MKC-PC-2003-2029: Assay of insulin in technosphere/insulin: collected from air sampling substrates

The High Performance Liquid Chromatographic (HPLC) method for the determination of insulin collected from air sampling substrates has been successfully validated.

The data generated in this study have met the required criteria for the selectivity, linearity, range, precision, accuracy, and sensitivity.

The intermediate precision (ruggedness) of the assay has also been demonstrated to the required standards.

The stability of the insulin Stock Standard within acceptable tolerances is 14 days when stored at 2 - 8°C.

The stability of insulin Calibration Standard solutions within acceptable tolerances is 3 days when stored at 2 - 8°C.

Recovery of insulin from the sampling substrates has been established within acceptable limits (100±5%) for substrate loadings of up to 1000 µg insulin.

MKC-PC-2003-2031: Method validation of an LC-MS/MS assay for the determination of fumaryl technosphere in dog plasma

The method presented here for the determination of Fumaryl Technosphere in dog plasma samples shows an acceptable linearity within the low calibration range of 0.25 ng/mL to 70 ng/mL and the high calibration range of 70 ng/mL to 20000 ng/mL, with good precision and accuracy. In addition, the method is specific and selective for Fumaryl Technosphere and its internal standard, (b) (4) Fumaryl Technosphere was found to be stable under the storage conditions investigated.

MKC-PC-2004-0021: Technosphere: HPLC analytical method validation for the assay of Technosphere: collected from air sampling substrates

The High Performance Liquid Chromatographic (HPLC) method for the determination of Technosphere collected from air sampling substrates has been successfully validated in this study.

The data generated in this study, based on STD solutions prepared in TDV, have met the required acceptance criteria for:

1. **Specificity**

   All components of the testing met their respective acceptance criteria.

2. **Linearity and range**

   Over the specified range of 1600 – 50 µg/mL, the unweighted regression model met the acceptance criteria.

3. **Precision**

   CV values were less than 2% (n = 6 samples) across the specified range.

4. **Accuracy**

   The unweighted regression model met the accuracy criteria on all three occasions. Consequently, Sample analysis may be performed using unweighted regression model in future analyses.

5. **Ruggedness**

   The results were within the acceptance criteria specified for each test indicating that the assay is not dependent on the analyst or column.

6. **Stability**

   For the stability assessment of Technosphere in stock and standard solutions, the established stability periods are summarized below:

Storage Temperature	Stability Period Established	
	Stock solutions	Calibration Standards
Room temperature	6 hours	6 hours
ca. 4°C	15 Days	Not established
Autosampler (4±2°C)	N/AP	24 hours

N/AP = Not applicable

7. **Recovery**

   Recovery of Technosphere from the sampling substrates has been established within acceptable limits (100±5%) for substrate loadings of up to 10 000 µg Technosphere.

Consequently, the method can be used at (b) (4) for the quantitative analysis of Technosphere collected from air sampling substrates.

MKC-PC-2006-0006: Mouse serum validation study: determination of human insulin in mouse serum by ELISA

An ELISA method ((b) (4) method 197-0501) has been validated for the quantitation of human insulin in mouse serum from 2 – 200 µU/mL.

Title: Mouse Serum Validation Study: Determination of Human Insulin in Mouse Serum by ELISA					
Report: 197-0501					
Species: Mouse					
Matrix: Serum					
Sample Volume: 20 µL					
Analyte	Assay Range	Intra-Batch Precision (%CV)	Intra-Batch Accuracy (%Diff)	Inter-Batch Precision (%CV)	Inter-Batch Accuracy (%Diff)
Human Insulin	2 to 200 µU/mL	1.8 to 9.4%	-2.9 to 8.6%	3.7 to 7.5%	0.1 to 3.0%

MKC-PC-2006-0007: Rat serum validation study: determination of human insulin in rat serum by ELISA

An ELISA method ((b) (4) method 197-0502) has been validated for the quantitation of human insulin in rat serum from 2 – 200 µU/mL.

Title: Rat Serum Validation Study: Determination of Human Insulin in Rat Serum by ELISA					
Report: 197-0502					
Species: Rat					
Matrix: Serum					
Sample Volume: 20 µL					
Analyte	Assay Range	Intra-Batch Precision (%CV)	Intra-Batch Accuracy (%Diff)	Inter-Batch Precision (%CV)	Inter-Batch Accuracy (%Diff)
Human Insulin	2 to 200 µU/mL	1.0 to 3.3 %	-5.2 to 11.5%	2.4 to 6.9%	-1.8 to 7.6%

MKC-PC-2006-0013: Dog serum validation study: determination of human insulin in dog serum by ELISA

An ELISA method ((b) (4) method 197-0503) has been validated for the quantitation of human insulin in dog serum from 2 – 100 µU/mL.

Title: Dog Serum Validation Study: Determination of Human Insulin in Dog Serum by ELISA					
Report: 197-0503					
Species: Dog					
Matrix: Serum					
Sample Volume: 20 µL					
Analyte	Assay Range	Intra-Batch Precision (%CV)	Intra-Batch Accuracy (%Diff)	Inter-Batch Precision (%CV)	Inter-Batch Accuracy (%Diff)
Human Insulin	2 to 100 µU/mL	2.5 to 6.5%	-9.4 to 15.6%	3.5 to 16.6%	-6.7 to 14.7%

MKC-PC-2006-0015: A cross validation of an HPLC method to determine levels of Technosphere® and Technosphere®/insulin formulated in DMSO

The High Performance Liquid Chromatographic (HPLC) method for the determination of Technosphere® has been successfully cross validated for the analysis of the Technosphere® content of Technosphere® and Technosphere®/Insulin solutions formulated in DMSO.

The data generated in this study, based on STD solutions prepared in DMSO, have met the required acceptance criteria for:

1. **Robustness**

   The method was successfully tested with diluted sample solutions containing up to 10% v/v DMSO.

2. **Stability**

   For the stability assessment of Technosphere® in samples of the Technosphere® and Technosphere®/Insulin formulated in DMSO are summarized below:

Storage Temperature	Stability Period Established for DMSO Formulations	
	Technosphere®	Technosphere®/Insulin
Room Temperature (protected from light)	24 hours	24 hours

The method can therefore be used at (b) (4) for the analysis of Technosphere® and Technosphere®/Insulin solutions formulated in DMSO.

MKC-PC-2006-0018: Stability determination and validation of an analytical method for the determination of Technosphere® particles and Technosphere®/insulin particles in PBS

A. Technosphere® Particles in PBS

In accordance with (b) (4) SOP OPAC0379 "Analytical Method Validation for Commercial Mammalian and Genetic Toxicology Studies" all acceptance criteria were met and the Analytical Method for the Determination of Technosphere® Particles in PBS was validated.

Technosphere® Particles in PBS was found to be stable for at least 24 hours when stored at room temperature, the results are summarized in Table 48.

Table 48: Room Temperature Stability Summary for Technosphere® Particles

Conc. of Form.	%of T=0 at Each Time Point (Hours)			
	0	8	12	24
0.5	100	104.9	108.2	105.7
5	100	105.2	99.8	105.4
20	100	97.2	96.4	97.4
50	100	99.9	105.8	100.5
110	100	103.2	104.2	104.4

Technosphere® Particles in PBS was found to be stable for at least 33 days when stored at 2-8°C, the results are summarized in Table 49.

Table 49: 2-8°C Stability Summary for Technosphere® Particles

Conc. of Form.	%of T=0 at Each Time Point (Days)				
	0	8	15	22	33
0.5	100	103.6	105.0	101.7	99.7
5	100	104.5	103.4	102.4	104.3
20	100	95.7	96.7	95.7	96.7
50	100	98.7	103.1	98.0	99.2
110	100	101.3	102.6	98.5	100.5

Table 50: -5 to -30°C Stability Summary for Technosphere® Particles

Conc. of Form.	%of T=0 at Each Time Point (Days)			
	0	8	33	62
0.5	100	103.5	98.2	104.6
5	100	104.6	104.5	103.8
20	100	97.9	96.1	95.2
50	100	98.6	97.8	103.6
110	100	100.7	100.9	101.5

B. Technosphere®/Insulin Particles in PBS

In accordance with (b) (4) SOP OPAC0379 "Analytical Method Validation for Commercial Mammalian and Genetic Toxicology Studies" all acceptance criteria were met and the Analytical Method for the Determination of Technosphere®/Insulin Particles in PBS was validated.

Technosphere®/Insulin Particles in PBS was found to be stable for at least 24 hours when stored at room temperature; the results are summarized in Table 51.

Table 51: Room Temperature Stability Summary for Technosphere®/Insulin Particles

Conc. of Form.	%of T=0 at Each Time Point (Hours)			
	0	8	20	24
0.5	100	112.4	109.5	110.3
5	100	101.1	99.8	99.2
20	100	99.1	98.9	98.4
50	100	103.4	101.3	95.5
110	100	101.2	99.5	101.5

Technosphere®/Insulin Particles in PBS was found to be stable for at least 33 days when stored at 2-8°C, the results are summarized in Table 52.

Table 52: 2-8°C Stability Summary for Technosphere®/Insulin Particles

Conc. of Form.	%of T=0 at Each Time Point (Days)				
	0	8	15	21	33
0.5	100	90.5	88.8	104.1	102.9
5	100	96.4	95.1	98.7	92.2
20	100	97.3	97.4	95.8	93.2
50	100	90.8	90.5	98.9	95.2
110	100	98.2	98.9	9.9	98.3

Technosphere®/Insulin Particles in PBS was found to be stable for at least 65 days when stored at -5 to -30°C, the results are summarized in Table 53.

Table 53: -5 to -30°C Stability Summary for Technosphere®/Insulin Particles

Conc. of Form.	%of T=0 at Each Time Point (Days)				
	0	8	15	33	65
0.5	100	90.8	87.2	105.7	85.4
5	100	96.1	95.7	93.6	93.1
20	100	96.9	97.5	97.6	93.9
50	100	91.5	88.1	94.1	85.0
110	100	100.3	99.2	97.5	94.9

MKC-PC-2007-0025: Validation of FDKP determination in mouse serum by LC/MC/MS (addendum 2)

An LC/MS/MS assay for the determination of FDKP in mouse serum was partially-validated from human serum (b) (4) 197-0602). Samples were spiked with internal standard (b) (4), processed by solid phase extraction and analyzed using reverse-phase HPLC Turbo Ion Spray® MS/MS detection. Positive ions for FDKP and internal standard (b) (4) were monitored in MRM mode. Drug-to-IS peak area ratios for the standards were used to create a linear calibration curve using $1/x^2$ weighted least-squares regression analysis. A detailed description of the method and sample preparations can be found in Appendix A. Experiments were conducted to evaluate the stability of FDKP in mouse serum at room temperature, through freeze/thaw cycles at -20 °C and -70 °C, and long-term storage stability. FDKP stability was assessed in processed samples (RT autosampler stability). Selectivity, extraction recovery, matrix effect, dilution tests, and injection carry-over were also evaluated.

MKC-PC-2008-0010: Analytical method validation for the HPLC assay of FDKP in PBS

The High Performance Liquid Chromatographic (HPLC) method for the determination of Fumaryl Diketopiperazine (FDKP) in Phosphate Buffer was successfully validated.

The data generated in this study, based on STD solutions prepared in Diluent, have met the required acceptance criteria for:

1. **Linearity**

   Over the specified nominal ranges 75 – 225 µg/mL, the unweighted and 1/concentration (1/C) and 1/concentration^2 (1/C^2) weighted calibration models met the acceptance criteria.

2. **Specificity**

   All components of the testing except for one injection in the carryover test met their respective acceptance criteria.

3. **Precision**

   CV% values for FDKP [redacted] (b) (4) were less than 2% (n = 3 samples) across the specified range.

4. **Accuracy**

   The single point calibration method and the unweighted, 1/concentration (1/C) and 1/concentration^2 (1/C^2) weighted regression models met the accuracy criteria on all three occasions.

   Consequently, sample analysis will initially be performed using the single point calibration method.

5. **Ruggedness**

   The results were within the acceptance criteria specified for each test indicating that the assay is independent of the analyst and HPLC column.

6. **Stability**

   For the stability assessment of the test article in stock and standard solutions, the established stability periods are summarized in Table D:

Table D: Summary of Stability Data

Storage Temperature	Stability Period Established	
	Stock solution	Working Standard
Room Temperature	24 hours	24 hours
4°C (range 2- 8°C)	14 days	14 days
Autosampler (8 ± 3°C)	NA	222 hours

NA = Not applicable.

7. **Impurities**

   All impurities [redacted] (b) (4) with a peak area % of (b) (4) and above were assessed along with the sum of impurity (b) (4) [redacted], total impurities, sum of FDKP [redacted] (b) (4) FDKP [redacted], [redacted] (b) (4), and % FDKP and was reported from the triplicate injection of a previously analysed FDKP solution (nominal concentration 150 µg/mL) prepared with the simulated FDKP bulk material. Further impurity assessment was conducted from three samples prepared with the Impurity enriched FDKP Lot No. FD08064, analysed in study 70569. Impurity assessment results of the impurity enriched FDKP Lot No. FD08064 was considered similar when compared to the Certificate of Analysis.

   The method can therefore be used at [redacted] (b) (4) for the analysis of FDKP in Phosphate Buffer dosing formulations.

2.6.4.3 Absorption

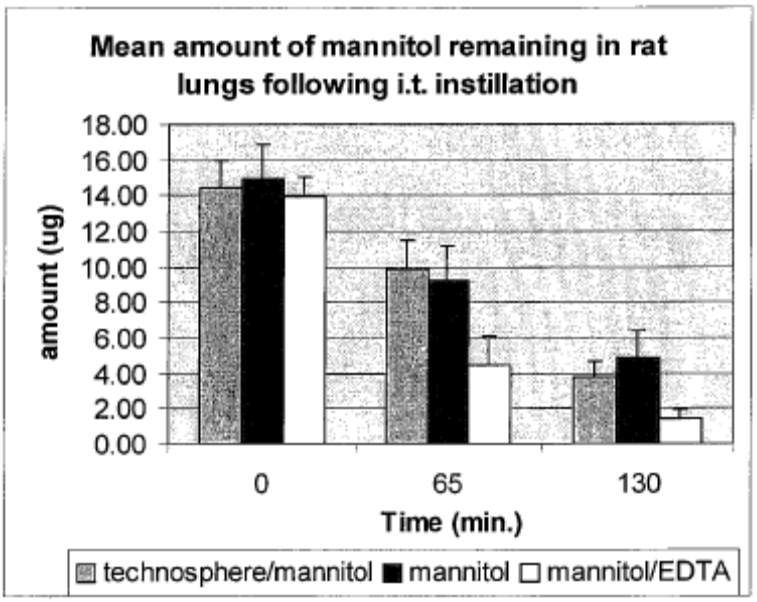
13 studies were provided d by the sponsor.

- PDC-PC-2000-2011: T in rat lung
- MKC-PC-2005-0003: 14-day PK SC study in CD-1 mice (T)
- MKC-PC-2006-0001: PK SC study in male dogs (TI)
- MKC-PC-2006-0002: PK SC study in minipigs (TI)
- MKC-PC-2006-0034: AME after a single IV in rats (T)
- MKC-PC-2006-0035: AME after a single IV in rats (TI)
- MKC-PC-2006-0036: AME after a single IV in dogs (T)
- MKC-PC-2007-0005: AME after a single IT, SC, or PO in rats (T)
- MKC-PC-2007-0009: AME after a single IT, SC, or PO in dogs (T)
- MKC-PC-2007-0010: AME after a single IT or PO in rats (TI)
- MKC-PC-2007-0011: AME after a single IV in dogs (T)
- MKC-PC-2007-0012: AME after a single IV in rats (TI)
- MKC-PC-2007-0013: AME after a single IV in rats (T)

PDC-PC-2000-0011: ^{3}H-Mannitol and Technosphere™ in Rat Lungs [(b) (4) (b) (4), Study No. RD.2000.01]

This study was to compare the rate of elimination of mannitol from rat lungs following IT administration with and without T. Since pulmonary administered TI could be absorbed more rapidly through the lung into the systemic circulation than insulin alone. One explanation for this might be due to opening of the tight junctions. This study was conducted to determine whether tight junctions play a role in Technosphere™ absorption enhancement. Three tritiated PBS, containing 1 mM mannitol (mannitol, T+mannitol, EDTA+mannitol), were given to anesthetized rats via IT instillation. After 0, 65, or 120 min, the rats were sacrificed and their lungs were removed and counted for radioactivity. Findings: There was no significant difference in the amount of ^{3}H-mannitol remaining in the lungs following all absorption periods with or without T. Therefore, it was unlikely that Technosphere™ opened or disrupted the tight junction region. On the other hand, the EDTA addition significantly enhanced the absorption of mannitol, theoretically by opening the tight junctions.

6.4. Comparison of the amount of 3H-mannitol remaining in the lung at 0, 65 and 130 min. post intratracheal administration



PDC-PC-2005-0003: Technosphere®-Particles: A 14-Day Subcutaneous PK Study in CD-1 Mice [By (b) (4) Study No. 7634]

This study was to generate the PK profile of T during the course of SC administration to CD-1 mice once daily for 14 days. The dose levels evaluated were 10, 25, and 75 mg/kg/day. The study population was subdivided into replicates (A and B). The mice of Replicate A were dosed only on Day 1 and subgroups of these animals (3/sex/timepoint) were bled terminally at 6 specified time points (10, 20, ,30, 45, 60, and 120 min) during the 24 hr period, following single SC administration of T or the VH (PBS). The mice of Replicate B were dosed on each of 14 days and were bled terminally at 6 specified time points during the 24 hr period, following the final Day 14 treatment. The animals were monitored for mortality and clinical signs, and weighted at intervals for dose calculation purposes.

Group Numbers	Group Designation	Dose Level (mg/kg/day)	Dose Volume* (mL/kg/day)	Dose Concentration (mg/mL)	Numbers of Animals	
					Males	Females
1	Control**	0	10	0	36	36
2	Low Dose	10	10	1	36	36
3	Mid Dose	25	10	2.5	36	36
4	High Dose	75	10	7.5	36	36

* The volume (10 mL/kg body weight) of each dose that was given to each relevant mouse each day was administered as a single bolus by subcutaneous injection.

** The mice of Group 1 received sterile Phosphate Buffered Saline (PBS).

Findings: The exposure was approx. dose proportional, and there did not appear to be any change in exposure (ACU_{last} or ACU_{inf}), when compared single dose to multiple dosing for 14 days. No sex difference was observed. The mean serum ACU_{inf} was approx. 4280,000 ng•min/mL for the high dose T animals, and the t½ ranged from 12.6 to 16.3 min. No apparent differences were observed due to the amount of the daily dose or the number for daily doses. In addition, daily SC

injection of T at doses up to 75 mg/kg/day was well-tolerated. The NOEL was considered as 75 mg/kg/day, and therefore acceptable for long-term repeated studies.

TABLE 1. FDKP Mean Serum Toxicokinetic Metrics Following Daily Subcutaneous Injection of Technosphere® Particles to Male and Female CD-1 Mice for up to 14 Days

	Sample Day	Group 2 (10 mg/kg/day)		Group 3 (25 mg/kg/day)		Group 4 (75 mg/kg/day)		
		Female	Male	Female	Male	Female	Male	M + F
C_{max} (ng/mL)	1	18070	20658	49409	48044	96247	99652	93558
	14	19258	16080	58554	52440	77714	100435	89075
t_{max} (min)	1	10	10	10	10	10	20	20
	14	15	15	15	15	15	15	15
AUC_{last} (ng*min/mL)	1	566198	586203	1376943	1303105	5401028	4168730	4784887
	14	539702	495473	1740710	1442827	3919243	4600399	4259755
AUC_{inf} (ng*min/mL)	1	570437	588032	1384071	1307162	5450536	4208961	4829572
	14	541789	499434	1752580	1446085	3929507	4642224	4283388
$t_{1/2}$ (min)	1	16.0	13.2	14.8	13.2	16.3	15.7	16.0
	14	13.6	16.1	15.3	12.8	12.6	16.0	13.8

TABLE 2. FDKP Mean Serum Concentrations Following Daily Subcutaneous Injections of Technosphere® Particles to Female and Male CD-1 Mice for up to 14 Days

Time (min)		10 mg/kg/day				25 mg/kg/day				75 mg/kg/day			
		Day 1		Day 14		Day 1		Day 14		Day 1		Day 14	
		Female	Male	Female	Male	Female	Male	Female	Male	Female	Male	Female	Male
Pre-dose	mean			7.30	117			BQL	BQL			6.12	11.6
	SD			8.31	198							0.657	4.37
5	mean	BQL	BQL			BQL	BQL			BQL	BQL		
	SD												
10	mean	18070	20658			49409	48044			96247	78856		
	SD	1944	1779			10551	11267			36998	10336		
15	mean			19258	16080			58554	52440			77714	100435
	SD			5807	639			18109	16080			58774	9488
20	mean	17461	19945			40422	41181			87464	99652		
	SD	1377	3178			9354	9389			3473	34132		
30	mean	9679	8933	10689	9571	22526	23346	33903	24250	89894	89072	72735	77600
	SD	2816	1970	3204	3102	6119	5192	8884	8541	8840	26141	57961	57017
45	mean	3027	4535	3810	2450	7139	8813	11536	8040	53439	58632	37145	61889
	SD	999	1230	783	1177	1104	2432	4853	1721	23010	15823	10312	24047
60	mean	2244	1314	803	1812	5427	2681	4392	4442	48426	14260	29022	25252
	SD	167	261	261	629	476	545	1604	329	33630	8418	6537	15819
120	mean	184	96.0	106	171	333	213	537	176	2110	1772	565	1816
	SD	117	32.0	10.7	90.7	220	156	612	130	1832	2422	310	1247

BQL = below the limit of quantization.
Blank spaces indicate that no blood sample was taken.

MKC-PC-2006-0001: PK of Human Insulin Following Subcutaneous Dose Administration to Non-Naïve Male Beagle Dogs [By (b) (4) Study No. LCL00006]

This study was to investigate the PK of Humalog insulin, regular insulin, and TI formulations (in suspension or solution), following the SC administration to non-naïve male beagle dogs. 18 male dogs were placed into 3 groups of 6 animals. For Dose Session 1, Group 1 animals received regular insulin via SC injection at 0.5 U/kg. Group 2 animals received Humalog via SC injection at 0.5 U/kg. Group 3 received TI, in sterile water, via SC injection at 0.5 U/kg. For Dose Session 2, Group 3 animals were given a 2-day washout period and assigned as Group 4 to receive TI, in 6.6% Tribasic phosphate solution/93/4% PBS, via SC injection at insulin dose of 0.5 U/kg.

Viabilities and clinical observations were recorded. Blood samples from all animals were collected via direct venipuncture of a cephalic vein, prior to dosing and at the following time points: 5, 10, 20, 30, 40 min, and 1, 2, 4, 6 hrs following SC administration. Findings: TI in suspension and in solution had higher insulin exposure (C_{max}, AUC_{last}, and AUC_{inf}) then either regular insulin or Humalog insulin following SC administration. The PK profile of insulin administered as TI in suspension showed that the shortest t_{max}, but was comparable to that of Humalog insulin. Humalog insulin had the shortest terminal ½ life. Overall, the kinetics of insulin over time was similar among Humalog insulin, TI suspension, and TI solution groups for the earlier portion of the graph, while the regular insulin curve showed slower kinetics and reached a lower C_{max}. Animals given regular insulin showed a slower insulin onset and a lower max circulating insulin level. Moreover, TI suspension produced higher FDKP exposure (C_{max}, AUC_{last}, and AUC_{inf}) than TI solution, following the SC administration. There was no significant difference in t_{max} for FDKP between TI suspension and solution. TI suspension had a shorter terminal ½ life for FDKP (72.51 min) than TI solution (94.77 min) following the SC administration.

TABLE 1. Insulin Mean Plasma Toxicokinetic Metrics Following a Single Subcutaneous Injection of Regular Insulin, Humalog® Insulin, Technosphere®/Insulin Suspension and Technosphere®/Insulin Solution to Male Beagle Dogs

	Grp. 1 Regular Insulin	Grp. 2 Humalog® Insulin	Grp. 3 T/I Suspension	Grp. 4 T/I Solution
C_{max} (µU/mL)	188.2	253.1	337.0	298.5
t_{max} (min)	36.7	28.3	26.7	38.3
AUC_{last} (µU *min/mL)	21,412.9	16,543.5	25,375.4	26,267.5
AUC_{inf} (µU *min/mL)	23,632.2	19,128.9	28,933.8	27,497.0
$t_{1/2}$ (min)	80.2	36.3	98.4	66.0

FIGURE 1. Insulin Mean Plasma Concentrations Following a Single Subcutaneous Injection of Regular Insulin, Humalog® Insulin, Technosphere®/Insulin Suspension and Technosphere®/Insulin Solution to Male Beagle Dogs

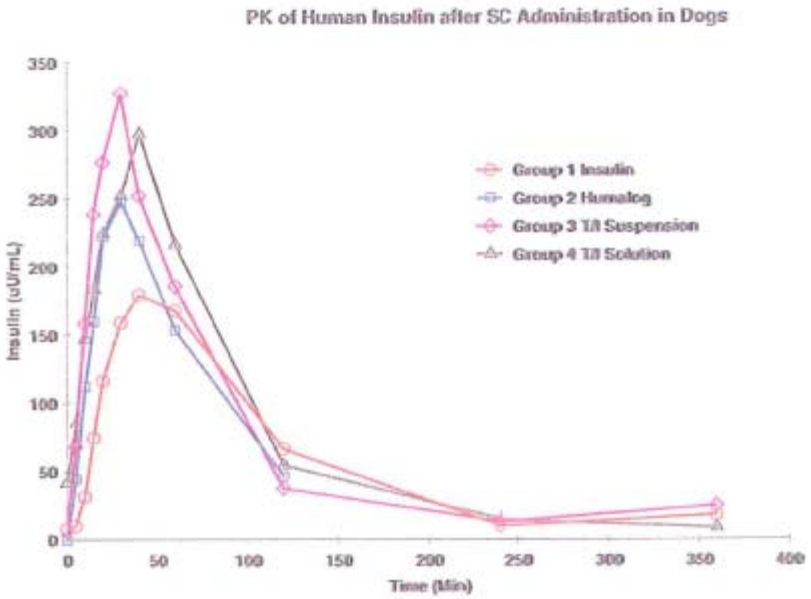
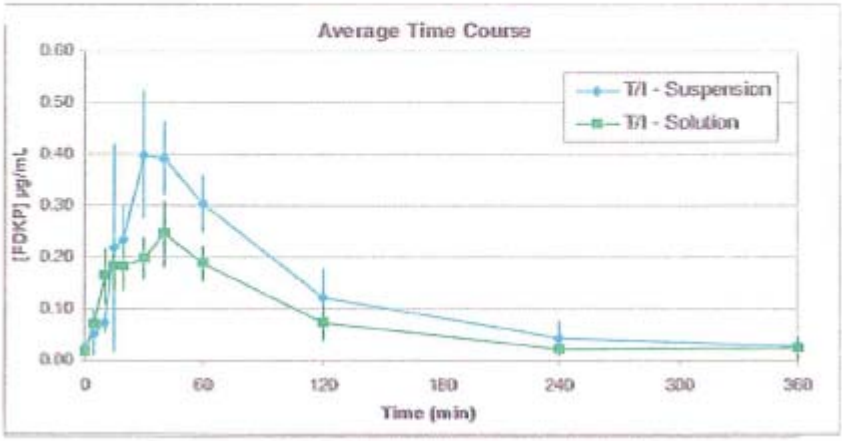


TABLE 3. FDKP Mean Plasma Toxicokinetic Metrics Following a Single Subcutaneous Injection of Technosphere®/Insulin Suspension and Technosphere®/Insulin Solution to Male Beagle Dogs

	Grp. 3 T/I Suspension	Grp. 4 T/I Solution
C_{max} (µg/mL)	0.47	0.25
t_{max} (min)	32.50	29.17
AUC_{last} (µg *min/mL)	44.41	27.53
AUC_{inf} (µg *min/mL)	47.54	31.84
$t_{1/2}$ (min)	72.51	94.77

FIGURE 2. FDKP Mean Plasma Concentrations Following a Single Subcutaneous Injection of Technosphere®/Insulin Suspension and Technosphere®/Insulin Solution to Male Beagle Dogs



MKC-PC-2006-0002: PK Study of Regular Insulin, Humalog® Insulin, and Technosphere®/Insulin Following Subcutaneous Administration of Minipigs [By (b) (4) Study No. 11803.01.01]

This study evaluated the PK profile of TI vs. regular and fast acting (Humalog) insulin, via SC injection to male pigs. Blood samples were collected for insulin and PDKP analysis at 0, 5, 10, 15, 20, 30, 40, 60, 90 min and at 2, 4, 6 hrs after dosing. Mortality and clinical observations were also recorded.

Group Number	Number of Animals	Test Article	Dose Volume (mL/kg)	Dose Level (U/kg)
1	4	Regular Insulin	0.0125	0.125
2	4	Humalog® Insulin	0.0125	0.125
3	4	Day 1: Technosphere®/Insulin (suspension) Day 3: Technosphere®/Insulin (solution)	0.0125	0.125

Findings: No clinical signs of toxicity or hypoglycemia were observed in all treatment groups. No treatment-related change in body weight was seen during dosing period (Days 1-3). For glucose levels, SC administration of 0.125 U/kg TI as a suspension (2/4 pigs) or a solution (3/4 pigs) was associated with slightly less consistent insulin responses and less steep drops in glucose value than those observed for insulin responses with SC administration of 0.125 U/kg regular insulin or Humalog. A trend for glucose levels to remain lower than baseline values at the 120, 240, and 360 min was seen for pigs given TI, but not for pigs given regular insulin or Humalog. The trend for continued lower glucose levels seen in pigs given TI was considered a potential test article related effect. In addition, no definitive between-group differences in glucose responses were seen between two TI formulations (solution or suspension), although a slightly higher incidence (3/4 vs. 2/4) of pigs with insulin responses was observed with the solution formulation than with the suspension formulation.

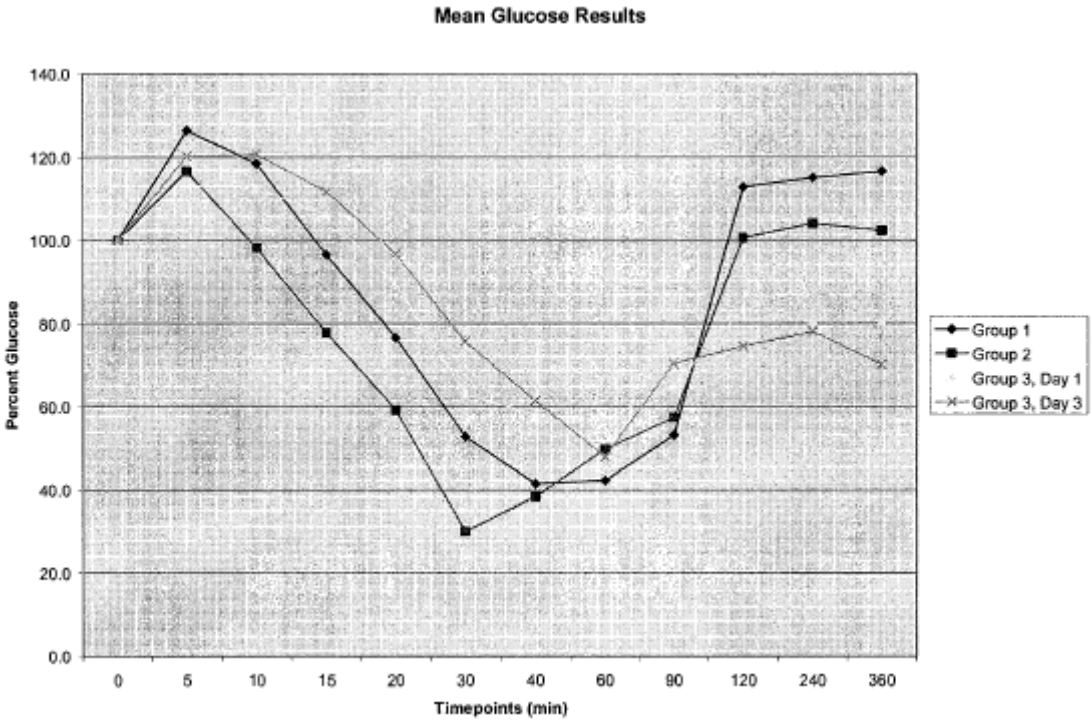


TABLE 1. Insulin Mean Plasma Toxicokinetic Metrics Following a Single Subcutaneous Injection of Regular Insulin, Humalog® Insulin and Technosphere®/Insulin to Male Gottingen Minipigs®

	Grp. 1 Regular Insulin	Grp. 2 Humalog® Insulin	Grp. 3 T/I Suspension	Grp. 4 T/I Solution
C_{max} (µU/mL)	115.00	86.17	85.00	106.25
t_{max} (min)	13.75	13.75	16.25	20.00
AUC_{last} (µU*min/mL)	10,150.62	4,516.12	11,836.25	16,866.25
AUC_{inf} (µU*min/mL)	15,237.05	6,147.99	18,407.82	26,582.30
$t_{1/2}$ (min)	187.30	194.50	208.71	222.85

TABLE 3. FDKP Mean Plasma Toxicokinetic Metrics Following a Single Subcutaneous Injection of Technosphere®/Insulin Suspension and Solution to Male Gottingen Minipigs®

	Grp. 3 T/I Suspension	Grp. 4 T/I Solution
C_{max} (µg/mL)	0.17	0.15
t_{max} (min)	35.00	25.00
AUC_{last} (µg*min/mL)	15.25	14.46
AUC_{inf} (µg*min/mL)	20.78	17.80
$t_{1/2}$ (min)	206.69	147.28

FIGURE 1. Insulin Mean Plasma Concentrations Following a Single Subcutaneous Injection of Regular Insulin, Humalog® Insulin and Technosphere®/Insulin to Male Gottingen Minipigs®

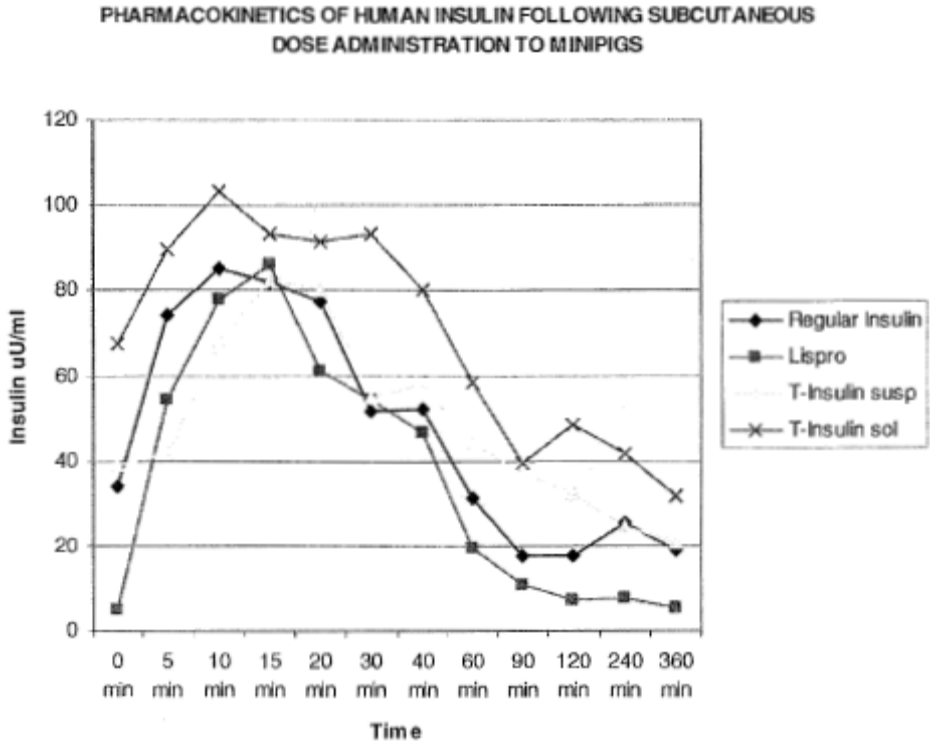
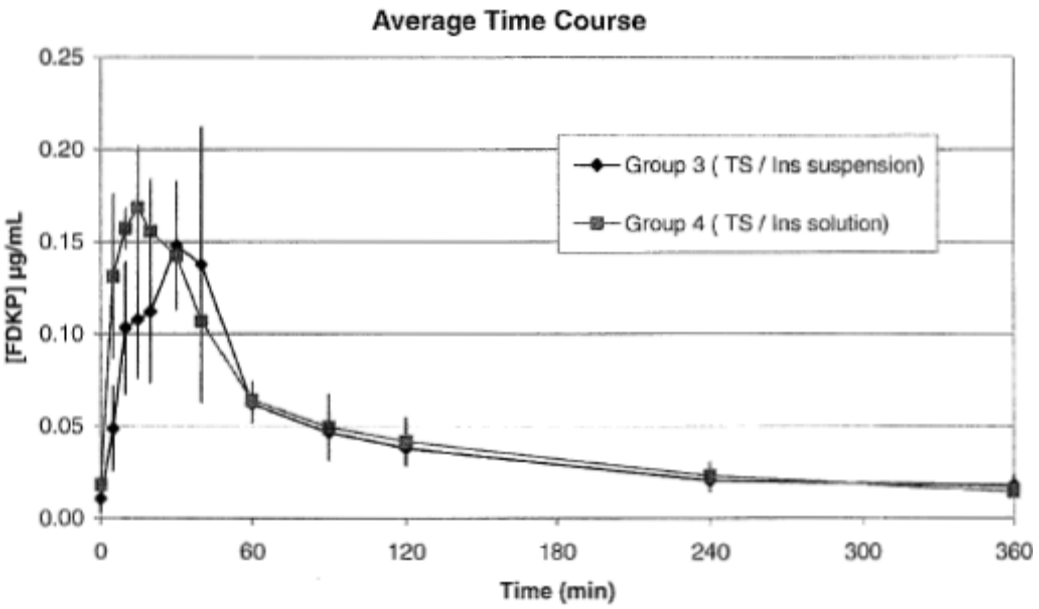


FIGURE 2. FDKP Mean Plasma Concentrations Following a Single Subcutaneous Injection of Technosphere®/Insulin Suspension and Solution to Male Gottingen Minipigs®



MKC-PC-2006-0034: Absorption, Metabolism, and Elimination Study of ^{14}C (Lysine Arm Labeled) FDKP Following a Single IV Administration in CRL:CD(SD) Rats [By (b) (4) Study No. N107962A]

The absorption, metabolism, and elimination of ^{14}C FDKP was examined following a single IV dose of ^{14}C FDKP to male and female Spraque-Dawley rats. The target dose level of the FDKP was 10 mg/kg with a target radioactivity (^{14}C-FDKP) dose of 200 µCi/ml for the Excreta group and 50 µCi/ml for the PK group. Urine, cage rinse, bile, feces (Excreta group) and blood (PK group) were collected at the protocol-specified times from each animal. Metabolic profiles were generated for selected urine, bile, and plasma samples when a concentration of radioactivity was sufficient to generate an adequate metabolic radiochromatographic profile.

Study Design – Core Study Animals

Group-Dose Route	Target Dose FDKP/Insulin[a] (mg/kg)	Target Concentration FDKP/Insulin[b] (mg/mL)	Dose Volume (mL/kg)	Animal ID Numbers[c]	
				Males	Females
1. IV PK	10	2	5	101-118	151-168
2. IV excreta	10	2	5	201-205	251-255

[a] The target radioactivity dose was 1 mCi/kg for the excreta group animals and 250 µCi/kg for the PK group animals.
[b] The target radioactivity concentration was 200 µCi/mL for the excreta group animals and 50 µCi/mL for the PK group animals.
[c] 5 rats/sex were used for bile/feces/urine collection through 96 hours post-dose. 18 rats per sex were used for repeated blood draws (maximum 3 blood draws/rat) for the PK sampling

Findings: the majority of the total radioactivity for male and female rats was excreted in the urine. The % administered dose recovered in the urine was 82.0±6.7% in males and 94.4±7.4% in females. Greater than 90% of the radioactivity found in the male and female rat urine was eliminated during the 0-6 hr collection period with the exception of one male (> 90% of

radioactivity excreted in 0-12 hrs) and two females (>90% of radioactivity excreted during the 6-12 hr collection period). The % administered excreted in the bile and feces was approx. 5-6% and 1-2% of the total radioactivity administered. Greater than 90% of the radioactivity was found in bile collected during the 0-3 hr collection period and in feces collected up to 72 hrs. These findings suggested that the major pathway of elimination occurred by the kidney with greater than 90% of the elimination radioactivity recovered in the urine. The liver represented a minor pathway of elimination as the percent of elimination radioactivity was low in the bile and feces.

In addition, there was no evidence that FDKP was metabolized as there were no new peaks in the metabolic profiles. This finding indicated that FDKP was eliminated unchanged. There was no evidence that FDKP was metabolized extensively as there were no peaks in the metabolic profiles that suggested even minor metabolites were found. Moreover, the ^{14}C FDKP plasma radioactive equivalent concentrations vs. time curves were biphasic, indicating that there was a distribution phase that preceded the elimination phase. Peak concentration at a 10 mg/kg FDKP target dose was 20.0 µg-eq/ml for males and 20.5 µg-eq/ml for females. The ½ life was 6.27 hrs and clearance value was 730 ml/hg/kg. Lastly, the findings of this study using FDKP alone were very similar to the findings with FDKP+insulin, taking into account of the 10 vs. 1 mg/kg difference. The 10X difference had no effect on clearance or ½ life but did result in a dose-proportional increase in C_{max} and AUC.

Table 11. Summary of PK Parameters Following a Single IV Administration[a]

Sex	Observed C_{max}[b] (ng/mL)	Elimination Rate Constant (hr^{-1})	Elimination Half-Life (hr)	CL-pred[c] (mL/hr/kg)	V_d-pred[c] (mL/kg)	AUC_{last} (ng/mL·hr)	Predicted AUC_{∞} (ng/mL·hr)
Males[d]	20000	0.165	4.21	700	4250	14200	14300
Females	20500	0.111	6.27	730	6590	13400	13700

a. Values are reported to three significant figures.
b. C_{max} was an observed concentration at the first time point (0.167 hours). Units could also be expressed as ng-eq/mL.
c. Clearance observed and V_d observed values were similar or the same.
d. Calculated parameters are not considered reliable since the best fit line for the terminal linear phase was 0.57, which was not within the acceptance criteria (0.85).

MKC-PC-2006-0035: Absorption, Metabolism, and Elimination Study of ^{14}C (Lysine Arm Labeled) FDKP/Insulin Following a Single IV Administration in CRL:CD(SD) Rats [By (b) (4) Study No. N107962B]

The absorption, metabolism, and elimination of ^{14}C FDKP was examined following a single IV dose of ^{14}C-FDKP/insulin to male and female Spraque-Dawley rats. The target dose level of the FDKP/insulin was 1/0.13 mg/kg (FDKP/insulin), with a target radioactivity (^{14}C-FDKP) dose of 100 µCi/ml and the target radioactivity concentration dose of 20 µCi/ml. Urine, cage rinse, bile, feces (Excreta group) and blood (PK group) were collected at the protocol-specified times from each animal. Metabolic profiles were generated for selected urine, bile, and plasma samples when a concentration of radioactivity was sufficient to generate an adequate metabolic radiochromatographic profile.

Study Design – Core Study Animals

Group-Dose Route	Target Dose FDKP/Insulin [a] (mg/kg)	Target Concentration FDKP/Insulin [b] (mg/mL)	Dose Volume (mL/kg)	Animal ID Numbers [c]	
				Males	Females
1 - IV PK	1/0.13	0.2/0.026	5	301-318	351-368
2 - IV excreta	1/0.13	0.2/0.026	5	401-405	451-455

a. The target radioactive dose was 100 µCi of ^{14}C-FDKP/kg.
b. The target radioactive concentration was 20 µCi/mL.
c. Five rats/sex were used for bile/feces/urine collection through 72 hours post-dose. 18 rats per sex were used for repeated blood draws (maximum three blood draws/rat) for the PK sampling.

Findings: The majority of the total radioactivity for male and female rats was excreted in the urine. The % administered dose recovered in the urine was 83.5±7.3% in males and 87.7±14.8% in females. Greater than 95% of the radioactivity found in the male and female rat urine was eliminated during the 1st 6 hrs after dose administrations. The % administered excreted in the bile and feces was approx. 3-6% and 2-3% of the total radioactivity administered. Greater than 90% of the radioactivity was found in the bile collected during the 0-3 hr collection period and in the feces collected during 48 hrs. These findings suggested that the major pathway of elimination occurred by the kidney with greater than 90% of the elimination radioactivity recovered in the urine. The liver represented a minor pathway of elimination as the percent of elimination radioactivity was low in the bile and feces.

In addition, there was no evidence that FDKP was metabolized as there were no new peaks in the metabolic profiles. This finding indicated that FDKP was eliminated unchanged. There was no evidence that FDKP was metabolized extensively as there were no peaks in the metabolic profiles that suggested even minor metabolites were found. Moreover, the ^{14}C-FDKP plasma radioactive equivalent concentrations vs. time curves were biphasic, indicating that there was a distribution phase that preceded the elimination phase. Peak concentration at a 1 mg/kg FDKP target dose were 1.66 µg-eq/ml for males and 1.52 µg-eq/ml for females. The elimination ½ life was 4.87(M)/4.15(F) hrs and clearance value was 839(M)/985(F) ml/hg/kg. There was no evidence of a gender-related effect. Lastly, the PK findings of this study using FDKP alone were very similar to the findings with FDKP+insulin, taking into account of the 10 vs. 1 mg/kg difference. The 10X difference had no effect on clearance or ½ life but did result in a dose-proportional increase in C_{max} and AUC.

Table 11. Summary of PK Parameters Following a Single IV Administration[a]

Sex	Observed C_{max}[b] (ng/mL)	Elimination Rate Constant (hr^{-1})	Elimination Half-Life (hr)	CL_pred[c] (mL/hr/kg)	V_d_Pred[c] (mL/kg)	AUC_{last} (ng/mL·hr)	Pred. AUC_{∞} (ng/mL·hr)
Males	1660	0.142	4.87	839	5890	1180	1190
Females	1520	0.167	4.15	985	5890	1000	1015

a. Values are reported to three significant figures.
b. C_{max} was an observed concentration at the first time point (0.167 hours). Units could also be presented as ng-eq/mL.
c. Clearance observed and V_d observed values were the same or similar.

MKC-PC-2006-0036: Absorption, Metabolism, and Elimination Study of ^{14}C (Lysine Arm Labeled) FDKP Following a Single IV Dose in Beagle Dogs [By (b) (4) Study No. N107963]

The absorption, metabolism, and elimination of ^{14}C FDKP was examined following a single IV dose of ^{14}C-FDKP to male and female Beagle dogs. The target dose level of the FDKP was 3 mg/kg with a target radioactivity (^{14}C-FDKP) dose of 75 µCi/Kg. Urine, cage rinse, bile, feces, and blood were collected at the protocol-specified times from each animal. Metabolic profiles were generated for selected urine, bile, and plasma samples when a concentration of radioactivity was sufficient to generate an adequate metabolic radiochromatographic profile.

Text Table 1. Study Design – Single Intravenous Dose Administration

Dose Route	Target Dose FDKP (mg/kg)[a]	Target Concentration FDKP[b] (mg/mL)	Dose Volume (mL/kg)	Animal ID Numbers	
				Males	Females
1. Intravenous	3	0.6	5	101, 103, 105	151, 153, 155

a. The target radioactive dose was 75 µCi of ^{14}C-FDKP/kg.
b. The target radioactive concentration was 15.0 µCi/mL.

Findings: the majority of the total radioactivity for male and female dogs was excreted in the urine. The % administered dose recovered in the urine was 96.8±2% in males and 94.8±2.8% in females. Greater than 95% of the radioactivity found in the male and female dog urine was eliminated during the 1st 12 hrs after dose administration. The % administered recovered in the bile was 0.7±0.2% in males and 0.5±0.3% in females. Greater than 95% of the radioactivity found in the male and female dog bile was eliminated during the 1st 6 and 12 hrs after dose administration. The % administered recovered in the feces was 0.3±0.2% in males and 0.7±0.4% in females. Greater than 95% of the radioactivity found in the male and female dog feces was eliminated during the 1st 84hrs after dose administration.

In addition, the urine, bile, and plasma metabolic profiles showed that FDKP was eliminated unchanged. There was no evidence that FDKP was metabolized extensively as there were no new peaks in the metabolic profiles that suggested even minor metabolites were formed. Moreover, the ^{14}C-FDKP plasma concentration vs. time profiles was biphasic. The time-dependent PK parameters, such as ½ life and clearance, showed that FDKP underwent rapid phase elimination. The ½ life values for this phase were 0.639±0.024 hrs for the males and 0.804±0.095 hrs for the females. On the other hand, the ½ life values for the slow phase elimination ere approx. 6 hrs for the males and 7 hrs for the females. Clearance values were approx. 270 ml/hr/kg for the males and 200 mg/hr/kg for the females. This was in agreement with the GFR for dogs (368 ml/hr/kg), indicating the kidney had a major role in clearing the drug. Furthermore, observed C_{max} values were 11.1±1.1 and 12.7±1.1 µg/ml for the males and females respectively, whereas ACU_{inf} values were 10400±1200 and 14300±2500 ng/ml•hr for the males and females respectively. There was no evidence of any sex differences.

Table 11. Summary of PK Parameters Following a Single IV Administration[a]

Sex	Animal ID	Observed C_{max} (ng/mL)	Elimination Rate Constant (hr^{-1})	Elimination Half-Life (hr)	Predicted Clearance (mL/hr/kg)	Predicted V_d (mL/kg)	AUC_{last} (hr*ng/mL)	Predicted AUC_{inf} (hr*ng/mL)
				Fast Elimination Phase				
Male	101R	9230	1.14	0.610	311	274	9000	9000
	103R	11200	1.12	0.619	287	256	9530	9530
	105	13000	1.01	0.687	224	222	12800	12800
	Mean	**11100**	**1.09**	**0.639**	**274**	**250**	**10400**	**10400**
	SE	**1100**	**0.04**	**0.024**	**26**	**15**	**1200**	**1200**
				Slow Elimination Phase				
Male	101R	9230	0.0935	7.41	310	3320	9000	9030
	103R	11200	0.301	2.30	286	951	9530	9540
	105	13000	0.0895	7.75	222	2480	12800	12900
	Mean	**11100**	**0.161**	**5.82**	**273**	**2250**	**10400**	**10500**
	SE	**1100**	**0.070**	**1.76**	**26**	**690**	**1200**	**1200**
				Fast Elimination Phase				
Female	151R	13300	1.03	0.673	226	219	12100	12100
	153R	14300	0.701	0.989	144	206	19300	19300
	155	10500	0.923	0.751	234	254	11500	11500
	Mean	**12700**	**0.885**	**0.804**	**201**	**226**	**14300**	**14300**
	SE	**1140**	**0.097**	**0.095**	**29**	**14**	**2500**	**2500**
				Slow Elimination Phase				
Female	151R	13300	0.116	5.95	225	1930	12100	12100
	153R	14300	0.0798	8.69	142	1780	19300	19500
	155	10500	0.0974	7.12	233	2390	11500	11600
	Mean	**12700**	**0.0979**	**7.25**	**200**	**2040**	**14300**	**14400**
	SE	**1140**	**0.0106**	**0.79**	**29**	**180**	**2500**	**2600**

a. Values are reported to three significant figures.

MKC-PC-2007-0005: Absorption, Metabolism, and Elimination Study of ^{14}C (Lysine Arm Labeled) FDKP Following a Single IV Dose (IT, SC, or PO) in CRL:CD(SD) rats [By (b) (4) Study No. N107964A]

The absorption, metabolism, and elimination of ^{14}C FDKP was examined following a single intratracheal (IT), oral (PO), or subcutaneous (SC) administration of ^{14}C-FDKP dose to male and female Spraque-Dawley rats. The target dose level of the FDKP, following IT, PO, and SC dosing, was 50 mg/kg, with a target radioactivity (^{14}C-FDKP) dose of 100 µCi/Kg for Excreta group rats and 50 µCi/Kg for PK group rats. Urine, cage rinse, bile (not collected for SC animals), feces, and blood were collected at the protocol-specified times from each animal. Metabolic profiles were generated for selected urine, bile, and plasma samples when a concentration of radioactivity was sufficient to generate an adequate metabolic radiochromatographic profile.

Dose Route Group	Target Dose FDKP[a] (mg/kg)	Target Concentration FDKP[b] (mg/mL)	Dose Volume (mL/kg)	Animal ID Numbers[c]	
				Males	Females
IT (Excreta group)	50	50	1	540-544	585-589
IT (PK group)	50	50	1	506-520	556-570
PO (Excreta group)	50	10	5	701-705	590-594[d]
PO (PK group)	50	10	5	706-720	756-770
SC (Excreta group)	50	10	5	640-644	680-684
SC (PK group)	50	10	5	606-620	656-670

a. The target radioactive dose was 100 µCi/kg for the excreta group animals and 50 µCi/kg for the PK group animals.
b. The target radioactive concentration was 250 µCi/mL (IT group females), 333 µCi/mL (IT group males) or 20 µCi/mL (PO and SC groups) for the excreta group animals and 125 µCi/mL (IT group females), 167 µCi/mL (IT group males) or 10 µCi/mL (PO and SC groups) for the PK group animals.
c. Five rats/sex were used for bile/feces/urine collection through 96 hours post-dose; 15 rats/sex were used for repeated blood draws (maximum three draws/ rat) for the PK sampling.
d. Low mass balance results required a second set of female rats to be tested.

Findings: **Feces:** For IT and PO groups, the majority of the total radioactivity for male and female rats was excreted in the feces. The % administered dose recovered in the feces from IT group rats was 85.3±18.5% in males and 72.2±16.9% in females. The % of administered dose recovered in the feces from PO group rats was 87.1±11.2% in males and 96.1±2.9% in females. For most animals in the IT and PO dose groups, greater than 95% of the radioactivity found in the male and female rat urine was eliminated by 48 hrs after dose administration. The % administered recovered in the feces from SC group rats was 13.3±4.8% in males and 16.5±7.5% in females. For most animals that received a SC dose, greater than 95% of the radioactivity found in feces was eliminated during the 1st 36 hrs after dose administration. **Urine:** For IT and PO groups, the minor of the total radioactivity was excreted in the urine. The % administered recovered in the urine from IT group rats was 6.42±2.30% in males and 12.3±10.9% in females. For most animals in IT group, greater than 95% of the radioactivity found in the male and female rats was eliminated during the 1st 48 hrs after dose administration. The % administered recovered in the urine from PO group rats was 4.83±3.11% in males and 2.30±1.09% in females. For most animals in PO group, greater than 95% of the radioactivity found in the male and female rats was eliminated by 60 hrs after dose administration. The % administered recovered in the urine from SC group rats was 83.2±14.9% in males and 83.4±9.5% in females. For most animals that received a SC dose, greater than 95% of the radioactivity found in urine was eliminated during the 1st 6 hrs after dose administration. **Bile:** The % administered recovered in the bile from IT group rats was 0.419±0.142% in males and 0.436±0.227% in females. For most animals in IT group, greater than 95% of the radioactivity found in bile was eliminated during the 1st 36 hrs after dose administration. The % administered recovered in the bile from PO group rats was 0.118±0.024% in males and 0.321±0.476% in females. For most animals in PO group, greater than 95% of the radioactivity found in the male and female rats was excreted by 60 hrs after dose administration. Therefore, based on these findings, the major pathway of elimination was GI tract following IT (equivocal) and PO administration and the kidneys following SC administration. Although the dose was administered intratracheally, the results indicated that the dose spilled over into the esophagus, thereby resulting in poor absorption and elimination by the GI tract.

In addition, the metabolic profiles of urine from the IT, PO, and SC rats, and bile from PO rats showed that FDKP was eliminated unchanged. There was no evidence that FDKP was

metabolized extensively as there were no new peaks in the metabolic profiles that suggested even minor metabolites were formed.

Moreover, for the IT dose group, the ^{14}C-FDKP plasma radioactivity equivalents concentration vs. time curves had highly variable absorption and elimination phase that included multiple peaks and sawtooth appearances. Tmax occurred at 1 hr for the males and females. The elimination ½ life values were 4.12(M)/2.74(F) hrs, and clearance values were 35.2(M)/26.8(F) ml/hr/kg. Systemic exposure PK parameters (C_{max} and AUC) were similar for males and females. Peak concentrations were 0.274(M)/0.455(F) µg-eq/ml. AUC∞ values were 1.42(M)/1.86(F) µg/ml•hr. There was no evidence for any sex differences. For the PO dose group, the ^{14}C-FDKP plasma radioactivity equivalents concentration vs. time profiles had a sawtooth appearance in the absorption phase and a flat appearance in the terminal linear phases that precluded any PK analysis, except for the observed PK endpoints. T_{max} and C_{max} were similar for box sexes and the values were approx. 1 hr and 0.440 µg-eq/ml, respectively. For the SC dose group, the ^{14}C-FDKP plasma radioactivity equivalents concentrations vs. time profiles were well-defined. T_{max} and ½ life values were 0.5 and 0.7 hrs, respectively for box sexes. Clearance values were 762(M)/727(F) ml/hr/kg. Peak concentrations (C_{max}) were 47.5(M)/47.8(F) µg-eq/ml. AUC∞ values were 65.6(M)/68.7(F) µg/ml•hr. There was no evidence for any sex differences. All in all, the SC route of administration compared to the IT and PO routes produced an earlier T_{max} and higher systemic exposure (C_{max} and AUC with > 10X). These findings were likely due to the poor absorption by the PO route.

Summary of PK Parameters Following a Single Administration, IT[a,b]

Sex	C_{max}[c] (ng/mL)	T_{max} (hr)	Elimination Rate Constant (hr^{-1})	Elimination Half-Life (hr)	Cl-F Predicted[d] (mL/hr/kg)	V_d-F Predicted[d] (mL/kg)	AUC_{last} (ng/mL·hr)	Pred. AUC_∞ (ng/mL·hr)
With Outliers								
Males	2930	0.760	0.444	1.56	13300	29900	3660	3760
Females	5080	0.167	0.253	2.74	16200	64000	2800	3100
Without Outliers								
Males	274	1.00	0.168	4.12	35200	209000	1020	1420
Females	455	1.00	0.253	2.74	26800	106000	1580	1860

a. Values are reported to three significant figures.
b. Values are reported with and without apparent outliers (see text).
c. Units could also be reported as ng-eq/mL.
d. Apparent clearance and V_d observed values were similar.

Summary of PK Parameters Following a Single Administration, PO[a,b]

Sex	C_{max}[c] (ng/mL)	T_{max} (hr)	Elimination Rate Constant (hr^{-1})	Elimination Half-Life (hr)	Cl-F Predicted[d] (mL/hr/kg)	V_d-F Predicted[d] (mL/kg)	AUC_{last} (ng/mL·hr)	Pred. AUC_∞ (ng/mL·hr)
Males	444	1.00	[0.0608]	[11.4]	[13500]	[223000]	[1390]	[3690]
Females	443	1.00	[0.0606]	[11.4]	[12600]	[209000]	[1580]	[3950]

a. Values are reported to three significant figures.
b. Bracketed values are suspect due to poorly defined terminal linear phase. See text.
c. Units could also be reported as ng-eq/mL.
d. Apparent clearance and V_d observed values were similar.

Summary of PK Parameters Following a Single Administration, SC[a]

Sex	C_{max} (ng/mL)[b]	T_{max} (hr)	Elimination Rate Constant (hr^{-1})	Elimination Half-Life (hr)	Cl-F Predicted[c] (mL/hr/kg)	Vd-F Predicted[c] (mL/kg)	AUC_{last} (ng/mL·hr)	Pred. AUC_{∞} (ng/mL·hr)
Male	47500	0.500	0.991	0.699	762	769	65600	65600
Female	47800	0.500	0.949	0.730	727	766	68700	68700

a. Values are reported to three significant figures.
b. Units could also be reported as ng-eq/mL.
c. Apparent clearance and V_d observed values were similar.

MKC-PC-2007-0009: Absorption, Metabolism, and Elimination Study of ^{14}C (Lysine Arm Labeled) FDKP Following a Single Dose (IT, SC, or PO) in Beagle Dogs [By (b) (4) Study No. N107965A]

The absorption, metabolism, and elimination of ^{14}C FDKP was examined following a single intratracheal (IT), oral (PO), or subcutaneous (SC) administration of ^{14}C-FDKP dose to male and female dogs. The target dose level of the FDKP, following IT, PO, and SC dosing was 50 mg/kg, with a target radioactivity (^{14}C-FDKP) dose of 50 µCi/Kg. Urine, cage rinse, bile, feces, and blood were collected at the protocol-specified times from each animal. Metabolic profiles were generated for selected urine, bile, and plasma samples when a concentration of radioactivity was sufficient to generate an adequate metabolic radiochromatographic profile.

Dose Route	Target Dose FDKP[a] (mg/kg)	Target Concentration FDKP[b] (mg/mL)	Dose Volume (mL/kg)	Animal ID Numbers	
				Males	Females
IT	50	50	1	201-203	251-253
PO	50	5	10	401-403	451-453
SC	50	50	1	301-303	351-353

a. The target radioactive dose was 50 µCi/kg.
b. The target radioactive concentration was 50 µCi/mL.

Findings: **Urine:** For IT and SC groups, the majority of the total radioactivity for male and female rats was excreted in the urine. The % administered dose recovered in the urine from IT group dogs was 80.2±21.8% in males and 68.9±11.6% in females. Greater than 89% of the radioactivity found in the urine was eliminated within 24 (M) and 36 (F) hrs after IT dose administration. The % of administered dose recovered in the urine from SC group dogs was 89.1±5.0% in males and 97.7±8.1% in females. Greater than 80% of the radioactivity found in the urine was eliminated during the 1st 18 hrs after dose administration. On the contrary, urine was a minor excretory route for PO group dogs. The % administered recovered in the urine from PO group dogs was 23.8±21.3% in males and 7.31±3.99% in females. Greater than 90% of the radioactivity found in feces was eliminated within 24-36 hrs after dose administration. **Feces:** For IT and SC groups, the minor of the total radioactivity was excreted in the feces. The % administered recovered in the feces from IT group rats was 16.3±22.0% in males and 18.9±9.2% in females. Greater than 93% of the radioactivity found in the feces of male and female dogs was eliminated within 84 hrs after dose administration. The % administered recovered in the urine from SC group dogs was 2.27±0.89% in males and 1.37±0.41% in females. Greater than 84% of the radioactivity found in the feces of male and female dogs was eliminated within 48 hrs after dose administration. On the other hand, feces were the major elimination route for the PO dose

group. The % administered recovered in the feces from PO group rats was 63.0±27.9% in males and 99.2±10.2% in females. Greater than 92% of the radioactivity found in feces was eliminated during the 1st 36 hrs after dose administration. **Bile:** Only traces of radioactivity were recovered in the bile for males (0.105±0.056%) and females (0.0411±0.0211%) that received an IT dose. Similarly, only a minimal % of the total radioactivity was excreted in the bile (approx. 0.06 and 0.04% of the administered PO dose was recovered in the male and female dog bile, respectively).

In addition, the urine metabolic profiles from the IT, PO, and SC groups indicated that FDKP was eliminated unchanged. The feces water extracts metabolic profiles from selected dog receiving PO dose indicated that FDKP was also eliminated unchanged. There was no evidence that FDKP was metabolized extensively as there were no new peaks in the metabolic profiles that suggested even minor metabolites were formed.

Moreover, for the IT, PO, or SC dose groups, the ^{14}C-FDKP plasma radioactivity equivalents concentration vs. time profiles had well-defined absorption, peak concentration, and elimination phases. Based on T_{max} values, absorption occurred faster following by the IT (0.75 and 0.92 hrs) and SC (1.6 and 1.3 hrs) routes than by the PO (2.3 and 1.7 hrs) route. IT route achieved an earlier T_{max} than SC route. Thus, the IT route of administration provided the fastest achievement of a max plasma concentration, although the differences following a SC administration was not clearly biologically relevant. Regardless route of administration, ½ life values were similar. This was expected since the dosage was the same for all routes and systemic concentrations achieved following each route did not culminate in rate limited elimination. Clearance values following PO administration differed by approx. an order of magnitude to the IT and SC groups. However, a direct comparison of clearance by route of administration was not possible unless the fraction absorbed following each route was known, since clearance was dependent on fraction absorbed for non-IV routes of administration.

Lastly, systemic exposure was greater following IT and SC administration than following PO administration. Based on C_{max} values, the highest levels were achieved after a SC administration (31.7 and 29.2 µg-eq/ml). The IT administration produced C_{max} values about ½ as much (16.3 and 15.4 µg-eq/ml) but the actual administrated dose was lower than what was administered SC. The lowest C_{max} values were obtained after the PO administration (4.52 and 1.73 µg-eq/ml), which indicated poorer absorption occurred by this route. The relative degree of systemic exposure among the different routs was supported by the AUC∞ estimates (IT: 90.5 and 87.2, PO: 37.5 and 15.7, and SC: 224 and 232 hr•µg/ml for males and females respectively). However, such estimates indicated a much larger systemic exposure occurred after the SC administration and the relative difference could be biological relevant.

Summary of PK Parameters Following a Single Administration, IT[a]

Gender	Animal ID	Observed C_{max} (ng/mL)[c]	Observed T_{max} (hrs)	Elimination Half-Life (hr)	Cl_F Predicted[b] (mL/hr/kg)	Vd_F Predicted[b] (mL/kg)	AUC_{last} (hr·ng/mL)	Predicted AUC_{∞} (hr·ng/mL)
Male	201	15400	0.750	4.52	316	2060	122000	125000
	202	15300	0.750	5.24	378	2860	69700	71400
	203	18300	0.750	5.03	647	4700	73400	75200
	Mean	**16300**	**0.750**	**4.93**	**447**	**3210**	**88300**	**90500**
	SE	**1000**	**0.000**	**0.21**	**101**	**780**	**16800**	**17300**
Female	251	16600	0.750	3.60	530	2750	58300	58900
	252	9950	1.00	4.85	548	3830	53800	55100
	253	19600	1.00	4.31	346	2150	145000	148000
	Mean	**15400**	**0.917**	**4.25**	**475**	**2910**	**85500**	**87200**
	SE	**2900**	**0.083**	**0.36**	**65**	**490**	**29500**	**30300**

a. Values are reported to three significant figures.
b. Apparent clearance and V_d observed values were similar.
c. Units could also be presented as ng-eq/mL.

Summary of PK Parameters Following a Single Administration, PO[a]

Gender	Animal ID	Observed C_{max} (ng/mL)[b]	Observed T_{max} (hrs)	Elimination Half-Life (hr)	Cl_F Predicted[c] (mL/hr/kg)	Vd_F Predicted[c] (mL/kg)	AUC_{last} (ng/mL·kg)	Predicted AUC_{∞} (hr·ng/mL)
Male	401	1020	3.00	9.18	4080	40200	10300	12000
	402	8360	2.00	5.06	631	17900	72500	76100
	403	4170	2.00	8.44	1970	54000	22300	24400
	Mean	**4520**	**2.33**	**7.56**	**2230**	**27500**	**35000**	**37500**
	SE	**2130**	**0.33**	**1.27**	**1000**	**14400**	**19000**	**19600**
Female	451	804	2.00	6.45	4010	37300	10900	12000
	452	2750	2.00	3.82	1980	10900	24300	24700
	453[d]	1640	1.00	10.8	4640	72600	8200	10500
	Mean	**1730**	**1.67**	**7.03**	**3540**	**40200**	**14500**	**15700**
	SE	**560**	**0.33**	**2.05**	**800**	**17900**	**5000**	**4500**

a. Values are reported to three significant figures.
b. Units could also be presented as ng-eq/mL.
c. Apparent clearance and V_d observed values were similar.
d. R^2 value was 0.81. If a user-defined terminal linear phase (R^2=0.99) was used, which involved omitting the 24 hour concentration time point, then the PK parameters that were affected for Animal 453 were: elimination half-life = 5.5 hours; apparent clearance = 5690 mL/hr/kg; apparent V_d = 45100 mL/kg; and AUC_{∞} = 8560 ng/mL·hr.

Summary of PK Parameters Following a Single Administration, SC[a]

Gender	Animal ID	Observed C_{max} (ng/mL)[b]	Observed T_{max} (hrs)	Elimination Half-Life (hr)	Cl_F Predicted[c] (mL/hr/kg)	Vd_F Predicted[c] (mL/kg)	AUC_{last} (ng/mL·hr)	Predicted AUC_{∞} (hr·ng/mL)
Male	301	31600	2.00	4.57	209	1380	220000	225000
	302	33800	2.00	6.21	194	1740	228000	241000
	303	29600	0.750	6.53	228	2150	194000	205000
	Mean	**31700**	**1.58**	**5.77**	**210**	**1760**	**214000**	**224000**
	SE	**1200**	**0.42**	**0.61**	**10**	**220**	**10000**	**10000**
Female	351	33400	1.00	6.58	164	1550	265000	284000
	352	25600	1.00	7.64	249	2750	173000	189000
	353	28600	2.00	3.71	210	1120	220000	223000
	Mean	**29200**	**1.33**	**5.98**	**208**	**1810**	**220000**	**232000**
	SE	**2300**	**0.33**	**1.17**	**25**	**490**	**27000**	**28000**

a. Values are reported to three significant figures.
b. Units could also be presented as ng-eq/mL.
c. Apparent clearance and V_d observed values were similar.

MKC-PC-2007-0010: Absorption, Metabolism, and Elimination Study of ^{14}C (Lysine Arm Labeled) FDKP/Insulin Following a Single IV Dose (IT or PO) in CRL:CD(SD) Rats [By (b) (4) Study No. N107964B]

The absorption, metabolism, and elimination of ^{14}C FDKP was examined following a single intratracheal (IT) administration of ^{14}C-FDKP/insulin dose to male and female Spraque-Dawley rats. The target dose level of the FDKP/insulin was 1.1/0.125 mg/kg, with a target radioactivity (^{14}C-FDKP) at 100 µCi/Kg for the Excreta group animals and 50 µCi/Kg for the PK group animals. Urine, cage rinse, bile, feces (Excreta group), and blood (PK group) were collected at the protocol-specified times from each animal. Metabolic profiles were generated for selected urine, bile, and plasma samples when a concentration of radioactivity was sufficient to generate an adequate metabolic radiochromatographic profile.

Study Design – Single Intratracheal Dose Administration

Group-Dose Route	Target Dose FDKP/Insulin[a] (mg/kg)	Target Concentration FDKP/Insulin[b] (mg/mL)	Dose Volume (mL/kg)	Animal ID Numbers[c]	
				Males	Females
1 - IT	1.1/0.125	1.1/0.125	1	801-820	851-870

a. The target radioactive dose was 100 µCi/kg for the Excreta group animals and 50 µCi/kg for the PK group animals.
b. The target radioactive concentration was 100 µCi/mL for the Excreta group animals and 50 µCi/mL for the PK group animals.
c. Five rats/sex were used for bile/feces/urine collection through 72 hours pre-dose; 15 rats per sex were used for repeated blood draws (maximum three draws/rat) for the pharmacokinetic sampling.

Findings: Based on the radioactivity recovery pattern following IT administration, it appeared that absorption did not occur via the lungs. The majority of the radioactivities for most animals were recovered in the feces and not in the urine or bile, indicating that the radioactivity recovery results were more typical of oral than pulmonary absorption. The % administered dose recovered in the feces was 55.3±17.9% in males and 70.6±29.1% in females. Greater than 90% of the radioactivity found in the male and female rat feces was eliminated within 48 hrs after dose administration. The % administered recovered in the urine was 6.0±2.1% in males and

16.2±4.4% in females. Greater than 90% of the radioactivity found in the male and female rat urine was eliminated within 48 hrs after dose administration. The % administered recovered in the bile was 0.755±0.551% in males and 0.793±0.607% in females. Greater than 90% of the radioactivity found in the male and female rat bile was eliminated within 60 hrs after dose administration.

In addition, the metabolic profiles showed that FDKP was eliminated unchanged. There was no evidence that FDKP was metabolized extensively as there were no new peaks in the metabolic profiles. Moreover, the ^{14}C-FDKP plasma radioactive equivalent concentration vs. time profiles had no absorption phase but had a sawtooth appearance. The latter concentration time points (≥ 4 hrs) allowed the terminal linear phases to be measured but the resulting PK parameters were questionable due to the uncertainty of the route of administration (PO vs. IT). The PK parameters for males and females were 96.3 and 83.4 ng-eq/ml (C_{max}), 1 hr (T_{max}), and 1.20 and 1.59 hrs (½ life), 4170 and 3410 ml/hr/kg (appearance clearance).

Summary of PK Parameters Following a Single IT Administration[a]

Sex	Observed C_{max}[b] (ng/mL)	Observed T_{max} (hr)	Elimination Rate Constant (hr^{-1})	Elimination Half-Life (hr)	CL_F Predicted[c] (mL/hr/kg)	V_d_F Predicted[c] (mL/kg)	AUC_{last} (ng/mL*hr)	Predicted AUC_{∞} (ng/mL*hr)
Male	96.3	1.00	0.575	1.20	4170	7250	257	264
Female	83.4	1.00	0.436	1.59	3410	7830	303	323

a. Values are reported to three significant figures.
b. Units could also be presented as ng-eq/mL.
c. Apparent Clearance and V_d observed values were similar.

MKC-PC-2007-0011: Absorption, Metabolism, and Elimination Study of ^{14}C (Fumaryl Arm Labeled) FDKP Following a Single IV Dose in Beagle Dogs [By (b) (4) Study No. N107967]

The absorption, metabolism, and elimination of ^{14}C (fumaryl arm labeled) FDKP was examined following a single IV administration of ^{14}C-FDKP dose to male and female Beagle dogs. The target dose level of the FDKP was 3 mg/kg, with a target radioactivity (^{14}C-FDKP) at 75 µCi/Kg. Urine, cage rinse, bile, feces, and blood were collected at the protocol-specified times from each animal. Metabolic profiles were generated for selected urine, bile and plasma samples when a concentration of radioactivity was sufficient to generate an adequate metabolic radiochromatographic profile.

Study Design – Single IV Dose Administration

Group-Dose Route	Target Dose FDKP[a] (mg/kg)	Target Concentration FDKP[b] (mg/mL)	Dose Volume (mL/kg)	Animal ID Numbers	
				Males	Females
1-Intravenous	3	0.6	5	101-103	151-153

a. The target radioactive dose was 75 µCi of ^{14}C-FDKP/kg.
b. The target radioactive concentration was 15.0 µCi/mL.

Findings: The majority of the total radioactivity for male and female dogs was excreted in the urine. The % administered dose recovered in the urine was 103±9% in males and 99.6±2.5% in females. Greater than 90% of the radioactivity found in the male and female dog urine was

eliminated 1st 12 hrs after dose administration. The % administered recovered in the bile was 0.4% in males and 0.2% in females. The % administered recovered in the feces was 0.4% in males and 0.5% in females. Greater than 84% and 83% of the radioactivity were found in the vile and feces collected 6 and 60 hrs after dose administration, respectively.

In addition, the metabolic profiles showed that FDKP was eliminated unchanged. There was no evidence that FDKP was metabolized extensively as there were no new peaks in the metabolic profiles. Moreover, the ^{14}C-FDKP plasma concentration vs. time profiles were biphasic. The time-dependent PK parameters, such as ½ life and clearance, showed that FDKP underwent rapid phase elimination. The ½ life values for this phase were 0.667±0.043 hrs for the males and 0.670±0.007 hrs for the females. Late phase elimination ½ life values approx. 6 hrs. However, for the slow (late) phase, the terminal linear portions of the profile were not characterized for the necessary number of ½ lives and the R^2 values were less than 0.85, suggesting that the PK parameters determined were from a poor regression. Clearance values were 342±32 ml/hr/kg for the males and 332±51 mg/hr/kg for the females, which were in close agreement with the GFR for dogs (368 ml/hr/kg), indicating kidney had a major role in clearing the drug. Furthermore, observed C_{max} values were 9.91±0.65and 12.1±1.3 µg/ml for the males and females respectively, whereas ACU_{inf} values were 9590±610 and 10500±1400 ng/ml•hr for the males and females respectively. However, to negate any effect of poor regression of the terminal linear phase, the observed AUC_{last} was used to evaluate systemic exposure. The AUC_{last} values were 9580±610 and 10500±1400 ng/ml•hr for the males and females, respectively. The AUC for the females were slightly larger than the males but the differences was not considered to be biologically relevant since the time-dependent PK parameters were similar. There was no evidence of any sex differences. Lastly, the PK parameter results for ^{14}C-fumaryl arm vs ^{14}C-lysine arm labeled FDKP study were similar.

Summary of PK Parameters Following a Single IV Administration[a]

Sex	Animal ID	Observed C_{max}[b] (ng/mL)	Elimination Rate Constant (hr^{-1})	Elimination Half-Life (hr)	Predicted Clearance (mL/hr/kg)	Predicted V_d (mL/kg)	AUC_{last} (ng/mL*hr)	Predicted AUC_∞ (ng/mL*hr)
Early Elimination Phase								
Male	101	10400	1.16	0.597	386	332	8730	8730
	102	10700	0.932	0.744	280	300	10800	10800
	103	8630	1.05	0.661	361	344	9240	9240
	Mean	**9910**	**1.05**	**0.667**	**342**	**326**	**9580**	**9580**
	SE	**650**	**0.07**	**0.043**	**32**	**13**	**610**	**610**
Late Elimination Phase[c]								
Male	101	--[d]	0.0944	7.34	385	4080	--[d]	--[d]
	102	--	0.149	4.66	279	1880	--	--
	103	--	0.109	6.35	361	3310	--	--
	Mean	--	**0.117**	**6.12**	**342**	**3090**	--	--
	SE	--	**0.016**	**0.78**	**32**	**650**	--	--

a. Values are reported to three significant figures.
b. Observed maximum concentration.
c. Characterized by a poor regression fit.
d. C_{max}, AUC_{last}, and AUC_∞ values are the same as reported for the early elimination phase.

Sex	Animal ID	Observed C_{max}[b] (ng/mL)	Elimination Rate Constant (hr^{-1})	Elimination Half-Life (hr)	Predicted Clearance (mL/hr/kg)	Predicted V_d (mL/kg)	AUC_{last} (ng/mL*hr)	Predicted AUC_∞ (ng/mL*hr)
Early Elimination Phase								
Female	151	9560	1.05	0.662	435	415	7700	7700
	152	12800	1.04	0.663	294	281	11100	11100
	153	13800	1.01	0.685	269	266	12500	12500
	Mean	**12100**	**1.03**	**0.670**	**333**	**321**	**10500**	**10500**
	SE	**1300**	**0.01**	**0.007**	**52**	**47**	**1400**	**1400**
Late Elimination Phase[c]								
Female	151	--[d]	0.0969	7.15	434	4480	--[d]	--[d]
	152	--	0.119	5.83	293	2470	--	--
	153	--	0.134	5.18	269	2010	--	--
	Mean	--	**0.116**	**6.06**	**332**	**2990**	--	--
	SE	--	**0.011**	**0.58**	**51**	**760**	--	--

a. Values are reported to three significant figures.
b. Observed maximum concentration.
c. Characterized by a poor regression fit.
d. C_{max}, AUC_{last}, and AUC_∞ values are the same as reported for the early elimination phase.

MKC-PC-2007-0012: Absorption, Metabolism, and Elimination Study of ^{14}C (Fumaryl Arm Labeled) FDKP/Insulin Following a Single IV Dose in CRL:CD(SD) [By (b) (4) Study No. N107966B]

The absorption, metabolism, and elimination of ^{14}C (fumaryl arm labeled) FDKP was examined following a single IV administration of ^{14}C-FDKP/insulin dose to male and female rats. The target dose level of the FDKP/insulin was 1/0.113 mg/kg, with a target radioactivity (^{14}C-FDKP) at 100 µCi/Kg for the Excreta group and PK group animals. All animals had jugular vein catheter for IV dosing, additionally Excreta group animals were bile-duct cannulated and PK group had femoral vein catheter (blood collection). Urine, cage rinse, bile, feces, and blood were collected at the protocol-specified times from each animal. Metabolic profiles were generated for selected urine, bile and plasma samples when a concentration of radioactivity was sufficient to generate an adequate metabolic radiochromatographic profile.

Study Design – Single IV Dose Administration

Group-Dose Route	Target Dose FDKP/Insulin[a] (mg/kg)	Target Concentration FDKP[b] (mg/mL)	Dose Volume (mL/kg)	Animal ID Numbers[c]	
				Males	Females
1-IV (Excreta group)	1/0.113	0.2/0.0226	5	201-205	251-255
2-IV (PK group)	1/0.113	0.2/0.0226	5	101-115	151-165

a. The target radioactive dose was 100 µCi/kg.
b. The target radioactive concentration was 20 µCi/mL.
c. Five rats/sex were used for bile/feces/urine collection through 96 hours post-dose; 15 rats/sex were used for repeated blood draws (maximum three draws/rat) for the PK sampling.

Findings: The majority of the total radioactivity for male and female dogs was excreted in the urine. The % administered dose recovered in the urine was 85.2±2.9% in males and 89.5±3.2% in females. Greater than 95% of the radioactivity found in the male and female rat urine was eliminated during the 1st 6 hrs after dose administration. The % administered recovered in the feces was 1.54±0.91% in males and 1.14±0.73% in females. Greater than 93% of the radioactivity was found in the feces collected 48 hrs after dose administration, respectively. The % administered recovered in the feces was 0.4% in males and 0.5% in females. The %

administered recovered in the bile was 4.81±2.95% in males and 6.11±3.23% in females. Greater than 98% of the radioactivity was eliminated during the 1st 6 hrs after dose administration.

In addition, the metabolic profiles showed that FDKP was eliminated unchanged. There was no evidence that FDKP was metabolized extensively as there were no new peaks in the metabolic profiles. The administration of insulin along with FDKP had no effect on metabolism and excretion of FDKP. Moreover, the ^{14}C-FDKP plasma radioactivity equivalents concentration vs. time curve was biphasic. Closer-selected time points were used to define the terminal linear phase since the WinNonlin algorithm did not make a distinction between the 2 phases. The peak concentrations at a 1 mg/kg FDKP target dose were 2.56 and 2.65 µg-eg/ml for males and females, respectively. The elimination ½ life and clearance values were similar between genders; 2.23 and 3.37 hrs and 625 and 611 ml/hr/kg for males and females, respectively. There was no evidence of a gender-related effect. Lastly, the findings of this study with FDKP/insulin were very similar the finings with FDKP alone, taking into account the 10 vs. 1 mg/kg dosage difference. The 10X difference had no effect on clearance or ½ life but did result in a dose proportional increase in C_{max} and AUC.

Summary of PK Parameters Following a Single IV Administration[a]

Gender	Observed C_{max}[b] (ng/mL)	Elimination Rate Constant (hr^{-1})	Elimination Half-Life (hr)	Cl_ pred[c] (mL/hr/kg)	V_d_pred[c] (mL/kg)	AUC_{last} (hr*ng/mL)	Predicted AUC_∞ (hr*ng/mL)
Male	2460	0.298	2.33	625	2100	1590	1600
Female	2650	0.206	3.37	611	2970	1630	1640

a. Values are reported to three significant figures.
b. C_{max} was an observed concentration at the first time point (0.167 hours).
c. Clearance observed and V_d observed values were the same or similar.

MKC-PC-2007-0013: Absorption, Metabolism, and Elimination Study of ^{14}C (Fumaryl Arm Labeled) FDKP Following a Single IV Dose in CRL:CD(SD) [By (b) (4) Study No. N107966A]

The absorption, metabolism, and elimination of ^{14}C (fumaryl arm labeled) FDKP was examined following a single IV administration of ^{14}C-FDKP dose to male and female rats. The target dose level of the FDKP/insulin was 10 mg/kg, with a target radioactivity (^{14}C-FDKP) of 1 µCi/Kg for the Excreta group animals and 500 µCi/Kg for PK group animals. Urine, cage rinse, bile, feces, and blood were collected at the protocol-specified times from each animal. Metabolic profiles were generated for selected urine, bile and plasma samples when a concentration of radioactivity was sufficient to generate an adequate metabolic radiochromatographic profile.

Study Design – Single IV Dose Administration

Group-Dose Route	Target Dose FDKP[a] (mg/kg)	Target Concentration FDKP[b] (mg/mL)	Dose Volume (mL/kg)	Animal ID Numbers[c]	
				Males	Females
IV (Excreta group)	10	2	5	201-205	251-255
IV (PK group)	10	2	5	101-115	151-165

a. The target radioactive dose was 1 mCi/kg for the Excreta group animals and 500 µCi/kg for the PK group animals.
b. The target radioactive concentration was 200 µCi/mL for the Excreta group animals and 100 µCi/mL for the PK group animals.
c. Five rats/sex were used for bile/feces/urine collection through 96 hours post-dose; 15 rats/sex were used for repeated blood draws (maximum three draws/rat) for the PK sampling.

Findings: The majority of the total radioactivity for male and female dogs was excreted in the urine. The % administered dose recovered in the urine was 94.7±6.2% in males and 92.9±4.3% in females. Greater than 95% of the radioactivity found in the male and female rat urine was eliminated during the 1st 12 hrs after dose administration. The % administered recovered in the feces was 1.32±1.05% in males and 1.62±1.54% in females. Greater than 91% of the radioactivity was found in the feces collected 72hrs after dose administration, respectively. The % administered recovered in the feces was 0.4% in males and 0.5% in females. The % administered recovered in the bile was 3.28±1.81% in males and 2.85±2.71% in females. Greater than 95% of the radioactivity was eliminated during the 1st 6 hrs after dose administration.

In addition, the metabolic profiles showed that FDKP was eliminated unchanged. There was no evidence that FDKP was metabolized extensively as there were no new peaks in the metabolic profiles. The administration of insulin along with FDKP had no effect on metabolism and excretion of FDKP. Moreover, the ^{14}C-FDKP plasma radioactivity equivalents concentration vs. time curves was biphasic. User-selected time points were used to define the terminal linear phase for the males (not the females) since the WinNonlin algorithm did not make a distinction between the 2 phases. The peak concentrations at a 10 mg/kg FDKP target dose were 22.7 and 25.9 µg-eg/ml for males and females, respectively. There was no evidence of a gender-related effect, except for longer elimination ½ life for males than females. Lastly, the findings of this study with FDKP alone were very similar the finings with FDKP insulin, taking into account the 1 vs. 10 mg/kg dosage differences. The 10X difference had no effect on clearance or ½ life but did result in a dose proportional increase in C_{max} and AUC.

Summary of PK Parameters Following a Single IV Administration[a]

Gender	Observed C_{max}[b] (ng/mL)	Elimination Rate Constant (hr^{-1})	Elimination Half-Life (hr)	Cl_pred[c] (mL/hr/kg)	V_d_pred[c] (mL/kg)	AUC_{last} (hr*ng/mL)	Predicted AUC_∞ (hr*ng/mL)
Male	22700	0.224	3.10	658	2940	15100	15200
Female	25900	0.531	1.30	615	1160	16200	16200

a. Values are reported to three significant figures.
b. C_{max} was an observed concentration at the first time point (0.167 hours). Units could also be expressed as ng-eq/mL
c. Clearance observed and V_d observed values were the same.

2.6.4.4 Distribution

PDC-PC-2002-0008 (memo to file: MKC-PC-2003-0007): PK, Tissue Distribution and Mass Balance of Radioactivity in Spraque-Dawley Rats Following a Single IT administration of Radiolabeled Technosphere®/Insulin [By (b) (4) Study No. 440035]

This study was to determine 1) the concentration and/or content of radioactivity in whole blood, urine, feces, cage wash, expired CO2, and carcass, and 2) the distribution in tissues, whole blood, and plasma, after a single IT administration of ^{14}C-Technosphere/^{3}H-insulin in rats, at a target dose level of 1.13 mg/kg (Groups 1 and 2) and 0,965 mg/kg (Groups 3 and 4) T and 0.113 mg/kg/ (Groups 1 and 2) and 0.097 mg/kg (Groups 3 and 4) insulin. For Groups 1 and 3 animals, blood samples were collected at selected time point prior to euthanasia. Following sacrifice, radioactivity concentration in tissues was obtained by quantitative whole-body autoradioluminography (QWBA). For Groups 2 and 4 animals, urine, feces, expired air, and cage rinses were collected over designated intervals up to 169 hrs post-dose. Carcasses were also solubilized in-toto at 168 hrs post dose for radioactivity concentration analysis. The radioactivity concentration in blood/plasma sample was measured.

The animals were assigned to study groups as follows:

Group No. Identification	Target Dose[a] (µCi/animal) from ^{14}C	Target Dose[a] (µCi/animal) from ^{3}H	Animal Numbers Males
1	10.525	130	1001-1033
2	10.525	130	2001-2005
3	8.2	101	3001-3016
4	8.2	101	4001-4003

[a] A target dose level of 0.113 mg/kg of Insulin and 1.13 mg/kg of Technosphere® were administered for Groups 1 and 2 animals and a target dose level of 0.097 mg/kg of Insulin and 0.965 mg/kg of Technosphere® were administered for Groups 3 and 4 animals.

Findings: For all groups, following IT administration of ^{14}C-T and ^{3}H-insulin, the observed radioactivity concentrations in whole blood were fairly low, indicating that little dose-related material was present in the systemic circulation at any one time. This might suggest that that absorption of test article-related material from the IT dosing was limited or that absorbed material was preferentially distributed to the tissues.

For Groups 1 and 3, PK parameters, the values for plasma, whole blood, and RBCs (where applicable), were calculated. See Table below. Also, the highest tissue radioactivity concentrations and largest $AUC_{0\text{-tlast}}$ were generally observed in the lung, trachea, and kidney for both ^{14}C and ^{3}H. Notable levels of radioactivity were also observed in the thyroid/parathyroid glands, in the walls of the GI tract, and in the urinary bladder wall. The smaller $AUC_{0\text{-tlast}}$ was generally observed in the eye, brain, spinal cord, seminal vesicle, and testis.

Parameter	Group 1				Group 3					
Sample:	Whole Blood		Plasma		Whole Blood		Plasma		RBC*	
	^{14}C	^{3}H	^{14}C	^{3}H	^{14}C	^{3}H	^{14}C	^{3}H	^{14}C	^{3}H
t_{max} (h)	0.33	24	0.33	24	0.08	48	0.08	48	0.33	48
C_{max} (µg eq/mL)	0.37	0.45	0.77	0.51	0.27	0.23	0.45	0.26	0.07	0.19
$AUC_{0\text{-last}}$ (µg eq·h/mL)	1.23	46.5	2.4	52.2	0.20	24.4	0.37	28.0	0.04	19.8

* Units for red blood cell (RBC) concentration are µg eq/g (C_{max}) and µg eq·h/g (AUC).

Moreover, the tissue to plasma ratios for Groups 1 and 3 remained constant over the study interval, indicating that radioactivity was cleared at approx. the same rate from plasma than from tissues. For lung and trachea (except for Group 3 for ^{14}C), the tissue to plasma values generally decreased over the study interval, suggesting a faster clearance from these tissues than from plasma. This was expected since the lung and trachea were the sites of administration.

For Groups 2 and 4, the excretion routes for ^{14}C were via urine (with approx. 33% and 25% recovered for Groups 2 and 4 respectively) and feces (with approx. 42% and 38% recovered for Groups 2 and 4 respectively) at 168 hrs post dose. Excretion of radioactivity in expired air accounted for < 1% for both groups. Recovery in the cage washes was < 3.0% for both groups. The excretion routes for ^{3}H were via urine (with approx. 31% and 30% recovered for Groups 2 and 4 respectively) and feces (with approx. 10.2% and 7.3% recovered for Groups 2 and 4 respectively) at 168 hrs post dose. Recovery in the cage washes was 2.1% and 0.79% for Groups 2 and 4, respectively. When compared to ^{14}C with ^{3}H, the majority of the recovered radioactivity was excreted by 72 hrs postdose with ^{14}C, whereas a more constant excretion was seen throughout the collection period up to 168 hrs with ^{3}H. A similar pattern of excretion was seen, regardless of the method of IT administration (e.g. liquid vs. powder).

Lastly, no labeled metabolites or degradation products were detected on the autoradioluminograms, indicating that the tritium might be linked to smaller molecules, which would not have been retained during the protein transfer from the gel to the nitrocellulose membrane. ^{3}H-insulin was detected only in the rat plasma at the 10- and 20- min time points. Therefore, radioactivity detected in plasma from time points 2 hr and higher was not linked to the parent ^{3}H-insulin.

2.6.4.5 Metabolism

PDC-PC-2000-0004: Inhibition of Cytochrome P4501A2, P4502C8, P4502D6, P4502C19, P4502C3A4 Catalytic Activities by Technosphere [By (b) (4), Study No. 000720b]

This study was to determine whether the selected test substances inhibit human cytochrome P450 activity. The inhibition study consisted of the determination of an IC_{50} for the test substance of each enzyme. Findings: Technosphere was essentially non-inhibitory at the highest concentration tested for all enzymes selected for this study.

Summary of IC_{50} values. Data represent duplicate determinations and are in units of micromolar.

	Test Substances			
Enzyme	Technosphere		Positive Control*	
CYP1A2	> 200	> 200	1.0	1.1
CYP2C8	> 200	> 200	4.7	4.9
CYP2C9	> 200	> 200	0.24	0.23
CYP2C19	> 200	> 200	1.2	1.6
CYP2D6 (AMMC)	> 200	> 200	0.004	0.004
CYP3A4 (BzRes)	> 200	> 200	0.14	0.13
CYP3A4 (BFC)	> 200	> 200	0.028	0.025

* - The positive controls used were furafylline (CYP1A2), quercetin (CYP2C8), sulfaphenazole (CYP2C9), tranylcypromine (CYP2C19), quinidine (CYP2D6) and ketoconazole (CYP3A4).

MKC-PC-2003-0007: Metabolite Stability and Profiling of 14C-Technosphere Particles Using Cryopreserved Hepatocytes from Rats, Dogs, Rabbits, Mice, and Human [By (b) (4), Study No. 400011]

This study was to evaluate the metabolic stability and profiling of T in cryopreserved primary hepatocytes from rats, dogs, mice, rabbits, and humans. First, two concentrations (5 and 50 µM) of T were incubated with cryopreserved primary human hepatocytes at various concentrations of viable cells/ml and for various time points to determine the optimal incubation conditions and the time-dependent nature of the metabolite profile of T. Next, at 2 time points (0 and 4 hrs) and 1 cell concentration (ca 400,000 viable cells/ml), T at 5 and 50 µM were incubated with cryopreserved primary hepatocytes from rats, dogs, mice, rabbits, and humans to compare the metabolic profile between species. Additionally, ^{14}C-T were incubated with cryopreserved primary hepatocytes from rats, dogs, mice, rabbits, and humans at 1, 5, and 50 µM for 0 and 4 hrs in order to facilitate the detection of metabolites that may have gone undetected in unlabeled incubations. Unlabeled samples were analyzed by HPLC equipped with a photodiode array detector. The presences of any novel peaks were quantitated for each chromatogram. Radiolabeled samples were analyzed by HPLC equipped with radiometric detection. Findings: No metabolites were detected across the species tested by HPLC equipped with a photodiode array detector. Also, no ^{14}C-labeled metabolites were detected in presence of rats, dogs, mice, rabbits, and human primary hepatocytes. The almost complete recovery or T and ^{14}C-T in all incubations, indicating that the test article was not metabolized by cryopreserved primary hepatocytes. This study showed that T was not a substrate for hepatocytes for any of the species tested.

MKC-PC-2004-0009: In Vitro Metabolism of FDKP in Human Microsomes [By (b) (4) Study No. 04774]

In vitro metabolism of FDKP in human liver microsomes (HLM) was examined. The metabolic stability of FDKP in HLM incubation was determined by LC/MS/MS in MRM mode. Screening for possible metabolites of FDKP was performed by highly sensitive LC/MS/MS method developed at (b) (4) Metabolites search was based on the possible metabolic pathways such as oxidation, hydroxylation, and hydrolysis. The calculated molecular and respective fragment ions of 14 proposed possible metabolites were search. Findings: FDKP was found to be stable after

incubations with HLM for up to 1 hr at 5 and 25 µM. having a mean recovery of > 95% as FDKP. However, no FDKP metabolites were detected. This study concluded that FDKP was stable under *in vitro* HML experimental conditions.

MKC-PC-2006-0014: Technosphere® and Technosphere®/Insulin: Evaluation of CYP450 Induction using Primary Cultures of Human Hepatocytes [By (b) (4) Study No. 7260-101]

This study tried to measure the extent of induction of CYP3A4, following exposure of human hepatocytes from three donors to T (FDKP) and TI and to compare the effects of the test articles with those of a prototypical inducer (rifampicin). Fresh human hepatocytes were exposed to 0.1, 1, or 10 µM FDKP containing in T or TI, rifampicin, or representative solvent control for 72 hrs. The doses were refreshed every 24 hrs during this exposure period. The CYP3A4 activities were evaluated by incubating testosterone for 1 hr and determining the rate of production of 6β-hydroxytestosterone using LC/MS/MS detection.

Compound	FDKP Concentration (µM)	Fold Induction		
		Donor 1	Donor 2	Donor 3
Technosphere®	0.1	1.80	1.35	1.08
	1	0.953	1.12	1.43
	10	0.832	1.18	1.32
Rifampicin (positive control)	50	21.0	6.33	5.63
Technosphere®/Insulin	0.1	1.42	0.760	0.927
	1	0.933	1.12	0.677
	10	1.86	1.48	0.877
Rifampicin (positive control)	50	14.0	5.47	4.40

Notes: All results are the mean of three replicates. Fold induction is calculated relative to an appropriate solvent control. An induction response >40% (Bjornsson *et al.*, 2003) of that of the prototypical inducer is considered significant.

Findings: Inducibility of CYP3A4 in human hepatocytes from all donors was demonstrated by rifampicin. Exposure of human hepatocytes to 0.1, 1, or 10 µM FDKP in T alone or TI did not result in notable induction of CYP3A4 activity in vitro. If the concentration of FDKP in the liver is maintained consistently at or below 10 µM for up to 72 hrs, no *in vitro* induction effect on CYP3A4 isoform activity would be anticipated in humans.

Fold induction caused by Technosphere® and Technosphere®/Insulin and prototypical inducer on testosterone 6β-hydroxylase activity (CYP3A4) in human hepatocytes

Compound	Concentration (µM)	Fold Induction[a]		
		Donor 1	Donor 2	Donor 3
Technosphere®	0.1	1.80	1.35	1.08
	1	0.953	1.12	1.43
	10	0.832	1.18	1.32
Rifampicin (positive control)	50	21.0	6.33	5.63
Technosphere®/Insulin	0.1	1.42	0.760	0.927
	1	0.933	1.12	0.677
	10	1.86	1.48	0.877
Rifampicin (positive control)	50	14.0	5.47	4.40

Note: All results are the mean of three replicates.
a Fold induction is calculated relative to an appropriate solvent control.

2.6.4.6 Excretion

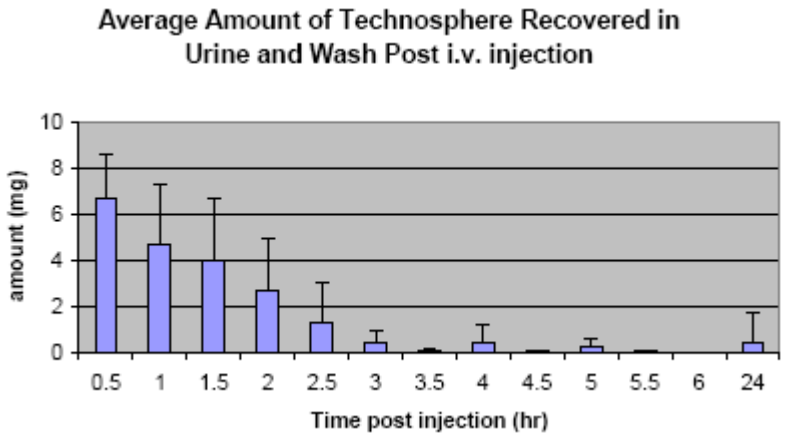
PDC-PC-2000-0007: Technosphere™ Excretion in Urine Following IV Administration to Rats [By PDC]

The study evaluated the urinary excretion of T in 9 male Spraque-Dawley rats, following the IV administration at 40 mg/kg T (FDKP in solution). FDKP was analyzed in urine samples collected at specific time points to 24 hrs postdose. The amount of FDKP in the urine was determined by HPLC with UV detection. Each amount was then added together to define the total amount excreted from each rat. Findings: Cumulative recoveries ranged from 64-117% of the total amount injected. The majority of FDKP was excreted in the urine within 3 hrs post dose. Mean % cumulative recovery of FDKP in the urine was 95.5%±18.4% after 24 hrs, suggesting that urinary excretion of FDKP was a major clearance mechanisms in rats.

Table 1 Recovery of FDKP in Urine (mg FDKP Recovered)

Time* (hrs)	2/1/2000	2/2/2000	2/3/2000	2/7/2000	2/8/2000	2/9/2000	2/10/2000	2/21/2000	2/24/2000
0.5	2.67	7.25	9.81	7.38	6.81	6.88	7.47	5.81	6.24
1	7.39	6.44	4.02	2.24	6.26	2.77	7.46	0.66	
1.5	4.35	3.45	1.73	9.96	3.07	2.13		5.24	1.93
2	4.99	1.39	4.80	0.91	1.07	0.71	6.31	0.90	
2.5	1.00	0.37		1.22	0.24	0.80		0.17	5.19
3	0.09	0.57	0.33	0.50	0.09	0.10		0.05	1.60
3.5	0.02	0.15	0.08	0.12	0.05	0.25		0.00	
4				0.11	0.00	0.08	1.95	0.00	0.29
4.5						0.04			
5	0.44	0.11	0.03	0.00	0.00		0.14	0.00	1.00
5.5						0.05			
6				0.00	0.00			0.00	
24	0.00	0.00	0.00	0.20	0.00	0.00	0.10	0.00	3.82
Cumulative Recovery (mg)	20.95	19.73	20.80	22.64	17.59	13.81	23.43	12.83	20.07
Cumulative Recovery (%)	104.75	98.65	104.00	113.2	87.95	69.05	117.15	64.15	100.35

*Hours post dose



MKC-PC-2004-0020: Metabolite Profile of Radioactivity in Urine and Recovery of Radioactivity in Feces from Spraque-Dawley Rats following a Single IT or SC administration of Radiolabeled FDKP [By (b) (4) Study No. 440105]

This study was to determine 1) the metabolite profile of ^{14}C-FDKP and major ^{14}C-FDKP metabolites in urine sample, and 2) the recovery of radioactivity in feces up to 7 days postdose, in

Spraque-Dawley rats after a single IT or SC administration of ^{14}C-T powder. Twelve male rats (Groups 1 and 2) were given ^{14}C-FDKP powder by IT administration via an insufflator, whereas 3 male rats (Group 3) were given ^{14}C-FDKP by sc injection. Blood sample were collected from animals at selected timepoints prior to euthanasia for possible metabolite profiling in plasma. Urine and feces (for Groups 2 and 3 animals only) were also collected over designated intervals up to 168 hrs post-dose. Following sacrifice, carcasses were discarded without further examination. The profile of ^{14}C-FDKP and major ^{14}C-labelled metabolites in urine samples were determined. Plasma samples were not examined due to suspected low concentrations of radioactivity in these samples. Findings: No difference was observed in the recovery of radioactivity in feces between the animals administered powder via IT instillation (Group 2, a dose level of 1.54 mg/kg ^{14}C-FDKP) and the animals administered a formulation subcutaneously (Group 3, a dose level of 1.45 mg/kg ^{14}C-FDKP). This study suggested that for Groups 2 and 3 animals, the radioactivity was absorbed into the general circulation and recovered in feces via biliary excretion. However, it was possible that some radioactivity was transferred into the GI tract through swallowing, following the IT instillation (Group 2). HPLC analysis also showed that in every urine sample except one, almost the entire radioactivity excreted from rats, following a single IT or SC administration of ^{14}C-FDKP, was the intake parent compound.

The animals were assigned to study group as follows:

Group No. Identification	Target Dose[a] (µCi/animal) from ^{14}C	Number of Animals
		Males
1	47	2
2	60	10
3	60	3

[a] A target dose level of 1.13 mg/kg (Group 1) or 1.66 mg/kg (Groups 2 and 3) of FDKP was administered.

2.6.4.7 Pharmacokinetic drug interactions

MKC-PC-2003-0018: Determination of Protein Binding of Technosphere in Various Species of Plasma [By (b) (4) Study No. TTP-MFN-M0002]

This study determined protein binding of FDKP in various species of plasma (human, canine, rat, rabbit, mouse, and monkey). Concentrations of FDKP at 200, 400, 800, and 1200 ng/ml were used to against the various species of animal plasma using 96-well equilibrium dialysis. The concentrations of FDKP were determined suing LC/MS/MS.

Percent Binding Results for Each Matrix

3a. Human Plasma

Sample	Amount plasma side (ng/mL)	Amount buffer side (ng/mL)	Difference plasma-buffer (ng/mL)	Total recovered (ng/mL)	% Binding	T_0 (ng/mL)	Average T_0/level (ng/mL)	Non-specific binding (NSB) (%)
200-1	84.8	107	-22.2	192	-12	170		-8
200-2	104	102	2	206	1	180	178	-16
200-3	88.6	101	-12.4	190	-7	183		-7
400-1	245	233	12	478	3	432		-17
400-2	156	229	-73	385	-19	398	409	6
400-3	183	202	-19	385	-5	398		6
800-1	367	411	-44	778	-6	859		11
800-2	376	405	-29	781	-4	894	872	10
800-3	407	413	-6	820	-1	862		6
1200-1	525	674	-149	1199	-12	1180		2
1200-2	596	707	-111	1303	-9	1240	1223	-7
1200-3	599	659	-60	1258	-5	1250		-3
			Average Binding		-6	**Average NSB**		-1

Data can be found in TTP-MFN-M0002, Protein Binding, Notebook 1, LDR 46.

3b. Canine Plasma

Sample	Amt plasma side (ng/mL)	Amt buffer side (ng/mL)	Difference plasma-buffer (ng/mL)	Total recovered (ng/mL)	% binding	T0 (ng/mL)	Avg T0 (ng/mL)	Non-specific binding (NSB) (%)
200-1	189	141	48	330	15	231		-46
200-2	190	134	56	324	17	235	226	-43
200-3	152	141	11	293	4	212		-30
400-1	255	238	17	493	3	401		-20
400-2	215	237	-22	452	-5	434	410	-10
400-3	235	239	-4	474	-1	396		-16
800-1	460	455	5	915	1	881		-11
800-2	441	412	29	853	3	822	823	-4
800-3	419	410	9	829	1	765		-1
1200-1	653	627	26	1280	2	1160		-10
1200-2	650	582	68	1232	6	1170	1160	-6
1200-3	639	627	12	1266	1	1150		-9
			Average Binding		4	**Average NSB**		-17

Data can be found in TTP-MFN-M0002, Protein Binding, Notebook 1, LDR 49.

3c. Rabbit Plasma

Sample	Amount plasma side (ng/mL)	Amount buffer side (ng/mL)	Difference plasma-buffer (ng/mL)	Total recovered (ng/mL)	% Binding	T_0 (ng/mL)	Average T_0/level (ng/mL)	Non-specific binding (NSB) (%)
200-1	114	99.7	14.3	214	7	222		-2
200-2	124	99	25	223	11	215	210	-6
200-3	125	106	19	231	8	193		-10
400-1	209	237	-28	446	-6	458		0
400-2	194	235	-41	429	-10	429	446	4
400-3	293	164	129	457	28	450		-3
800-1	390	443	-53	833	-6	793		0
800-2	432	448	-16	880	-2	863	835	-5
800-3	472	397	75	869	9	850		-4
1200-1	597	678	-81	1275	-6	1250		-2
1200-2	639	609	30	1248	2	1220	1247	0
1200-3	597	670	-73	1267	-6	1270		-2
			Average Binding (%)		2	Average NSB (%)		-2

Data can be found in TTP-MFN-M0002, Protein Binding, Notebook 1, LDR 52.

3d. Rat Plasma

Sample	Amount plasma side (ng/mL)	Amount buffer side (ng/mL)	Difference plasma-buffer (ng/mL)	Total recovered (ng/mL)	% Binding	T_0 (ng/mL)	Average T_0/level (ng/mL)	Non-specific binding (NSB) (%)
200-1	107	104	3	211	1	217		3
200-2	116	115	1	231	0	214	217	-7
200-3	127	115	12	242	5	219		-12
400-1	249	282	-33	531	-6	386		-29
400-2	239	206	33	445	7	416	412	-8
400-3	228	220	8	448	2	435		-9
800-1	347	490	-143	837	-17	805		3
800-2	354	499	-145	853	-17	928	865	1
800-3	380	461	-81	841	-10	861		3
1200-1	583	579	4	1162	0	1330		13
1200-2	579	729	-150	1308	-11	1350	1340	2
1200-3	587	686	-99	1273	-8	1340		5
			Average binding (%)		-4	Average NSB (%)		-3

Data can be found in TTP-MFN-M0002, Protein Binding, Notebook 1, LDR 55.

3e. Monkey Plasma

Sample	Amount plasma side (ng/mL)	Amount buffer side (ng/mL)	Difference plasma-buffer (ng/mL)	Total recovered (ng/mL)	% Binding	T_0 (ng/mL)	Average T_0/level (ng/mL)	Non-specific binding (NSB) (%)
200-1	126	118	8	244	3	191		-23
200-2	99.8	98.4	1.4	198	1	183	198	0
200-3	132	105	27	237	11	221		-19
400-1	209	210	-1	419	0	507		13
400-2	199	240	-41	439	-9	460	482	9
400-3	220	222	-2	442	0	479		8
800-1	428	528	-100	956	-10	1020		2
800-2	384	453	-69	837	-8	940	980	15
800-3	364	435	-71	799	-9	923		18
1200-1	500	706	-206	1206	-17	1280		-2
1200-2	572	595	-23	1167	-2	1330	1178	1
1200-3	542	748	-206	1290	-16	1240		-10
			Average Binding (%)		-5	Average NSB (%)		1

Data can be found in TTP-MFN-M0002, Protein Binding, Notebook 1, LDR 58.

3 f. Mouse Plasma

Sample	Amount plasma side (ng/mL)	Amount buffer side (ng/mL)	Difference plasma-buffer (ng/mL)	Total recovered (ng/mL)	% Binding	T_0 (ng/mL)	Average T_0/level (ng/mL)	Non-specific binding (NSB) (%)
200-1	227	< 0	NA	NA	NA	< 0		NA
200-2	187	< 0	NA	NA	NA	< 0	NA	NA
200-3	374	< 0	NA	NA	NA	< 0		NA
400-1	168	< 0	NA	NA	NA	38.7		NA
400-2	267	< 0	NA	NA	NA	24.9	26	NA
400-3	393	< 0	NA	NA	NA	15.7		NA
800-1	276	69.1	206.9	345	60	504		29
800-2	386	89.3	296.7	475	62	494	488	3
800-3	316	101	215	417	52	465		14
1200-1	454	281	173	735	24	1040		29
1200-2	345	489	-144	834	-17	1070	1031	19
1200-3	280	329	-49	609	-8	983		41
			Average binding (%)		-1	Average NSB (%)		30
			1200 ng/mL			1200 ng/mL		

Note: Due to interference of the FDKP peak at the lower levels, only data at 1200 ng/mL considered reliable.
Data can be found in TTP-MFN-M0002, Protein Binding, Notebook 1, LDR 61.

2.6.4.8 Other Pharmacokinetic Studies

MKC-PC-2006-0008: Pharmacokinetics of Technosphere/Insulin Following Subcutaneous, Intravenous and Oral Administration to Male and Female Spraque-Dawley Rats [By (b) (4) , Study No. LCL00007-06-574]

This study was to study the effect on blood glucose levels and the PK profiles of TI and T following the SC, IV, and PO administration to male and female Spraque-Dawley rats. Rats were placed into 9 groups (6M/6F per group). For Dose Session I, Group 1 animals received T at a target dose of 100 mg/kg whereas Group 2 animals received at a target dose of 50 mg/kg via an

IV bolus injection via the jugular vein catheter. For Dose Session II, Group 3 animals received TI at a target dose of 1.0 U/kg insulin and 0.32 mg FDKP, via an IV bolus injection via the jugular vein catheter. Group 4 animals received T at a target dose of 11 mg/kg, via a sc injection. For Dose Session III, Group 5 animals received T at 50 mg/kg via a sc injection. Group 6 animals received TI at a target dose of 2.5 U/kg insulin and 0.8 mg/kg FDKP, via SC injection. For Dose Session IV, Group 7 animals received T at a target dose of 11 mg/kg via oral gavage. Group 8 animals received at a target dose of 50 mg/kg via oral gavage. For Dose Session V, Group 9 animals received TI at a target dose of 156 U/kg insulin and 50 mg/kg FDKP via oral gavage. Viabilities and clinical observations were recorded. All rats were sacrificed at the conclusion of the in-life study. Blood samples were collected prior to dosing and 5, 10, 15, 20, 30, 40 min, and 1, 2, 4, and 6 hrs following the SC, IT, or PO administration.

Session *	Group Number	Number of Rats **	Test Article	Insulin Dose Level (~U/kg)	Technosphere® Dose Level (~mg/kg)	Dose Volume (~mL/kg)	Technosphere® Dose Concentration (mg/mL)	Vehicle[a]	Dose Route
I 3/15/06	1	12	T	N/A	11	1	11	solution	IV-Bolus
	2	12	T	N/A	50	1	50	solution	
II 3/16/06	3	12	T/I	1.0	0.32	1	0.32	solution	
	4	12	T	N/A	11	1	11	solution	Subcutaneous
III 3/17/06	5	12	T	N/A	50	1	50	solution	
	6	12	T/I	2.5	0.8	1	0.8	solution	
IV 3/21/06	7	12	T	N/A	11	2	5.5	solution	Oral gavage
	8	12	T	N/A	50	2	25	solution	
V 3/22/06	9	12	T/I	156	50	2	50	solution	

Conc. = Concentration. T= Technosphere® T/I= Technosphere®/ Insulin (the technosphere particles will contain the insulin dose) N/A= not applicable
*All animals will be treated once on day one. Session number only indicates how many animals will be dosed each day.
** All animals will be implemented with dual jugular vein cannulas to facilitate intravenous dosing and blood collection (6 males and 6 females per group)
[a]Solution of T consists of 5.5, 11, 25 or 50 mg/mL of T at pH 7.4 +/- 0.2
Solution of T/I consists of 0.32, 0.8, or 50 mg/mL T/I at pH 7.4 +/- 0.2

Findings on glucose analysis: No animals became hypoglycemic, therefore no Dextrose treatments were given.

Findings on TK analysis: The exposure of FDKP increased with dose, following IV, SC, or oral administration as either a solution of T or TI. There were no significant differences in exposure of FDKP between males and females. Exposure to the rats to human insulin following the TI administration via the IV and SC routs showed sex differences of greater exposure in the male rats than the female rats. There were only a few of the rats that had quantifiable human insulin within oral administration group of TI.

TABLE 1. Combined (Male plus Female) Mean FDKP Plasma Toxicokinetic Metrics Following a Single Administration of Technosphere® Microparticles (T) or Technosphere/Insulinin (T/I) to Male and Female Sprague-Dawley Rats by IV Bolus, or the Subcutaneous (SC) or Oral (PO) Routes (n=6/sex)

		IV Bolus			SC			PO*		
	mg/kg	11 (T)	50 (T)	0.32 (T/I)	11 (T)	50 (T)	0.80 (T/I)	5.5 (T)	25 (T)	50 (T/I)
C_{max} (ng/mL)	Mean	32625	120678	677	15408	76600	851	73.2	315	440
	SD	2926	18595	80.6	3124	9758	208	61.6	282	952
	% CV	8.97	15.4	11.9	20.3	12.7	24.5	84.2	89.4	217
t_{max} (min)	Mean	5.0	5.42	5.0	12.9	12.5	14.6	75.8	89.6	186
	SD	0	1.44	0	4.98	3.37	1.44	82.7	100	110
	% CV	0	26.6	0	38.6	27.0	9.90	109	111	59.1
AUC_{last} (ng*min/mL)	Mean	862812	2978454	15417	899075	4445940	48024	12449	36193	26320
	SD	203817	459693	2652	101796	327498	6934	3989	12526	15181
	% CV	23.6	15.4	17.2	11.3	7.37	14.4	32.0	34.6	57.7
AUC_{inf} (ng*min/mL)	Mean	862996	2978769	15563	899245	4446599	48334	14085[a]	47050[b]	NA[c]
	SD	203860	459704	2647	101836	327470	7296	5631	12209	
	% CV	23.6	15.4	17.0	11.3	7.36	15.1	40.0	25.9	
$t_{1/2}$ (min)	Mean	28.1	21.4	18.4	27.4	25.9	28.8	104[a]	118[b]	NA[c]
	SD	5.92	1.11	2.93	2.38	2.85	3.90	15.6	33.1	
	% CV	21.1	5.16	15.9	8.7	11.0	13.6	15.0	28.1	

* All PO mean concentration metrics are skewed by occasional single concentration in individual rats that are more than 3 SD above the mean.
a) n=5 as the concentration data from 7 rats did not allow the calculation of the terminal metrics. b) n=4 as the concentration data from 8 rats did not allow the calculation of the terminal metrics. c) NA, as the concentration data from all 12 rats did not allow the calculation of the terminal metrics.

TABLE 2. Mean Insulin Serum Toxicokinetic Metrics Following a Single Administration of Technosphere/Insulin (T/I) to Male and Female Sprague-Dawley Rats by IV Bolus, or the Subcutaneous (SC) or Oral (PO) Routes (n=6/sex)

		IV Bolus (1 U/kg)			SC (2.5 U/kg)			PO (156 U/kg)*		
	Sex	Male	Female	M + F	Male	Female	M + F	Male[a]	Female[b]	M + F[c]
C_{max} (uU/mL)	Mean	3222	2466	2844	1310	1260	1285	228	82.7	154
	SD	1601	1765	1654	583	416	484	83.8	30.8	91.2
	% CV	99.4	143	116	89.1	66.0	75	73.6	74.6	118
t_{max} (min)	Mean	5.00	5.00	5.0	14.2	13.3	13.8	28.3	80.0	46.8
	SD	0.00	0.00	0.0	2.04	4.08	3.11	12.6	56.6	37.8
	% CV	0.0	0.0	0.0	28.8	61.2	45.2	88.8	141	162
AUC_{last} (uU*min/mL)	Mean	28653	22689	25671	62140	42216	52178	7549	6937	5808
	SD	12039	12787	12244	22324	14498	20745	2332	8235	4448
	% CV	84.0	113	95.4	71.9	68.7	79.5	61.8	237.4	153
AUC_{inf} (uu*min/mL)	Mean	28781	22788	25784	62947	42578	52762	7605	NC	NA
	SD	12024	12767	12231	22595	14485	20990	2284		
	% CV	83.6	112	94.9	71.8	68.0	79.6	50.1		
$t_{1/2}$ (min)	Mean	8.03	7.23	7.63	28.8	19.4	24.1	10.2	NC	NA
	SD	1.20	2.37	1.84	4.34	3.87	6.28	8.56		
	% CV	30.0	65.6	48.3	30.2	39.9	52.1	168		

a) N = 3, b) N = 2, c) N = 5
NC – not calculable due to insufficient data to determine terminal kinetics. NA not applicable because there are no values for the female rats.

MKC-PC-2006-0009: Pharmacokinetics of Technosphere/Insulin Following Crossover Subcutaneous and Oral Administration to Non-Naïve Male and Female Beagle Dogs [By (b) (4) Study No. LCL00008-06-555]

This study was to study the effect on blood glucose levels and the PK profiles of TI and T following the SC or PO administration to non-naïve male and female beagle dogs. Dogs were placed into 2 groups (3M/3F per group). The dogs received a single oral or sc dose via oral gavage or an interscapula region of the dorsal thorax once on 3 occasions. For Dose Session I,

Group 1 animals received at a target dose of 11 mg/kg via SC injection. The Group 2 animals received T at a target dose of 50 mg/kg via SC injection. For Dose Session II, Group 1 animals received T at a target dose of 11 mg/kg via oral gavage. Group 2 animals received T at a target dose of 50 mg/kg via oral gavage. For Dose Session III, Group 1 animals received TI at a target dose of 0.25 U/kg insulin and 0.08 mg/kg FDKP via SC injection. Group 2 animals received TI at a target dose of 156 U/kg insulin and 50 mg/kg FDKP via SC injection. Viabilities and clinical observations were recorded. All rats were sacrificed at the conclusion of the in-life study. Blood samples were collected prior to dosing and 5, 10, 15, 20, 30, 40 min, and 1, 2, 4, and 6 hrs following SC and PO administrations.

A. Study Design Table

Session	Group	No.[b]	Test Article	Insulin Dose Level (~U/kg)	Technosphere® Dose Level (mg/kg)	Dose Volume (mL/kg)	Technosphere ® Dose Conc. (mg/mL)	Vehicle[a]	Dose Route
I	1	6	T	N/A	11	0.1	110	solution	SQ
3/13/06	2	6	T	N/A	50	0.45	110	solution	SQ
II	2	6	T	N/A	11	5	2.2	solution	Oral
3/15/06	1	6	T	N/A	50	5	10	solution	Oral
III	1	6	T/I	0.25	0.08	0.1	0.8	solution	SQ
3/17/06	2	6	T/I	156	50	5	10	solution	Oral

No. = Number Conc. = Concentration Vol. = Volume T= Technosphere® T/I= Technosphere®/ Insulin (the technosphere will contain the insulin dose) N/A = not applicable

[a]Solution of T consists of 110, 2.2, or 10 mg/mL of T at pH 7.4+/- 0.2. Solution of T/I consists of 0.8 or 10 mg/mL T/I at pH7.4+/- 0.2.

[b]The sample size will consist of 3 males and 3 females per dose group

Findings for glucose analysis: In Dose Session I, Groups 1 and 2, there was an effect on blood glucose levels from dosing to the 6 hr time point. In Dose Session II, Group 1 males, there was a transient increase in blood glucose levels from dosing to the 1 hr time point. The blood glucose levels then decreased to within 3.5% of the baseline average by the 6 hr time point. For Group 1 females, there was no effect on blood glucose levels from dosing to the 6 hr time point. For Group 2 males, there was no effect on blood glucose levels from dosing to the 6 hr time point. For Group 2 females, there was a transient increase in blood glucose levels from dosing to the 2 hr time point. The blood glucose levels then decreased to 25% below baseline average by the 6 hr time point. In Dose Session III, Group 1 males, there was a dramatic decrease in blood glucose levels starting at the 5 min time point and continued until the 1 hr time point, where the blood glucose level was 34.9% of the baseline average. The blood glucose levels then began to increase and were 90.1% of the baseline average by the 6 hr time point. For Group 1 females, there was a dramatic decrease also in blood glucose levels starting at the 15 min time point and continued to drop until the 30 min time point, where blood glucose level was 48.8% of the baseline average. The blood glucose levels then began to increase and were 86.3% of the baseline average by the 6 hr time point. For Group 2 males, there was a transient decrease in blood glucose levels from dosing to the 40 min time point. The blood glucose level then increased to within 0.5% of the baseline average by the 6 hr time point. For Group 2 females, there was a transient decrease in blood glucose levels from dosing to the 30 min time point. The blood glucose levels then increased to within 88.3% of the baseline average by the 6 hr time point.

Findings for TK analysis: The exposure in dogs to FDKP following either SC or PO administration of FDKP as T or TI increased in approx. a dose proportional manner. There were no significant differences in exposure of FDKP between males and females. The relative bioavailability of the PO vs. SC is approx. 3%. The mean t_{max} following oral administration was

approx 85 min with a large variability. The mean t_{max} following sc administration was approx. 45 min for the lower doses and 70 min for the 50 mg/kg dose of T. Where calculable, the mean plasma t½ values ranged from 44 to 57 min for FDKP by the sc route and 84-110 min following the oral route. In addition, the interpretation of the insulin concentration data was confounded by the cross-reactivity of endogenous dog insulin with the ELISA used to measure the administered human insulin. After adjusting with the time zero insulin (baseline), the sc administration of TI (approx. 0.25 U/kg) produced a C_{max} of 59 and 94 uU/ml in the females and males respectively, with a tmax of approx. 30 min.

TABLE 1. Combined (Male plus Female) Mean FDKP Plasma Toxicokinetic Metrics Following a Single Administration of FDKP Solution (T) in Male and Female Beagle Dogs by the Subcutaneous (SC) or Oral (PO) Routes (n=3/sex)

		Group 1(T) (11 mg/kg)		Group 2 (T) (50 mg/kg)	
		SC	PO	SC	PO
C_{max} (ng/mL)	Mean	18879	332	54314	2821
	SD	4260	121	7509	3437
	%CV	22.6	36.5	13.8	121.8
t_{max} (min)	Mean	48.3	95.0	70.0	78.3
	SD	13.3	39.9	24.5	81.1
	%CV	27.5	42.0	35.0	103.5
AUC_{last} (ng*min/mL)	Mean	2431511	70894	9250033	383551
	SD	607190	28615	1724728	303836
	%CV	25.0	40.4	18.6	79.2
AUC_{inf} (ng*min/mL)	Mean	2453899	86835	9459410	456182[a]
	SD	625177	37274	1830242	357605
	%CV	25.5	42.9	19.3	78.4
$t_{1/2}$ (min)	Mean	47.9	109.5	56.6	92.8[a]
	SD	6.75	15.10	14.3	15.1
	%CV	14.1	13.8	25.2	16.3

a) N=5 as the concentration data from one female dog did not allow the calculation of the terminal metrics.

TABLE 2. Combined (Male plus Female) Mean FDKP Plasma Toxicokinetic Metrics Following a Single Administration of Technosphere/Insulin Solution (T/I) in Male and Female Beagle Dogs by the Subcutaneous (SC) or Oral (PO) Routes (n=3/sex)

		Group 1 SC (0.08 mg/kg)	Group 2 PO (50 mg/kg)
C_{max} (ng/mL)	Mean	**122**	**1753**
	SD	34	1046
	% CV	28.3	59.7
t_{max} (min)	Mean	**43.3**	**85.0**
	SD	13.7	82.9
	% CV	31.5	97.5
AUC_{last} (ng*min/mL)	Mean	**16395**	**323530**
	SD	3945	142970
	% CV	24.1	44.2
$NAUC_{last}$	Mean	**204938**	**6471**
AUC_{inf} (ng*min/mL)	Mean	**2453899**	**386908**[a]
	SD	625177	
	% CV	25.5	
$t_{1/2}$ (min)	Mean	**43.5**	**84.4**[a]
	SD	5.53	
	% CV	12.7	

a) N=1 only one female dog had FDKP concentration data that allowed the calculation of the terminal metrics.

TABLE 3. Mean Baseline Corrected Insulin Serum Toxicokinetic Metrics Following a Single Administration of Technosphere/Insulin Solution (T/I) in Male and Female Beagle Dogs by the Subcutaneous (SC) Route (n=3/sex)

		Male	Female	Combined M + F
C_{max} (uU/mL)	Mean	**93.0**	**59.0**	**76.0**
	SD	46.2	18.6	36.6
	% CV	49.7	31.5	48.1
t_{max} (min)	Mean	**30.0**	**28.3**	**29.2**
	SD	10.0	12.6	10.2
	% CV	33.3	44.4	35.0
AUC_{last} (uU*min/mL)	Mean	**10085**	**7163**	**8624**
	SD	2041	3226	2896
	% CV	20.2	45.0	33.6
AUC_{inf} (uU*min/mL)	Mean	**12367**[a]	**8219**	**9878**
	SD	475	4129	3707
	% CV	3.8	50.2	37.5
$t_{1/2}$ (min)	Mean	**40.8**[a]	**55.8**	**49.8**
	SD	11.7	14.6	14.4
	% CV	28.8	26.1	29.0

a) N=2 dogs with evaluable terminal kinetics

2.6.5 PHARMACOKINETICS TABULATED SUMMARY

[Pivotal studies pertinent to the primary indication and core pharmacology studies relevant to the primary pharmacodynamic effect, as available and as provided by the sponsor]

Note from the Reviewer: Usually this section refers to studies demonstrating MOA. However, the sponsor included an extensive tabulated summary, which most these studies were non-GLP. Only PK overview tabulated summary is included here.

2.6.5.1 Pharmacokinetic Overview

Test Article: FDKP

Individual Study Reports						Location
Type of Study/Description	GLP[a]	Test System	Method of Administration	Testing Facility	Study No.	Module
Absorption						
Pharmacokinetics of ^{14}C-FDKP after single dose	No	SD rat	iv	(b) (4)	MKC-PC-2007-0013 (b) (4) N107966A)	4.2.2.2
Pharmacokinetics of ^{14}C-FDKP/Insulin after single dose	No	SD rat	iv		MKC-PC-2007-0012 (b) (4) N107966B)	4.2.2.2
Pharmacokinetics of ^{14}C-FDKP after single dose	No	SD rat	iv		MKC-PC-2006-0034 ((b) (4) N107962A)	4.2.2.2
Pharmacokinetics of ^{14}C-FDKP/Insulin after single dose	No	SD rat	iv		MKC-PC-2006-0035 (b) (4) N107962B)	4.2.2.2
Administration of regular insulin, Humolog® insulin and Technosphere Insulin	No	Minipig	sc		MKC-PC-2006-0002 (b) 11803.01.01)	4.2.2.2
Pharmacokinetics after single dose	No	SD rat	sc, po, intratracheal		MKC-PC-2007-0005 (N107964A)	4.2.2.2
Absorption after a single dose	No	SD rat	Intratracheal		MKC-PC-2007-0010 (N107964B)	4.2.2.2
Pharmacokinetics and effect on blood glucose	No	SD rat	sc, po, intratracheal		MKC-PC-2006-0008 ((b) (4) LCL00007)	4.2.2.7
Pharmacokinetics and effect on blood glucose	No	SD rat	Inhalation		MKC-PC-2006-0024 ((b) 75022) (4)	4.2.1.1
Pharmacokinetics and effect on blood glucose	No	Beagle dog	sc, po		MKC-PC-2006-0009 ((b) (4) LCL00008)-	4.2.2.7

Individual Study Reports						Location
Type of Study/Description	**GLP[a]**	**Test System**	**Method of Administration**	**Testing Facility**	**Study No.**	**Module**
Pharmacokinetics and effect on blood glucose	No	Beagle dog	Inhalation	(b) (4)	MKC-PC-2006-0023 (b) (4) 65016)	4.2.1.1
Pharmacokinetics of ^{14}C-(lysine) FDKP after single dose	No	Beagle dog	iv		MKC-PC-2006-0036 (b) (4) N1079963)	4.2.2.2
Pharmacokinetics of ^{14}C-(fumaryl) FDKP after single dose	No	Beagle dog	iv		MKC-PC-2007-0011 (b) (4) N107967)	4.2.2.2
Pharmacokinetics of human insulin	No	Beagle dog	sc		MKC-PC-2006-0001 (b) (4) LCL00006)	4.2.2.2
Distribution						
Absorption, distribution and excretion	Yes	SD rat	Intratracheal		MKC-PC-2002-0008 (b) (4) 440035)	4.2.2.3
Plasma protein binding of FDKP	Yes	Mouse, rat, rabbit, dog, monkey, human	In vitro		MKC-PC-2003-0018 (TTP-MFN-M0002)	4.2.2.6
Pharmacokinetics in pregnant rats and lacteal secretion	Yes	Female Sprague Dawley rat	sc		MKC-PC-2004-0008 (b) (4) LCL00003)	4.2.3.5.3
Placental transfer	Yes	Female Sprague Dawley rat	sc		MKC-PC-2006-0010 (b) (4) LCL00009)	4.2.3.5.1
Metabolism						
In vitro metabolism of ^{14}C-Technosphere/ Technosphere Particles	No	Cryopreserved hepatocytes from rat, dog, rabbit, mice and human	In vitro		MKC-PC-2003-0007 (b) (4) 400011)	4.2.2.4

Individual Study Reports						Location
Type of Study/Description	**GLP[a]**	**Test System**	**Method of Administration**	**Testing Facility**	**Study No.**	**Module**
In vitro metabolism of FDKP	No	Human liver microsomes	In vitro	(b) (4)	MKC-PC-2004-0009 (b) (4) 01214) Project # 04774	4.2.2.4
Cytochrome P450 induction	No	Human hepatocytes	In vitro		MKC-PC-2006-0014 (b) (4) 7260-101)	4.2.2.4
Cytochrome P450 inhibition	No	Recombinant human cytochrome P450 isozymes	In vitro		PDC-PC-2000-0004 (b) (4) 000720b)	4.2.2.4
Excretion						
Metabolism and excretion	Yes	Sprague-Dawley rat	Intratracheal and sc		MKC-PC-2004-0020 (b) (4) 440105)	4.2.2.4
Pharmacokinetics - Other						
^{3}H-Mannitol absorption in rat lungs	No	Rat	Intratracheal instillation		PDC-PC-2000-0001	4.2.2.2
Bioanalytical methods						
Method validation of LC-MS/MS assay for the determination of Fumaryl Technosphere in rat serum	Yes	Rat serum	NA		MKC-PC-2003-0028 (b) (4) 01008) 03057 Project #	4.2.2.1

Individual Study Reports						Location
Type of Study/Description	GLP[a]	Test System	Method of Administration	Testing Facility	Study No.	Module
LC-MS/MS assay validation of FDKP in K_2EDTA human plasma	Yes	Human plasma	NA	(b) (4)	MKC-PC-2006-0040 (b) (4) 197-0504)	4.2.2.1
Long term frozen storage stability of FDKP in human serum	Yes	Human serum	NA		MKC-PC-2006-0012 (b) (4) 197-0602)	4.2.2.1
LC-MS/MS assay validation of FDKP in mouse serum	Yes	Mouse serum	NA		MKC-PC-2007-0025 (b) (4) 197-0706)	4.2.2.1
LC-MS/MS assay validation of FDKP in dog plasma	Yes	Dog plasma	NA		MKC-PC-2003-0031 (b) (4) 03059)	4.2.2.1
Determination of human insulin in dog serum by ELISA	Yes	Dog serum	NA		MKC-PC-2006-0013 (b) (4) 197-0503)	4.2.2.1
Determination of human insulin in rat serum by ELISA	Yes	Rat serum	NA		MKC-PC-2006-0007 (b) (4) 197-0502)	4.2.2.1
Determination of human insulin in mouse serum by ELISA	Yes	Mouse serum	NA		MKC-PC-2006-0006 (b) (4) 197-0501)	4.2.2.1
Analytical performance of the Linco Lispro Insulin Radioimmunoassay (RIA)	Yes	Human serum	NA		MKC-PC-2006-0042	Module 5
Insulin: bioanalytical assay validation	Yes	Human serum	NA		MKC-PC-2006-0041	Module 5

Individual Study Reports						Location
Type of Study/Description	GLP[a]	Test System	Method of Administration	Testing Facility	Study No.	Module
Cross-validation of an HPLC method to determine levels of Technosphere and Technosphere Insulin formulated in dimethylsulfoxide	Yes	DMSO stock solutions	NA	(b) (4)	MKC-PC-2006-0015 (b) (4) 40069)	4.2.2.1
Stability and method validation for Technosphere and Technosphere Insulin	Yes	Phosphate buffered saline	NA		MKC-PC-2006-0018 (b) (4)	4.2.2.1
HPLC analytical method validation for the assay of insulin (in Technosphere) collected from air sampling substrates	Yes	Air sampling substrates	NA		MKC-PC-2003-0029 (b) (4) 4227)	4.2.2.1
HPLC analytical method validation for Technosphere collected from air sampling substrates	Yes	Air sampling substrates	NA		MKC-PC-2004-0021 (b) (4) 4304)	4.2.2.1
Validation of a HPLC method for the determination of Technosphere on filters	Yes	Air sampling filters	NA		MKC-PC-2003-0027 (b) (4) 180085	4.2.2.1

[a] An entry of "Yes" indicates that the study includes a GLP compliance statement.

Abbreviations: ^{14}C, carbon-14; DMSO, dimethylsulfoxide; GLP, Good Laboratory Practice; HPLC, high performance liquid chromatography; iv, intravenous; LC-MS/MS, liquid chromatography-tandem mass spectroscopy; NA, not applicable; No., number; po, oral; sc, subcutaneous; SD, Sprague Dawley.

2.6.6 TOXICOLOGY

2.6.6.1 Overall toxicology summary

General toxicology: The toxicity of TI and/or T alone was evaluated in single-dose studies in rats and dogs by the PO route and in repeat-dose studies in mice, rats, and dogs by the SC, PO, or inhalation routes.

Single dose studies showed that T was well-tolerated acutely at the limit dose of 5 g/kg administered by the PO route. The focus of this review is the inhalational route since this is the intended clinical route.

Repeat dose studies were done with both TI and T alone in mice (SC route only), rats (inhalation route only), dogs (inhalation, PO, and SC routes). The rat inhalational studies were primarily nose only and dog inhalational studies were nose and mouth. In all studies, FDKP administered as Technosphere particles (T) and TI were well tolerated. There were no target organs affected by chronic exposure across species. The primary effects were pharmacologic in nature and related to high doses of insulin.

Study Number	Study Title	Test Article(s)[a]	Written Summary	Tabular Summary
MKC-PC-2006-0011	28-Day Study in rasH2 Hybrid Mice	T, TI	2.6.6.3.1	2.6.7.6
MKC-PC-2005-0011	13-Week Study in CD-1 Mice	T, TI	2.6.6.3.2	2.6.7.7A
PDC-PC-1996-0002	4-Week Study in Rats	T	2.6.6.3.3	2.6.7.7B
PDC-PC-1999-0002	26-Week Study in Rats	T	2.6.6.3.4	2.6.7.7C
MKC-PC-2003-0002	26-Week Study in Rats	T, TI	2.6.6.3.5	2.6.7.7D
PDC-PC-1996-0001	Preliminary 5-Day Study in Dogs	T	2.6.6.3.6	2.6.7.6
MKC-PC-2003-0022	5-Day Study in Dogs	T, TI	2.6.6.3.7	2.6.7.6
PDC-PC-1997-0001	4-Week Study in Dogs	T	2.6.6.3.8	2.6.7.7E
PDC-PC-1994-0001	4-Week Study in Dogs	T	2.6.6.3.9	2.6.7.6
PDC-PC-2000-0003	13, 26, or 39-Week Study in Dogs	T, TI	2.6.6.3.10	2.6.7.7F
MKC-PC-2003-0004	39-Week Study in Dogs	T, TI	2.6.6.3.11	2.6.7.7G

[a] T=Technosphere particles; TI=Technosphere Insulin

In **mice**, T and TI were tested in TgrasH2 for 28 days and CD-1 mice for 13 weeks by the SC administration. The transgenic mouse study was also used to identify doses for the subsequent carcinogenicity study. The CD-1 mouse study was used to support repeat-dose exposure as second rodent specie. There was evidence of hypoglycemia at high insulin doses in both studies.

- Transgenic mouse study: Up to 75 mg/kg/day T and up to 35 mg/kg/day TI was given to mice by the SC route for 28 days. Because there were early deaths, likely due to hypoglycemia, additional lower dose TI groups were added. Blood glucose levels were variable in these transgenic mice following TI administration. In addition, T alone was well-tolerated and did not affect glucose levels. Moreover, insulin and FDKP C_{max} and exposure ($AUC_{0\text{-}inf}$) increased with increasing doses in males and females. High circulating insulin levels were achieved within 15 min post dosing. The transgenic mouse study revealed that the NOAEL for TI was 5 mg/kg/day, and the NOAEL for T was 75 mg/kg/day.

- CD-1 mouse study: T (10 and 75 mg/kg/day) and TI (10 and 35 mg/kg/day) were given to mice by sc route for 13 weeks. At 35 mg/kg/day TI, deaths were seen and considered as secondary to hypoglycemia. There was an increased body weight which was apparently treatment-related. Blood glucose levels were lowered substantially in these CD-1 mice following TI administration. Additionally, T alone was well-tolerated and did not impact glucose levels. The CD-1 mouse study showed that the NOAEL for TI was 10 mg/kg/day and the NOAEL for T was 75 mg/kg/day.

Note from the reviewer**:** Re: **26 week rat** study vs. **39 week dog** study: When compared to rat inhalation study, there were no decreased body weights in dogs, and there were also no increased

lung organ weights observed in dogs in a T dose-dependent fashion. In addition, no increased proliferating activity in bronchial cells was detected in dogs treated with T and TI. With regards to histopathological findings, a local effect of T inhalation in the nasal cavities (i.e. eosinophilic globules and epithelial degeneration; unrecoverable) was seen in rats, whereas a local effect of T inhalation in the lungs (i.e. alveolar and/or bronchial interstitial neutrophil infiltrate; recoverable) was observed in dogs. Moreover, T could also lead to degeneration and necrosis of the myocardium and infiltration of mononuclear cells, which was not recoverable in rats, whereas T (or TI) could lead to thymic atrophy, and hypocellularity of the seminiferous epithelium, germ cell degenerations in dogs.

In **rats**, three inhalation studies up to 26 weeks were conducted. For T, it was well-tolerated with no apparent dose-limiting toxicities observed. In 4-week study, there was no change in body weight. However, in 13- and 26-week studies, there was a slight decrease in male body weights. For TI, there was a dose-related decrease in blood glucose levels. In addition, upon necropsy, pulmonary organ weights were apparently increased in a T dose-related fashion. However, there were no gross or histological correlates. There were also histological findings, such as eosinophilic globules and epithelial degeneration (olfactory, respiratory epithelium and maxillary sinus) with higher severity and incidence, noted in the nasal cavities of T groups, indicating a local effect of T inhalation. These changes did not show recovery after 30 days following discontinuation of treatment. Approx. 50% males treated with T had degeneration and necrosis of the myocardium and infiltration of mononuclear cells. The cardiac effects were not recoverable.

- The PCNA (Proliferating Cell Nuclear Antigen) analysis (non-GLP) was performed to assess epithelial cell activity in selected sites of the respiratory tree. A slight numerical imbalance was observed in the upper airway bronchial cells in the T and TI groups at week 26. However, the magnitude of change was below the threshold, i.e. > 3x increase over background. This change was likely related to duration of exposure and upper airway particulate impaction, since alveolar airway cells were not affected. No histological changes of epithelial hyperplasia or metaplasia were observed in any of the respiratory tree tissues. In addition, insulin was not likely to cause these changes because these changes also occurred in animals not receiving insulin, and were limited to upper airways. In addition, no histological changes of hyperplasia or metaplasia indicative of biologically significant cell proliferation were observed.

- The rat inhalation studies revealed that the NOAEL was < 11.7 mg/kg/day for T and 1.9 mg/kg (males) or 1.3 mg/kg (females) for TI. Hyperplasia and metaplasia are seen in various organs including lung but not in a T dose-dependent manner. Other T related toxicities were seen, including nasal and heart effects with mortality so NOAEL would be lower than 11.7 mg/kg/day. However, only one dose of T was tested in this 26 week rat study. *Note: NOAEL of 11.7 mg/kg/day was determined by the sponsor.*

In **dogs**, six studies up to 39 weeks were conducted. For T, no effects on clinical signs, body weight, feed consumption, or hematology/clinical chemistry parameters were observed up to 15-17 mg/kg/day. Measurable plasma FDKP levels (C_{max} – 4.56-12.15 µg/ml) were observed.

- In 5-day study, TI was well-tolerated and had dose-dependent decreases in blood glucose levels. In 30-day study, 50 mg/kg/day T was well tolerated following via PO administration. A greater incidence of occasional emesis and change in feces was observed in animals receiving T. In 4-week study, up 40.3 mg/kg/day T via the inhalation route was well-tolerated. The NOAEL for T was 40.3 mg/kg (mean C_{max} of 18.6 µg/ml and mean AUC_{0-24h}

of 55 μg•h/ml). In 26-week study, 1.9 mg/kg/day TI produced an increase in the female body weight.

- In one 39-week study, Up to 38 mg/kg/day T produced no toxicologically significant effects. In another 39-week study, both TI (0.36 mg/kg/day for males and 1.92 mg/kg/day for females) and T (2.4 mg/kg/day for males and 10.9 mg/kg/day for females) were well tolerated. Minimal toxicity was observed at the high TI dose and was characterized by neutrophil infiltrate of the lung that regressed following 8 weeks of recovery. In addition, TK analyses showed that T and TI were absorbed quickly and C_{max} was achieved within 20 min after the end of dosing. The NOAEL for T was at the highest dose of 10.9 mg/kg/day and for TI was at the highest dose of 1.92 mg/kg/day.

Genetic toxicology: In vitro studies with TI and T (bacterial reverse mutational and mammalian chromosomal aberration w/ S9) and in vivo studies with T only (mouse micronucleus) showed that there was no mutagenic potential and no apparent genetic toxicity with either T alone, or TI in valid studies. Note from the reviewer: The sponsor did not give an explanation why a mouse micronucleus assay was not done with TI. This might be due to the fact that the genotoxic potential of insulin is well established (negative).

Carcinogenicity: Two studies were done in rats (via inhalation administration for 104 weeks) and transgenic mice (via SC administration for 6 months).

- In mice, they were given T at 25 and 75 mg/kg/day and TI at 2.5 and 5 mg/kg/day (in males) and 0.6 and 1.25 mg/kg/day (in females) for 6 months via the sc route. In rats, they were given T up to 46 mg/kg/day and TI up to 1.23 mg/kg/day (in males) and 0.76 mg/kg/day (in females) for 24 months via the inhalation route. In both studies, the survival rates were not different among groups. Hypoglycemia-related mortality was observed at the higher doses in the transgenic mouse study. The observed dose-limiting toxicity was related to hypoglycemia at high insulin doses. No evidence for carcinogenicity due to T alone or TI in either species.

- In the mouse study, exposure to FDKP was dose proportional and there were no sex differences or evidence of accumulation. Exposure to TI was also dose-proportional for males but greater than dose-proportional for females, with no evidence of accumulation. In addition, no evidence of increased oncogenicity or microscopic pathological effects related to T alone or TI in both male and female transgenic mice.

- In the rat study, both T and TI were well-tolerated by inhalation. Exposure to FDKP increased with increasing dose in all treated groups. There was no definitive TK analysis was not definitive due to the variability in circulating insulin concentrations measured in TI treatment groups. There were no indications of carcinogenic potential of either T alone or TI in both males and females. In addition, lung PCNA analysis showed that alveolar and bronchiolar cells were not different across all groups.

Reproductive toxicology: Reproductive and development toxicity studies were done in rats and rabbits via the sc route. Only T alone was studied to avoid potential confounding effects of insulin-induced hypoglycemia. In rats, there were no teratogenic findings (during organogenesis) and no impairment of fertility or effects on offspring. However, in rabbits, there was maternal toxicity as well as teratogenic findings (i.e. major malformation) during organogenesis.

- Exposure to FDKP was confirmed in pregnant animals; where the exposure was substantial in pregnant rats and rabbits during the critical gestation periods for both species. In rats given 100 mg/kg/day T, AUC_{last} was 8,335,854 ng•min/mL on GD7. In rabbits given 2 mg/kg/day T, AUC_{last} was 482,240 ng•min/mL on GD19. *In a clinical setting, the projected human AUC would be approx. 46,000 ng•min/ml, with the projected human daily exposure of 105 mg TI* (b) (4)

- Utero-exposure was confirmed in a separate rat fetal TK study. This study determined fetal blood FDKP levels in rats after maternal exposure to sc injection of T (0, 10, 30, and 100 mg/kg/day) on presumed GD 6 through 17, indicating dose-proportional fetal exposure at concentrations. Also, the FDKP was still present at least 24 hrs after exposure to the dam.

- In rats, there was no maternal toxicity. The NOAEL in pregnant rats was 100 mg/kg/day, whereas the fetal NOAEL was 100 mg/kg/day. In rabbits, there was a death at the high dose of 100 mg/kg/day. There was also body weight loss at lower doses of 2 mg/kg/day, indicating maternal toxicity. The NOAEL in pregnant rabbits was < 2 mg/kg/day, whereas the fetal NOAEL was 100 mg/kg/day.

Special toxicology: Local tolerance toxicity, Immunotoxicology, PS80 toxicity, photoxicity, environmental toxicology, industrial toxicology of FDKP intermediates, and FDKP impurities qualification study were evaluated for T and/or TI.

- Local tolerance of T and TI was evaluated in a primary skin irritation study in rabbits and as part of a guinea pig dermal hypersensitivity study (T only). In rabbits, both T and TI were similar in dermal response, with early signs of irritancy abating after 24 hrs. Both T and TI were considered non-irritating, using U.S. FDA criteria. In guinea pigs, there was no dermal sensitization after single intradermal injections of T (5% wt /volume), followed by dermal application (25% wt/wt) with dermal application challenges twice, every 2 weeks.

- An immunotoxicity assessment was conducted in rats exposed to T or TI by inhalation administration once daily for 28 days. These animals were evaluated for absolute and relative numbers of lymphocytes, and the ability to mount an antibody response to antigen (T-cell dependent). Up to 44 mg/kg/day (males) and 47 mg/kg/day (females) T and up to 1.33 mg/kg/day (males) and 0.79 mg/kg/day (females) TI were achieved. In addition, a separate subgroup received a dose of 75 mg/kg/day daily by the SC administration. These animals had no significant changes in lymphocyte subpopulations or response to T-cell dependent antigen. However, a decrease in NK cells was noted after the SC administration but was no known clinical relevance in the absence of the control group by the same route of exposure.

- The toxicity of PS80 (polysorbate 80), a component of TI, was studies in rats given T containing (b) (4) PS80 for 28 days. The NOAEL was the high dose of 43 mg/kg/day T (1.11. mg/kg/day PS80). The safety margin for PS80 administered by pulmonary delivery at the maximum anticipated human dose was 24.7 fold, based on mg/kg basis for TI.

- The photoxicity of T and TI were study in mouse 3T3 fibroblasts cells, in the presence or absence of UVA light, which was assessed by neutral red uptake. Neither TI nor T particles was considered to be phototoxic at the highest concentration (1000 mg/L) tested.

- The exotoxicity potential of FDKP was studied in freshwater fishes and algae at a maximum concentration of 100 mg/L, in the presence of Daphnia magna. FDKP showed no exotoxic

potential. In addition, FDKP can be biodegraded in the presence of microorganisms in sewage treatment plant. It is not likely to bioaccumulate or result in terrestrial exposures due to its low octanol-water partition coefficient.

- Seven FDKP impurities [redacted] (b) (4) were monitored during manufacturing development. These impurities were qualified in core toxicology studies and in an additional 28-day impurity qualification study with three major impurities [redacted] (b) (4) by the SC administration. There were no systemic toxicities with these major impurities. Others such as [redacted] (b) (4) were not tested in this 28-day study, but had been qualified previously at 16-, 10-, and 7.5-fold the maximum anticipated human dose, respectively. [redacted] (b) (4) was only not tested because it could be removed during TI formulation. Moreover, Ames mutagenicity studies for these individual FDKP impurities were not conducted due to solubility issues. However, mutagenicity studies with FDKP containing the impurities were negative.

Study Number	Study Title	Test Article(s)[a]	Written Summary	Tabular Summary
MKC-PC-2007-0019	Primary Skin Irritation Study in Rabbits	T, TI	2.6.6.7.1	2.6.7.16
PDC-PC-2001-0001	Dermal Sensitization Study in Guinea Pigs	T	2.6.6.8.1	2.6.7.17
MKC-PC-2008-0020	28-Day Immunotoxicity Study in Rats	T, TI	2.6.6.8.2	2.6.7.17
MKC-PC-2008-0006	28-Day Toxicity Study with 3 Known Impurities in Rats	T	2.6.6.8.3	2.6.7.17
MKC-PC-2008-0005	28-Day Toxicity Study of PS80-Enriched Technosphere® Particles in Rats	T	2.6.6.8.4	2.6.7.17
MKC-PC-2007-0004	Phototoxicity Assay in Balb/c 3T3 Mouse Fibroblasts	T, TI	2.6.6.8.5	2.6.7.17
MKC-PC-2008-0016	Primary Dermal Irritation/Corrosion Study with 3 FDKP Intermediates in Rabbits	Intermediates	2.6.6.8.10.1	2.6.7.17
MKC-PC-2008-0018	Dermal Sensitization Study in Guinea Pigs with 3 FDKP Intermediates	Intermediates	2.6.6.8.10.2	2.6.7.17
MKC-PC-2008-0019	Acute Oral Toxicity Study with 3 FDKP Intermediates in Rats	Intermediates	2.6.6.8.10.3	2.6.7.17
MKC-PC-2008-0023	Primary Ocular Irritation/Corrosion Study with 3 FDKP Intermediates in Rabbits	Intermediates	2.6.6.8.10.4	2.6.7.17
MKC-PC-2008-0021	FDKP Partition Coefficient	T	2.6.6.8.8	2.6.7.17
MKC-PC-2008-0001	Acute Toxicity to *Daphnia Magna*	T	2.6.6.8.6	2.6.7.17
MKC-PC-2008-0002	Algal Growth Inhibition Assay	T	2.6.6.8.7	2.6.7.17
MKC-PC-2008-0024	Ready Biodegradability: Modified Sturm Test	T	2.6.6.8.9	2.6.7.17

[a] T=Technosphere; TI=Technosphere Insulin

2.6.6.2 Single-dose toxicity

3 studies were submitted by the sponsor:

- PDC-PD-1993-0001: acute oral tox rat study

- PDC-PC-1993-0002: acute oral tox rat study
- PDC-PD-1004-0002: acute oral tox dog study

PDC-PC-1993-0001 - Acute, Single Dose Oral Toxicity in Rats; Limit Test

Young Spraque-Dawley adult rats (5/sex/group) were given a single dose on Day 1 at 5 g/kg FDKP (25% w/v) or 20 ml/kg (0.1 M) citric acid as VH control by gavage. These animals were observed for clinical signs for 14 days after dosing. Body weights were obtained on the day of the dosing and at the end of the 14-day observation period. Findings: There were no clinical signs and no changes in body weight related to TI or VH control. The acute oral LD_{50} for 0.1M citric acid was > 20 ml/kg and for FDKP was > 5 g/kg, in both male and female rats.

PDC-PC-1993-0002 – Acute Oral Toxicity Study in Rats (Limit Test) with Technosphere Calcitonin

Young Spraque-Dawley adult rats (5/sex/group) were given a single dose on Day 1 at 50 mg/kg FDKP by gavage. These animals were observed for clinical signs for 14 days after dosing. Body weights were obtained on the day of the dosing and at the end of the 14-day observation period. Serum chemistry analysis was done before the study and at the end of the 14-day observation period. Animals were sacrificed at the end of the study and examined grossly at necropsy. Histopathology was also done on heart, lung, liver, kidneys, esophagus, stomach, duodenum, jejunum, ileum, colon, rectum, mesenteric lymph node, bone with marrow, and all gross lesions. Findings: There were no FDKP treatment related clinical signs, changes in body weight, serum chemistry parameters, gross, or histopathology findings. The acute oral LD_{50} for FDKP was > 50 mg/kg in both male and female rats.

PDC-PC-1994-0002 – Acute Oral Toxicity Study of Technosphere in Dogs (Limit Test)

Beagle dogs at age of 5 months (2/sex/group) were given a single oral dose on Day 1 at 5 g/kg FDKP (paced in gelatin capsules). These animals were observed for clinical signs for 14 days after dosing. Body weights were obtained on the day of the dosing and at the end of the 14-day observation period. Animals were sacrificed at the end of the study and examined grossly at necropsy. Findings: There were no mortalities in this study. However, a moderate amount of emesis, containing traces of test material, was seen at 5 and/or 24 hrs after dosing. Firm/liquid feces were also seen, with trace amounts of test material present, within the 1st 24 hrs after dosing. There were no test article-related gross findings at necropsy.

2.6.6.3 Repeat-dose toxicity

11 studies were submitted by the sponsor:

- PDC-PC-1994-0001: 4-week study in dogs
- PDC-PC-1996-0001: 5-day study in dogs
- PDC-PC-1996-0002: 4-week study in rats
- PDC-PC-1997-0001: 4-week study in dogs
- *PDC-PC-1999-0002: 26-week study in rats*
- *PDC-PC-2000-0003: 13-, 26-, 39-week study in dogs*
- *MKC-PC-2003-0002: 26-week study in rats (pivotal)*
- *MKC-PC-2003-0004: 39-week study in dogs (pivotal)*

- MKC-PC-2003-0022: 5-day study in dogs
- MKC-PC-2005-0011: 13-week study in CD-1 mice
- MKC-PC-2006-0011: 28-day study in rasH2 Hybrid mice

PDC-PC-1994-0001: Technosphere And T/Calcitonin (5% Load), 4 Week Toxicity Study in Dogs, Inhalation and Subcutaneous [By (b) (4) Study No. 94443]

Sixteen dogs (8 M/8 F) were assigned into 4 groups and administered with VH control (saline), T, or T+Calcitonin at either 5 mg/kg or 50 mg/kg for 30 days.

Dose Groups	Dose Suspensions mg/mL	Daily Dose mg/kg	Number of Males	Number of Females
Vehicle Control Saline pH 5.7	0	0	2 (1 & 2)	2 (3 & 4)
Technosphere™	50	50	2 (5 & 6)	2 (7 & 8)
Technosphere™ plus Calcitonin	5	5	2 (9 & 10)	2 (11 & 12)
Technosphere™ plus Calcitonin	50	50	2 (13 & 14)	2 (15 & 16)

Animal numbers appear in parentheses.

Findings: All dogs survived to their terminal sacrifice. No-test article related toxicity was noted in any of the antemortem parameters measured in these dogs. However, a greater incidence of occasional emesis and changes in feces were evident in animals receiving T (Groups 2, 3, and 4). Similarly, no test-article related postmortem changes were found in these dogs. However, there were few gross changes, including small right thyroid in one VH control male, tan/pink mottling of the left cranial and left cardiac lobes of the lung in one 50 mg/kg T male, and red/congested axillary lymph node in one 50 mg/kg T male. There were also some microscopic tissues changes, such as peribronchiolar and granulomatous inflammation, thymus atrophy, and axillary lymph hemorrhage. Nonetheless, these gross and microscopic changes observed in this study were not related to T or TI treatment. All in all, the study indicated that T and T+Calcitonin did not produce pathologic changes in beagle dogs.

PDC-PC-1996-0001: Preliminary 5-Day Inhalation Feasibility Study in Dogs on Technosphere [By (b) (4) Study No. I96009, reviewed by Herman Lee and his review was dated 08/20/02 in DARRTS]

This inhalation feasibility study was performed in 2 beagle dogs (1 M/1 F). These dogs were given FDKP powder daily for 5 days (15.18 mg/kg BW/day for the male and 16.74 mg/kg BW/day for the female). Findings: The FDKP was well-tolerated. There were no mortalities and no toxicologically changes in clinical observations, body weight gain, food consumption, hematology, and clinical chemistry parameters. In addition, TK analysis showed the evidence of FDKP exposure with a C_{peak}, ranging from 4.56 to 15.0 µg/ml, approx. 15 min after the end of exposure. The values of the females were approx. 3X as high as the plasma levels of males.

PDC-PC-1996-0002: Fumaryl-Technosphere (Powder) Four-Week Inhalation (Nose-Only) Toxicity Study in the Rat [By (b) (4) Study No. I96032, reviewed by Herman Lee and his review was dated 08/20/02 in DARRTS]

A total of 140 (70 M/70 F) rats were dosed daily for 4 weeks with an inhalation exposure system for dusts. The dust was administered 60, 150, and 180 min. The effectively achieved doses were calculated to be 0.5 (low dose), 14.4 (mid dose), and 61.7 (high dose) FDKP mg/kg BW/day.

Number of rats in groups:

Group	male rats	female rats
1	10 + 5	10 + 5
2	10 + 5	10 + 5
3	10 + 5	10 + 5
4	10 + 10 + 5	10 + 10 + 5

GROUP	NO. OF ANIMALS m/f	APPLICATION TIME [min.]	ESTIMATED INHALED DOSE OF FUMARYL-TECHNOSPHERE™ [mg/kg BW/d]	ACHIEVED INHALED DOSE OF FUMARYL-TECHNOSPHERE™ [mg/kg BW/d]	ANIMAL NUMBER MALE - NO. FEMALE - NO.
G1 (AIR CONTROL)	15/15	180	0	-	101 - 115 151 - 165
G2 (LOW DOSE)	15/15	60	0.8	0.5	201- 215 251 –2 65
G3 (MID DOSE)	15/15	150	10	14.4	301 – 315 351 – 365
G4 (HIGH DOSE)	25/25	180	120	61.7	401 – 425 451 – 475

Findings: There were 7 deaths but these were not caused by the test article. All deaths were from TK animals due to blood sampling or cardiac puncture. There were no test article-related changes in clinical signs, body weight, food consumption, ophthalmology, hematology, clinical chemistry, and urinalysis parameters, observed in these animals. In addition, there were no test-article related organ weight changes, as well as gross or histopathological findings observed in these rats.

PDC-PC-1997-0001: Fumaryl-Technosphere™/ Fumaryl-Technosphere™ about 5% Salmon Calcitonin 4-Week Toxicity Study in Dogs, Inhalation and Subcutaneous Administration [By (b) (4) Study No. I97003]

A total of 36 (18 M/18 F) Beagle dogs were dosed for 4 weeks. The animals from Groups 2, 3, and 5, were dosed once a day for 8 min whereas the animals from Groups 1 and 4 were dosed three times a day for 21 min each.

Groups	Animals Number (incl. Pharmaco-kinetic)	Identification	Test Article	Target Dose (mg/kg)	Route of Administration	Duration of Exposure (min.)
1	3 m 3 f	101-103 151-153	Air	/	Inhalative	63**
2	3 m 3 f	201-203 251-253	Fumaryl-Technosphere™	0.4	Inhalative	8
3	3 m 3 f	301-303 351-353	Fumaryl-Technosphere™	4.0	Inhalative	8
4	3 m 3 f	401-403 451-453	Fumaryl-Technosphere™	40.0	Inhalative	63**
5	3 m 3 f	501-503 551-553	Salmon Calcitonin-Technosphere™	4.0	Inhalative	8
6	3 m 3 f	601-603 651-653	Salmon Calcitonin	0.1/0.05*	Subcutaneously	-

Findings: There was no compound-related mortality. Clinical signs, including emesis, were seen in Group 4 females and in Group 5 males and females. Emesis was probably caused by the high amount of dust, causing local and unspecific irritation in the pharynx. Females were more affected. In addition, there were no changes in mean body weight gain and mean food consumption in animals treated with Technosphere alone. However, a severe decrease in body weight due to reduced food intake was observed in animals treated with Salmon Calcitonin Technosphere (Groups 5 and 6). There was also were no changes in ECG, and ophthalmology, urinalysis, bone marrow parameters. Moreover, for hematology, animals treated with T showed no compound-related changes. However, animals treated with T+Calcitonin or Calcitonin showed a tendency to slightly increased platelet counts. Male animals from this group also showed slightly decreased thrombin times. For clinical chemistry, animals treated with T revealed no dose or time dependent changes that could be attributed to the test article. However, animals treated with T+Calcitonin or Calcitonin had minor changes, such as slight reductions of APT in Groups 5 and 6 females, decreased bilirubin levels in Group 6 males and females, and slight decrease of total protein in Group 6 animals. Furthermore, there were no gross pathological findings and organ weight changes that could be attributed to test compound. No treatment-related histopathological findings were seen in the respiratory tract or other organ systems. Lesions at the sc injection site were indicative of mild to moderate local toxicity of the compound. Lastly, the NOAEL of T was 40.3 mg/kg, with overall mean C_{max} of 18.6 µg/ml and $AUC_{0\text{-}24h}$ of 55 µg*h/ml.

MKC-PC-2003-0022: Technosphere®/Insulin: A 5-Day Dose Range Finding Inhalation Toxicity Study in Beagle Dogs [By (b) (4) Study No. 6031]

This study was conducted to help select target doses for a subsequent 39-week inhalation study in the dog (pivotal study #2). The animals were exposed to T or TI for 15 min via an oronasal face mask once daily for 5 days. Estimated achieved dosages were 0, 0.0265, 0.0894, and 0.1996 mg Insulin/kg/day for Groups 1, 2, 3, and 4, respectively.

Dose Group / Treatment	Dose Level of Insulin U/kg/day[C]/ (mg/kg/day)		Overall Dose Level of Technosphere® or Technosphere®/Insulin (mg/kg/day)[D]	No. of Animals	
	Target[A]	Achieved	Theoretical	Males	Females
1 (Control)	0[B]	0[B]	1.966	1	1
2 (Low Dose)	1.092/ (0.0379)	0.764/ (0.0265)	0.265	1	1
3 (Mid Dose)	3.277/ (0.1137)	2.576/ (0.0894)	0.894	1	1
4 (High Dose)	5.461/ (0.1895)	5.666/ (0.1966)	1.966	1	1

[A] = Target value was calculated based on an estimated body weight of 8 kg.
[B] = Control animals were exposed at a target aerosol concentration of Technosphere® gravimetrically equivalent to that of the High Dose group.
[C] = Target and achieved dose levels were converted to International al Unit (U) using a conversion factor of 1U insulin = 0.0347 mg of Insulin.
[D] = Theoretical dose levels for Technosphere®/Insulin (Groups 2 to 4) were calculated assuming that the achieved insulin dosage was 10-% and the rest of 90% was Technosphere®.

Findings: There were no mortalities during the study and there were no adverse clinical signs observed. No changes in body weights and food consumption in treated animals. There were dose-dependent decreases in plasma glucose levels, following exposure in mid and high dose groups (Groups 3 and 4). However, levels steadily increased to within 20% of predose levels by 4 hrs post exposure. TK analyses showed that high dose TI was able to produce measurable plasma exposure to T (as measured by plasma FDKP) and to Insulin. For both plasma FDKP and insulin, female dogs seemed to receive a greater exposure than the male dogs. A decrease in exposure to both FDKP and Insulin by Day 5 was also observed in the mid and high dose groups (Groups 3 and 4). However, only 1 dog/sex/group, the significance of these findings could not be established.

MKC-PC-2005-0011: A 13-Week Study of the Comparative Toxicokinetic Profile and of the Comparative Toxicity of Technosphere®-Particles and of Technosphere®-Insulin in CD-1 Mice [By (b) (4) Study No. 7679]

This study was conducted in mice given T or TI daily for 13 weeks via SC injection. The test article doses evaluated were 10 and 75 mg/kg/day T and 10 and 35 mg/kg/day TI. The SC injection was the route of administration chosen because it was the route by which TI would be evaluated in a subsequent carcinogenicity study in mice.

Group Number	Group Designation	Dose Level*** (mg/kg/day)	Dose Volume* (mL/kg/day)	Dose Concentration (mg/mL)	Numbers of Animals			
					Main Study		Toxicokinetic Phase	
					Males	Females	Males	Females
1	Control**	0	10	0	10	10	9	9
2	Low Dose T®	10	10	1	10	10	54	54
3	High Dose T®	75	10	7.5	10	10	54	54
4	Low Dose T®I	10	10	1	10	10	54	54
5	High Dose T®I	35	10	3.5	10	10	54	54

T® - Technosphere®-Particles
T®I - Technosphere®-Insulin
* The volume (10 mL/kg body weight) of each dose given to each relevant mouse each day was administered as a single bolus by subcutaneous injection.
** The mice of Group 1 received sterile Phosphate Buffered Saline (PBS).

Findings: Nine mice were found dead. Since these animals belonged to high dose TI group (35 mg/kg/day), their deaths were considered to be related to a hypoglycemic response. Besides hypoglycemic responses, there were no compound-related adverse clinical signs. The body weight, food consumption, hematology, and clinical chemistry parameters, were unaffected by the T or TI treatment. With regards to blood glucose levels, 10 and 35 mg/kg/day TI resulted in a lowering of blood glucose to approx. 50% (or less) when compared to control and pre-treatment levels. This effect was comparable at both doses and showed no sign of reversal during the 120 min post-dosing sampling period. In addition, there were no effects on organ weight and macroscopic/microscopic findings when treated with T or TI. As for TK analysis in mice, after SC injection up to 13 weeks, exposure to FDKP was approx. dose-proportional. Based on the similarity of the exposure to FDKP in Group 2 and 4, FDKP exposure did not appear to be affected by the presence of insulin with T. On the other hand, insulin exposure increased with an increase in dose, although less than dose-proportionally. Exposure was also slightly higher in the male mice, although generally less than 20% greater than seen in the female mice.

Overall, daily SC injection of T (up to 75 mg/kg/day) produced no adverse effects on any of the parameters evaluated. Daily SC injection of TI (up to 35 mg/kg/day) caused deaths in a total of 6 animals receiving 35 mg/kg/day and 3 animals at the same dose levels euthanized for humane reasons. Decreased blood glucose levels were observed in both 10 and 35 mg/kg/day groups treated with TI. This was, however, known as a pharmacological effect of insulin. No other adverse effect in any of the other parameters evaluated. Also, insulin exposure, as measured by C_{max} and AUC, was less than dose proportional, with slightly higher exposures in males when compared to females. In the high dose group, the insulin exposures were slightly decreased after repeated exposure when compared to a single dose. Lastly, based on the mortality observed in mice receiving TI at a dose of 35 mg/kg/day, the NOAEL for TI was 10 mg/kg/day. Based on lack of changes in any of the parameters evaluated, the NOEL for T was 75 mg/kg/day.

MKC-PC-2006-0011: 28-Day Repeated Dose Subcutaneous Toxicity Study in Model 00178-W CB6F1/Jic-TgrasH2@Tac Hybrid Mice [By (b) (4) Study No. AB29DV/AB29DW.2S3R (b) (4)

This study was designed for the selection of doses for the subsequent pivotal TgrasH2 6-month carcinogenicity original and supplemental study. Animals were treated with VH alone (PBS), T or TI, daily for 28 days via sc injection. The dose levels for the T were 10, 35, 75 mg/kg/day whereas the dose levels for the TI were 2.5, 5, 10, 15, and 35 mg/kg/day.

Group Number	Test Article	Nominal Dose (mg/kg/day)	Main Group N/Sex	Toxicokinetic Group N/Sex
1	Vehicle (PBS)	0	10	5
2	Technosphere® Particles	10	10	20
3	Technosphere® Particles	35	10	20
4	Technosphere® Particles	75	10	20
5	Technosphere®/Insulin	10	10	20
6	Technosphere®/Insulin	35	10	20
7	Vehicle (PBS)	0	10	5
8	Technosphere®/Insulin	2.5	10	20
9	Technosphere®/Insulin	5	10	20
10	Technosphere®/Insulin	15	10	20

Findings: 22 moralities were seen following treatment with TI (1 at 2.5 mg/kg, 2 at 10 mg/kg, 3 at 15 mg/kg/day, and 16 at 35 mg/kg) among main and TK animals. Male groups had increased mortalities of 3.3%, 3.3%, and 16.7% following treatment with 10, 15, and 35 mg/kg/day TI, respectively. Female groups had increased mortalities of 3.3%, 6.7%, and 33.3% following treatment with 10, 15, and 35 mg/kg/day TI, respectively. Mortality in TI-treated animals was presumed to be the result of severe hypoglycemia. During cageside and hands-on observations, lethargy was noted in 1 male at 10 mg/kg/day TI and in 1 female at 35 mg/kg/day T. A hunched appearance was also noted in 1 female at 35 mg/kg/day TI. During unscheduled observations, comatose, prostrate, labored/dyspnea, and lethargy were noted in 1 TK animal at 15 mg/kg/day TI. In addition, treatment with T or TI did not have effects on body weights, body weight gains, and weekly and total food consumption (Groups 2-6 vs. Group 1, and Groups 8-10 vs. Group 7) in either sex. Several hematology (i.e. decreased WBC, lymphocytes, and monocytes in some males and decreased RBC, hemoglobin with increased platelets and lymphocytes in some females), and clinical chemistry (i.e. increased Na+ and Cl- in Group 2 male [vs. Group 1] and increased K+ and AST in Groups 8 and 10 females [vs. Group 7]) parameters were significantly different in treated groups when compared to the corresponding VH group. However, these changes were considered, by the sponsor, to be biologically insignificant due to lack of dose dependency and corresponding microscopic findings. Furthermore, there were no changes in organ weights in males or females across all does groups. No macroscopic and microscopic findings were observed in treated animals.

As for TK analysis, high circulating insulin levels were achieved within 15 min after sc injection of TI. Insulin, FDKP C_{max} and $AUC_{0\text{-}inf}$ increased exponentially with increasing doses in male and female mice. At dose < 15 mg/kg, the relationship between C_{max} and dose was linear. The doses > 15 mg/kg, C_{max} increases were not linear in both males and females. Multiple dosing of TI lead to higher systemic insulin exposure in females compared to males in 3/5 dose groups evaluated (5, 10, and 35 mg/kg/day). All in all, based on this study, the suggested MTD for use in a 26 week oncogenecity transgenic mice was 10 mg/kg/day for TI. The NOAEL for TI in this study was 5 mg/kg/day. The NOAEL and MTD for the T were determined to be 75 mg/kg/day.

PDC-PC-1999-0002: A 26-Week Inhalation Toxicity Study of Fumaryl Technosphere™ (b) (4) in the Albino Rat [By (b) (4) Study No. 91740, reviewed by Herman Rhee and his review was dated 08/20/02 in DARRTS]

Rats (141 M/141 F) were given FDKP daily via inhalation for 26 week. The animals in the Group 1 were exposed daily to conditioned room air for the same duration as the high dose group (Group 3). The animals in the treated groups were exposed daily to a powder formulation of FDKP (b) (4) The theoretical achieved doses were 5.3 and 51.3 for Groups 2 and 3, respectively.

Group No. Identification	Target Dose Level (mg/kg/day)	Main Study* Males	Main Study* Females	Recovery Animals Males	Recovery Animals Females	Toxicokinetic Males	Toxicokinetic Females
1 Air control	0	1001-1030	1501-1530	1031-1035	1531-1535	-	-
2 Low dose	5	2001-2030	2501-2530	2031-2035	2531-2535	2036-2038	2536-2538
3 High dose	50	3001-3030	3501-3530	3031-3035	3531-3535	3036-3038	3536-3538
4 Health screen	-	4001-4010	4501-4510	-	-	-	-

* The first 15 rats/sex/group of the main study animals were euthanized following 13 weeks of treatment. The remaining 15 rats/sex/group were euthanized following 26 weeks of treatment.

Group	Gender	RMV (L/min)	Active conc'n (mg/L)	Exposure duration (min)	MMAD (µm)	Deposition Fraction	Body weight (kg)	Theoretical Achieved Dose (mg/kg/day)
Low	Male Female	0.205 0.137	0.276	60	2.2	0.65	0.4490 0.2630	4.9 5.6 **5.3** [a]
High	Male Female	0.197 0.134	2.04	60	2.7	0.85	0.4249 0.2561	48.2 54.4 **51.3** [a]

a Mean of Males and Females.

Findings: There were no treatment related deaths, clinical signs, or ocular changes. A slight decrease in mean body weight was observed in the high dose males, starting from Week 6 and was still evident following the 4-week recovery period. However, there was no toxicologically significant effect on food consumption, clinical pathology parameters (hematology, clinical biochemistry, and urinalysis), or organ weight (except minor adrenal weight change). In addition, there were no treatment-related macroscopic findings. Minor increases in the number of alveolar macrophages and phagocytic activities were noted (see table below). Such increases, however, were considered to be a normal physiological response to the inhalation of particulate matter than a toxic effect. These increases were absent after 4-week recovery. Other histological changes included foci of tension lipidosis in livers, renal pelvic dilation, erythrocytosis in lymph nodes, and thymic, possibly agonal, focal hemorrhage. These changes were considered, by the sponsor, to be spontaneous in origin. Overall, daily 60 min exposure of rats to FDKP [redacted] (b) (4) for 26 weeks resulted in slightly decreased body weights at the high dose. Therefore, the NOEL was determined to be < 48 mg/kg/day.

	Males			Females		
Group No.	1	2	3	1	2	3
13 weeks:						
Number of animals examined	15	15	15	15	15	15
Alveolar macrophage accumulation	-	5	12	-	-	5
26 weeks:						
Number of animals examined	15	16	15	15	15	16
Alveolar macrophage accumulation	1	4	11	-	2	8

Herman Rhee's review on this study:

Conclusion: NOAEL appeared to be >5 mg/kg/day in rats, which is approximately 25 times of the recommended human dose. There were significant reductions in body weight during the entire study period even after the recovery period without significant changes in food consumption in male rats.

Gross and Histopathology: There were pale areas in the liver and dark foci in occasional lymph nodes and the thymus. The sponsor considered that they appeared to be incidental as reported in 13-week studies. Other histological changes seen were foci of lipidosis in livers, renal pelvic dilatation, erythrocytosis in lymph nodes and thymic hemorrhage appeared to be spontaneous in origin. No histological changes were seen that might account for the minor adrenal weight change reported in a 26-week study and most of the gross changes were observed in control air group as shown below. The severity scores in the histopathological findings at Week 27 and after recovery period were also summarized in the table below.

Fumaryl Technosphere™ Effects on Histology after 26-Week Treatment & Recovery in Rats												
Treatment Duration	Findings at 26-Week Treatment						Findings After 4-Week Recovery					
Groups	Control		LD		HD		Control		LD		HD	
Sex	M	F	M	F	M	F	M	F	M	F	M	F
Epididymis: Oligo/Aspermia	1						1					
Lymph node mandibular: Plasmacytosis	1											
Lymph node tracheobronchial hemorrhage			2									
Reproductive: Testes-atrophy	1						1					
Pituitary: Cyst	1											
Pituitary: Hyperplasia at pars distalis	1						1					
Liver: Tension lipidos		1										
Thymus: Atrophy/necrosis – lymphoid		1										
1, 2, and 3 indicate minimal, slight, and moderate histopathological findings, respectively. Blank indicates negative finding and other tissues such as kidney and urinary bladder were examined.												

PDC-PC-2000-0003: 13, 26, or 39 Week Inhalation Toxicity Study of Fumaryl Technosphere (b) (4) or Insulin with Fumaryl Technosphere (b) (4) Powder Formulation in the Beagle Dog [By (b) (4) Study No. 91739, reviewed by Herman Rhee and his reviews were dated 08/20/02 and 09/17/02 in DARRTS]

The animals in the Group 1 were exposed daily to conditioned room air for the same duration as the high dose group (Group 3). The animals in Groups 2 and 3 were exposed daily to a Technosphere powder. Individual doses for Group 4 animals were calculated based on the most recent body weight to achieve a target dose of 5 U insulin/kg/day. The targeted doses for FDKP for Groups 2 and 3 were 5.2 and 40.5 mg/kg/day respectively.

Group No. Identification	Target Dose Level (mg/kg/day)	Toxicology Animals*		Recovery Animals	
		Males	Females	Males	Females
1 Air control	0	101-106	151, 152, 160, 154, 155, 161	107-109	157-159
2 Low dose	5	201-206	251,260,261, 254-256	207-209	257-259
3 High dose	50	310,302-306	351, 352, 360,354-356	307-309	357-359
4 Insulin	5U/Kg/day	401-403	451-453	404-405	454-455

* 3 dogs/sex/group of the main study were euthanized following 13 weeks of treatment (Groups 1 to 3) and 3 dogs/sex in Group 4 were euthanized following 26 weeks of treatment. The remaining 3 dogs/sex in Groups 1 to 3 were euthanized following 39 weeks of treatment. The recovery animals were maintained untreated for a period of 4 weeks following the appropriate treatment period.

Findings: There was no deaths and no treatment-related clinical signs, ocular, or cardiovascular changes during the treatment period. There was an increased body weights in females exposed to TI. Food consumption and clinical chemistry parameters were not affected by treatment. There were also no treatment-related changes in the hematology parameters. However, higher eosinophil counts were observed in 3 treated males during the main study when compared to control animals. Due to a huge individual variability, this finding was not considered treatment related. In addition, there were no treatment related changes in organ weights (except an increase in lung weights in males after 13 weeks, a trend decrease in kidney weights in males and females after 13 weeks, and an increase in adrenal gland weights after 39 weeks) and pathological findings (except occasional changes in localized foci or inflammation sites of the respiratory tract). Moreover, spontaneous changes (i.e. congestion or hemorrhage in lymph node sinuses, localized areas of inflammation within the lungs, accompanied by fibrosis and increased width of subpleural alveolar septae, pituitary cysts, and thymic atrophy) were observed in animals from all treatment and recovery groups. However, these findings were considered, by the sponsor, within the normal range of common findings for dogs of this age. Overall, daily exposure of FDKP to dogs for 26 weeks produced an increase in the body weight of females but not in the males. However, daily exposure of FDKP (as high as 42.0 mg/kg/day) to dogs for 39 weeks produced no toxicologically significant effects.

Herman Rhee's review on this study:

Study Title: A 13-Week Inhalation Toxicity Study of (b) (4) Fumaryl Technosphere™ alone or with Insulin in Dogs

Conclusion: Fumaryl Technosphere™ had no remarkable treatment-related effect in 13-week toxicity study in dogs. NOAEL must be at or near 42 mg/kg/day (achieved dose of Fumaryl Technosphere™ in dogs).

Gross and Histopathology:
There were no drug-related gross lesions observed in any of the treated animals. No direct and treatment-related histopathological changes were seen in the respiratory tract or in the lung. A few gross findings appeared to be sporadic and not drug-treated related. Spontaneous lesions were observed in control and treated animals with similar incidences and severity, which appeared to be incidental(Please see the two tables below). There were no gender specific histopathological findings.

PROJECT NO.: 91739

INCIDENCE OF ANIMALS WITH MICROSCOPIC FINDINGS BY ORGAN/GROUP/SEX

WEEK 13

MALE

DOSE GROUP DESTINATION		1	2	3
NUMBER OF ANIMALS EXAMINED		3	3	3
L.NODE MANDIBULAR	EXAMIN:	3	3	3
L.NODE MESENTERIC	EXAMIN:	3	3	3
LARYNX	EXAMIN:	3	3	3
- Inflammation		-	-	1
LIVER	EXAMIN:	3	3	3
LUNG	EXAMIN:	3	3	3
- Inflammation		2	2	-
- Inflammation: bronchoalveolar		-	-	1
- Inflammation: pleura		-	-	1
- Macrophage accumulation		1	-	1

Re: IND 61,729
Technosphere
(Insulin Inhalation System)
Serial Number: 007
Project# 91739
Week 13

Week 13										
Study Group		1		2		3		4		
Dose (mg/kg b.w./day)		Air		5		50		5 + 5 U insulin		
Animals (male, female)		m	f	m	f	m	f	m	f	
Number of animals sacrificed		3	3	3	3	3	3	0	0	
Cardiovascular System										
	hematocyst	A								
	ectopic thyroid									
Respiratory System										
	lung-inflammation					S				
	bronchus-inflammation					S				
	larynx-inflammation					S				
Liver	congestion									
Reproductive System										
	Testes-atrophy									
	Epididymis-oligo/azospermia									
	Ovary-cyst									
Endocrine System										
	pitutary-cyst					T				
	parathyroid-cyst									
	thymus-atrophy									
Lymphatic System										
	mediastinal node-hemorrhage									
	mediastinal node-pigment deposit									
	cervical lymph node-hyperplasia									
	tracheobronchial lymph node-hemorrhage	A				S				
	deep cervical lymph node-hemorrhage									
	mesenteric lymph node-hemorrhage									
	mesenteric lymph node-hyperplasia									
	spleen-inflammation									
Integumentary System										
	skin-crust									
	skin-ulceration									

Capital and small letters indicate male and female dogs, respectively. All are moderate in severity. * indicates severity score was marked.

Study Title: A 39-Week Inhalation Toxicity Study of (b) (4) Fumaryl Technosphere™ alone and with Insulin in Dogs including 4-Week Recovery Study

Conclusion: Fumaryl Technosphere™ had no remarkable treatment-related effect in 13- or 39-week toxicity study in dogs except an increase in adrenal weight in HD male group. NOAEL must be at or near 42 mg/kg/day (achieved dose of Fumaryl Technosphere™ in dogs).

Gross and Histopathology:
No clear treatment-related histopathological changes were seen in the respiratory tract or in the lung. A few gross findings appeared to be sporadic and not drug-treated related. Spontaneous lesions were observed in control and treated animals with similar incidences and severity, which were documented in two tables below. In the group 4 (5 mg technosphere and 5 U insulin), more dogs had histopathological findings with high severity scores in both sex. This may indicate that the incidences are more common with insulin than with technosphere. There were no gender specific histopathological findings during the 39-week study and after additional 4-week recovery period as shown below.

Histopathological Findings in 39-Week Toxicologic Studies in Dogs(#91739)								
Treatment Duration	39-Week Treatment				Recovery at Week-43			
Dose in mg/kg/day	Air	5	50	5 +*	Air	5	50	5 +*
Number of dogs examined	6	6	6	6	6	6	6	4
Cardiac/Hematocyst		1%		1@				1
"/Ectopic thyroid					1			
Respiratory/Lung inflammation				1		1		
"/Bronchus-inflammation								
"/Larynx-inflammation								
Liver congestion						1		
Reproductive/Testes-atrophy				1@				
"/Epididymis-obligo/azospermia	1		1	1@				
"/Ovary-cyst					1			
Endocrine/Pituitary-cyst					1	1		
"/Parathyroid-cyst								
"/Thymus-atrophy		1	1			2	1	
Lymphatic/Mediastinal node-hemorrhage	3	3%~	3	2&		1	1	1$
"/Mediastinal node-pigment deposit								
"/Cervical lymph node-hyperplasia				1				
"/Tracheobronchial lymph node-hemo^		1%						1$
/Deep cervical lymph node-hemorrhage		1~						
"/Mesenteric lymph node-hemorrhage								
"/Mesenteric lymph node-hyperplasia		1						
"/Spleen-inflammation		1&		1&		1		
Integumentary/Skin-crust	1							
"/Skin-ulceration			1					
*This dose groups had insulin (5 Units) on top of 5 mg/kg/day technosphere and were examined at Weeks 26 and 30 for recovery. ^Hemorrhage. The same signs such as @, $ or & indicate the same animal.								

Re: IND 61,729
Technosphere
(Insulin Inhalation System)
Serial Number: 007
Project# 91739
Week 26

Week 26											
Study Group			1		2		3		4		
Dose (mg/kg b.w./day)			Air		5		50		5 + 5 U insulin		
Animals (male, female)			m	f	m	f	m	f	m	f	
Number of animals sacrificed			0	0	0	0	0	0	3	3	
Cardiovascular System											
	hematocyst								DD		
	ectopic thyroid										
Respiratory System											
	lung-inflammation									dd	
	bronchus-inflammation										
	larynx-inflammation										
Liver	congestion										
Reproductive System											
	Testes-atrophy								DD		
	Epididymis-oligo/azospermia								DD *		
	Ovary-cyst										
Endocrine System											
	pitutary-cyst										
	parathyroid-cyst										
	thymus-atrophy										
Lymphatic System											
	mediastinal node-hemorrhage								BB	aa *	
	mediastinal node-pigment deposit										
	cervical lymph node-hyperplasia									cc	
	tracheobronchial lymph node-hemorrhage										
	deep cervical lymph node-hemorrhage										
	mesenteric lymph node-hemorrhage										
	mesenteric lymph node-hyperplasia										
	spleen-inflammation								BB		
Integumentary System											
	skin-crust										
	skin-ulceration										

Capital and small letters indicate male and female dogs, respectively. All are moderate in severity. * indicates severity score was marked.

Recovery Study: Recovery group animals previously exposed to (b) (4) powder of technosphere with or without insulin showed that there were no gross treatment-related changes. Small number of histological changes that were seen in animals from all treated and recovery groups were within normal range of common findings for dogs of the age. The minor changes included congestion or hemorrhage in lymph node sinuses, localized area of inflammation within the lungs with fibrisis, pituitary cysts, or thymic atrophy as indicated previously and in a table below.

Re: IND 61,729
Technosphere
(Insulin Inhalation System)
Serial Number: 007
Project# 91739
Week 30 (recovery)

Week 30 (recovery)									
Study Group		1		2		3		4	
Dose (mg/kg b.w./day)		Air		5		50		5 + 5 U insulin	
Animals (male, female)		m	f	m	f	m	f	m	f
Number of animals sacrificed		0	0	0	0	0	0	2	2
Cardiovascular System									
	hematocyst							FF	
	ectopic thyroid								
Respiratory System									
	lung-inflammation								
	bronchus-inflammation								
	larynx-inflammation								
Liver	congestion								
Reproductive System									
	Testes-atrophy								
	Epididymis-oligo/azospermia								
	Ovary-cyst								
Endocrine System									
	pitutary-cyst								
	parathyroid-cyst								
	thymus-atrophy								
Lymphatic System									
	mediastinal node-hemorrhage								ff
	mediastinal node-pigment deposit								
	cervical lymph node-hyperplasia								
	tracheobronchial lymph node-hemorrhage							FF	
	deep cervical lymph node-hemorrhage								
	mesenteric lymph node-hemorrhage								
	mesenteric lymph node-hyperplasia								
	spleen-inflammation								
Integumentary System						✓			
	skin-crust								
	skin-ulceration								

Capital and small letters indicate male and female dogs, respectively. All are moderate in severity. * indicates severity score was marked.

Pivotal Study #1

Study title: A 26-Week Inhalation Toxicity Study of A Powder Technosphere®/Insulin Formulation In The Albino Rat With A 13-Week Interim Kill and A 30-Day Recovery Period (MKC-PC-2003-2002)

Key study findings:

- Exposure to T (11.7 mg/kg/day) and TI (as high as 3.46 IU/kg/day in females and 5.18 IU/kg/day in males) were stable and respirable. T and TI were well-tolerated by the rats after 26 weeks of exposure.
- Hypoglycemic responses were observed, leading to the death of 1 high dose male, which was considered pharmacological responses to TI.
- There were no other clinical signs that were considered of toxicological significance that could be attributed to T or TI treatment.
- Decreased body weights and/or body weight gains were noted in T control (Group 2) and high dose TI (Group 4) males and females, in a T-dose dependent manner. Decreased body weights (i.e. approx. 8% on Day 183) were also observed in females from the T control group (Group 2). In addition, slightly decreased food consumptions were observed in T control (Group 2) females when compared to air control Group 1. Decreased body wt was consistently seen with T treatment in rodents.
- No treatment related changes were observed on ophthalmology, hematology, clinical chemistry, and urinalysis parameters. No change in monthly insulin levels that could be attributed to treatment.

- Slightly increased glucose levels, prior to exposure, were observed in high-dose TI males and females (Group 4) during the Month 6. Glucose levels at a dose of 3.46 (F)/5.18 (M) IU/kg/day were slightly to significantly decreased in rats from both sex on Days 1 (main group and newly added TK group single-dosed on Study Day 176), 90, and 182 following the exposure.
- The T administration correlated with an increase in pulmonary weight in a dose-related manner when compared to control (Group 2 [5.9%] > Group 4 [3.4%] > Group 3 [2.9%]) at Week 26. However, the toxicological significance could not be clearly confirmed as this dose did not correlate with any gross or microscopic findings.
- There were no macroscopic changes observed that could be attributed to treatment. Most of microscopic findings, such as eosinophilic globules and epithelial degeneration in the nasal cavities, seen in M and F given T and TI were not dose-dependent. Higher severity and incidence of these findings were observed in the T control group, suggesting that the changes observed in the T/TI animals were likely due to the inhalation of the T particulate. In addition, the presence of insulin did not influence either the incidence or severity of these lesions. Moreover, histological alterations were limited to those consistent with impaction on the nasal epithelium. There were no signs of recovery in the males and a slight decrease in the incidence of the findings was observed in females.
- Minimal to slight myocardial degeneration/necrosis was seen in 50% T-treated males at 11.7 mg/kg/day.
- There was no dose-dependent lung histopathology observed with T or TI. The PCNA analysis in the lung sections showed that an increase in proliferation rate of bronchial cells was observed at Weeks 13 and 26 in the TI-treated groups. However, there were signs of recovery in high-dose TI animals (Group 4) following a 4-week recovery period. This was considered as a pharmacological effect of the insulin. In addition, there were no increases in alveolar cells PCNA.
- For TK analysis of insulin, C_{max} and $AUC_{0\text{-}tlast}$ values were generally slightly higher in females when compared to males after corrected for insulin dose. There was a slight increase detected following repeated TI exposure, however such increase was not considered to be the result of accumulation to steady-state levels, due to the relatively short terminal 1/2 life seen and the general lack of quantifiable levels at pre-dose. For TK analysis of FDKP, as measured by mean serum C_{max} and AUC_{last} as a function of dose across a single blood sampling day, showed an increase with dose, but was less than dose proportional.
- A dose-dependent increase (1.4-1.6 fold) in CYP3A1/2 activity was seen in low-dose and high-dose TI (Groups 3 and 4) male rats.
- The NOAEL for TI (Technosphere+ insulin) was 5.18/3.46 (MF) IU/kg/day or 1.91/1.28 (M/F) mg/kg/day.
- The NOAEL for T (Technosphere) was < 11.7 mg/kg/day due to 50% male having myocardial degeneration/necrosis and 3 deaths post-dosing.

Study no.: PCS-MTL Study No. 78235
Volume #, and page #: Module 4.2.3.2 repeat-dose-tox, 1468 pages
Conducting laboratory and location: (b) (4)

Date of study initiation: April 2003.
GLP compliance: Yes. Few exceptions were listed below.

- Prestudy filter analysis performed at MannKind Corporation (non-GLP); as this was for prestudy atmosphere characterization only and not for animals' treatment, this is not considered to have any impact on the study data.
- Proliferating Cell Nuclear Antigen (PCNA) evaluation; the data provided by this evaluation can be used as investigational only. Actual GLP evaluation would need to be performed in order to support this study or program.
- Test article analysis was performed under Good Manufacturing Procedures at MannKind Corporation.
- QA reports for TK report (FDKP) were not provided to test facility study director.

QA report: yes (x) no ( )
Drug, lot #, and % purity: See below.

2.1. Control Article

Identity	Technosphere®
Batch/Lot No.	D035U.03.002, D035U.03.003, D035U.03.005, D035U.03.006
Retest Date	December 31, 2004, February 28, 2005, March 31, 2005, March 31, 2005
Description	White powder
Purity	At least 99%
Storage Conditions	Room temperature, dark and desiccate
Handling Precautions	Lab coat, gloves, goggles and mask (respirator was worn during treatment)
Supplier	MannKind Corporation

2.2. Test Article

Identity	Technosphere®/Insulin
Batch/Lot No.	D043R.03.003, D043R.03.004, D043RB.03.001, D043RB.03.002
Retest Date	February 28, 2005, March 31, 2005, June 30, 2005, July 2005 (Expiry date for lot D043RB.03.002)
Description	White powder
Purity	9.0 to 9.1% Insulin
Storage Conditions	-20°C, dark, over desiccant
Handling Precautions	Lab coat, gloves, goggles and mask (respirator was worn during treatment)
Supplier	MannKind Corporation

Methods

Doses: See table below.

Group No. Identification	Technosphere Target Dose Level (mg/kg/day)	Insulin Target Dose Level (IU/kg/day)	Animal Number			
			Males			
			13 Week Phase*	26 Week Phase*	Recovery	Toxicokinetic**
1 Air control	0	0	1001-1015	1016-1030	1031-1035	1036-1047
2 Technosphere® control	11.2	0	-	2001-2015	-	2016-2027
3 Technosphere®/insulin	1.12	3	3001-3015	3016-3030	-	3031-3042
4 Technosphere®/insulin	1.87	5	4001-4015	4016-4030	4031-4035	4036-4048
5 Health screen	-	-	5001-5010	-	-	-
			Females			
Identification			13 Week Phase*	26 Week Phase*	Recovery	Toxicokinetic**
1 Air control	0	0	1501-1515	1516-1530	1531-1535	1536-1547
2 Technosphere® control	11.2	0	-	2501-2515	-	2516-2527
3 Technosphere®/insulin	0.37	1	3501-3515	3516-3530	-	3531-3542
4 Technosphere®/insulin	1.12	3	4501-4515	4516-4530	4531-4535	4536-4547
5 Health screen	-	-	5501-5510	-	-	-

* At the end of the 13- and 26-week periods, 10 animals/sex/group were randomly selected for conduct of terminal procedures.
** No study parameters other than body weight were recorded for toxicokinetic animals.

Species/strain: Spraque-Dawley CD® (Crl: CD® (SD) BR) rats
Number/sex/group or time point (main study):
15/sex/group (#1, #3, and #4) for 13 week or 26 week phase
15/sex/group (#2) for 26 week phase only
10/sex/group (#5) for 13 week phase only
Route, formulation, volume, and infusion rate: Inhalation, exposure daily
180 min for Groups 1 and 2
35 and 100 min for Groups 3 and 4 females respectively
110 and 180 min for Groups 3 and 4 males respectively
Satellite groups used for toxicokinetics or recovery:
TK: 6/sex/group; Recovery: 5/sex/group
Age: 9 to 10 weeks of age
Weight: Between 309-393 g (males) and 153-258 g (females)
Sampling times: See Observations and Times.
Unique study design or methodology (if any): TK and 30-day recovery period, inhalation by nose only

Observations and times:

Mortality: Twice daily
Clinical signs: Twice daily
Body weights: Weekly
Food consumption: Weekly
Ophthalmoscopy: Once prior to the start of treatment and again on Weeks 13 and 26.
EKG: Not done.
Hematology: During Week 13 and again at study termination (Weeks 13 and 26 phases)

Parameters examined:

*+ activated partial thromboplastin time
blood cell morphology
erythrocyte indices (MCV, MCH, MCHC and RDW)
hematocrit
hemoglobin
mean platelet volume
platelet count
*+ prothrombin time
red blood cell count
reticulocyte count
white blood cell count (total, absolute and percent differential)

+ At termination and for pre-euthanized animals.

Clinical chemistry: Same as above.

Parameters examined:

A/G ratio (calculated)		
alanine aminotransferase		
albumin		
alkaline phosphatase	#	glucose
aspartate aminotransferase		inorganic phosphorus
blood urea nitrogen		potassium
calcium	#+*	serum insulin
chloride		sodium
cholesterol		total bilirubin
creatinine		total protein
globulin (calculated)		triglycerides

Performed monthly on the first 5 rats/sex/group prior to dosing.
+ Serum insulin not performed on health screen animals.
* Serum insulin was analyzed by PCS-MTL's Immunochemistry group.

Urinalysis: Same as above.

Parameters examined	microscopy of centrifuged deposit
	nitrite
bilirubin	pH
blood	protein
color and appearance	specific gravity
glucose	urobilinogen
ketones	volume

Gross pathology: Upon completion of the treatment and recovery periods.

Organ weights: At the end of the study.

For each main study animal euthanized at completion of treatment and recovery periods, the following organs were dissected free of fat and weighed:

adrenal glands	
brain	pituitary
heart	prostate
kidneys	spleen
liver	thymus
lungs	thyroid lobes and parathyroid glands
ovaries/testes	uterus

Histopathology: Adequate Battery: yes (x), no ( ) - explain: See below for tissues collected.
Peer review: yes ( ), no (x) – Not mentioned by the sponsor.

	abnormalities
a	animal identification
	adrenals
	aorta (thoracic)
**	bone and marrow (sternum)
	brain (cerebrum, cerebellum, midbrain and medulla oblongata)
	bronchi
	cecum
	cervix
	colon
	duodenum
*	epididymides
	esophagus
*	eyes
	Harderian glands
	heart (including section of aorta)
	ileum
	jejunum
	kidneys
	larynx
	lacrimal glands
	liver (sample of 2 lobes)
++	lungs(all lobes)
	lymph nodes (tracheobronchial, mandibular and mesenteric)
+	mammary gland (inguinal)
	skeletal muscle
	skin (inguinal)
	spinal cord (cervical)
**++	nasal cavities and sinuses (3 levels)
	spleen
*+	optic nerves
	stomach
	ovaries
*	testes
	pancreas
	thymus
	pharynx
+	thyroid lobes (and parathyroids)
	pituitary
	tongue
	prostate
	trachea
a	rectum
	urinary bladder
	salivary gland
	uterine horns
	sciatic nerve
	uterus
	seminal vesicles
	vagina

*	Fixed in Zenker's fluid (euthanized animals only).
**	Bone decalcified prior to sectioning.
+	Examined histopathologically if present in routine sections of eyes (optic nerves), thyroid lobes (parathyroid glands), or skin (mammary gland).
++	Infused with neutral buffered 10% formalin (all animals).
[a]	Retained but not processed.

Groups 1, 2 and 4: Tissues listed under tissue preservation
Group 3: Respiratory tissues (lungs, larynx, pharynx, bronchi, trachea and nasal cavities and sinuses, tracheobronchial lymph nodes)
All gross lesions

TK/Cytochrome P450 Analyses:

- P450 analysis: At termination of the 13 Week phase. Activities of CYP1A1/2, 2B1/2, 3A1/2, and 2E1 were determined.
- TK analysis: Blood samples were collected on Days 1 and 90, Day 176 (7 time points), and on Day 182 (6 time points)

The animals bled on Day 176 (following a single dose administration, see above) after the start of dosing are as follows:

Group	Pre-dose	2 min post-Rx	10 min post-Rx	40 min post-Rx	3 hours post-Rx	8 hours post-Rx	24 hours post-Rx
1	1042-1044 1542-1544	1045-1047 1545-1547	1042-1044 1542-1544	1045-1047 1545-1547	1042-1044 1542-1544	1045-1047 1545-1547	1042-1044 1542-1544
2	2022-2024 2522-2524	2025-2027 2525-2527	2022-2024 2522-2524	2025-2027 2525-2527	2022-2024 2522-2524	2025-2027 2525-2527	2022-2024 2522-2524
3	3037-3039 3537-3539	3040-3042 3540-3542	3037-3039 3537-3539	3040-3042 3540-3542	3037-3039 3537-3539	3040-3042 3540-3542	3037-3039 3537-3539
4	4043-4045 4542-4544	4046-4048 4545-4547	4043-4045 4542-4544	4046-4048 4545-4547	4043-4045 4542-4544	4046-4048 4545-4547	4043-4045 4542-4544

The animals bled on Day 182 after the start of dosing are as follows:

Group	Pre-dose	2 min post-Rx	10 min post-Rx	40 min post-Rx	3 hours post-Rx	8 hours post-Rx
1	1036-1038 1536-1538	1039-1041 1539-1541	1036-1038 1536-1538	1039-1041 1539-1541	1036-1038 1536-1538	1039-1041 1539-1541
2	2016-2018 2516-2518	2019-2021 2519-2521	2016-2018 2516-2518	2019-2021 2519-2521	2016-2018 2516-2518	2019-2021 2519-2521
3	3031-3033 3531-3533	3034-3036 3534-3536	3031-3033 3531-3533	3034-3036 3534-3536	3031-3033 3531-3533	3034-3036 3534-3536
4	4036-4038 4536-4538	4039-4041 4539-4541	4036-4038 4536-4538	4039-4041 4539-4541	4036-4038 4536-4538	4039-4041 4539-4541

Note from the reviewer: Anti-drug antibody detection was not found in this study.

Results

Mortality: On Day 2, one high-dose TI male (No. 4038) had neurological dysfunction with 3 mg/dL glucose (historical range was 104-223 mg/dL). On Day 56, one high-dose TI female (No. 4531) had a moderate mass and severe lesion on the abdominal region. On Day 73, three T control group (Nos. 2504, 2508, and 2517) died immediately after dosing. These three animals

were weak, cold to touch, and had decreased activity. On Day 86, one high-dose TI male (No. 4008) died after ophthalmology assessment. On Day 176, one high-dose TK female (No. 4542) died after blood collection. On Day 182, one Air Control female (No. 1539) and one high-dose male (No. 4042) died also after blood collections. These signs were typical of hypoglycemic shock in TI-treated animals. However, the deaths in T-treated animals suggest that the T dose at 11.7 mg/kg/day exceeds the MTD. Note from the reviewer: Based on cardiac histopathology, it indicates that these deaths might be cardiac related since these animals were given insulin.

Clinical signs: Clinical signs included protruding eyeballs in 2 low-dose TI females (Group 3) on Days 52/59 and in 2 high-dose TI females (Group 4) on Days 73/80/87. The sponsor considered this was not related to treatment even though this was not observed in the control animals. In addition, other clinical signs included thin fur cover and/or staining of the fur in various regions, scabbing and/or dried skin or scab of the tail, and broken teeth. These observations were sporadic and were considered unrelated to treatment.

TABLE NO. 5 INCIDENCE OF CLINICAL OBSERVATIONS MALES PROJECT NO. 78235

GROUP 1 - AIR CONTROL
GROUP 2 - TECHNOSPHERE CONTROL
GROUP 3 - LOW DOSE TECHNOSPHERE®/INSULIN 3/1 IU/kg/day
GROUP 4 - HIGH DOSE TECHNOSPHERE®/INSULIN 5/3 IU/kg/day

Count of Frequency Number	Group / Sex			
Clinical Sign\Site	1m	2m	3m	4m
Number of animals per group	**35**	**15**	**30**	**35**
Broken Toe Nail\Digit Forepaw Left	2	1	0	0
Broken Toe Nail\Digit Forepaw Right	1	0	1	2
Broken Toe Nail\Digit Hindpaw Right	1	0	0	0
Broken Toe Nail\Forepaw Left	1	1	0	1
Broken Toe Nail\Forepaw Right	0	1	1	2
Broken Toe Nail\Hindpaw Left	0	0	0	1
Broken Toe Nail\Hindpaw Right	0	0	1	1
Found Dead	0	0	0	1
Fur Staining Green\Muzzle	0	0	0	1
Fur Staining Red\Hindpaw Left	1	0	0	0
Fur Staining Red\Hindpaw Right	1	0	0	0
Fur Staining Red\Muzzle	2	0	0	2
Fur Staining Red\Periorbital Right	1	0	0	0
Fur Thin Cover\Abdominal	1	1	1	0
Fur Thin Cover\Dorsal Cervical	0	0	1	0
Fur Thin Cover\Forelimb Left	3	4	4	2
Fur Thin Cover\Forelimb Right	3	3	4	1
Fur Thin Cover\Forepaw Left	8	3	7	6
Fur Thin Cover\Forepaw Right	8	4	6	6
Fur Thin Cover\Inguinal Right	1	0	0	0

Count of Frequency Number	Group / Sex			
Clinical Sign\Site	1m	2m	3m	4m
Number of animals per group	**35**	**15**	**30**	**35**
Fur Thin Cover\Ventral Cervical	0	0	1	0
Fur Ungroomed	4	1	1	4
Material Liquid Red	1	0	0	0
Prepuce Discharge Mucoid	0	0	2	0
Skin Dry\Tail	3	2	1	1
Skin Lesion\Tail	0	0	1	1
Skin Papule\Forepaw Right	0	1	0	0
Skin Papule\Tail	4	0	3	2
Skin Red\Forelimb Left	0	0	1	1
Skin Red\Forelimb Right	0	0	2	1
Skin Red\Forepaw Left	0	0	1	0
Skin Red\Forepaw Right	0	0	1	0
Skin Red\Hindpaw Left	0	0	0	1
Skin Red\Left	0	0	0	1
Skin Red\Pinna Left	0	0	1	0
Skin Red\Pinna Right	0	0	1	0
Skin Red\Tail	5	2	2	2
Skin Scab\Footpad Left	0	1	0	0
Skin Scab\Footpad Right	0	1	1	0
Skin Scab\Forelimb Right	0	0	1	0

Count of Frequency Number	Group / Sex			
Clinical Sign\Site	1m	2m	3m	4m
Number of animals per group	**35**	**15**	**30**	**35**
Skin Scab\Forepaw Left	0	1	2	0
Skin Scab\Forepaw Right	0	0	3	2
Skin Scab\Tail	6	2	2	3
Swollen\Tail	1	0	0	0
Tail Bent	2	0	1	2
Tail Severed	0	0	0	1
Teeth Broken	1	2	4	4
Teeth Missing	1	0	0	1
Terminal Euthanasia	35	15	30	34

TABLE NO. 5 INCIDENCE OF CLINICAL OBSERVATIONS FEMALES PROJECT NO. 78235

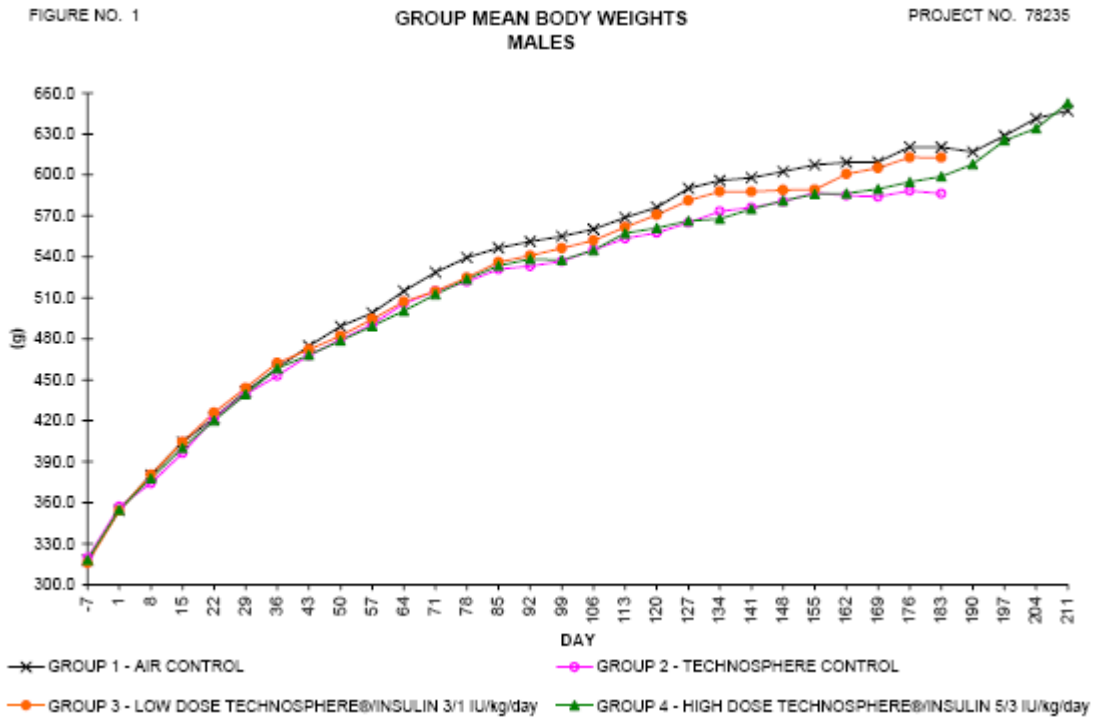
GROUP 1 - AIR CONTROL
GROUP 2 - TECHNOSPHERE CONTROL
GROUP 3 - LOW DOSE TECHNOSPHERE®/INSULIN 3/1 IU/kg/day
GROUP 4 - HIGH DOSE TECHNOSPHERE®/INSULIN 5/3 IU/kg/day

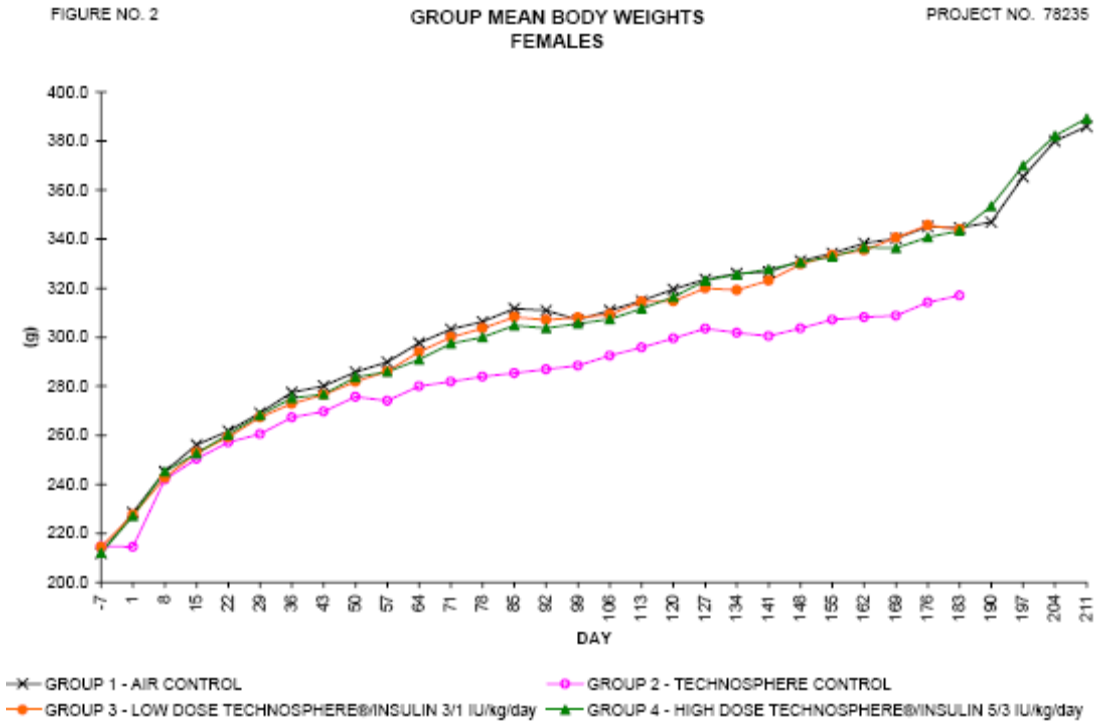
Count of Frequency Number	Group / Sex			
Clinical Sign\Site	1f	2f	3f	4f
Number of animals per group	**35**	**15**	**30**	**35**
Activity Decreased	0	2	0	1
Broken Toe Nail\Forepaw Right	0	0	0	2
Cold to Touch	0	2	0	0
Convulsions Non-Sustained	0	1	0	0
Dehydrated	0	0	0	1
Eye Partly Closed\Right	0	0	0	1
Eyeball Protruding\Left	0	0	2	2
Eyeball Protruding\Right	0	0	2	2
Found Dead	0	2	0	0
Fur Staining Black\Periorbital Right	0	0	0	1
Fur Staining Red\Cranium	1	0	0	0
Fur Staining Red\Muzzle	1	0	1	1
Fur Staining Red\Periorbital Left	0	0	0	1
Fur Staining Red\Periorbital Right	0	0	0	1
Fur Thin Cover\Abdominal	1	0	1	1
Fur Thin Cover\Forelimb Left	2	3	2	3
Fur Thin Cover\Forelimb Right	2	3	2	4
Fur Thin Cover\Forepaw Left	3	2	1	6
Fur Thin Cover\Forepaw Right	3	2	3	8
Fur Thin Cover\Hindlimb Left	0	0	1	2

Count of Frequency Number	Group / Sex			
Clinical Sign\Site	1f	2f	3f	4f
Number of animals per group	**35**	**15**	**30**	**35**
Fur Thin Cover\Hindlimb Right	0	0	1	2
Fur Thin Cover\Lumbar	0	0	0	1
Fur Thin Cover\Muzzle	0	0	1	0
Fur Ungroomed	0	0	0	1
Malocclusion	0	0	0	1
Mass No.01\Abdominal	0	0	0	1
Muscle Tone Decreased	0	0	0	1
Skin Black\Mass No.01 Site	0	0	0	1
Skin Blue\Muzzle	0	0	2	0
Skin Dry\Forelimb Left	0	1	0	0
Skin Dry\Forepaw Left	0	1	0	0
Skin Dry\Tail	2	0	6	1
Skin Lesion w/ Discharge\Tail	0	1	0	0
Skin Lesion\Abdominal	0	0	0	1
Skin Lesion\Mouth	0	0	1	0
Skin Lesion\Tail	0	1	0	0
Skin Papule\Forelimb Right	0	1	0	0
Skin Papule\Tail	1	4	0	3
Skin Red\Digit Forepaw Right	1	0	0	0
Skin Red\Forelimb Left	0	1	0	0
Skin Red\Forelimb Right	0	1	0	0

Count of Frequency Number	Group / Sex			
Clinical Sign\Site	1f	2f	3f	4f
Number of animals per group	**35**	**15**	**30**	**35**
Skin Red\Forepaw Left	0	1	0	0
Skin Red\Forepaw Right	1	1	0	0
Skin Red\Hindpaw Right	0	1	0	0
Skin Red\Tail	0	2	2	3
Skin Scab\Digit Forepaw Right	0	0	1	0
Skin Scab\Eyelid Left	1	0	0	0
Skin Scab\Footpad Left	0	1	0	0
Skin Scab\Footpad Right	1	1	0	0
Skin Scab\Forelimb Left	0	2	0	0
Skin Scab\Forepaw Left	0	2	0	1
Skin Scab\Forepaw Right	1	1	0	1
Skin Scab\Periorbital Right	0	0	0	1
Skin Scab\Tail	5	3	4	5
Swollen\Tail	0	1	0	0
Tail Bent	1	0	1	0
Teeth Broken	3	1	2	3
Teeth Missing	1	1	1	0
Terminal Euthanasia	35	13	30	34
Thin	1	0	0	1
Unscheduled Euthanasia	0	0	0	1
Weak	0	2	0	0

Body weights: During main study period, slight decreases in body weights and/or body weight gains were noted in all groups in the males in a T dose-related manner, when compared to air control group. There were statistically significant decreases in body weights in T-treated females (Group 2), when compared to the air control group from Week 10 onwards. During recovery period, high-dose TI males (Group 4) regained weight and body weights were similar to the control animals (Group 1). There were no differences in the females' body weights during the recovery period, when compared to controls. Lastly, other statistically significances were seen sporadically and were not considered to be any toxicological significance.





Food consumption: During main study period, significantly lower food consumption was noted from Week 1 onwards in Group 2 females, when compared to air control Group1. On the other hand, increased food consumption was noted in Groups 3 and 4 females, when compared to T control Group 2 females throughout the study. In addition, no change in food consumption was observed in T or TI-treated males. During recovery period, there were no differences in food

consumption noted. Lastly, other statistically significances were seen sporadically and were not considered to be of any toxicological significance.

Ophthalmoscopy: There were no treatment-related ocular findings noted at Weeks 13 and 26.

EKG: Not performed.

Hematology: At Week 13, slight increases in WBC and lymphocytes in T control Group 2 males and females were noted when compared to air control Group 1. However, the levels were within historical reference range and were not considered to be any of toxicological significance.

Hematology	Sex	Time	Air control	T control	TI low	TI high
↓ BASO (%) WBC Differential Count	M	Week 13		0.38 ±0.11	0.29 ±0.13	0.28 ±0.11
↓ WBC ($10^3/\mu L$)	M	Week 13		10.100 ±2.131	8.415 ± 2.414	8.398 ± 2.047
↓ LYMP (n) WBC Differential Count Absolute	M	Week 13		8.002 ±1.765	6.344 ±2.179	
↓ MONO (n) WBC Differential Count Absolute	M	Week 13		0.275 ±0.095		0.190 ±0.067
↓ BASO (n) WBC Differential Count Absolute	M	Week 13		0.040 ±0.013	0.026 ±0.016	0.024 ±0.012
↑ MPV (fL)	M	Week 13	7.48 ±0.29	7.72 ±0.27	7.95 ±.38	8.23 ±0.38
↓ EOS (%) WBC Differential Count	F	Week 13		2.21 ±1.14		1.67 ±0.45
↓ EOS (n) WBC Differential Count Absolute	F	Week 13		0.135 ±0.044		0.098 ±0.030
MPV (fL)	F	Week 13	7.85 ±0.56	7.82 ±0.26	8.10 ±0.28	8.40 ±0.43
↑ MCHC (g/dL)	F	Week 13	31.74 ±0.71		32.19 ±0.53	32.15 ±0.73
↓ RETIC (%)	F	Week 13		2.09 ±0.42		1.63 ±0.51
↑ PT (sec)	M	Week 26	15.27 ±0.88	16.45 ±1.21		
↑ MPV (fL)	F	Week 26	7.26 ±0.39			7.78 ±0.31
↑ PT (sec)	F	Week 26	14.70 ±0.59	15.36 ±0.53		
↑ MONO (%) WBC Differential Count	M	Recovery	2.20 ±0.41			2.96 ±.30
↓ ESO (%) WBC Differential Count	M	Recovery	2.64 ±0.40			1.82 ±0.68
↓ EOS (%) WBC Differential Count	F	Recovery	3.14 ±0.72			1.98 ±0.55

↑ WBC (10^3/µL)	F	Recovery	2.124 ±0.500			3.862 ±1.036
↑ LYMP (n) WBC Differential Count Absolute	F	Recovery	1.448 ±0.322			2.822 ±0.899
↑ MCHC (g/dL)	F	Recovery	33.72 ±0.49			34.46 ±0.29

* generated by the reviewer.

Clinical chemistry/Urinalysis: At Week 13, increases in AST and ALT (2X higher than other animals) were noted in two low-dose TI animals (1 male and 1 female). This was not observed in high-dose TI animals (Group 4). At Week 26, decrease in cholesterol level was observed in T control group, when compared to air control Group 1. However, this was within historical reference ranges and was not considered any toxicological significance. Moreover, there were no changes in clinical chemistry parameters noted at the end of the recovery period. Lastly, there was no T or TI treatment-related change in urinalysis parameters at Weeks 13 and 26, and at the end of the recovery period.

Clinical Chemistry	Sex	Time	Air control	T control	TI low	TI high
↑ BUN (mg/dL)	M	Week 13	15.93 ±2.54	18.71 ±2.69		
↓ CREA (mg/dL)	M	Week 13		0.66 ±0.08		0.60 ±0.06
CA (mg/dL)	M	Week 13	10.96 ±0.85	12.00 ±1.17	10.94 ±.75	11.05 ±0.65
NA (mEq/L)	M	Week 13	148.0 ±3.5	154.1 ±4.4	149.0 ±3.3	149.1 ±2.5
↓ BUN (mg/dL)	F	Week 13		20.93 ±2.18	18.12 ±2.60	
↓ CREA (mg/dL)	F	Week 13		0.68 ±0.04	0.64 ±0.05	
GLUC (mg/dL)	F	Week 13	108.2 ±25.4	83.8 ±6.0	108.6 ±25.9	107.3 ±28.5
↓ NA (mEq/L)	F	Week 13		148.4 ±1.6	146.7 ±2.2	
PHOS (mg/dL)	F	Week 13	6.732 ±1.042	5.728 ±0.971	6.600 ±1.107	6.604 ±0.945
GLUC (mg/dL)	M	Week 26	144.8 ±29.7	192.0 ±69.4	175.5 ±25.3	
CHOL (mg/dL)	M	Week 26	71.9 ±16.9	54.9 ±23.9	71.9 ±14.1	
↑ NA (mEq/L)	M	Week 26	145.9 ±2.6			148.6 ±3.1
↑ CL (mEq/L)	M	Week 26	105.1 ±2.2	108.6 ±2.9	108.1 ±2.2	108.6 ±2.2
↑ GLUC (mg/dL)	F	Week 26	129.7 ±19.3		162.0 ±18.2	150.9 ±34.1

↓ TP (g/dL)	F	Week 26	7.84 ±0.72	7.03 ±0.53		
↓ ALB (g/dL)	F	Week 26	5.53 ±0.5	4.93 ±0.49		
↑ NA (mEq/L)	F	Week 26	145.0 ±1.3			147.7 ±2.4
↑ CA (mg/dL)	F	Recovery	10.32 ±0.50			10.94 ±0.30

* generated by the reviewer.

Glucose levels: Slight increases in glucose levels were observed in high-dose TI males and females (Group 4) at Month 6, when compared to control Groups 1 and 2. At the end of the recovery, levels remain slightly higher in Group 4, compared to control Group 1. Lower glucose levels were seen in low- and high-dose TI males and females (Groups 3 and 4) when compared to control groups on Days 1, 90, 176, and 182 at the selected time points.

TABLE NO. 10 GROUP MEAN (S.D.) (N) CLINICAL BIOCHEMICAL ANALYSES PROJECT
GLUCOSE LEVELS (mg/dL)
TOXICOKINETIC ANIMALS
DAY 1 - MALES

GROUP 1 - AIR CONTROL
GROUP 2 - TECHNOSPHERE CONTROL
GROUP 3 - LOW DOSE TECHNOSPHERE®/INSULIN 3/1 IU/kg/day
GROUP 4 - HIGH DOSE TECHNOSPHERE®/INSULIN 5/3 IU/kg/day

Group Sex	Summary Information	Time Point 1 - 3 min post dose	20 min post dose
1m	Mean	158.0	154.5
	S.D.	24.2	2.1
	N	3	2
2m	Mean	183.0	199.0
	S.D.	10.4	22.1
	N	3	3
3m	Mean	105.0 a	122.0
	S.D.	30.4	13.9
	N	3	3
4m	Mean	121.0	114.0 a
	S.D.	38.7	55.6
	N	3	3

TABLE NO. 10 GROUP MEAN (S.D.) (N) CLINICAL BIOCHEMICAL ANALYSES PROJECT I
GLUCOSE LEVELS (mg/dL)
TOXICOKINETIC ANIMALS
DAY 1 - FEMALES

GROUP 1 - AIR CONTROL
GROUP 2 - TECHNOSPHERE CONTROL
GROUP 3 - LOW DOSE TECHNOSPHERE®/INSULIN 3/1 IU/kg/day
GROUP 4 - HIGH DOSE TECHNOSPHERE®/INSULIN 5/3 IU/kg/day

Group Sex	Summary Information	Time Point 1 - 3 min post dose	20 min post dose
1f	Mean	183.0	178.0
	S.D.	19.7	3.5
	N	3	3
2f	Mean	156.0	171.0
	S.D.	15.9	39.3
	N	3	3
3f	Mean	108.0 Ba	95.0 Aa
	S.D.	15.6	9.6
	N	3	3
4f	Mean	119.0 B	82.5 Ba
	S.D.	21.7	27.6
	N	3	2

TABLE NO. 10 GROUP MEAN (S.D.) (N) CLINICAL BIOCHEMICAL ANALYSES PROJECT
GLUCOSE LEVELS (mg/dL)
TOXICOKINETIC ANIMALS
DAY 90 - MALES

GROUP 1 - AIR CONTROL
GROUP 2 - TECHNOSPHERE CONTROL
GROUP 3 - LOW DOSE TECHNOSPHERE®/INSULIN 3/1 IU/kg/day
GROUP 4 - HIGH DOSE TECHNOSPHERE®/INSULIN 5/3 IU/kg/day

Group Sex	Summary Information	Time Point 1 - 3 min post dose	20 min post dose
1m	Mean	192.0	246.0
	S.D.	72.0	42.5
	N	3	3
2m	Mean	201.0	203.0
	S.D.	12.0	45.0
	N	3	3
3m	Mean	134.0	131.0
	S.D.	32.1	52.8
	N	3	3
4m	Mean	168.0	181.0
	S.D.	37.5	87.4
	N	3	3

TABLE NO. 10 GROUP MEAN (S.D.) (N) CLINICAL BIOCHEMICAL ANALYSES PROJECT
GLUCOSE LEVELS (mg/dL)
TOXICOKINETIC ANIMALS
DAY 90 - FEMALES

GROUP 1 - AIR CONTROL
GROUP 2 - TECHNOSPHERE CONTROL
GROUP 3 - LOW DOSE TECHNOSPHERE®/INSULIN 3/1 IU/kg/day
GROUP 4 - HIGH DOSE TECHNOSPHERE®/INSULIN 5/3 IU/kg/day

Group Sex	Summary Information	Time Point 1 - 3 min post dose	20 min post dose
1f	Mean	171.0	234.0
	S.D.	6.0	42.0
	N	3	3
2f	Mean	164.0	256.5
	S.D.	40.8	171.8
	N	3	2
3f	Mean	112.0	98.0
	S.D.	26.2	3.5
	N	3	3
4f	Mean	104.0	78.0
	S.D.	35.8	31.2
	N	3	3

TABLE NO. 10 GROUP MEAN (S.D.) (N) CLINICAL BIOCHEMICAL ANALYSES PROJECT
GLUCOSE LEVELS (mg/dL)
TOXICOKINETIC ANIMALS
DAY 176 - MALES

GROUP 1 - AIR CONTROL
GROUP 2 - TECHNOSPHERE CONTROL
GROUP 3 - LOW DOSE TECHNOSPHERE®/INSULIN 3/1 IU/kg/day
GROUP 4 - HIGH DOSE TECHNOSPHERE®/INSULIN 5/3 IU/kg/day

Group Sex	Summary Information	Time Point 2 min post dose	10 min post dose	40 min
1m	Mean	164.0	142.0	
	S.D.	6.2	30.8	
	N	3	3	
2m	Mean	154.0	168.0	
	S.D.	25.2	9.0	
	N	3	3	
3m	Mean	98.0 A	79.5 Ab	
	S.D.	31.5	10.6	
	N	3	2	
4m	Mean	92.0 A	65.0 Ab	
	S.D.	31.8	25.9	
	N	3	3	

TABLE NO. 10 GROUP MEAN (S.D.) (N) CLINICAL BIOCHEMICAL ANALYSES PROJECT
GLUCOSE LEVELS (mg/dL)
TOXICOKINETIC ANIMALS
DAY 176 - FEMALES

GROUP 1 - AIR CONTROL
GROUP 2 - TECHNOSPHERE CONTROL
GROUP 3 - LOW DOSE TECHNOSPHERE®/INSULIN 3/1 IU/kg/day
GROUP 4 - HIGH DOSE TECHNOSPHERE®/INSULIN 5/3 IU/kg/day

Group Sex	Summary Information	Time Point 2 min post dose	10 min post dose	40 min
1f	Mean	165.0	151.0	
	S.D.	12.0	38.0	
	N	3	3	
2f	Mean	153.0	161.0	
	S.D.	16.7	11.4	
	N	3	3	
3f	Mean	100.0 Aa	116.0	
	S.D.	24.2	36.2	
	N	3	3	
4f	Mean	105.0 A	102.0	
	S.D.	24.6	34.6	
	N	3	3	

TABLE NO. 10 GROUP MEAN (S.D.) (N) CLINICAL BIOCHEMICAL ANALYSES PROJECT
GLUCOSE LEVELS (mg/dL)
TOXICOKINETIC ANIMALS
DAY 182 - MALES

GROUP 1 - AIR CONTROL
GROUP 2 - TECHNOSPHERE CONTROL
GROUP 3 - LOW DOSE TECHNOSPHERE®/INSULIN 3/1 IU/kg/day
GROUP 4 - HIGH DOSE TECHNOSPHERE®/INSULIN 5/3 IU/kg/day

Group Sex	Summary Information	Time Point 2 min post dose	10 min post dose	40 min
1m	Mean	126.0	115.0	
	S.D.	0.0	12.1	
	N	3	3	
2m	Mean	118.0	133.0	
	S.D.	3.5	6.2	
	N	3	3	
3m	Mean	65.0 Dc	52.0 Bb	
	S.D.	6.9	6.2	
	N	3	3	
4m	Mean	65.0 Dc	62.0 Ab	
	S.D.	6.2	28.8	
	N	3	3	

TABLE NO. 10 GROUP MEAN (S.D.) (N) CLINICAL BIOCHEMICAL ANALYSES PROJECT
GLUCOSE LEVELS (mg/dL)
TOXICOKINETIC ANIMALS
DAY 182 - FEMALES

GROUP 1 - AIR CONTROL
GROUP 2 - TECHNOSPHERE CONTROL
GROUP 3 - LOW DOSE TECHNOSPHERE®/INSULIN 3/1 IU/kg/day
GROUP 4 - HIGH DOSE TECHNOSPHERE®/INSULIN 5/3 IU/kg/day

Group Sex	Summary Information	Time Point 2 min post dose	10 min post dose	40 min
1f	Mean	109.0	120.0	
	S.D.	7.5	7.9	
	N	3	3	
2f	Mean	121.0	136.5	
	S.D.	3.5	6.4	
	N	3	2	
3f	Mean	74.0 Aa	48.0 Cc	
	S.D.	18.3	7.9	
	N	3	3	
4f	Mean	59.0 Bb	40.0 Cc	
	S.D.	20.0	9.6	
	N	3	3	

Significantly different from control group (group 1) value: A - P ≤ 0.05 B - P ≤ 0.01 C - P ≤ 0.001 (Dunnett)
D - P ≤ 0.05 E - P ≤ 0.01 F - P ≤ 0.001 (Dunn)
Significantly different from control group (group 2) value: a - P ≤ 0.05 b - P ≤ 0.01 c - P ≤ 0.001 (Dunnett)
d - P ≤ 0.05 e - P ≤ 0.01 f - P ≤ 0.001 (Dunn)

Gross pathology: There were no macroscopic findings that could be attributed to T or TI treatment.

Incidence of Necropsy Findings by Organ/Group/Sex Incidence of Necropsy Findings by Organ/Group/Sex
Treatment Period Treatment Period
13 Weeks 13 Weeks
MALE FEMALE

ORGAN/FINDING	DOSE GROUP:	1	3	4
	ANIMAL EXAMINED:	10	10	10
ADRENAL	:			
- -Area dark-	:	-	1	-
KIDNEY	:			
- -Area depressed-	:	-	1	-
- -Dilatation pelvis-	:	-	-	1
LIVER	:			
- -Area pale-	:	5	2	2
LUNG	:			
- -Spongy-	:	-	-	1
L. NODE MANDIBULAR	:			
- -Foci dark-	:	1	-	-
RECTUM	:			
- -Parasite-	:	2	1	-
TRACHEA	:			
- -Fluid pale clear-	:	-	-	1

Incidence of Necropsy Findings by Organ/Group/Sex
Treatment Period
26 weeks
MALE

ORGAN/FINDING	DOSE GROUP:	1	2	3	4
	ANIMAL EXAMINED:	10	2	10	11
ADRENAL	:				
- -Area dark-	:	-	-	1	1
- -Discoloration dark-	:	-	1	-	-
KIDNEY	:				
- -Dilatation pelvis-	:	-	-	-	1
LIVER	:				
- -Area pale-	:	3	-	2	3
LUNG	:				
- -Area dark-	:	-	-	-	1
LYMPH NODE	:				
- -Foci dark-	:	-	-	1	-
L. NODE MANDIBULAR	:				
- -Foci dark-	:	-	-	1	1
L.N.TRACHEOBRONCHIAL	:				
- -Area dark-	:	-	-	-	1
PITUITARY	:				
- -Enlargement-	:	3	-	2	3
RECTUM	:				
- -Parasite-	:	-	-	1	2
STOMACH	:				
- -Area dark-	:	-	2	-	1
SUBCUTANEOUS TISSUE	:				
- -Mass-	:	-	-	-	1
THYMUS	:				
- -Area dark-	:	-	1	-	-
- -Foci dark-	:	-	1	-	-

Incidence of Necropsy Findings by Organ/Group/Sex
Treatment Period
26 weeks
FEMALE

ORGAN/FINDING	DOSE GROUP:	1	2	3	4
	ANIMAL EXAMINED:	10	10	10	10
ADRENAL	:				
- -Foci dark-	:	1	1	1	1
- -Foci pale-	:	-	-	1	1
CECUM	:				
- -Area dark-	:	-	-	-	1
FAT	:				
- -Mass-	:	1	-	-	-
ILEUM	:				
- -Area raised-	:	-	-	1	-
KIDNEY	:				
- -Area pale-	:	-	-	1	-
- -Dilatation pelvis-	:	1	1	-	1
- -Discoloration dark-	:	-	-	-	1
LIVER	:				
- -Area dark-	:	-	2	-	-
- -Area pale-	:	3	2	3	3
LUNG	:				
- -Area dark-	:	-	-	1	1
- -Area pale-	:	1	1	1	1
- -Area raised-	:	-	1	-	-
- -Mass-	:	-	-	1	-
LYMPH NODE	:				
- -Area dark-	:	1	1	1	-
- -Discoloration dark-	:	1	1	-	-
- -Foci dark-	:	-	-	-	1
L. NODE MANDIBULAR	:				
- -Area dark-	:	-	-	-	1
- -Enlargement-	:	-	1	-	1
- -Foci dark-	:	1	1	2	1
L.NODE MESENTERIC	:				
- -Enlargement-	:	-	-	-	1

ORGAN/FINDING	DOSE GROUP:	1	2	3	4
	ANIMAL EXAMINED:	10	10	10	10
ADRENAL	:				
- -Area dark-	:	1	-	-	-
- -Foci dark-	:	-	2	2	-
- -Foci pale-	:	1	-	1	-
CECUM	:				
- -Area dark-	:	-	1	-	-
- -Area raised-	:	-	-	1	1
KIDNEY	:				
- -Adhesion-	:	-	-	-	1
- -Area depressed-	:	-	-	-	1
LIVER	:				
- -Area dark-	:	2	-	-	1
- -Area depressed-	:	1	-	-	-
- -Area pale-	:	1	1	2	4
- -Area raised-	:	1	-	1	-
LUNG	:				
- -Area pale-	:	-	1	-	-
LYMPH NODE	:				
- -Area dark-	:	3	-	1	1
- -Discoloration dark-	:	-	-	-	1
- -Enlargement-	:	-	-	-	1
L. NODE MANDIBULAR	:				
- -Area dark-	:	-	2	2	-
- -Discoloration dark-	:	1	-	-	-
- -Enlargement-	:	-	-	-	1
- -Foci dark-	:	2	3	1	2
L.N.TRACHEOBRONCHIAL	:				
- -Area dark-	:	1	-	2	-
- -Enlargement-	:	1	-	-	-
- -Foci dark-	:	2	-	-	-
- -One not found-	:	1	-	1	-
- -Small-	:	1	-	-	1

ORGAN/FINDING	DOSE GROUP:	1	2	3	4
	ANIMAL EXAMINED:	10	10	10	10
L.N.TRACHEOBRONCHIAL	:				
- -Enlargement-	:	1	1	2	2
- -One not found-	:	-	1	-	-
PROSTATE	:				
- -Area dark-	:	-	1	-	-
- -Foci pale-	:	1	2	1	-
SPLEEN	:				
- -Area raised-	:	-	1	-	-
- -Cyst-	:	-	-	1	-
STOMACH	:				
- -Area dark-	:	1	-	-	-
- -Area depressed-	:	1	-	1	-
- -Foci dark-	:	-	-	1	-
THYMUS	:				
- -Foci dark-	:	1	2	-	1
- -Small-	:	1	1	1	-
THYROID	:				
- -Enlargement-	:	-	-	1	-
- -Foci pale-	:	1	-	1	1
URINARY BLADDER	:				
- -Area pale-	:	-	-	1	-

Incidence of Necropsy Findings by Organ/Group/Sex
Recovery Period

MALE

ORGAN/FINDING	DOSE GROUP:	1	2	3	4
	ANIMAL EXAMINED:	10	10	10	10
OVARY	:				
- -Cyst-	:	1	-	-	1
PITUITARY	:				
- -Enlargement-	:	5	6	3	4
SPLEEN	:				
- -Cyst-	:	1	-	-	1
STOMACH	:				
- -Area depressed-	:	1	-	-	-
SUBCUTANEOUS TISSUE	:				
- -Mass-	:	-	-	1	-
- -Thickening-	:	-	-	1	-
TAIL	:				
- -Area raised-	:	-	1	-	-
THYMUS	:				
- -Foci dark-	:	-	1	1	-
- -Small-	:	-	-	2	1
UTERUS	:				
- -Cyst-	:	-	1	-	-

Incidence of Necropsy Findings by Organ/Group/Sex
Recovery Period

FEMALE

ORGAN/FINDING	DOSE GROUP: 1	4
	ANIMAL EXAMINED: 5	5
ADRENAL		
- -Foci pale-	-	1
LACRIMAL GLAND		
- -Foci dark-	1	-
- -Foci pale-	1	-
LIVER		
- -Area pale-	3	-
LUNG		
- -Area dark-	-	1
L. NODE MANDIBULAR		
- -Foci dark-	1	1
L.N.TRACHEOBRONCHIAL		
- -Enlargement-	1	1
- -Foci dark-	-	1
RECTUM		
- -Parasite-	1	-
SEMINAL VESICLE		
- -Area dark-	1	-
SPLEEN		
- -Cyst-	-	1
TESTIS		
- -Enlargement-	2	-
- -Small-	1	-
- -Soft-	3	-
THYMUS		
- -Foci dark-	1	-
- -Small-	1	-
URINARY BLADDER		
- -Dilatation-	1	-

ORGAN/FINDING	DOSE GROUP: 1	4
	ANIMAL EXAMINED: 5	5
ADRENAL		
- -Area dark-	3	-
- -Enlargement-	1	-
- -Foci dark-	-	1
KIDNEY		
- -Discoloration dark-	-	1
LIVER		
- -Area dark-	-	1
- -Area pale-	2	1
L.N.TRACHEOBRONCHIAL		
- -Area dark-	1	-
- -Not found-	-	1
OVARY		
- -Cyst-	-	1
PITUITARY		
- -Enlargement-	3	3
STOMACH		
- -Foci dark-	1	-
THYMUS		
- -Foci dark-	1	1
- -Small-	1	-

Organ weights:

There was a significant increase in absolute pulmonary weights in males receiving 2.94 IU/kg/day in the 13 week phase compared to the air control group. At Week 26, there was a significant increase in relative pulmonary weights for the T control group (Group 2) males (14%) and females (15%). Such increases in relative pulmonary weights were also observed in TI males receiving 5.18 IU/kg/day (11%) when compared to air control group (Group 1). However, there was a significant decrease (12 to 14%) in relative pulmonary weights in TI females receiving 1.14 and 3.46 IU/kg/day when compared to the T control group (Group 2). These data suggested that the Technosphere administration correlated with an increase in pulmonary weight, and such increase was dose-related (Group 2 > Group 4 > Group 3). However, such effects were not consistent among absolute and relative measures. In addition, effects in males at 5.18 IU/kg/day did not indicate a progression of increase in lung weight from 13 to 26 weeks of treatment. The insulin combined with Technosphere did not potentiate effects on lung weight. Moreover, the significant organ weigh difference observed in the lung in all phases was without macroscopic or microscopic correlative change. These weight differences were not evident after recovery period. Lastly, other differences, including those that reached statistical significance, were not considered toxicological significance. Note from the reviewer: Increased relative lung weight seen in M and F in T and high TI groups. This seems to indicate that the presence of Technosphere particles could result in increased relative lung weight, corrected for body weight.

Organ Weights	Sex	Time	Air control	T control	TI low	TI high

↑ Lung (g)	M	13 weeks	1.5677 ±0.07335			1.7044 ±0.15782
↑ Lung Relative to body wt (%)	M	26 Weeks	0.29250 ±0.036241	0.33392 ±0.036241		0.32357 ±0.025428
↓ Prostate (g)	M	26 weeks	1.5982 ±0.17718			1.4158 ±0.07873
↑ Adrenal Relative to body wt (%)	F	26 weeks	0.01972 ±0.004530	0.02435 ±0.003981		0.02071 ±0.002786
↑ Lung Relative to body wt (%)	F	26 weeks	0.40511 ±0.036898	0.46577 ±0.029980	0.40042 ±0.034027	0.41008 ±0.028485

* generated by the reviewer.

Histopathology: There were findings related to the T or TI in the nasal cavities, such as eosinophilic globules and epithelial degeneration. The main treatment related finding was an increased cytoplasmic accumulation of eosinophilic globules and occurred in 4 areas: sustentacular cells of olfactory epithelium, respiratory epithelial cells in areas bordering the olfactory epithelium, respiratory epithelial cells in the dorsal portion of the maxillary sinus, and occasionally, in the nasal seromucos glands in the lamina propria.

At Week 13, there was accumulation of eosinophilic globules observed in the epithelia of the ethmoid turbinates located in the posterior portion of the nasal cavity (histologic section level 3). There was an effect in the high-dose females both in terms of incidence (1/2 in T control group, 1/10 at 1.14 IU/kg/day, and 8/11 at 3.46 IU/kg/day) and severity (minimal in T control females and 1.14 IU/kg/day groups, and minimal to slight at 3.46 IU/kg/day). In addition, even though there was no T control for direct comparison in males, there was an apparent increase in severity from minimal to slight in males at 2.94 IU/kg/day compare to minimal to moderate at 5.28 IU/kg/day. Moreover, there was also an increase in the incidence and the severity of the lesion in males compared to the females (1/10 and 8/11 in females compared to 9/10 males in Groups 3 and 4 respectively, and minimal to slight in females compared to minimal to moderate in males).

At Week 26, there was accumulation of eosinophilic globules observed in the epithelia of the ethmoid turbinates (histologic section level 3), and the lesion also extended anteriorly involving the septum and dorsal meatus of the nasoturbinate area (histologic section level 2 and a less extent, level 1). In addition, when compared with 13 week phase, there was an apparent increased incidence in the occurrence of the finding in females (5/10 and 10/10 compared to 1/10 and 8/11 in the 13 week phase at 1.14 and 3.46 IU/kg/day, respectively). However, when taken into account with the incidence in the T control (10/10), such effect appeared to be due to T itself rather than TI. Moreover, there was an increase in severity in males (minimal to slight compared to minimal to moderate at 2.94 IU/kg/day, and minimal to moderate compared to minimal to marked at 5.18 IU/kg/day). The magnitude of this severity was not progressive with duration of treatment relative to the severity observed at 13-weeks of treatment.

All in all, similar findings were observed from both 13-week and 26-week phases. There was still a dose-related increase in incidence in females but also a slight increase in males. There was a dose-related increase in severity in both sexes. There was also an increase in the incidence and the severity of the lesion in males compared to the females.

In addition, Group 3 (low-dose TI) males received the same dose level as Group 4 (high-dose TI) females. When compared these groups, there was a trend towards an increase in severity in

males. Minimal to slight epithelial degeneration was occasionally described in the T and TI animals. However, a specific distribution pattern could not be established. Epithelial degeneration could also be observed in 1 male from Group 4 in each phase. There was an increased incidence of this finding in the 2/10 males and 2/10 females from T control group (Group 2).

Moreover, the greatest severity of eosinophilic globules accumulation was seen in the T control group (Group 2). The lesion distribution was similar to the Group 3 and Group 4 in the 26-week phase, which included histologic section levels 3, 2, and to a lesser extent, level 1. In this Group 2, the severity of the lesion was greater in males, although the distribution of the lesion appeared to be more extensive in females (the lesion extended to level 1 in most females). Since rats were obligate nose breathing animals, the presence of globules was consistent with the physiological impaction of particulate material on the nasal cavity. The presence of insulin did not seem to influence either the incidence or severity of these lesions. Furthermore, histological alterations were limited to those consistent with impaction on the nasal epithelium. No drug-related alterations were observed in the lower respiratory trace, particularly in the deep lung.

At the end of the recovery period, accumulation of eosinophilic globules was still present. The lesion distribution was similar to the 26-week phase. There was no evidence of recovery in males, as the incidence and severity of the finding was similar to the 26-week phase group. However, there was a decrease in the severity of the lesion in males.

Other microscopic findings were considered incidental and not associated with T or TI treatment. Note from the reviewer: HD T (11.7 mg/kg/d) was not assessed at 13 weeks.

Incidence of Animals with Microscopic Findings by Organ/Group/Sex
Treatment Period
13 Weeks
MALE

DOSE GROUP DESTINATION NUMBER OF ANIMALS EXAMINED		1 10	3 10	4 10
ADRENAL	EXAMIN:	10	1	10
- Congestion		-	1	-
AORTA	EXAMIN:	10	-	10
BONE MARROW	EXAMIN:	10	-	10
- Hypocellularity: hematopoietic		-	-	1
BONE-STERNUM	EXAMIN:	10	-	10
BRAIN	EXAMIN:	10	-	10
BRONCHUS	EXAMIN:	10	10	10

DOSE GROUP DESTINATION NUMBER OF ANIMALS EXAMINED		1 10	3 10	4 10
CAVITY NASAL/SINUSES	EXAMIN:	10	10	10
- Eosinophilic globules: olfactory epithelium		-	9	9
- Eosinophilic globules: respiratory epithelium		-	4	5
- Degeneration: olfactory epithelium		-	-	1
CECUM	EXAMIN:	10	-	10
- Parasite		1	-	-
- Inflammation		-	-	1

DOSE GROUP DESTINATION NUMBER OF ANIMALS EXAMINED		1 10	3 10	4 10
COLON	EXAMIN:	10	-	10
- Parasite		-	-	1
DUODENUM	EXAMIN:	10	-	10
EPIDIDYMIS	EXAMIN:	10	-	10
ESOPHAGUS	EXAMIN:	10	-	10
EYE	EXAMIN:	10	-	10
FAT	EXAMIN:	10	10	10
HARDERIAN GLAND	EXAMIN:	10	-	10

DOSE GROUP DESTINATION NUMBER OF ANIMALS EXAMINED		1 10	3 10	4 10
HEART	EXAMIN:	10	-	10
- Infiltration: mixed cell		3	-	1
- Degeneration and/or necrosis: myocardial		1	-	-
ILEUM	EXAMIN:	10	-	10
JEJUNUM	EXAMIN:	10	-	10
KIDNEY	EXAMIN:	10	1	10
- Pyelitis/pyelonephritis		1	-	-
- Dilatation: pelvis		-	-	1
- Basophilia: tubular		1	-	1

DOSE GROUP DESTINATION NUMBER OF ANIMALS EXAMINED		1 10	3 10	4 10
LACRIMAL GLAND	EXAMIN:	10	-	10
- Infiltration: mononuclear cell		-	-	1
LARYNX	EXAMIN:	10	10	10
LIVER	EXAMIN:	10	2	10
- Tension lipidosis		5	2	1
LUNG	EXAMIN:	10	10	10
- Hemorrhage		-	1	2
- Histiocytosis		6	1	2
- Inflammation: interstitial		3	1	2

DOSE GROUP DESTINATION NUMBER OF ANIMALS EXAMINED		1 10	3 10	4 10
L. NODE MANDIBULAR	EXAMIN:	10	-	10
- Erythrocytosis/hemorrhage		1	-	-
L.NODE MESENTERIC	EXAMIN:	10	-	10
- Erythrocytosis/hemorrhage		1	-	-
L.N.TRACHEOBRONCHIAL	EXAMIN:	10	9	10
- Erythrocytosis/hemorrhage		-	2	-
MAMMARY GLAND	EXAMIN:	5	-	6
MUSCLE SKELETAL	EXAMIN:	10	-	10
NERVE SCIATIC	EXAMIN:	10	-	10

DOSE GROUP DESTINATION NUMBER OF ANIMALS EXAMINED		1 10	3 10	4 10
NERVES OPTIC	EXAMIN:	10	-	10
PANCREAS	EXAMIN:	10	-	10
- Atrophy: acinar cell		3	-	3
PARATHYROID GLAND	EXAMIN:	8	-	10
PHARYNX	EXAMIN:	10	10	10
PITUITARY	EXAMIN:	10	-	10
PROSTATE	EXAMIN:	10	-	10
- Inflammation		4	-	1
RECTUM	EXAMIN:	2	1	-
- Parasite		1	1	-

DOSE GROUP DESTINATION NUMBER OF ANIMALS EXAMINED		1 10	3 10	4 10
SALIV.GL. MANDIBULAR	EXAMIN:	10	-	10
SEMINAL VESICLE	EXAMIN:	10	-	10
SKIN	EXAMIN:	10	-	10
SPINAL CORD CERVICAL	EXAMIN:	10	-	10
SPLEEN	EXAMIN:	10	-	10
STOMACH	EXAMIN:	10	-	10
TAIL	EXAMIN:	10	10	10
TESTIS	EXAMIN:	10	-	10
THYMUS	EXAMIN:	10	-	10
- Hemorrhage		2	-	2

DOSE GROUP DESTINATION NUMBER OF ANIMALS EXAMINED		1 10	3 10	4 10
THYROID	EXAMIN:	10	-	10
TONGUE	EXAMIN:	10	-	10
TRACHEA	EXAMIN:	10	10	10
URINARY BLADDER	EXAMIN:	10	-	10

Incidence of Animals with Microscopic Findings by Organ/Group/Sex
Treatment Period
13 Weeks
FEMALE

DOSE GROUP DESTINATION NUMBER OF ANIMALS EXAMINED		1 10	2 2	3 10	4 11
ADRENAL	EXAMIN:	10	2	1	11
- Hemorrhage		-	1	-	1
- Congestion		-	1	1	-
AORTA	EXAMIN:	10	2	-	11
BONE MARROW	EXAMIN:	10	2	-	11
- Hypercellularity: myeloid		-	-	-	1
BONE-STERNUM	EXAMIN:	10	2	-	11
BRAIN	EXAMIN:	10	2	-	11
BRONCHUS	EXAMIN:	10	2	10	11

DOSE GROUP DESTINATION NUMBER OF ANIMALS EXAMINED		1 10	2 2	3 10	4 11
CAVITY NASAL/SINUSES	EXAMIN:	10	2	10	11
- Inflammation		-	-	1	-
- Eosinophilic globules: olfactory epithelium		-	1	1	8
- Eosinophilic globules: respiratory epithelium		-	1	-	1
CECUM	EXAMIN:	10	2	-	11
- Parasite		3	-	-	-
COLON	EXAMIN:	10	2	-	11

DOSE GROUP DESTINATION NUMBER OF ANIMALS EXAMINED		1 10	2 2	3 10	4 11
DUODENUM	EXAMIN:	10	2	-	11
ESOPHAGUS	EXAMIN:	10	2	-	11
EYE	EXAMIN:	10	2	-	11
FAT	EXAMIN:	10	2	10	11
HARDERIAN GLAND	EXAMIN:	10	2	-	11
HEART	EXAMIN:	10	2	-	11
- Infiltration: mixed cell		1	-	-	-
ILEUM	EXAMIN:	10	2	-	11
JEJUNUM	EXAMIN:	10	2	-	11

DOSE GROUP DESTINATION NUMBER OF ANIMALS EXAMINED		1 10	2 2	3 10	4 11
KIDNEY	EXAMIN:	10	2	-	11
- Pyelitis/pyelonephritis		2	-	-	1
- Dilatation: pelvis		1	-	-	1
- Basophilia: tubular		1	-	-	2
- Cast: hyaline		-	-	-	1
LACRIMAL GLAND	EXAMIN:	10	2	-	11
- Infiltration: mononuclear cell		-	1	-	-
LARYNX	EXAMIN:	10	2	10	11
- Ulceration		-	1	-	-

DOSE GROUP DESTINATION NUMBER OF ANIMALS EXAMINED		1 10	2 2	3 10	4 11
LIVER	EXAMIN:	10	2	2	11
- Tension lipidosis		1	-	1	3
- Hematopoiesis: extramedullary		-	-	-	1
LUNG	EXAMIN:	10	2	10	11
- Hemorrhage		1	1	1	1
- Histiocytosis		4	-	1	1
- Mineralization: vascular		1	-	-	-
LYMPH NODE	EXAMIN:	-	-	1	-
- Erythrocytosis/hemorrhage: sinusal		-	-	1	-

DOSE GROUP DESTINATION NUMBER OF ANIMALS EXAMINED		1 10	2 2	3 10	4 11
L. NODE MANDIBULAR	EXAMIN:	10	2	1	11
- Erythrocytosis/hemorrhage		-	-	1	1
L.NODE MESENTERIC	EXAMIN:	10	2	-	11
L.N.TRACHEOBRONCHIAL	EXAMIN:	10	2	10	11
- Erythrocytosis/hemorrhage		2	1	1	2
MAMMARY GLAND	EXAMIN:	9	1	-	11
- Adenocarcinoma		-	-	-	1
MUSCLE SKELETAL	EXAMIN:	10	2	-	10
NERVE SCIATIC	EXAMIN:	10	2	-	11

DOSE GROUP DESTINATION NUMBER OF ANIMALS EXAMINED		1 10	2 2	3 10	4 11
NERVES OPTIC	EXAMIN:	10	2	-	11
OVARY	EXAMIN:	10	2	-	11
PANCREAS	EXAMIN:	10	2	-	11
PARATHYROID GLAND	EXAMIN:	7	1	-	9
PHARYNX	EXAMIN:	10	2	10	11
PITUITARY	EXAMIN:	10	2	2	11
RECTUM	EXAMIN:	-	-	1	2
SALIV.GL. MANDIBULAR	EXAMIN:	10	2	-	11
SKIN	EXAMIN:	10	2	-	11

DOSE GROUP DESTINATION NUMBER OF ANIMALS EXAMINED		1 10	2 2	3 10	4 11
SPINAL CORD CERVICAL	EXAMIN:	10	2	-	11
- Degeneration: nerve fiber		-	1	-	-
SPLEEN	EXAMIN:	10	2	-	11
- Hematopoiesis/extramedullary: increased		-	-	-	1
STOMACH	EXAMIN:	10	2	-	11
- Erosion: glandular mucosa		-	-	-	1
- Congestion		-	2	-	-
TAIL	EXAMIN:	10	2	10	11
THYMUS	EXAMIN:	10	2	-	11
- Hemorrhage		-	2	-	1

DOSE GROUP DESTINATION NUMBER OF ANIMALS EXAMINED		1 10	2 2	3 10	4 11
THYROID	EXAMIN:	10	2	-	11
TONGUE	EXAMIN:	10	2	-	11
TRACHEA	EXAMIN:	10	2	10	11
URINARY BLADDER	EXAMIN:	10	2	-	11
- Infiltration: mononuclear cell		1	-	-	-
- Inflammation		-	-	-	1
UTERUS	EXAMIN:	10	2	-	11
VAGINA	EXAMIN:	10	2	-	11

Incidence of Animals with Microscopic Findings by Organ/Group/Sex
Treatment Period
26 Weeks
MALE

DOSE GROUP DESTINATION NUMBER OF ANIMALS EXAMINED		1 10	2 10	3 10	4 10
ADRENAL	EXAMIN:	10	10	2	10
- Hyperplasia: medullary		-	1	-	-
- Hypertrophy: cortical		1	1	-	2
- Congestion		-	-	1	-
- Degeneration: cystic		1	1	-	-
AORTA	EXAMIN:	10	10	-	10
BONE MARROW	EXAMIN:	10	10	-	10
BONE-STERNUM	EXAMIN:	10	10	-	10
BRAIN	EXAMIN:	10	10	-	10

DOSE GROUP DESTINATION NUMBER OF ANIMALS EXAMINED		1 10	2 10	3 10	4 10
BRONCHUS	EXAMIN:	10	10	10	10
CAVITY NASAL/SINUSES	EXAMIN:	10	10	10	10
- Exudate		-	-	1	-
- Inflammation		-	1	2	3
- Eosinophilic globules: olfactory epithelium		-	10	8	10
- Eosinophilic globules: respiratory epithelium		-	8	4	8
- Eosinophilic globules: maxillary sinus		-	1	-	-
- Degeneration: transitional epithelium		-	1	-	-

DOSE GROUP DESTINATION NUMBER OF ANIMALS EXAMINED		1 10	2 10	3 10	4 10
CAVITY NASAL/SINUSES	CONT'D.	10	10	10	10
- Degeneration: respiratory epithelium		-	2	-	1
CECUM	EXAMIN:	10	10	-	10
COLON	EXAMIN:	10	10	-	10
- Parasite		-	1	-	-
DUODENUM	EXAMIN:	10	10	-	10
EPIDIDYMIS	EXAMIN:	10	10	-	10
ESOPHAGUS	EXAMIN:	10	10	-	10
EYE	EXAMIN:	10	10	-	10
- Atrophy: retina		-	-	-	1

DOSE GROUP DESTINATION NUMBER OF ANIMALS EXAMINED		1 10	2 10	3 10	4 10
HARDERIAN GLAND	EXAMIN:	10	10	-	10
HEART	EXAMIN:	10	10	-	10
- Degeneration and/or necrosis: myocardial		1	5	-	1
- Infiltration: mononuclear cell		2	5	-	2
ILEUM	EXAMIN:	10	10	1	10
JEJUNUM	EXAMIN:	10	10	-	10
- Diverticulum		1	-	-	-

DOSE GROUP DESTINATION NUMBER OF ANIMALS EXAMINED		1 10	2 10	3 10	4 10
KIDNEY	EXAMIN:	10	10	1	10
- Dilatation: pelvis		1	1	-	1
- Basophilia: tubular		2	1	1	3
- Cast: hyaline		1	-	-	-
- Hyaline droplet: tubular		-	1	-	-
LACRIMAL GLAND	EXAMIN:	10	10	-	10
LARYNX	EXAMIN:	10	10	10	10
- Granuloma		-	1	-	1

DOSE GROUP DESTINATION NUMBER OF ANIMALS EXAMINED		1 10	2 10	3 10	4 10
LIVER	EXAMIN:	10	10	3	10
- Tension lipidosis		1	2	1	1
- Infiltration: mononuclear cell		-	-	-	1
- Vacuolation: centrilobular		-	1	-	-
- Congestion		-	1	-	-
- Degeneration: cystic		-	1	-	-
LUNG	EXAMIN:	10	10	10	10
- Hemorrhage		-	-	1	-
- Histiocytosis		2	2	3	3
- Fibrosis: pleura		-	-	1	-

DOSE GROUP DESTINATION NUMBER OF ANIMALS EXAMINED		1 10	2 10	3 10	4 10
LYMPH NODE	EXAMIN:	2	2	1	1
- Erythrocytosis/hemorrhage: sinusal		2	2	1	1
L. NODE MANDIBULAR	EXAMIN:	10	10	2	10
- Erythrocytosis/hemorrhage		-	1	2	2
L.NODE MESENTERIC	EXAMIN:	10	10	-	10
- Infiltration: histiocytic cell		-	-	-	1
L.N.TRACHEOBRONCHIAL	EXAMIN:	10	10	10	10
MAMMARY GLAND	EXAMIN:	5	6	-	8
MUSCLE SKELETAL	EXAMIN:	10	10	-	10
- Degeneration: myofiber		-	-	-	1

DOSE GROUP DESTINATION NUMBER OF ANIMALS EXAMINED		1 10	2 10	3 10	4 10
NERVE SCIATIC	EXAMIN:	10	10	-	10
- Degeneration: nerve fiber		-	1	-	1
NERVES OPTIC	EXAMIN:	10	10	-	10
PANCREAS	EXAMIN:	10	10	-	10
- Atrophy: acinar cell		4	4	-	-
- Hyperplasia: islet cell		-	-	-	1
PARATHYROID GLAND	EXAMIN:	9	9	-	3
PHARYNX	EXAMIN:	10	10	10	10
PITUITARY	EXAMIN:	10	10	-	10
- Cyst		1	2	-	-

DOSE GROUP DESTINATION NUMBER OF ANIMALS EXAMINED		1 10	2 10	3 10	4 10
PROSTATE	EXAMIN:	10	10	1	10
- Inflammation		6	4	-	1
- Hemorrhage		1	-	-	-
SALIV.GL. MANDIBULAR	EXAMIN:	10	10	-	10
SEMINAL VESICLE	EXAMIN:	10	10	-	10
SKIN	EXAMIN:	10	10	-	10
SPINAL CORD CERVICAL	EXAMIN:	10	10	-	10
SPLEEN	EXAMIN:	10	10	1	10
- Cyst: capsular		-	-	1	-
- Inflammation		-	1	-	-

DOSE GROUP DESTINATION NUMBER OF ANIMALS EXAMINED		1 10	2 10	3 10	4 10
STOMACH	EXAMIN:	10	10	2	10
- Hemorrhage		-	-	1	-
TESTIS	EXAMIN:	10	10	-	10
THYMUS	EXAMIN:	10	10	1	10
- Hemorrhage		2	2	-	5
THYROID	EXAMIN:	10	10	2	10
TONGUE	EXAMIN:	10	10	-	10
TRACHEA	EXAMIN:	10	10	10	10
URINARY BLADDER	EXAMIN:	10	10	1	10
- Edema		-	-	1	-

Incidence of Animals with Microscopic Findings by Organ/Group/Sex
Treatment Period
26 Weeks
FEMALE

DOSE GROUP DESTINATION NUMBER OF ANIMALS EXAMINED		1 10	2 10	3 10	4 10
ADRENAL	EXAMIN:	10	10	3	10
- Accessory cortical tissue		1	-	-	-
- Hypertrophy: cortical		3	1	-	1
- Congestion		1	5	-	1
- Degeneration: cystic		2	1	-	2
AORTA	EXAMIN:	10	10	-	10
BONE MARROW	EXAMIN:	10	10	-	10
BONE-STERNUM	EXAMIN:	10	10	-	10
BRAIN	EXAMIN:	10	10	-	10

DOSE GROUP DESTINATION NUMBER OF ANIMALS EXAMINED		1 10	2 10	3 10	4 10
BRONCHUS	EXAMIN:	10	10	10	10
CAVITY NASAL/SINUSES	EXAMIN:	10	10	10	10
- Inflammation		1	-	2	1
- Hemorrhage		-	-	1	-
- Eosinophilic globules: olfactory epithelium		-	10	5	10
- Eosinophilic globules: respiratory epithelium		-	10	-	1
- Eosinophilic globules: maxillary sinus		-	5	-	-
- Degeneration: olfactory epithelium		-	2	-	-

DOSE GROUP DESTINATION NUMBER OF ANIMALS EXAMINED		1 10	2 10	3 10	4 10
CECUM	EXAMIN:	10	10	1	10
- Hemorrhage		-	1	1	-
COLON	EXAMIN:	10	10	-	10
- Parasite		1	1	-	1
DUODENUM	EXAMIN:	10	10	-	10
ESOPHAGUS	EXAMIN:	10	10	-	10
EYE	EXAMIN:	10	10	-	10
- Fold/rosette: retina		1	-	-	-
HARDERIAN GLAND	EXAMIN:	10	10	-	10

DOSE GROUP DESTINATION NUMBER OF ANIMALS EXAMINED		1 10	2 10	3 10	4 10
HEART	EXAMIN:	10	10	-	10
- Degeneration and/or necrosis: myocardial		-	1	-	-
- Infiltration: mononuclear cell		1	2	-	-
ILEUM	EXAMIN:	10	10	-	10
JEJUNUM	EXAMIN:	10	10	-	10

DOSE GROUP DESTINATION NUMBER OF ANIMALS EXAMINED		1 10	2 10	3 10	4 10
KIDNEY	EXAMIN:	10	10	-	10
- Pyelitis/pyelonephritis		-	-	-	1
- Dilatation: pelvis		-	1	-	-
- Basophilia: tubular		-	-	-	2
- Cast: hyaline		-	2	-	1
- Infiltration: mononuclear cell		-	1	-	-
- Inflammation		-	-	-	1
LACRIMAL GLAND	EXAMIN:	10	10	-	10
- Infiltration: mononuclear cell		1	-	-	-

DOSE GROUP DESTINATION NUMBER OF ANIMALS EXAMINED		1 10	2 10	3 10	4 10
LARYNX	EXAMIN:	10	10	10	10
- Hemorrhage		-	-	1	-
- Inflammation		-	1	-	-
LIVER	EXAMIN:	10	10	3	10
- Angiectasis		1	-	-	-
- Tension lipidosis		2	1	3	2
- Inflammation		-	1	-	-
- Congestion		1	-	-	-

DOSE GROUP DESTINATION		1	2	3	4
NUMBER OF ANIMALS EXAMINED		10	10	10	10
LUNG	EXAMIN:	10	10	10	10
- Hemorrhage		-	1	-	-
- Histiocytosis		2	1	-	-
LYMPH NODE	EXAMIN:	3	-	1	3
- Erythrocytosis/hemorrhage: sinusal		3	-	1	2
L. NODE MANDIBULAR	EXAMIN:	10	10	2	10
- Erythrocytosis/hemorrhage		1	3	2	1
L.NODE MESENTERIC	EXAMIN:	10	10	-	10
L.N.TRACHEOBRONCHIAL	EXAMIN:	10	10	10	10
- Erythrocytosis/hemorrhage		3	2	2	-

DOSE GROUP DESTINATION		1	2	3	4
NUMBER OF ANIMALS EXAMINED		10	10	10	10
MAMMARY GLAND	EXAMIN:	9	10	1	10
- Ectasia: ducts and/or alveoli		1	1	1	-
MUSCLE SKELETAL	EXAMIN:	10	10	-	10
NERVE SCIATIC	EXAMIN:	10	10	-	10
- Degeneration: nerve fiber		1	-	-	1
NERVES OPTIC	EXAMIN:	10	10	-	10
OVARY	EXAMIN:	10	10	-	10
PANCREAS	EXAMIN:	10	10	-	10
- Atrophy: acinar cell		-	1	-	-

DOSE GROUP DESTINATION		1	2	3	4
NUMBER OF ANIMALS EXAMINED		10	10	10	10
PARATHYROID GLAND	EXAMIN:	6	4	-	8
PHARYNX	EXAMIN:	10	10	10	10
PITUITARY	EXAMIN:	10	10	3	10
- Cyst		-	1	1	-
SALIV.GL. MANDIBULAR	EXAMIN:	10	10	-	10
SKIN	EXAMIN:	10	10	-	10
- Hemorrhage		-	2	-	-
SPINAL CORD CERVICAL	EXAMIN:	10	10	-	10
SPLEEN	EXAMIN:	10	10	-	10
- Cyst: capsular		1	-	-	-

DOSE GROUP DESTINATION NUMBER OF ANIMALS EXAMINED		1 10	2 10	3 10	4 10
STOMACH	EXAMIN:	10	10	-	10
TAIL	EXAMIN:	-	1	-	-
- Abscess		-	1	-	-
THYMUS	EXAMIN:	10	10	2	10
- Hemorrhage		-	-	1	-
THYROID	EXAMIN:	10	10	-	10
- Cyst: squamous		2	-	-	-
TONGUE	EXAMIN:	10	10	-	10
TRACHEA	EXAMIN:	10	10	10	10

DOSE GROUP DESTINATION NUMBER OF ANIMALS EXAMINED		1 10	2 10	3 10	4 10
URINARY BLADDER	EXAMIN:	10	10	-	10
- Inflammation		-	-	-	1
UTERUS	EXAMIN:	10	10	-	10
- Cyst		-	1	-	-
- Dilatation		-	-	-	2
VAGINA	EXAMIN:	10	10	-	10

RECOVERY PERIOD

Incidence of Animals with Microscopic Findings by Organ/Group/Sex
Recovery Period

MALE

DOSE GROUP DESTINATION NUMBER OF ANIMALS EXAMINED		1 5	4 5
ADRENAL	EXAMIN:	5	5
- Vacuolation: cortical		1	-
- Hypertrophy: cortical		1	-
AORTA	EXAMIN:	5	5
BONE MARROW	EXAMIN:	5	5
BONE-STERNUM	EXAMIN:	5	5
BRAIN	EXAMIN:	5	5
BRONCHUS	EXAMIN:	5	5

DOSE GROUP DESTINATION NUMBER OF ANIMALS EXAMINED		1 5	4 5
DUODENUM	EXAMIN:	5	5
EPIDIDYMIS	EXAMIN:	5	5
- Oligo/aspermia		3	-
ESOPHAGUS	EXAMIN:	5	5
EYE	EXAMIN:	5	5
HARDERIAN GLAND	EXAMIN:	5	5
HEART	EXAMIN:	5	5
- Infiltration: mononuclear cell		-	1
- Fibrosis		1	1

DOSE GROUP DESTINATION		1	4
NUMBER OF ANIMALS EXAMINED		5	5
DUODENUM	EXAMIN:	5	5
EPIDIDYMIS	EXAMIN:	5	5
- Oligo/aspermia		3	-
ESOPHAGUS	EXAMIN:	5	5
EYE	EXAMIN:	5	5
HARDERIAN GLAND	EXAMIN:	5	5
HEART	EXAMIN:	5	5
- Infiltration: mononuclear cell		-	1
- Fibrosis		1	1

DOSE GROUP DESTINATION		1	4
NUMBER OF ANIMALS EXAMINED		5	5
LARYNX	EXAMIN:	5	5
- Hemorrhage		1	-
LIVER	EXAMIN:	5	5
- Tension lipidosis		2	-
LUNG	EXAMIN:	5	5
- Hemorrhage		1	2
- Histiocytosis		2	1
L. NODE MANDIBULAR	EXAMIN:	5	5
- Erythrocytosis/hemorrhage		1	2

DOSE GROUP DESTINATION		1	4
NUMBER OF ANIMALS EXAMINED		5	5
PARATHYROID GLAND	EXAMIN:	5	4
PHARYNX	EXAMIN:	5	5
PITUITARY	EXAMIN:	5	5
PROSTATE	EXAMIN:	5	5
- Inflammation		1	1
SALIV.GL. MANDIBULAR	EXAMIN:	5	5
SEMINAL VESICLE	EXAMIN:	5	5
SKIN	EXAMIN:	5	5
SPINAL CORD CERVICAL	EXAMIN:	5	5

DOSE GROUP DESTINATION		1	4
NUMBER OF ANIMALS EXAMINED		5	5
ILEUM	EXAMIN:	5	5
JEJUNUM	EXAMIN:	5	5
KIDNEY	EXAMIN:	5	5
- Basophilia: tubular		1	3
- Cast: hyaline		1	-
- Hyaline droplet: tubular		-	1
- Dilatation: tubular		-	1
LACRIMAL GLAND	EXAMIN:	5	5
- Inflammation		1	-
- Deposits: pigment		1	-

DOSE GROUP DESTINATION		1	4
NUMBER OF ANIMALS EXAMINED		5	5
L.NODE MESENTERIC	EXAMIN:	5	5
L.N.TRACHEOBRONCHIAL	EXAMIN:	5	5
- Erythrocytosis/hemorrhage		-	1
MAMMARY GLAND	EXAMIN:	3	5
MUSCLE SKELETAL	EXAMIN:	5	5
NERVE SCIATIC	EXAMIN:	5	5
NERVES OPTIC	EXAMIN:	5	5
PANCREAS	EXAMIN:	5	5
- Atrophy: acinar cell		2	4
- Hyperplasia: islet cell		3	1

DOSE GROUP DESTINATION		1	4
NUMBER OF ANIMALS EXAMINED		5	5
SPLEEN	EXAMIN:	5	5
- Cyst: capsular		-	1
STOMACH	EXAMIN:	5	5
TESTIS	EXAMIN:	5	5
- Degeneration/atrophy: seminiferous epithelium		3	-
- Dilatation: tubular		1	-
THYMUS	EXAMIN:	5	5
- Hemorrhage		1	-

DOSE GROUP DESTINATION		1	4
NUMBER OF ANIMALS EXAMINED		5	5
THYROID	EXAMIN:	5	5
- Cyst: squamous		1	-
TONGUE	EXAMIN:	5	5
TRACHEA	EXAMIN:	5	5
URINARY BLADDER	EXAMIN:	5	5

Incidence of Animals with Microscopic Findings by Organ/Group/Sex
Recovery Period

FEMALE

DOSE GROUP DESTINATION		1	4
NUMBER OF ANIMALS EXAMINED		5	5
ADRENAL	EXAMIN:	5	5
- Degeneration: cystic		2	-
AORTA	EXAMIN:	5	5
BONE MARROW	EXAMIN:	5	5
BONE-STERNUM	EXAMIN:	5	5
BRAIN	EXAMIN:	5	5
BRONCHUS	EXAMIN:	5	5

DOSE GROUP DESTINATION		1	4
NUMBER OF ANIMALS EXAMINED		5	5
EYE	EXAMIN:	5	5
HARDERIAN GLAND	EXAMIN:	5	5
HEART	EXAMIN:	5	5
- Degeneration and/or necrosis: myocardial		1	-
- Infiltration: mononuclear cell		1	-
- Fibrosis		1	-
ILEUM	EXAMIN:	5	5
JEJUNUM	EXAMIN:	5	5

DOSE GROUP DESTINATION		1	4
NUMBER OF ANIMALS EXAMINED		5	5
CAVITY NASAL/SINUSES	EXAMIN:	5	5
- Eosinophilic globules: olfactory epithelium		-	5
- Eosinophilic globules: respiratory epithelium		-	2
CECUM	EXAMIN:	5	5
COLON	EXAMIN:	5	5
DUODENUM	EXAMIN:	5	5
ESOPHAGUS	EXAMIN:	5	5

DOSE GROUP DESTINATION		1	4
NUMBER OF ANIMALS EXAMINED		5	5
KIDNEY	EXAMIN:	5	5
- Chronic progressive nephropathy		-	1
- Pyelitis/pyelonephritis		-	1
- Basophilia: tubular		1	1
- Cast: hyaline		1	-
- Dilatation: tubular		-	1
LACRIMAL GLAND	EXAMIN:	5	5
LARYNX	EXAMIN:	5	5

DOSE GROUP DESTINATION		1	4
NUMBER OF ANIMALS EXAMINED		5	5
LIVER	EXAMIN:	5	5
- Tension lipidosis		2	1
- Congestion		-	1
LUNG	EXAMIN:	5	5
- Histiocytosis		-	1
L. NODE MANDIBULAR	EXAMIN:	5	5
L.NODE MESENTERIC	EXAMIN:	5	5
L.N.TRACHEOBRONCHIAL	EXAMIN:	5	5
- Erythrocytosis/hemorrhage		1	-

DOSE GROUP DESTINATION		1	4
NUMBER OF ANIMALS EXAMINED		5	5
PHARYNX	EXAMIN:	5	5
PITUITARY	EXAMIN:	5	5
SALIV.GL. MANDIBULAR	EXAMIN:	5	5
SKIN	EXAMIN:	5	5
SPINAL CORD CERVICAL	EXAMIN:	5	5
SPLEEN	EXAMIN:	5	5
STOMACH	EXAMIN:	5	5
- Hemorrhage		1	-

DOSE GROUP DESTINATION		1	4
NUMBER OF ANIMALS EXAMINED		5	5
MAMMARY GLAND	EXAMIN:	5	5
MUSCLE SKELETAL	EXAMIN:	5	5
NERVE SCIATIC	EXAMIN:	5	5
NERVES OPTIC	EXAMIN:	5	5
OVARY	EXAMIN:	5	5
- Cyst: luteal		-	1
PANCREAS	EXAMIN:	5	5
- Atrophy: acinar cell		-	1
PARATHYROID GLAND	EXAMIN:	2	5

DOSE GROUP DESTINATION		1	4
NUMBER OF ANIMALS EXAMINED		5	5
THYMUS	EXAMIN:	5	5
- Hemorrhage		1	1
- Atrophy: lymphoid		1	-
THYROID	EXAMIN:	5	5
- Cyst: squamous		1	-
TONGUE	EXAMIN:	5	5
TRACHEA	EXAMIN:	5	5
URINARY BLADDER	EXAMIN:	5	5
UTERUS	EXAMIN:	5	5
- - Dilatation		-	1

DOSE GROUP DESTINATION		1	4
NUMBER OF ANIMALS EXAMINED		5	5
VAGINA	EXAMIN:	5	5

Note from the reviewer:

- There were pancreas and kidney findings in males during recovery: pancreas may be related to insulin whereas kidney may relate to FDPK.
- There was no recovery of nasal cavity finding 30 days post-dose in females but not in males.
- There were also more kidney effects showing up in the recovery groups more than treated group. This might be due to long t ½ life of FDKP.

Toxicokinetics:

- Insulin: Dose dependent exposure (as indicated by C_{max} and $AUC_{0\text{-tlast}}$ parameters) was observed in almost all cases from low to high dose level (2.94 to 5.18 IU/kg/day for males, 1.14 to 3.46 IU/kg/day for females). It was dose-proportional increases for insulin exposure in males and less than dose-proportional increases for females following a single dose. Similar results were observed on Day 182, although exposure in males was less than proportional. In addition, C_{max} and $AUC_{0\text{-tlast}}$ values were generally higher in females compared to males at each dose level after correcting for the lower dose received in females.

Moreover, peak insulin levels were generally observed between the 2 and 10 min post exposure. A slight increase could be observed following repeated powder TI exposure. However, this increase was not considered to be the result of accumulation to steady-state levels, due to the relatively short terminal half-life and the general lack of quantifiable levels at pre-dose.

APPENDIX NO. 16 Serum Insulin C_{max} and $AUC_{0\text{-}tlast}$ to the Albino Rat PROJECT NO. 78235
TABLE NO. TK-3 and the Proportional Change of Each Parameter Relative to the Target
Low Dose Following Nose-Only Inhalation Exposure of Technosphere®/Insulin

Day 1 (Males)

Group No.	Insulin Target Dose Level (IU/kg/day)	C_{max} (µU/mL)	$AUC_{0\text{-}tlast}$ (µU•min/mL)	Dose Proportionality	C_{max} Ratio	$AUC_{0\text{-}tlast}$ Ratio
3	3	89.5	4054	1	1	1
4	5	105	7001	1.67	1.18	1.73

Day 182 (Males)

Group No.	Insulin Target Dose Level (IU/kg/day)	C_{max} (µU/mL)	$AUC_{0\text{-}tlast}$ (µU•min/mL)	Dose Proportionality	C_{max} Ratio	$AUC_{0\text{-}tlast}$ Ratio
3	3	238	6683	1	1	1
4	5	125	8020	1.67	0.524	1.20

APPENDIX NO. 16 Serum Insulin C_{max} and $AUC_{0\text{-}tlast}$ to the Albino Rat PROJECT NO. 78235
TABLE NO. TK-3 and the Proportional Change of Each Parameter Relative to the Target
Low Dose Following Nose-Only Inhalation Exposure of Technosphere®/Insulin

Day 1 (Females)

Group No.	Insulin Target Dose Level (IU/kg/day)	C_{max} (µU/mL)	$AUC_{0\text{-}tlast}$ (µU•min/mL)	Dose Proportionality	C_{max} Ratio	$AUC_{0\text{-}tlast}$ Ratio
3	1	91.1	3039	1	1	1
4	3	101	2150	3	1.11	0.708

Day 182 (Females)

Group No.	Insulin Target Dose Level (IU/kg/day)	C_{max} (µU/mL)	$AUC_{0\text{-}tlast}$ (µU•min/mL)	Dose Proportionality	C_{max} Ratio	$AUC_{0\text{-}tlast}$ Ratio
3	1	106	1982	1	1	1
4	3	143	11105	3	1.34	5.60

- Technosphere®: Exposure to Technosphere® particles was determined by measurement of its major component, FDKP. FDKP exposure (as measured by mean serum C_{max} and $AUC_{0\text{-}tlast}$) showed an increase with dose, but was less than dose proportional. The analysis for FDKP in serum on Days 1 and 90 appeared equivalent with the exception of the Group 2 (T control) females where the C_{max} and $AUC_{0\text{-}tlast}$ were nearly three times greater on Day 90. The C_{max} and $AUC_{0\text{-}tlast}$ on Day 176 (equivalent Day 1) and Day 182 did not show any

significant difference in single vs. multiple daily dosing up to 182 days and no differences between the sexes. In addition, the t_{max} occurred shortly after the conclusion of dosing, with a median value of 10 min post-dose. AUC_{inf} and $T_{1/2}$ could not be determined for Days and 90 due to insufficient data. For the equivalent Day 1 (Day 176), the $t_{1/2}$ of FDKP ranged from 48-80 min. For Day 182, $t_{1/2}$ of FDKP ranged from 76-121 min.

TABLE 2. FDKP Mean-Serum Toxicokinetic Metrics Following Daily Inhalation of Technosphere® Particles (T) or Technosphere®/Insulin (T/I) in Male and Female Sprague Dawley Rats for up to 182 Days

	Study Day	Group 2 (T) (FDKP mg/kg/day)			Group 3 (T/I) (FDKP mg/kg/day)		Group 4 (T/I) (FDKP mg/kg/day)	
		Female (12.80)	Male (10.5)	Normalized M+F[e] (11.7)	Female (0.404)	Male (1.05)	Female (1.28)	Male (1.91)
C_{max} (ng/mL)	1[a]	3503	4712		1231	1143	1221	1206
	89[a]	9013	6649		981	1637	2357	1718
	1 (176)[b]	4169	3465		785	804	1063	1161
	182[c]	5502	4220	4866	785	931	1532	1198
t_{max} (min)	1[a]	10.0	5.0		1.0	10.0	10.0	10.0
	89[a]	20.0	10.0		20.0	10.0	40.0	20.0
	1 (176)[b]	2.0	2.0		2.0	10.0	10.0	10.0
	182[c]	2.0	2.0		40.0	2.0	10.0	10.0
AUC_{last} (ng*min/mL)	1[a]	119435	149669		30416	37186	38038	38599
	89[a]	321775	199979		36623	54195	60835	60869
	1 (176)[b]	682590	363087		145798	84627	129880	232266
	182[c]	703445	514362	608069	97895	140457	176373	191492
AUC_{inf} (ng*min/mL)	1[a]	NR[d]	NR[d]		NR[d]	NR[d]	NR[d]	NR[d]
	89[a]	NR[d]	NR[d]		NR[d]	NR[d]	NR[d]	NR[d]
	1 (176)[b]	683028	363819		NR[d]	84769	129954	232369
	182[c]	743274	524954	632174	98691	150929	178264	197082
$t_{1/2}$ (min)	1[a]	NR[d]	NR[d]		NR[d]	NR[d]	NR[d]	NR[d]
	89[a]	NR[d]	NR[d]		NR[d]	NR[d]	NR[d]	NR[d]
	1 (176)[b]	50.6	79.9		NR[d]	48.2	66.1	62.6
	182[c]	109.7	87.0		69.0	121.0	76.2	93.8

a) Blood samples taken through 40 minutes post dose.
b) Equivalent Day 1, see section 3.1 for explanation, blood samples collected through 24 hours post dose
c) Blood samples collected through 8 hours post last dose
d) NR - not reportable, insufficient data to determine terminal kinetics.
e) Exposure values for the males and females were divided by their respective dose and then multiplied by a mean dose of 11.7. The mean of these values is reported as normalized male+ female.

Other:

- PCNA analyses in lung sections:

No difference in the proliferation rate of alveolar cells in any group or any occasion. However, there were significant changes in the proliferation rate of bronchial cells in Groups 3 and 4 animals. This was consistent with anatomical topography of bronchial cells being cephalad to the

alveolar cells since bronchial cells were the first to be exposed and to absorb TI. In addition, the increase could be attributed only to insulin since the amount of T was lower in Group 4 than in the T control group (Group 2). Such increase in the proliferation rate of bronchial cells was seen at Week 13 and continued through Week 26. All groups showed significant increases by Week 26. During the recovery phase, the PCNA values of Group 4 males and females significantly decreased, indicating a recovery trend.

Chronic insulin administration has known to have an adaptive effect on late phase G1, together with IGF-1, thereby increasing the proliferation rate of renewing cell type such as the bronchial cells. Upon discontinuation of insulin, recovery is evident, as seen in Group 4 animals. The increase in the proliferation rate of bronchial cells with daily TI administration to rats via inhalation did not reflect evidence of risk for neoplastic transformation of bronchial cells. Rather, it reflected adaptive effects.

Text Table 3a. Mean Percent PCNA Labeled Alveolar Cells in Male Rats

	Duration of Exposure		
Group Identification	Percent Labeled Alveolar Cells at 13 Weeks	Percent Labeled Alveolar Cells at 26 Weeks	Percent Labeled Alveolar Cells at 30 Weeks (last 4 weeks recovery)
Group 1	0.08	0.08	0.08
Air Control	± 0.041	± 0.032	± 0.027
Group 2	NA	0.08	NA
Tech/Control		± 0.024	
Group 3	0.08	0.10	NA
Tech/Ins (3 IU/kg/d)	± 0.032	± 0.034	
Group 4	0.11	0.11	0.11
Tech/Ins (5 IU/kg/d)	± 0.058	± 0.038	± 0.075

NA, not applicable; no statistically significant differences exist when comparing any of the groups.

Text Table 3b. Mean Percent PCNA Labeled Alveolar Cells in Female Rats

	Duration of Exposure		
Group Identification	Percent Labeled Alveolar Cells at 13 Weeks	Percent Labeled Alveolar Cells at 26 Weeks	Percent Labeled Alveolar Cells at 30 Weeks (last 4 weeks recovery)
Group 1	0.07	0.07	0.07
Air Control	± 0034	± 0.032	± 0.025
Group 2	NA	0.07	NA
Tech/Control		± 0.025	
Group 3	0.08	0.08	NA
Tech/Ins (1 IU/kg/d)	± 0.032	± 0.026	
Group 4	0.10	0.09	0.11
Tech/Ins 3 IU/kg/d)	± 0.051	± 0.045	± 0.046

NA, not applicable; no statistically significant differences exist when comparing of any of the groups.

Text Table 1a. Mean Percent PCNA Labeled Bronchial Cells in Male Rats

	Duration of Exposure		
Group Identification	Percent Labeled Bronchial Cells at 13 Weeks	Percent Labeled Bronchial Cells at 26 Weeks	Percent Labeled Bronchial Cells at 30 Weeks (last 4 weeks recovery)
Group 1	2.10	2.38	1.99
Air Control	± 0.866	± 0.757	± 0.708
Group 2	NA	3.21*	NA
Tech (11.2 mg/kg/d) /Control		± 0.437	
Group 3	3.60**[a]	3.76***[a]	NA
Tech (1.05 mg/kg/d) /Ins (3 IU/kg/d)	± 0.592	± 0.820	
Group 4	4.20**[b]	5.14***[bc]	3.58*[d]
Tech (1.91 mg/kg/d) /Ins (5 IU/kg/d)	± 0.891	± 1.322	± 0.973

NA, not applicable;
*, p <0.05; **p <0.01; and ***, p <0.001 compared to group 1;
a, no statistical significance when the two groups 3 are compared over time (13 vs 26 weeks);
b, no statistical significance when the two groups 4 are compared over time (13 vs 26 weeks);
c, significant at p<0.05 when compared to group 2;
d, no significance, but demonstrates a recovery trend compared to values at either 13 or 26 wks.

Text Table 1b. Mean Percent PCNA Labeled Bronchial Cells in Female Rats

	Duration of Exposure		
Group Identification	Percent Labeled Bronchial Cells at 13 Weeks	Percent Labeled Bronchial Cells at 26 Weeks	Percent Labeled Bronchial Cells at 30 Weeks (4 weeks recovery)
Group 1	1.96	2.02	1.79
Air Control	± 0.864	± 0.843	± 0.447
Group 2	NA	2.53	NA
Tech (11.2 mg/kg/d) /Control		± 0.428	
Group 3	3.32**[a]	3.03**[a]	NA
Tech (0.41 mg/kg/d) /Ins (1 IU/kg/d)	± 0.725	± 0.456	
Group 4	3.61***[b]	4.55***[bc]	2.82*[d]
Tech (1.28 mg/kg/d) /Ins (3 IU/kg/d)	± 0.777	± 0.450	±0.795

NA, not applicable;
*, p <0.05; **p <0.01; and ***, p <0.001 compared to group 1;
a, no statistical significance when the two groups 3 are compared over time (13 vs 26 weeks);
b, significant at p <0.05 when the two groups 4 are compared over time (13 vs 26 weeks);
c, significant at p<0.01 when compared to group 2;
d, significantly low at p<0.05 compared to values at either 13 or 26 weeks, demonstrating recovery.

Note from the reviewer: Based on the safety pharmacology study, 50 mg/kg T and 0.32 mg/kg TI did not affect pulmonary functions. Increased bronchial cell proliferations were seen in LD/HD TI groups (male: 3/5 IU/kg insulin & 1.12/1.87 mg/kg FDKP; female: 1/3 IU/kg insulin & 0.37/1.12 mg/kg FDKP). The exposure levels of T and/or TI in this study were much lower than the doses used in the pulmonary safety pharmacology study.

- P450 quantification:

There were no statistically significant differences observed in total cytochrome P450 content in males and females. However, a statistically significant increase (1.4-1.6 fold) in CYP3A1 activity was seen in low- and high-dose TI male (Groups 3 and 4) rats (2.94 IU/kg/day and 5.18 IU/kg/day respectively). The CYP3A1 activity increase was dose-dependent in male rats and also gender specific as no statistically significant differences was seen with female rats. In addition, there were no significant differences in male and female rats with others P450s, including CYP1A1/2, CYP2B1/2, and CYP2E1, at any dose levels when compared with air control groups.

Table 1: Mean Total Cytochrome P450 and Cytochrome P450 dependent Enzyme Activities in Rat Liver Microsomes Following Treatment with Technosphere®/Insulin at Various Insulin Target Dose Levels.

Sex	Group No. Treatment	Insulin Target Dose Level (IU/kg/day)	P450 (nmol/mg protein)	EROD Activity (nmol/min/mg protein)	PROD Activity (nmol/min/mg protein)	CZXH Activity (nmol/min/mg protein)	TESH Activity (nmol/min/mg protein)
Male	1-Air Control	0	0.964 ± 0.174	0.164 ± 0.045	0.0078 ± 0.0016	1.465 ± 0.246	2.943 ± 1.042
	3-Technosphere ®/insulin	3	1.099 ± 0.217	0.187 ± 0.038	0.0088 ± 0.0016	1.657 ± 0.217	4.263^ ± 0.696
	4-Technosphere ®/insulin	5	1.065 ± 0.104	0.188 ± 0.066	0.0089 ± 0.0023	1.836 ± 0.176	4.667^^ ± 0.592
Female	1-Air Control	0	0.721 ± 0.307	0.250 ± 0.047	0.0078 ± 0.0012	2.214 ± 0.714	1.802 ± 0.811
	3-Technosphere ®/insulin	1	0.983 ± 0.248	0.247 ± 0.037	0.0082 ± 0.0015	2.092 ± 0.269	1.450 ± 0.215
	4-Technosphere ®/insulin	3	0.926 ± 0.080	0.225 ± 0.034	0.0087 ± 0.0008	1.796 ± 0.531	1.309 ± 0.280

All values are expressed as mean ± SD
^ = $p<0.05$ Significantly different from control group (group 1) ($p<0.05$ Dunnett's test)
^^ = $p<0.01$ Significantly different from control group (group 1) ($p<0.01$ Dunnett's test)

EROD = 7-ethoxyresorufin *O*-deethylase activity (CYP1A1/2 activity)
PROD = 7-pentoxyresorufin *O*-dealkylase activity (CYP2B1/2 activity)
CZXH = Chlorzoxazone 6-hydroxylase activity (CYP2E1 activity)
TESH = Testosterone 6β-hydroxylase activity (CYP3A1/2 activity)

Pivotal Study #2

Note from the reviewer: **PDC-PC-2000-003** (13, 26, and 39 week inhalation dog study with T and TI) was described above and was not included as pivotal studies. That study used oronasal administration by mask and duration of exposure depended on the dose administered. This study was done by previous sponsor, PDC. **MKC-PC-2003-2004** is chosen as the pivotal study for dog for consistency reasons because this study is not previously reviewed and was conducted by the current sponsor, MKC.

Study title: Technosphere®/Insulin: A 39-Week Inhalation Toxicity Study in Beagle Dogs Followed by a 8-Week Recovery Period (MKC-PC-2003-2004)

Key study findings:

- Exposure to T and TI were stable and respirable. See below for estimated achieved dosages.

Estimated dosages	Group 1 (Air)	Group 2 (T low)	Group 3 (T high)	Group 4 (TI low)	Group 5 (TI high)
T (mg/kg/day)	0	0.39	10.9	0.39	1.92
Insulin (mg/kg/day)	0	0	0	0.0407	0.2198

- T and TI were well-tolerated by the dogs.
- Two mortalities (one T low male and one Air Control male) were observed during the course of the study. Neither was considered to be related to treatment.
- Clinical signs such as increased respiration, increased activity, and salivation, were observed in both treated and control groups. These signs were transient. Hypoglycemic events were observed in two high-dose TI animals (on days of TK sampling; Day 15 and Week 20). Those events occurred because the altered feeding regimen to prevent interference from endogenous insulin. These animals recovered following administration of dextrose and no such events were observed on any other days.
- Body weights, food consumption, ophthalmoscopy, EKG, hematology, clinical chemistry, and urinalysis parameters were not affected by T or TI treatment. This differs from rat findings described in the pivotal study #1.
- There were dose-dependent decreases in serum glucose values in the TI groups. The most significant decreases in serum glucose levels occurred 15 to 30 min post end of exposure in the high-dose TI group. In addition, decreases in glucose levels for the low-dose TI group were not as significant, with decreases observed immediately post exposure. Glucose levels for both groups, then steadily increased, reaching pre-dose levels at 240 min post end of the exposure.
- Organ weights, macroscopic findings, or cell proliferation activity was not affected by T or TI treatment. This differs from rat findings described in the pivotal study #1.
- There were minimal to mild alveolar and/or bronchial-interstitial neutrophil infiltrate of the lungs in both TI groups (1/8 for low-dose TI group and 3/8 for high-dose TI group) after 39 weeks of treatment. These findings regressed following 8 weeks of recovery. These suggest lung irritation in a T dose-dependent manner.
- In addition, minimal to mild thymic atrophy was seen in 6/8 high-dose T group at Week 39. Minimal to moderate hypocellularity of the seminiferous epithelium and minimal to mild germ cell degeneration were seen in T or TI group (1/4 for low-dose T and 2/4 for high-dose TI) at Week 39. The sponsor considered these findings were commonly observed in young Beagle dogs kept under laboratory conditions.
- Furthermore, testes and epididymal findings appeared in the TI group above concurrent controls.
- TK analyses showed that systemic exposure to insulin and FDKP was rapid and C_{max} was normally achieved within 20 min after the end of dosing.
- For FDKP, the mean t_{max} values ranged from 7.5 to 37.5 min post end of dosing. These were similar between days, sexes, and dose, and were similar between T and TI groups. The mean terminal ½ life ranged from 52.9 to 83.9 min, when calculable. Within the T groups, FDKP levels were relatively consistent for the 39 weeks of exposure and between sexes. The mean AUC_{last} and AUC_{inf} were slightly less than dose-proportional. Within the high-dose T group, mean AUC_{last} were 395,379 (males) and 493,440 (females) ng•min/mL.

- For insulin, in the high-dose TI group, the terminal ½ life of insulin ranged from a mean of 16.9 to 26.7 min and mean AUC_{last} was 4278 (males) and 3998 (females) µU•min/mL. In the low-dose TI group, too many samples were below the lower limit of quantification.
- The NOAEL for T (FDKP) was 10.9 mg/kg/day, whereas the NOAEL for TI was 0.2198 mg/kg/day.

Study no.: (b) (4) Study No. 2973
Volume #, and page #: Module 4.2.3.2 repeat-dose-tox, 1312 pages
Conducting laboratory and location: (b) (4)
Date of study initiation: Nov 14th, 2003
GLP compliance: Yes.
QA report: yes (x) no ( )
Drug, lot #, and % purity: See below.

Test Article:

Identity:	Technosphere® Insulin
Batch/Lot No.:	D043RD-03-001
Description:	White powder
Expiry:	03-2005
Purity (HPLC assay):	11.7% insulin (range 10.3-13.7%)
Storage Conditions:	Freezer (-10°C to -30°C)
Handling Precautions:	Standard laboratory procedures during routine handling and the wearing of a cartridge respirator during dosing.
Supplier:	MannKind BioPharmaceuticals One Casper Street, Danbury, CT 06810, USA

Vehicle / Control Article:

Identity:	Technosphere® Batch/Lot No.: D035V.03.010 / D035V.03.011 / D035V.03.012 (also referenced as D035U.03.010 / D035U.03.011 / D035U.03.012)
Description:	White powder
Expiry Date:	09-2005 / 10-2004 / 11-2004
Purity (HPLC assay):	99.5 / 99.4 / 99.5%
Storage Conditions:	Room temperature
Handling Precautions:	Standard laboratory procedures during routine handling and the wearing of a cartridge respirator during dosing.
Supplier:	MannKind BioPharmaceuticals One Casper Street, Danbury, CT 06810, USA

Methods

Doses: T_{low} - 2.4 mg/kg/day and T_{high} - 10.9 mg/kg/day
TI_{low} - 0.39 mg/kg/day and TI_{high} - 1.92 mg/kg/day

Grp	Treatment	Dose (mg/kg/day)[b]	Sacrificed Week 26		Sacrificed Week 39		Recovery Animals	
			M	F	M	F	M	F
1	Air Control		4	4	4	4	2	2
2	Technosphere particles (Low dose):	2.4			4	4		
3	Technosphere particles (High dose)	10.9			4	4		
4	TI (Low dose)	0.39/0.04 [a]			4	4		
5	TI (High dose)	1.92/0.22 [a]	4	4	4	4	2	2

[a] Shown are doses for TI in (mg/kg/day) / Insulin (mg/kg/day)

[b] Mean of males and females

Abbreviations: F = Female; Grp = group; M = Male; TI = Technosphere® Insulin

Species/strain: Beagle dogs (*Canis familiaris*)
Number/sex/group or time point (main study): 32/sex for 26 or 39 weeks
Route, formulation, volume, and infusion rate: Inhalation (15 min daily exposure)
Satellite groups used for toxicokinetics or recovery: Recovery: 2/sex (Groups 1 and 5)
Age: Between 7 and 8 months old
Weight: Between 6.8-9.9 kg (males) and 5.4-8.0 kg (females)
Sampling times: See Observations and Times.
Unique study design or methodology (if any): 8-week recovery period, dosing is administered by nose/mouth inhalation. Dogs were exposed for 15 min daily with T or TI.

Observations and times:

Mortality: Once daily during the pre-treatment phases and twice daily (a.m. and p.m.) thereafter.
Clinical signs: Once daily and twice daily on dosing days.
Body weights: Once prior to randomization and weekly thereafter.
Food consumption: Once daily.
Ophthalmoscopy: Once during the acclimation period and during Weeks 12/13, 26, and 39.
EKG: Once during the acclimation period and during Weeks 13, 26, and 39.
Hematology: Once during the pretreatment period and during Weeks 13, 26, and 39 of treatment, and again during the last week of the recovery period.

Hematology*	**Parameters Examined****	
	Red blood cell count	Mean Corpuscular Hemoglobin@
	Hematocrit	Mean Corpuscular Volume
	Hemoglobin	Morphology of cells
	White blood cell count	Platelet count
	WBC differential (absolute)	Reticulocyte count++
	Mean Corpuscular Hemoglobin Concentration@	

* EDTA was used as an anticoagulant.
** Various parameters were automatically recorded by the instrumentation, but are not reported.
@ Calculated parameters
++ Smears were prepared but not examined.

Coagulation*	**Parameters Examined****
	Activated partial thromboplastin time
	Prothrombin time

* Citrate was used as an anticoagulant.
** Various parameters were automatically recorded by the instrumentation, but are not reported.

Clinical chemistry: Same as above.

Clinical Chemistry* (serum)	**Parameters Examined**	
	A/G ratio (calculated)	Creatinine
	Alanine aminotransferase	Globulin (calculated)
	Albumin	Glucose
	Alkaline phosphatase	Phosphorus (inorganic)
	Aspartate aminotransferase	Potassium
	Bilirubin (total)	Sodium
	Calcium	Total protein
	Chloride	Triglycerides
	Cholesterol (total)	Urea

* gel clotting activator tubes were used

- Plasma level determinations (for glucose, insulin, and T levels): Days 1, 15, 22 and during Week 20, and the last day of treatment. Following time points: predose, immediately postdose, and 15, 30, 60, 120, and 240 min postdose.

Urinalysis: Same as above.

Urinalysis	**Parameters Examined****	
	Bilirubin	Protein
	Blood	Sediment microscopy
	Color and appearance	Specific gravity
	Glucose	Urobilinogen
	Ketones	Volume
	pH	

** Various parameters were automatically recorded by the instrumentation, but are not reported.

Gross pathology: The day following their respective last dose (Weeks 26 and 39) and following the 8 week recovery period.

Organ weights: At the end of the study.

Adrenals	Pituitary
Brain	Prostate
Heart	Spleen
Kidneys	Testes
Liver	Thymus
Respiratory tract from the nares	Thyroid lobes (with Parathyroids)
Ovaries	Uterus

Histopathology: Adequate Battery: yes (x), no ( ) - explain: See below for tissues collected.
Peer review: yes ( ), no (x) – Not mentioned by the sponsor.

Abnormal Tissues	Pancreas
Adrenals	Pharynx
Animal Identification****	Pituitary
Aorta (Thoracic)	Prostate
Brain (Cerebral Cortex, Midbrain, Cerebellum and Medulla)	Salivary Gland (Mandibular)
	Mammary Gland (Inguinal) +
Carina	Nasal Cavity (4 sections)#
Cecum	Nasopharynx
Colon	Optic Nerves *
Epididymides *	Ovaries
Esophagus	Sciatic Nerve

Eyes *	Skeletal Muscle
Femur & Marrow	Skin & Subcutis (Inguinal)
Gallbladder	Small Intestine, Duodenum
Heart ***	Small Intestine, Ileum
Kidneys	Small Intestine, Jejunum
Lacrimal glands	Spinal Cord (Cervical)
Larynx (2 sections)	Spleen
Liver (sample of two lobes)	Sternum & Marrow
Lungs (left anterior, left middle, left posterior, right posterior, right middle, right anterior and accessory)**	Stomach
	Testes*
	Thymus
	Thyroid lobes with parathyroids +
Lymph Node, Mandibular	Tongue
Lymph Node, Mesenteric	Trachea (and bronchi)
Lymph Node Bronchial	Urinary Bladder
Lymph Node Retropharyngeal	Uterus (horns and body)
	Vagina

*	Fixed in Zenker's fluid (euthanized animals only).
**	For each euthanized animal, one small lobe was tied off, detached and inflated with formalin vapor by bubbling air through 10% neutral buffered formalin. Formalin vapor fixation continued until the lobe was considered, visually, to be fully inflated. The lobe was then tied off, placed in a jar of fixative and capped with the ligature thread trapped in the lid. The jar was then inverted with the lobe held proximal to the lid to ensure immersion in fixative. The remaining lobes were then filled with liquid fixative, ligated and immersed in a container of fixative and capped.
***	Sections of both ventricles and atria, septum with papillary muscle.
****	Preservation only.
+	Parathyroids and mammary gland were examined microscopically only if present in routine sections.
#	Decalcified.

Others:

- PCNA analyses in lung sections: At the Week 26, 39, and following the 8-week recovery period.

Note from the reviewer: No anti-drug antibody assessment was found.

Results

Mortality: Two mortalities during the course of the study. One low-dose T control male was found dead on Day 19 due to a hemorrhage caused during TK sampling. Another Air Control male was found dead on Day 128 due to procedure-related stress during restraint.

Clinical signs: Signs of vocalization, increased respiration, increased activity and salvation were observed in treated and control groups. These were transient in duration and as they are commonly observed during inhalation dog exposure.

- Hypoglycemic events were observed for two high-dose TI groups following exposure on Day 15 and Week 20. Both occurred on days of TK sampling, which the feeding regimen was altered to prevent interference from endogenous insulin. These two animals recovered

following administration of dextrose and no such hypoglycemic events were observed any other days.

Body weights: No adverse effects on body weights were seen. Body weight profiles were comparable across dose groups and between sexes.

FIGURE 1

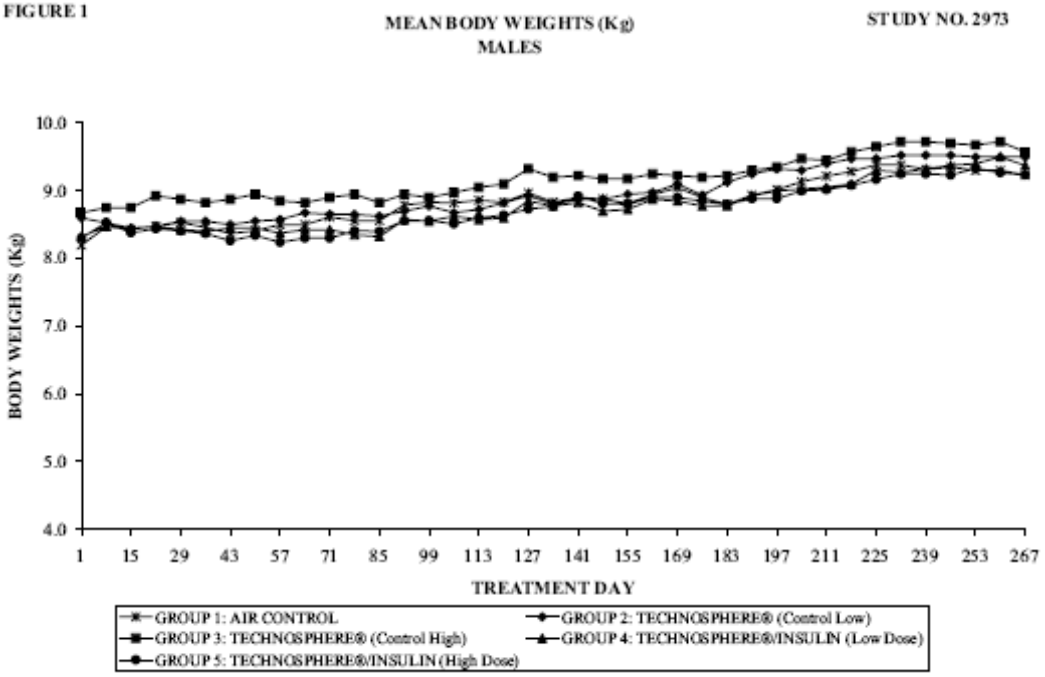
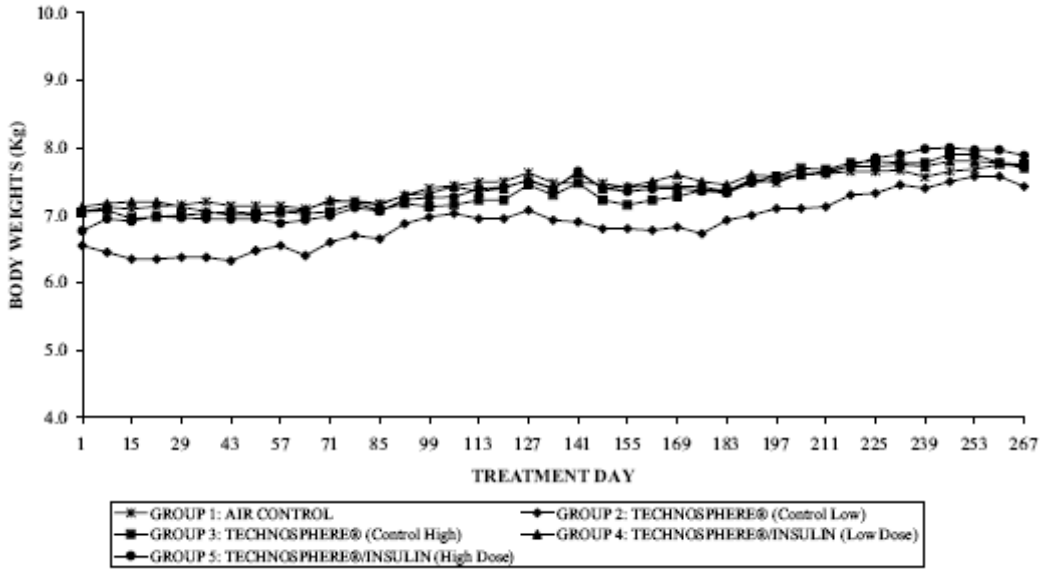


FIGURE 2



Food consumption: There were no treatment related effects on food consumption.

Ophthalmoscopy: There were no ophthalmoscopic findings that were treatment-related.

EKG: EKG parameters were unaffected by treatment. There were no observable effects on the morphology of the P-QRS-T complexes, PR, and QT intervals, as well as QRS complex duration in any animals of any groups compared to pre-treatment. All values, including mean heart rates, showed minor random variations. No rhythm abnormalities were noted.

Hematology: There were no treatment related trends in T or TI treated animals when compared to Air Control. Clotting times were also unaffected by treatment with T or TI.

Hematology	Sex	Time	Air control	T low	T high	TI low	TI high
↓ HGB (g/L)	F	Week 13	155 ±6.3	139 ±7.8			
↓ HCT (L/L)	F		0.48 ±0.028	0.42 ±0.038			
↓ MCV (fL)	F		70.9 ±2.28	67.0 ±2.99			
↑ PLT ($x10^9$/L)	F		323 ±49.2	423 ±65.9			
↑ PLT ($x10^9$/L)	F	Week 26	322 ±61.6	417 ±78.1			
↓ MCH (g/L)	F	Week 39	23.9 ±0.63	23.0 ±0.37			
Coagulation	**Sex**	**Time**	**Air control**	**T low**	**T high**	**TI low**	**TI high**
↑ APTT (sec)	F	Pretreatment	12.9 ±0.93				14.4 ±1.70

* generated by the reviewer. The absence of data in the boxes indicates no significant change.

Clinical chemistry: There were no treatment related effects in clinical chemistry parameters. However, the liver enzymes (ALP and ALT) were generally decreased, but not statistically significant, in TI treated males (Groups 4 and 5) throughout the study, when compared to Air Control (Group 1). However, similar differences from Air Control were also noted pretreatment. The wide intra-group variations were noted in these parameters at all sampling occasions and the values were considered to be within normal biological variation. In addition, cholesterol levels were significantly lower in Tlow-treated females (Group 2) at the Week 26, but the values were comparable to pretreatement values. This value was considered unrelated to treatment.

Clinical Chemistry	Sex	Time	Air control	T low	T high	TI low	TI high
↑ ALP (U/L)	F	Pretreatment	67 ±16.0		100 ±40.8		
↑ ALT (U/L)	F	Pretreatment	26 ±7.0	69 ±37.8			
↓ CHOL (mmol/L)	F	Week 26	4.75 ±0.790	3.36 ±0.548			

* generated by the reviewer.

Glucose:

Glucose levels were comparable between all time points throughout the treatment period. There was also no difference between males and females.

The VH control groups which did not receive insulin and received T alone (Groups 2 and 3) remained relatively unchanged over the 240 min sampling period.

Decrease in serum glucose levels for TI low animals (Group 4) were not as significant, with decreased observed immediately post exposure of up to 6%, with the most significant decreases between 19 to 34 %.

In addition, dose-dependent decreases in serum glucose levels were seen in TI high animals (Group 5): a decrease in serum glucose was detected immediately post exposure with decreases from predose values of between 14 to 22 %. The most significant decreases in serum glucose levels occurred 15 to 30 min post end of exposure. The decreases at these time points were comparable between sexes, with decreases from predose values of between 57 and 65% for males and 57 to 70 % for females.

Lastly, glucose levels for both TI high and TI low groups then steadily increased, reaching predose levels at the final sampling time point of 240 min post end of exposure.

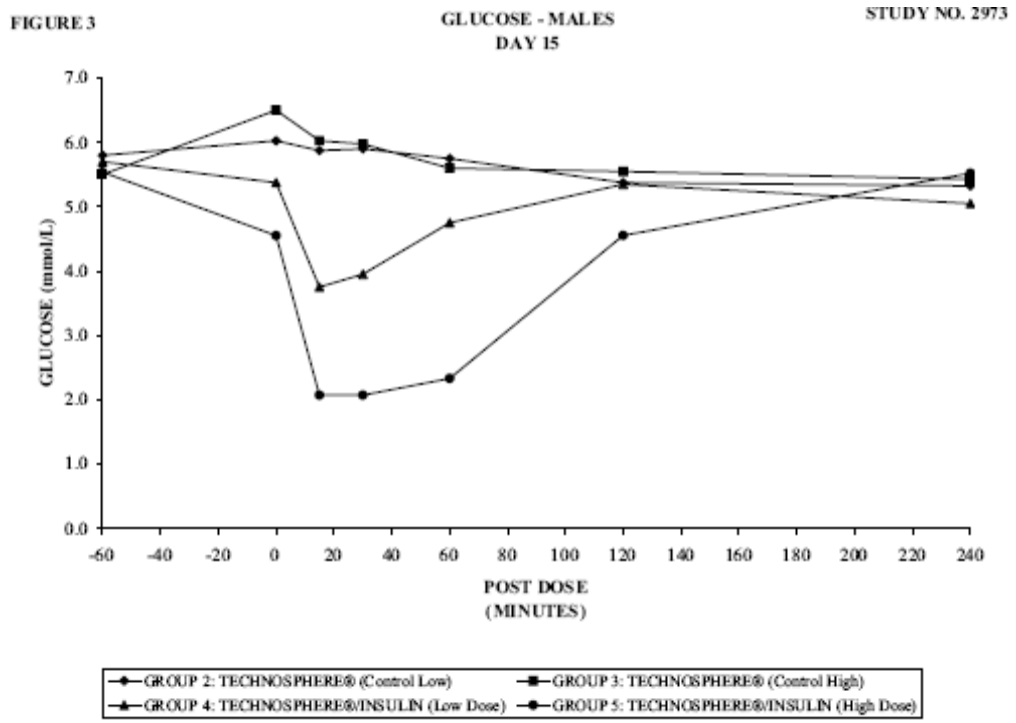


FIGURE 4

GLUCOSE - FEMALES
DAY 15

STUDY NO. 2973

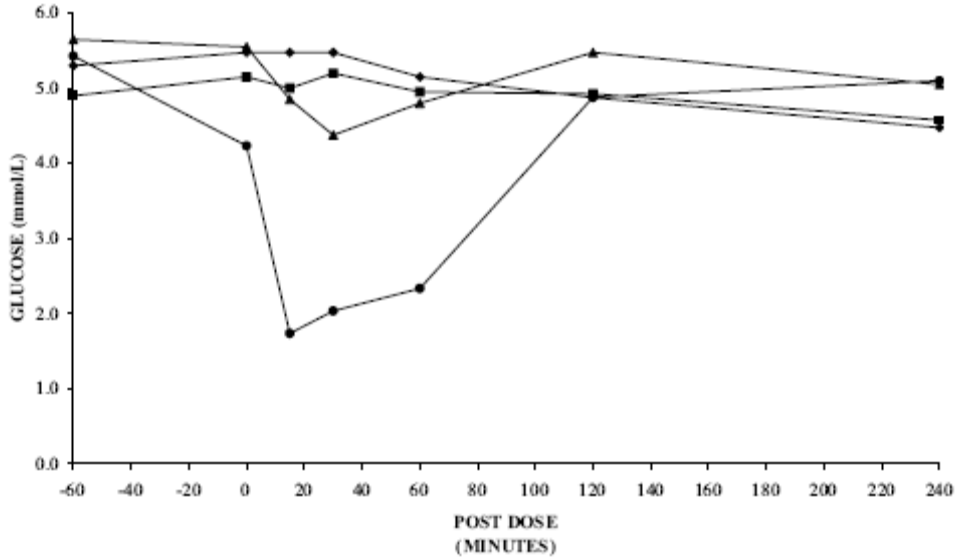
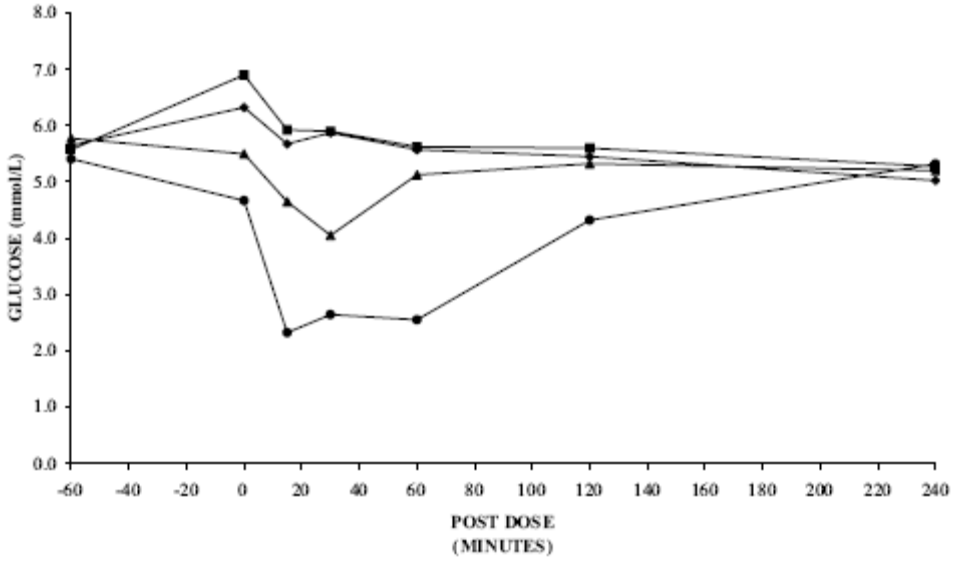


FIGURE 5

GLUCOSE - MALES
DAY 22

STUDY NO. 2973



GROUP 2: TECHNOSPHERE® (Control Low)
GROUP 3: TECHNOSPHERE® (Control High)
GROUP 4: TECHNOSPHERE®/INSULIN (Low Dose)
GROUP 5: TECHNOSPHERE®/INSULIN (High Dose)

FIGURE 6

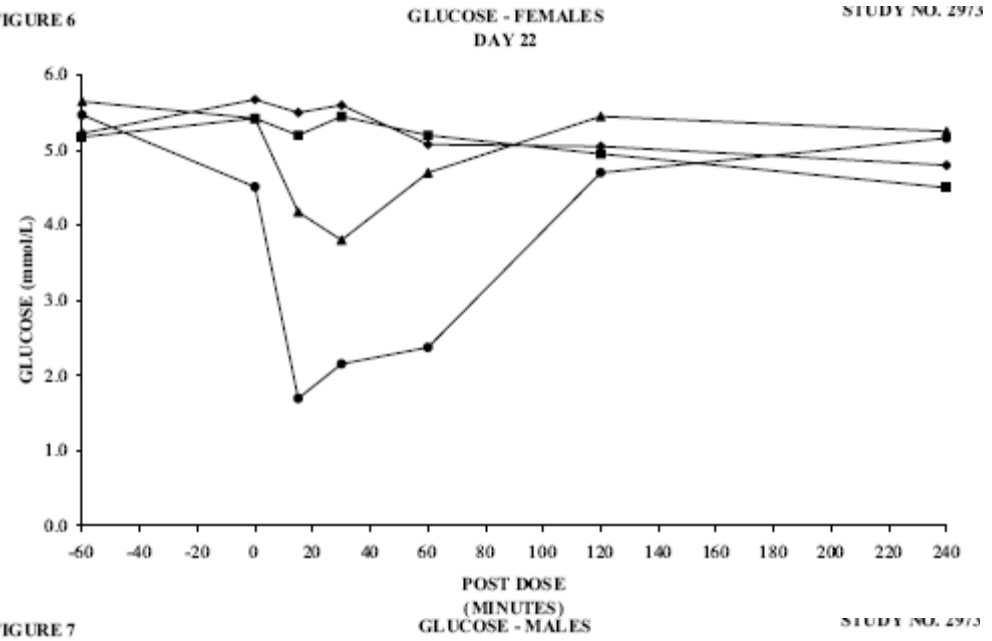


FIGURE 7

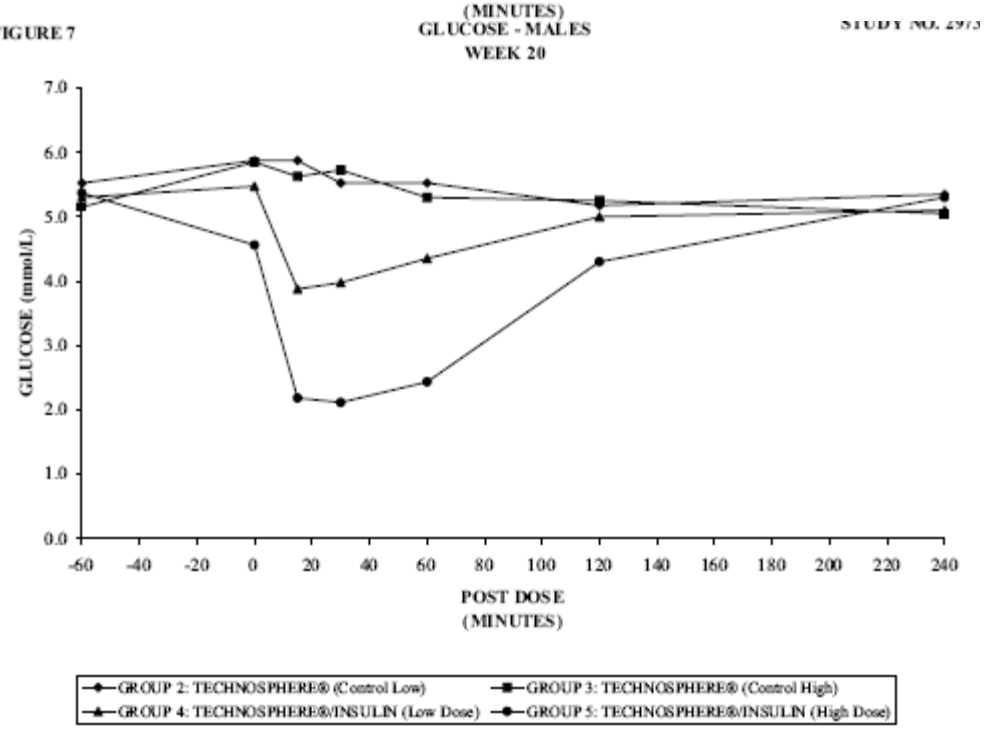


FIGURE 8

GLUCOSE - FEMALES
WEEK 20

STUDY NO. 2973

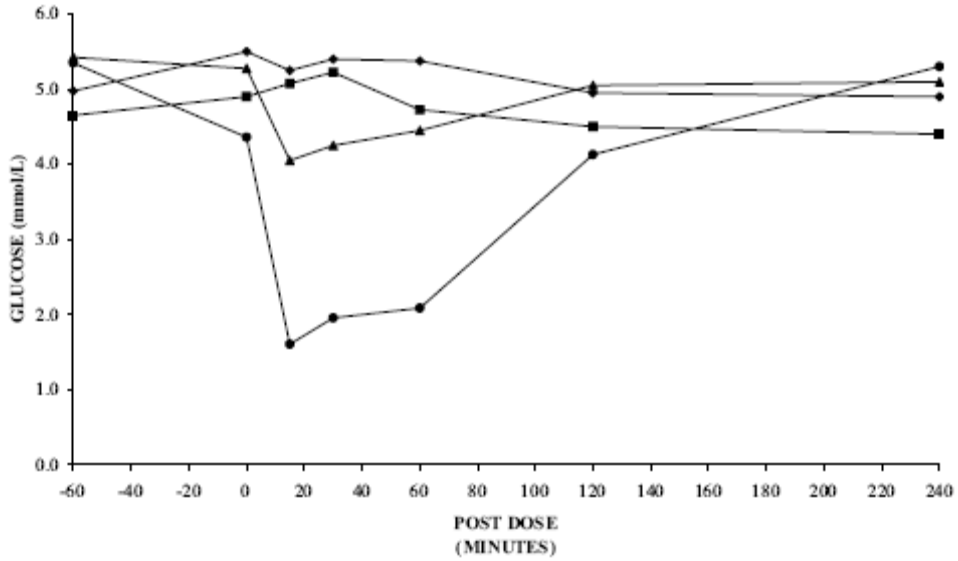
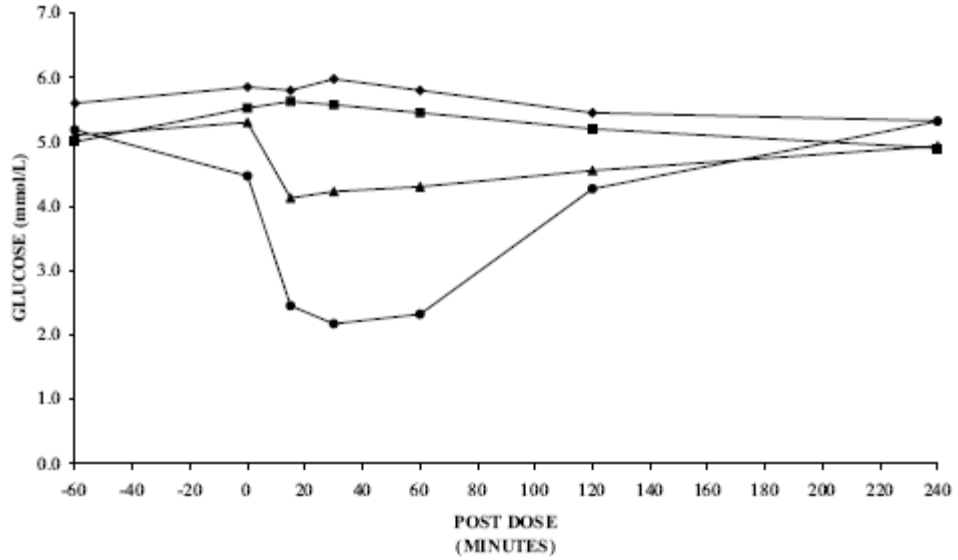


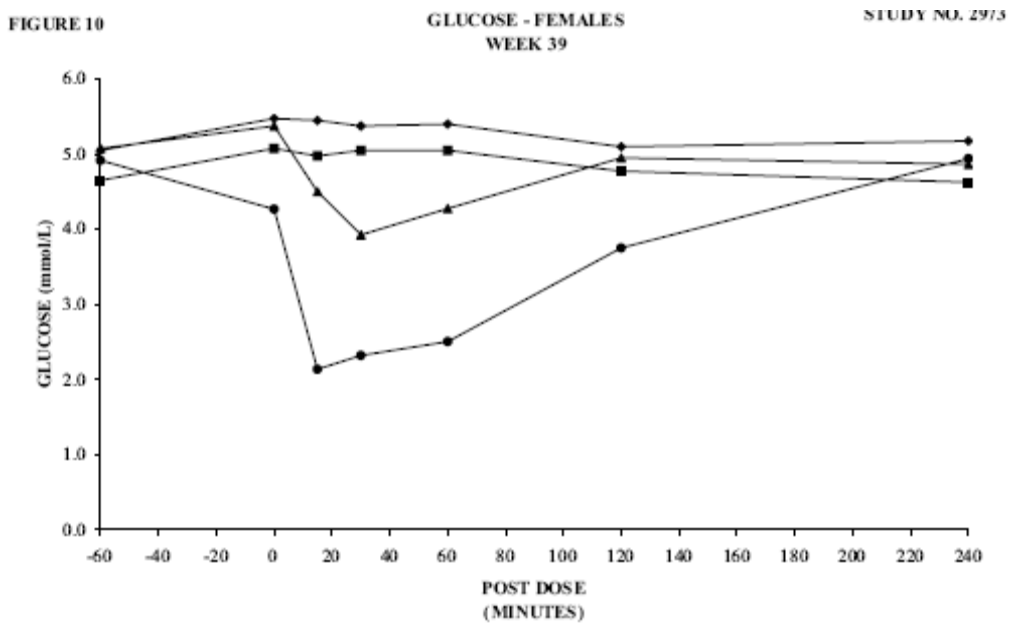
FIGURE 9

GLUCOSE - MALES
WEEK 39

STUDY NO. 2973



GROUP 2: TECHNOSPHERE® (Control Low)
GROUP 3: TECHNOSPHERE® (Control High)
GROUP 4: TECHNOSPHERE®/INSULIN (Low Dose)
GROUP 5: TECHNOSPHERE®/INSULIN (High Dose)



Urinalysis: Urinalysis parameters were not affected by treatment with T or TI.

Urinalysis	Sex	Time	Air control	T low	T high	TI low	TI high
↓ Specific Gravity	F	Pretreatment	1.064 ±0.0090			1.034 ±0.0083	

* generated by the reviewer.

Gross pathology: No remarkable lesions were noted. There were a variety of spontaneous changes were seen across all groups and sexes with no indication of an effect of treatment. The sponsor noted that these findings were consistent with changes commonly observed in dogs of their ages kept under laboratory conditions. See tables below for details (provided by the sponsor).

GROUP INCIDENCE: NECROPSY - ALL DATA

DAY 183

Kill type: TERMINAL

Tissue / Observation	Group:	1	5	1	5
	Sex:	M	M	F	F
	Number:	3	4	4	4
LYMPH NODE, RETROPHARYNGEAL					
DARK AREA		0	0	0	1
LUNG					
ADHESION		0	1	0	0
CYST		0	0	0	1
DEPRESSED AREA		1	0	0	0
PALE FOCUS		0	0	1	0
LYMPH NODE					
DARK DISCOLORATION		0	0	1	0
SMALL INTESTINE, DUODENUM					
MOTTLED		0	1	1	0
RAISED AREA		0	1	0	0
SMALL INTESTINE, ILEUM					
MOTTLED		0	1	0	0
SMALL INTESTINE, JEJUNUM					
MOTTLED		0	1	1	0
SPLEEN					
SMALL		0	0	1	1
STOMACH					
DARK AREA		0	1	0	0
THYMUS					
SMALL		0	1	1	0

GROUP INCIDENCE: NECROPSY - ALL DATA **DAY 274**

Kill type: TERMINAL

Tissue / Observation	Group:	1	2	3	4	5	1	2	3	4	5
	Sex:	M	M	M	M	M	F	F	F	F	F
	Number:	4	4	4	4	4	4	4	4	4	4
ADRENAL											
ENLARGEMENT		0	0	0	0	0	0	0	0	0	1
CECUM											
DARK AREA		1	0	0	0	0	0	0	0	0	0
MOTTLED		0	1	1	0	0	0	0	1	0	0
COLON											
MOTTLED		1	0	1	0	0	0	0	1	0	2
ESOPHAGUS											
FROTHY MATERIAL		0	1	0	0	0	0	0	0	0	0
GALLBLADDER											
PALE FOCUS		0	0	0	0	1	0	0	0	0	0
KIDNEY											
DARK DISCOLORATION		0	1	2	1	0	0	0	0	3	0
IRREGULAR SURFACE		0	0	0	0	0	0	0	1	0	0
PALE DISCOLORATION		0	0	1	0	0	0	0	0	0	0
LIVER											
ADHESION		0	0	0	0	0	0	0	1	0	0
LYMPH NODE, MESENTERIC											
DARK DISCOLORATION		1	0	0	0	0	0	0	1	0	0
MOTTLED		0	2	1	1	0	1	0	0	1	1
LYMPH NODE, RETROPHARYNGEAL											
DARK AREA		1	3	1	1	2	0	2	3	2	4
MOTTLED		0	1	1	0	0	0	0	0	1	2
LUNG											
ADHESION		0	1	0	0	0	0	1	0	0	1
CYST		0	1	0	0	0	0	0	0	0	1
DARK AREA		2	0	0	1	0	0	0	0	0	1
DEFORMED		0	0	0	0	0	0	1	0	0	0
DEPRESSED AREA		1	0	0	0	0	0	0	0	0	0
MOTTLED		1	0	0	0	0	0	0	0	0	0
PALE AREA		1	2	0	1	1	0	2	0	1	1
PALE FOCUS		0	0	1	0	0	0	1	0	0	1
LYMPH NODE											
DARK DISCOLORATION		0	1	1	0	1	0	0	0	0	1
ENLARGEMENT		0	1	0	0	0	0	0	0	0	0
MOTTLED		1	1	2	0	0	0	0	0	0	0
MAMMARY GLAND											
MOTTLED		0	0	0	0	0	0	1	0	0	0
THICKENING		0	0	0	0	0	1	1	0	0	0

OVARY										
CYST	-	-	-	-	-	0	0	1	0	0
PANCREAS										
PALE DISCOLORATION	0	0	1	0	1	0	0	0	0	0
PITUITARY										
CYST	0	0	0	0	0	1	0	0	1	1
SMALL INTESTINE, DUODENUM										
DARK DISCOLORATION	0	0	1	0	0	0	0	0	0	0
MOTTLED	0	1	0	1	0	1	0	1	0	2
RAISED AREA	0	0	0	0	1	0	0	0	0	0
SMALL INTESTINE, ILEUM										
DARK AREA	0	0	0	1	0	0	0	0	0	1
MOTTLED	0	0	0	0	0	0	0	1	0	0
SMALL INTESTINE, JEJUNUM										
DARK AREA	0	0	1	0	0	0	0	1	0	0
DARK DISCOLORATION	0	1	0	0	0	1	0	0	0	1
MOTTLED	0	1	0	0	0	0	0	0	0	0
SPLEEN										
ENLARGEMENT	0	0	0	0	1	0	0	0	0	0
PALE AREA	0	1	1	0	0	1	0	0	1	0
PALE FOCUS	0	1	0	0	1	0	0	0	1	1
SMALL	0	1	1	0	1	1	0	2	2	1
STOMACH										
DARK DISCOLORATION	0	1	0	0	0	0	0	0	0	0
DARK FOCUS	0	0	0	0	0	0	0	1	0	0
MOTTLED	0	0	0	0	0	0	0	0	0	1
PALE AREA	0	0	0	0	0	0	0	0	1	0
PALE FOCUS	0	0	0	0	0	0	0	0	1	0
THYMUS										
DARK AREA	0	1	0	0	0	1	0	0	0	0
DARK FOCUS	0	1	1	2	0	1	0	0	1	0
MOTTLED	0	0	0	0	0	0	0	1	0	0
SMALL	0	0	0	0	0	0	0	1	1	0
THYROID LOBE										
DARK DISCOLORATION	0	0	0	0	0	0	0	1	1	2
FIRM	0	0	0	0	0	0	0	0	0	1
URINARY BLADDER										
DARK DISCOLORATION	0	0	0	0	0	0	0	0	0	1
DARK FOCUS	0	0	1	0	0	0	0	0	0	1
VAGINA										
MOTTLED	-	-	-	-	-	0	0	1	2	0

GROUP INCIDENCE: NECROPSY - ALL DATA

Kill type: RECOVERY

Tissue / Observation	Group:	1	5	1	5
	Sex:	M	M	F	F
	Number:	2	2	2	2
CECUM					
MOTTLED		1	0	0	0
COLON					
MOTTLED		0	2	0	0
ESOPHAGUS					
FROTHY MATERIAL		1	0	0	0
LIVER					
PALE AREA		1	0	0	0
LYMPH NODE, MESENTERIC					
MOTTLED		1	0	0	0
LYMPH NODE, RETROPHARYNGEAL					
DARK AREA		1	0	1	0
LUNG					
ADHESION		0	0	1	0
DARK AREA		0	0	0	1
DEPRESSED AREA		0	0	1	1
PALE AREA		2	0	0	0
PALE FOCUS		1	0	0	0
LYMPH NODE					
MOTTLED		1	0	0	0
PITUITARY					
CYST		0	0	0	1
SMALL INTESTINE, JEJUNUM					
DARK DISCOLORATION		0	0	1	0
SPLEEN					
ENLARGEMENT		0	0	0	1
PALE FOCUS		0	0	1	0
SMALL		1	0	0	0
SKIN & SUBCUTIS					
WOUND		0	1	0	0
STOMACH					
PALE DISCOLORATION		1	0	0	0
THYMUS					
DARK FOCUS		0	0	1	0
SMALL		1	0	0	0
TRACHEA					
DEFORMED		1	0	0	0
THYROID LOBE					
DARK DISCOLORATION		1	0	0	0
VAGINA					
RAISED AREA		-	-	0	1

GROUP INCIDENCE: NECROPSY - ALL DATA

Kill type: FOUND DEAD

Tissue / Observation	Group:	1
	Sex:	M
	Number:	1
CECUM		
MOTTLED		1
COLON		
MOTTLED		1
GALLBLADDER		
GELATINOUS MATERIAL		1
THICKENING		1
KIDNEY		
DARK DISCOLORATION		1
LYMPH NODE, BRONCHIAL		
DARK DISCOLORATION		1
LUNG		
NON-COLLAPSING		1
LYMPH NODE		
DARK DISCOLORATION		1
SMALL INTESTINE, DUODENUM		
DARK DISCOLORATION		1
SMALL INTESTINE, ILEUM		
DARK DISCOLORATION		1
SMALL INTESTINE, JEJUNUM		
DARK DISCOLORATION		1
SPLEEN		
SMALL		1
THYMUS		
DARK DISCOLORATION		1
THYROID LOBE		
DARK DISCOLORATION		1

Organ weights: There were no treatment-related changes on organ weights.

There was a significant increase in adrenal weight in females from TI groups at Week 39, however, the increases were not proportional to dose or sex and the weights were considered within normal variation. In addition, there was a significant decrease in brain weight in high-dose TI females at Week 26. This decrease was not seen in the males or at Week 39 or following 8 weeks of recovery. Moreover, there were minor, but not significant, differences in liver, prostate and spleen weights in males at Week 39 from low-dose T control and/or high-dose TI. However these differences showed no dose relationship.

Organ Wt	Sex	Time	Air control	T low	T high	TI low	TI high
↑ Adrenals (g)	F	Day 274	1.141 ±0.0955			1.514 ±0.2362	1.516 ±0.2748

↑ Adrenals (%/body wt)	F	Day 274	0.0149 ±0.00188			0.0190 ±0.00197	0.0188 ±0.00243
↓ Brain (%/body wt)	F	Day 183	1.0641 ±0.07871				0.9172 ±0.02450

* generated by the reviewer.

TABLE NO. 8 — ORGAN WEIGHTS - ABSOLUTE — STUDY NO. 2973

SUMMARY OF MEANS (g)
DAY 183
MALES

GROUP 1: AIR CONTROL — GROUP 5: TECHNOSPHERE®/INSULIN (High Dose)

GROUP NO.		BODY WT.	ADRENALS	BRAIN	HEART	KIDNEYS	LIVER	PITUITARY
1	MEAN	9067	1.504	77.9	77.9	49.7	274.3	0.0599
	SD	1320.4	0.4903	6.02	3.11	3.44	29.80	0.01051
	N	3	3	3	3	3	3	3
5	MEAN	9000	1.314	77.6	78.8	53.0	274.0	0.0626
	SD	752.8	0.1266	3.56	6.40	3.74	12.30	0.00634
	N	4	4	4	4	4	4	4

GROUP NO.		PROSTATE	RESPIRATORY TRACT (Nare)	SPLEEN	TESTES	THYMUS	THYROID LOBES & PARATHYROIDS
1	MEAN	6.07	106.9	74.5	11.01	5.64	0.850
	SD	1.408	5.12	43.56	0.985	2.7841	0.160
	N	3	3	3	3	3	3
5	MEAN	5.36	109.1	69.3	12.18	4.60	0.760
	SD	1.700	12.65	15.22	0.744	2.2243	0.1337
	N	4	4	4	4	4	4

TABLE NO. 8 — ORGAN WEIGHTS - ABSOLUTE — STUDY NO. 2973

SUMMARY OF MEANS (g)
DAY 183
FEMALES

GROUP 1: AIR CONTROL — GROUP 5: TECHNOSPHERE®/INSULIN (High Dose)

GROUP NO.		BODY WT.	ADRENALS	BRAIN	HEART	KIDNEYS	LIVER	OVARIES
1	MEAN	7325	1.136	77.7	65.7	39.0	238.6	0.910
	SD	518.8	0.2256	3.76	9.13	6.96	17.20	0.2317
	N	4	4	4	4	4	4	4
5	MEAN	7375	1.295	67.6 +	62.7	36.1	251.0	1.078
	SD	221.7	0.2236	2.10	4.49	2.90	28.43	0.3666
	N	4	4	4	4	4	4	4

GROUP NO.		PITUITARY	RESPIRATORY TRACT (Nare)	SPLEEN	THYMUS	THYROID LOBES & PARATHYROIDS	UTERUS
1	MEAN	0.0679	91.1	43.6	3.02	0.667	2.74
	SD	0.00994	8.96	24.29	1.338	0.1253	0.387
	N	4	4	4	4	4	3
5	MEAN	0.0557	87.9	49.1	3.79	0.599	4.31
	SD	0.01790	8.08	16.99	0.745	0.0656	1.398
	N	4	4	4	4	4	3

+ = Statistically significant when compared with Group 1 at $p \leq 0.05$ (t test)

TABLE NO. 8 ORGAN WEIGHTS - ABSOLUTE STUDY NO. 2973

SUMMARY OF MEANS (g)

DAY 274

MALES

GROUP 1: AIR CONTROL

GROUP 2: TECHNOSPHERE® (Control Low) GROUP 4: TECHNOSPHERE®/INSULIN (Low Dose)

GROUP 3: TECHNOSPHERE® (Control High) GROUP 5: TECHNOSPHERE®/INSULIN (High Dose)

GROUP NO.		BODY WT.	ADRENALS	BRAIN	HEART	KIDNEYS	LIVER	PITUITARY
1	MEAN	9425	1.289	80.4	82.1	51.0	298.0	0.0685
	SD	1384.1	0.1764	4.88	12.89	3.50	20.86	0.01066
	N	4	4	4	4	4	4	4
2	MEAN	9575	1.484	74.4	84.5	54.6	343.5	0.0689
	SD	1078.2	0.1347	3.47	9.47	5.62	43.79	0.00884
	N	4	4	4	4	4	4	4
3	MEAN	9700	1.335	74.6	84.6	48.6	285.6	0.0651
	SD	1036.0	0.1895	3.62	11.17	8.02	7.33	0.00527
	N	4	4	4	4	4	4	4
4	MEAN	9475	1.217	77.7	81.7	50.9	309.8	0.0657
	SD	1302.2	0.3234	3.90	12.84	6.05	59.36	0.01785
	N	4	4	4	4	4	4	4
5	MEAN	9600	1.237	76.0	76.5	53.1	306.7	0.0652
	SD	2249.4	0.1906	2.24	10.59	11.65	48.43	0.00886
	N	4	4	4	4	4	4	4

GROUP NO.		PROSTATE	RESPIRATORY TRACT (Nare)	SPLEEN	TESTES	THYMUS	THYROID LOBES & PARATHYROIDS
1	MEAN	5.07	83.7	66.9	12.40	4.28	0.622
	SD	3.402	4.90	33.97	2.169	2.811	0.0409
	N	4	4	4	4	4	4
2	MEAN	5.29	80.1	62.4	11.71	3.97	0.622
	SD	1.785	3.87	48.43	3.888	1.968	0.1953
	N	4	4	4	4	4	4
3	MEAN	5.82	75.5	70.2	12.33	2.72	0.650
	SD	3.075	4.14	37.50	2.586	1.078	0.2554
	N	4	4	4	4	4	4
4	MEAN	5.28	76.6	78.1	14.60	3.96	0.606
	SD	0.931	17.07	16.29	2.782	1.901	0.1279
	N	4	4	4	4	4	4
5	MEAN	6.95	78.3	93.6	11.58	2.84	0.669
	SD	4.492	11.62	45.64	2.063	1.082	0.2609
	N	4	4	4	4	4	4

TABLE NO. 8 ORGAN WEIGHTS - ABSOLUTE STUDY NO. 2973

SUMMARY OF MEANS (g)

DAY 274

FEMALES

GROUP 1: AIR CONTROL

GROUP 2: TECHNOSPHERE® (Control Low) GROUP 4: TECHNOSPHERE®/INSULIN (Low Dose)

GROUP 3: TECHNOSPHERE® (Control High) GROUP 5: TECHNOSPHERE®/INSULIN (High Dose)

GROUP NO.		BODY WT.	ADRENALS	BRAIN	HEART	KIDNEYS	LIVER	OVARIES
1	MEAN	7700	1.141	72.2	64.0	36.0	222.5	1.014
	SD	326.6	0.0955	1.62	6.29	3.21	24.64	0.3428
	N	4	4	4	4	4	4	4
2	MEAN	7500	1.185	72.1	70.2	40.5	263.7	1.163
	SD	804.2	0.0653	3.93	4.30	1.11	25.44	0.3588
	N	4	4	4	4	4	4	4
3	MEAN	7825	1.296	74.5	63.0	35.7	243.3	0.955
	SD	457.3	0.1587	7.64	6.45	4.86	36.23	0.2199
	N	4	4	4	4	4	4	4
4	MEAN	7950	1.514 *	73.0	67.9	35.4	280.6	0.825
	SD	574.5	0.2362	3.74	2.25	1.40	18.98	0.0721
	N	4	4	4	4	4	4	4
5	MEAN	8025	1.516 *	74.3	68.7	38.0	264.4	1.207
	SD	699.4	0.2748	7.95	9.59	5.62	48.86	0.3867
	N	4	4	4	4	4	4	4

GROUP NO.		PITUITARY	RESPIRATORY TRACT (Nare)	SPLEEN	THYMUS	THYROID LOBES & PARATHYROIDS	UTERUS
1	MEAN	0.0599	60.6	41.6	2.70	0.580	2.42
	SD	0.01044	6.30	14.73	0.305	0.0973	NA
	N	4	4	4	4	4	1
2	MEAN	0.0585	67.9	54.8	2.73	0.600	3.02
	SD	0.00591	4.38	15.86	1.054	0.0988	NA
	N	4	4	4	4	4	1
3	MEAN	0.0672	66.8	68.2	2.64	0.479	2.94
	SD	0.00578	2.53	46.42	1.209	0.1012	0.453
	N	4	4	4	4	4	2
4	MEAN	0.0691	64.2	42.5	3.57	0.608	3.06
	SD	0.00677	5.45	18.32	2.030	0.0768	0.641
	N	4	4	4	4	4	3
5	MEAN	0.0719	67.6	42.4	3.42	0.481	NA
	SD	0.01340	17.48	11.41	0.660	0.0725	NA
	N	4	4	4	4	4	0

NA = Not applicable

* = Statistically significant when compared with Group 1 at $p \leq 0.05$ (Dunnett's)

TABLE NO. 8 ORGAN WEIGHTS - ABSOLUTE STUDY NO. 2973
SUMMARY OF MEANS (g)
END OF RECOVERY
MALES

GROUP 1: AIR CONTROL GROUP 5: TECHNOSPHERE®/INSULIN (High Dose)

GROUP NO.		BODY WT.	ADRENALS	BRAIN	HEART	KIDNEYS	LIVER	PITUITARY
1	MEAN	9100	1.265	81.4	86.6	53.7	282.7	0.0612
	SD	141.4	0.1810	1.70	2.05	13.72	11.24	0.00912
	N	2	2	2	2	2	2	2
5	MEAN	9200	0.977	72.8	76.4	48.6	288.5	0.0634
	SD	141.4	0.1718	0.57	7.71	4.10	0.07	0.01004
	N	2	2	2	2	2	2	2

GROUP NO.		PROSTATE	RESPIRATORY TRACT (Nare)	SPLEEN	TESTES	THYMUS	THYROID LOBES & PARATHYROIDS
1	MEAN	6.76	79.5	82.9	14.35	2.19	0.688
	SD	2.687	1.77	33.94	2.517	1.146	0.3769
	N	2	2	2	2	2	2
5	MEAN	4.72	77.1	84.6	15.03	5.44	0.626
	SD	0.106	5.09	12.66	5.565	2.800	0.1598
	N	2	2	2	2	2	2

TABLE NO. 8 ORGAN WEIGHTS - ABSOLUTE STUDY NO. 2973
SUMMARY OF MEANS (g)
END OF RECOVERY
FEMALES

GROUP 1: AIR CONTROL GROUP 5: TECHNOSPHERE®/INSULIN (High Dose)

GROUP NO.		BODY WT.	ADRENALS	BRAIN	HEART	KIDNEYS	LIVER	OVARIES
1	MEAN	8100	1.258	75.2	67.8	37.1	269.2	0.928
	SD	282.8	0.3111	5.73	4.31	3.46	20.51	0.3486
	N	2	2	2	2	2	2	2
5	MEAN	8550	1.401	75.0	69.0	38.2	237.1	1.149
	SD	919.2	0.1188	7.92	8.20	4.31	33.30	0.2404
	N	2	2	2	2	2	2	2

GROUP NO.		PITUITARY	RESPIRATORY TRACT (Nare)	SPLEEN	THYMUS	THYROID LOBES & PARATHYROIDS	UTERUS
1	MEAN	0.0641	65.5	103.1	4.86	0.533	3.03
	SD	0.00212	3.18	0.07	0.976	0.1527	NA
	N	2	2	2	2	2	1
5	MEAN	0.0734	67.9	90.2	4.03	0.576	4.14
	SD	0.01881	7.92	59.40	0.905	0.1096	NA
	N	2	2	2	2	2	1

NA = Not applicable

TABLE NO. 9 ORGAN WEIGHTS - RELATIVE TO TERMINAL BODY WEIGHT STUDY NO. 2973
SUMMARY OF MEANS (%)
DAY 183
MALES

GROUP 1: AIR CONTROL GROUP 5: TECHNOSPHERE®/INSULIN (High Dose)

GROUP NO.		ADRENALS	BRAIN	HEART	KIDNEYS	LIVER	PITUITARY
1	MEAN	0.0171	0.8764	0.8686	0.5552	3.0433	0.0007
	SD	0.00768	0.17516	0.10327	0.08127	0.28302	0.00006
	N	3	3	3	3	3	3
5	MEAN	0.0146	0.8650	0.8814	0.5930	3.0545	0.0007
	SD	0.00135	0.06132	0.11300	0.07774	0.18003	0.00012
	N	4	4	4	4	4	4

GROUP NO.		PROSTATE	RESPIRATORY TRACT (Nare)	SPLEEN	TESTES	THYMUS	THYROID LOBES & PARATHYROIDS
1	MEAN	0.0670	1.1927	0.8055	0.1242	0.0602	0.0096
	SD	0.01368	0.14322	0.40032	0.02822	0.02075	0.00244
	N	3	3	3	3	3	3
5	MEAN	0.0590	1.2267	0.7669	0.1361	0.0502	0.0085
	SD	0.01565	0.24838	0.12982	0.01480	0.02295	0.00160
	N	4	4	4	4	4	4

TABLE NO. 9 ORGAN WEIGHTS - RELATIVE TO TERMINAL BODY WEIGHT STUDY NO. 2973
SUMMARY OF MEANS (%)
DAY 183
FEMALES

GROUP 1: AIR CONTROL GROUP 5: TECHNOSPHERE®/INSULIN (High Dose)

GROUP NO.		ADRENALS	BRAIN	HEART	KIDNEYS	LIVER	OVARIES
1	MEAN	0.0155	1.0641	0.8944	0.5317	3.2607	0.0126
	SD	0.00249	0.07871	0.07025	0.08035	0.15844	0.00405
	N	4	4	4	4	4	4
5	MEAN	0.0176	0.9172 +	0.8507	0.4903	3.4037	0.0146
	SD	0.00318	0.02450	0.06619	0.04433	0.37748	0.00506
	N	4	4	4	4	4	4

GROUP NO.		PITUITARY	RESPIRATORY TRACT (Nare)	SPLEEN	THYMUS	THYROID LOBES & PARATHYROIDS	UTERUS
1	MEAN	0.0009	1.2442	0.5853	0.0422	0.0091	0.0364
	SD	0.00008	0.10022	0.30386	0.02140	0.00173	0.00633
	N	4	4	4	4	4	3
5	MEAN	0.0008	1.1916	0.6716	0.0512	0.0081	0.0584
	SD	0.00024	0.11063	0.24468	0.00859	0.00102	0.01979
	N	4	4	4	4	4	3

+ = Statistically significant when compared with Group 1 at $p \leq 0.05$ (t test)

TABLE NO. 9 ORGAN WEIGHTS - RELATIVE TO TERMINAL BODY WEIGHT STUDY NO. 2973
SUMMARY OF MEANS (%)
DAY 274
MALES

GROUP 1: AIR CONTROL
GROUP 2: TECHNOSPHERE® (Control Low)
GROUP 3: TECHNOSPHERE® (Control High)
GROUP 4: TECHNOSPHERE®/INSULIN (Low Dose)
GROUP 5: TECHNOSPHERE®/INSULIN (High Dose)

GROUP NO.		ADRENALS	BRAIN	HEART	KIDNEYS	LIVER	PITUITARY
1	MEAN	0.0138	0.8663	0.8714	0.5484	3.1885	0.0007
	SD	0.00140	0.13665	0.04822	0.07822	0.24628	0.00008
	N	4	4	4	4	4	4
2	MEAN	0.0158	0.7838	0.8831	0.5710	3.6185	0.0007
	SD	0.00352	0.08516	0.02947	0.02426	0.56703	0.00010
	N	4	4	4	4	4	4
3	MEAN	0.0140	0.7767	0.8714	0.5044	2.9742	0.0007
	SD	0.00323	0.10730	0.06261	0.09407	0.38002	0.00014
	N	4	4	4	4	4	4
4	MEAN	0.0127	0.8284	0.8635	0.5392	3.2529	0.0007
	SD	0.00193	0.08940	0.07286	0.03697	0.27950	0.00013
	N	4	4	4	4	4	4
5	MEAN	0.0134	0.8240	0.8132	0.5661	3.2639	0.0007
	SD	0.00330	0.18882	0.11012	0.12897	0.52134	0.00008
	N	4	4	4	4	4	4

GROUP NO.		PROSTATE	RESPIRATORY TRACT (Nare)	SPLEEN	TESTES	THYMUS	THYROID LOBES & PARATHYROIDS
1	MEAN	0.0509	0.8971	0.6879	0.1321	0.0442	0.0067
	SD	0.02727	0.08989	0.24752	0.01995	0.02400	0.00119
	N	4	4	4	4	4	4
2	MEAN	0.0544	0.8418	0.6246	0.1209	0.0407	0.0064
	SD	0.01502	0.06763	0.44825	0.03406	0.01791	0.00171
	N	4	4	4	4	4	4
3	MEAN	0.0589	0.7829	0.7057	0.1266	0.0280	0.0067
	SD	0.02577	0.06871	0.33457	0.02085	0.01064	0.00206
	N	4	4	4	4	4	4
4	MEAN	0.0558	0.8058	0.8265	0.1536	0.0408	0.0065
	SD	0.00662	0.12275	0.13663	0.01547	0.01558	0.00154
	N	4	4	4	4	4	4
5	MEAN	0.0672	0.8474	0.9356	0.1244	0.0295	0.0069
	SD	0.03785	0.23702	0.40439	0.02961	0.01014	0.00154
	N	4	4	4	4	4	4

TABLE NO. 9 ORGAN WEIGHTS - RELATIVE TO TERMINAL BODY WEIGHT STUDY NO. 2973
SUMMARY OF MEANS (%)
DAY 274
FEMALES

GROUP 1: AIR CONTROL
GROUP 2: TECHNOSPHERE® (Control Low)
GROUP 3: TECHNOSPHERE® (Control High)
GROUP 4: TECHNOSPHERE®/INSULIN (Low Dose)
GROUP 5: TECHNOSPHERE®/INSULIN (High Dose)

GROUP NO.		ADRENALS	BRAIN	HEART	KIDNEYS	LIVER	OVARIES
1	MEAN	0.0149	0.9388	0.8326	0.4685	2.8914	0.0132
	SD	0.00188	0.02918	0.09436	0.04559	0.32121	0.00433
	N	4	4	4	4	4	4
2	MEAN	0.0159	0.9687	0.9429	0.5443	3.5614	0.0156
	SD	0.00141	0.11765	0.10575	0.05861	0.63006	0.00517
	N	4	4	4	4	4	4
3	MEAN	0.0165	0.9568	0.8107	0.4577	3.1145	0.0123
	SD	0.00124	0.14045	0.12186	0.07119	0.48667	0.00306
	N	4	4	4	4	4	4
4	MEAN	0.0190 *	0.9227	0.8593	0.4474	3.5455	0.0105
	SD	0.00197	0.10135	0.09237	0.04741	0.37449	0.00168
	N	4	4	4	4	4	4
5	MEAN	0.0188 *	0.9259	0.8562	0.4725	3.3109	0.0151
	SD	0.00243	0.04760	0.09775	0.04196	0.67121	0.00488
	N	4	4	4	4	4	4

GROUP NO.		PITUITARY	RESPIRATORY TRACT (Nare)	SPLEEN	THYMUS	THYROID LOBES & PARATHYROIDS	UTERUS
1	MEAN	0.0008	0.7901	0.5450	0.0352	0.0075	0.0332
	SD	0.00013	0.10307	0.21333	0.00457	0.00120	NA
	N	4	4	4	4	4	1
2	MEAN	0.0008	0.9120	0.7244	0.0361	0.0081	0.0419
	SD	0.00008	0.10143	0.18307	0.01242	0.00142	NA
	N	4	4	4	4	4	1
3	MEAN	0.0009	0.8539	0.8618	0.0342	0.0061	0.0372
	SD	0.00010	0.02248	0.58498	0.01626	0.00127	0.00898
	N	4	4	4	4	4	2
4	MEAN	0.0009	0.8092	0.5490	0.0450	0.0077	0.0393
	SD	0.00010	0.06699	0.28420	0.02435	0.00142	0.01159
	N	4	4	4	4	4	3
5	MEAN	0.0009	0.8394	0.5269	0.0426	0.0060	NA
	SD	0.00008	0.18457	0.13662	0.00761	0.00049	NA
	N	4	4	4	4	4	0

NA = Not applicable
* = Statistically significant when compared with Group 1 at $p \leq 0.05$ (Dunnett's)

TABLE NO. 9 ORGAN WEIGHTS - RELATIVE TO TERMINAL BODY WEIGHT STUDY NO. 2973
SUMMARY OF MEANS (%)
END OF RECOVERY
MALES

GROUP 1: AIR CONTROL GROUP 5: TECHNOSPHERE®/INSULIN (High Dose)

GROUP NO.		ADRENALS	BRAIN	HEART	KIDNEYS	LIVER	PITUITARY
1	MEAN	0.0140	0.8945	0.9514	0.5914	3.1055	0.0007
	SD	0.00219	0.00474	0.03734	0.15988	0.07531	0.00014
	N	2	2	2	2	2	2
5	MEAN	0.0106	0.7915	0.8294	0.5280	3.1357	0.0007
	SD	0.00170	0.01831	0.07106	0.03649	0.04738	0.00014
	N	2	2	2	2	2	2

GROUP NO.		PROSTATE	RESPIRATORY TRACT (Nare)	SPLEEN	TESTES	THYMUS	THYROID LOBES & PARATHYROIDS
1	MEAN	0.0745	0.8734	0.9140	0.1579	0.0240	0.0076
	SD	0.03069	0.03302	0.38721	0.03012	0.01223	0.00424
	N	2	2	2	2	2	2
5	MEAN	0.0513	0.8377	0.9202	0.1629	0.0594	0.0068
	SD	0.00191	0.04243	0.15175	0.05798	0.03132	0.00163
	N	2	2	2	2	2	2

TABLE NO. 9 ORGAN WEIGHTS - RELATIVE TO TERMINAL BODY WEIGHT STUDY NO. 2973
SUMMARY OF MEANS (%)
END OF RECOVERY
FEMALES

GROUP 1: AIR CONTROL GROUP 5: TECHNOSPHERE®/INSULIN (High Dose)

GROUP NO.		ADRENALS	BRAIN	HEART	KIDNEYS	LIVER	OVARIES
1	MEAN	0.0155	0.9296	0.8360	0.4585	3.3211	0.0114
	SD	0.00332	0.10317	0.02404	0.05876	0.13718	0.00389
	N	2	2	2	2	2	2
5	MEAN	0.0164	0.8773	0.8065	0.4461	2.7676	0.0137
	SD	0.00042	0.00170	0.00919	0.00247	0.09199	0.00431
	N	2	2	2	2	2	2

GROUP NO.		PITUITARY	RESPIRATORY TRACT (Nare)	SPLEEN	THYMUS	THYROID LOBES & PARATHYROIDS	UTERUS
1	MEAN	0.0008	0.8092	1.2731	0.0603	0.0066	0.0384
	SD	0.00000	0.06760	0.04533	0.01421	0.00212	NA
	N	2	2	2	2	2	1
5	MEAN	0.0009	0.7938	1.0987	0.0480	0.0067	0.0450
	SD	0.00007	0.00728	0.81282	0.01577	0.00057	NA
	N	2	2	2	2	2	1

NA = Not applicable

Histopathology:

Overall, there were no findings at Week 26 or following 8 weeks of recovery. But there were histopathological changes in respiratory tract tissues that following 39 weeks of treatment, which were considered to be related to treatment.

- Week 26 (Day 183):

There were higher incidences of epithelial metaplasia, mixed or mononuclear cell infiltrate and lymphoid aggregations in the upper respiratory tract (i.e. nasal cavity, larynx, trachea, carina, and

bronchi) were seen in Air Control group (Group 1) and high-dose TI group (Group 5) when compared to unexposed controls. The sponsor suggested that these were commonly seen in inhalation studies and were indicative of an adaptive change within the respiratory tract rather than overt toxicity. Note from the reviewer: The absence of neoplastic findings in the rodent carcinogenicity studies conforms this.

In addition, there were other histopathology findings, including minimal to mild bronchial and epithelial metaplasia in TI-treated males, minimal to mild larynx epithelial metaplasia in TI-treated females, lung mononuclear cell infiltrate in TI-treated males and females, pituitary cysts in TI-treated males, mild to moderate decreases in spleen blood volume of TI-treated males, and minimal to mild thyroid c-cell complexes in TI-treated females.

- Week 39 (Day 274):

Minimal to mild alveolar and/or bronchial-interstitial neurtophil infiltrate of the lungs were seen in TI groups (Groups 4 and 5) following 39 weeks of treatment. However, the incidence of these findings were low (1/8 and 3/8 animals respectively). These findings completely regressed following 8 weeks of recovery (Group 5).

Minimal to mild thymic atrophy was seen in 6/8 high-dose T control (Group 3) animals at Week 39. This finding was inconsistent between doses of T and was commonly observed in dogs of their ages. The sponsor considered as unlikely to be related to treatment with T.

Minimal to moderate hypocellularity of seminiferous epithelium and minimal to mild germ cell degeneration were noted in 1/4 animals in the low-dose T control group (Group 2) and in 2/4 animals in high-dose TI group (Group 5) at Week 39. Germ cell degeneration was also observed in Air Control (Group 1). Minimal to severe hypospermia/aspermia was noted treated groups (esp. ¾ in Group 5) when compared to Air Control group. Furthermore, there were other histopathology findings, including minimal to mild colon congestion/hemorrhage in low and high-dose TI groups (Groups 4 and 5, not recoverable in females).

Note from the reviewer: Target organs were nasal cavity and heart in rats, whereas target organs in dogs were lung, thymus, testis, and epididymis in dogs. Microscopic findings were T related in a dose-dependent manner. Historical control was not provided by the sponsor.

GROUP INCIDENCE: HISTOPATHOLOGY - ALL DATA **DAY 183**

Kill type: TERMINAL

Tissue / Observation	Group:	1	5	1	5
	Sex:	M	M	F	F
	Number:	3	4	4	4
EPIDIDYMIS					
Number examined		3	4	-	-
HYPOSPERMIA/ASPERMIA		1	1	-	-
mild		0	1	-	-
moderate		1	0	-	-
BRONCHI					
Number examined		3	4	4	4
METAPLASIA, EPITHELIAL		1	2	1	0
minimal		0	2	1	0
mild		1	0	0	0
KIDNEY					
Number examined		3	4	4	4
DILATATION,TUBULAR		0	1	0	0
minimal		0	1	0	0
BASOPHILIA,TUBULAR		1	0	2	1
minimal		1	0	2	1
CELL INFILTRATE, MONONUCLEAR		0	0	1	0
minimal		0	0	1	0
LARYNX					
Number examined		3	4	4	4
METAPLASIA, EPITHELIAL		1	2	2	4
minimal		1	1	1	3
mild		0	1	1	1
LYMPHOID AGGREGATIONS		0	0	1	0
minimal		0	0	1	0
LYMPH NODE, BRONCHIAL					
Number examined		3	4	4	4
HEMOSIDEROSIS		1	1	1	1
minimal		1	1	1	1
CONGESTION AND/OR HEMORRHAGE		1	2	2	2
minimal		0	2	2	2
mild		1	0	0	0
CELL INFILTRATE, NEUTROPHIL		0	0	0	1
minimal		0	0	0	1
LYMPH NODE, MESENTERIC					
Number examined		3	4	4	4
CONGESTION AND/OR HEMORRHAGE		2	2	1	2
minimal		1	2	0	1
mild		1	0	1	1
LYMPH NODE, MANDIBULAR					
Number examined		3	4	4	4
CONGESTION AND/OR HEMORRHAGE		1	0	0	0
mild		1	0	0	0

GROUP INCIDENCE: HISTOPATHOLOGY - ALL DATA **DAY 183**

Kill type: TERMINAL

Tissue / Observation	Group:	1	5	1	5
	Sex:	M	M	F	F
	Number:	3	4	4	4
LYMPH NODE, RETROPHARYNGEAL					
Number examined		3	4	4	4
CONGESTION AND/OR HEMORRHAGE		1	0	0	1
mild		1	0	0	0
moderate		0	0	0	1
LUNG					
Number examined		3	4	4	4
HEMOSIDEROSIS		1	2	1	1
minimal		0	2	0	1
mild		1	0	1	0
EMPHYSEMA		0	0	0	1
present		0	0	0	1
MICROGRANULOMA		1	1	1	1
minimal		1	1	0	0
mild		0	0	1	1
FIBROSIS,PLEURAL		0	1	0	0
minimal		0	1	0	0
PNEUMONIA,INTERSTITIAL		1	1	0	0
minimal		1	0	0	0
mild		0	1	0	0
CELL INFILTRATE, NEUTROPHIL		1	2	1	0
minimal		1	2	1	0
CELL INFILTRATE, MIXED		0	0	1	0
mild		0	0	1	0
MACROPHAGES, ALVEOLAR		1	1	2	1
minimal		0	0	2	0
mild		1	1	0	1
CELL INFILTRATE, MONONUCLEAR		1	2	2	3
minimal		0	1	0	3
mild		1	1	2	0
METAPLASIA, EPITHELIAL		0	0	0	1
mild		0	0	0	1
MACROPHAGES, SUBPLEURAL		0	0	1	0
mild		0	0	1	0
OBSTRUCTION, BRONCHIOLAR		0	0	1	0
minimal		0	0	1	0
LYMPH NODE					
Number examined		0	0	1	0
CONGESTION AND/OR HEMORRHAGE		0	0	1	0
moderate		0	0	1	0
CARINA					
Number examined		3	4	4	4
CELL INFILTRATE, NEUTROPHIL		1	0	0	0
minimal		1	0	0	0
METAPLASIA, EPITHELIAL		1	1	1	0
mild		1	1	1	0
LYMPHOID AGGREGATIONS		3	3	1	1

GROUP INCIDENCE: HISTOPATHOLOGY - ALL DATA **DAY 183**

Kill type: TERMINAL

Tissue / Observation	Group:	1	5	1	5
	Sex:	M	M	F	F
	Number:	3	4	4	4
CARINA (continued)					
LYMPHOID AGGREGATIONS (continued)					
minimal		2	3	1	1
mild		1	0	0	0
NASAL CAVITY					
Number examined		3	4	4	4
CELL INFILTRATE, MIXED		1	1	0	0
minimal		1	1	0	0
CELL INFILTRATE, MONONUCLEAR		1	0	0	1
minimal		1	0	0	1
METAPLASIA, EPITHELIAL		0	1	2	1
minimal		0	1	1	1
mild		0	0	1	0
LYMPHOID AGGREGATIONS		0	0	0	1
minimal		0	0	0	1
PHARYNX					
Number examined		3	4	4	4
LYMPHOID AGGREGATIONS		0	1	1	1
minimal		0	1	1	0
mild		0	0	0	1
PITUITARY					
Number examined		3	4	4	4
CYST		0	2	1	0
mild		0	1	1	0
moderate		0	1	0	0
CALCIFICATION,FOCAL		1	0	0	0
minimal		1	0	0	0
SMALL INTESTINE, DUODENUM					
Number examined		3	4	4	4
NOTE		0	1	0	0
present		0	1	0	0
CONGESTION AND/OR HEMORRHAGE		0	1	0	0
minimal		0	1	0	0
SMALL INTESTINE, ILEUM					
Number examined		3	4	4	4
CONGESTION AND/OR HEMORRHAGE		0	1	0	0
minimal		0	1	0	0
SMALL INTESTINE, JEJUNUM					
Number examined		3	4	4	4
CONGESTION AND/OR HEMORRHAGE		0	1	0	0
minimal		0	1	0	0

GROUP INCIDENCE: HISTOPATHOLOGY - ALL DATA **DAY 183**

Kill type: TERMINAL

Tissue / Observation	Group:	1	5	1	5
	Sex:	M	M	F	F
	Number:	3	4	4	4
SPLEEN					
Number examined		3	4	4	4
HEMOSIDEROSIS		0	0	2	2
minimal		0	0	1	2
mild		0	0	1	0
EXTRAMEDULLARY HEMATOPOIESIS		0	1	0	0
minimal		0	1	0	0
BLOOD VOLUME, DECREASED		0	2	3	2
mild		0	2	2	2
moderate		0	0	1	0
SKIN & SUBCUTIS					
Number examined		3	4	4	4
CELL INFILTRATE, MONONUCLEAR		0	0	1	0
minimal		0	0	1	0
STOMACH					
Number examined		3	4	4	4
CONGESTION AND/OR HEMORRHAGE		0	1	0	0
minimal		0	1	0	0
THYMUS					
Number examined		3	4	4	4
ATROPHY		0	1	2	1
minimal		0	0	0	1
mild		0	1	1	0
moderate		0	0	1	0
TRACHEA					
Number examined		3	4	4	4
METAPLASIA, EPITHELIAL		0	1	0	0
minimal		0	1	0	0
TESTIS					
Number examined		3	4	-	-
HYPOCELLULARITY		3	1	-	-
minimal		1	1	-	-
mild		2	0	-	-
THYROID LOBE					
Number examined		3	4	4	4
CYST		0	1	0	0
mild		0	1	0	0
C-CELL COMPLEXES		0	0	1	3
minimal		0	0	0	2
mild		0	0	1	1

GROUP INCIDENCE: HISTOPATHOLOGY - ALL DATA

DAY 274

Kill type: TERMINAL

Tissue / Observation	Group:	1	2	3	4	5	1	2	3	4	5
	Sex:	M	M	M	M	M	F	F	F	F	F
	Number:	4	4	4	4	4	4	4	4	4	4
CECUM											
Number examined		4	4	4	4	4	4	4	4	4	4
CONGESTION AND/OR HEMORRHAGE		1	0	1	0	0	1	0	1	0	1
minimal		0	0	1	0	0	1	0	1	0	1
mild		1	0	0	0	0	0	0	0	0	0
COLON											
Number examined		4	4	4	4	4	4	4	4	4	4
DEGENERATION,VACUOLAR		0	0	0	0	1	0	0	0	0	0
mild		0	0	0	0	1	0	0	0	0	0
CONGESTION AND/OR HEMORRHAGE		1	0	1	0	0	0	0	1	1	2
minimal		1	0	0	0	0	0	0	0	1	1
mild		0	0	1	0	0	0	0	1	0	1
EPIDIDYMIS											
Number examined		4	4	4	4	4	-	-	-	-	-
HYPOSPERMIA/ASPERMIA		0	2	1	1	3	-	-	-	-	-
minimal		0	0	1	1	1	-	-	-	-	-
mild		0	1	0	0	2	-	-	-	-	-
severe		0	1	0	0	0	-	-	-	-	-
HISTIOCYTOSIS		0	0	0	0	1	-	-	-	-	-
mild		0	0	0	0	1	-	-	-	-	-
DEGENERATION, GERM CELLS		0	1	1	0	0	-	-	-	-	-
minimal		0	0	1	0	0	-	-	-	-	-
mild		0	1	0	0	0	-	-	-	-	-
ESOPHAGUS											
Number examined		4	4	4	4	4	4	4	4	4	4
CELL INFILTRATE, MONONUCLEAR		0	1	0	0	0	0	0	0	0	0
minimal		0	1	0	0	0	0	0	0	0	0
HEART											
Number examined		4	4	4	4	4	4	4	4	4	4
CELL INFILTRATE, MONONUCLEAR		0	0	1	0	0	0	0	0	0	0
minimal		0	0	1	0	0	0	0	0	0	0
BRONCHI											
Number examined		4	4	4	4	4	4	4	4	4	4
CELL INFILTRATE, MIXED		0	0	0	1	0	1	0	0	1	0
minimal		0	0	0	0	0	1	0	0	1	0
mild		0	0	0	1	0	0	0	0	0	0
METAPLASIA, EPITHELIAL		1	1	1	2	1	0	1	1	3	2
minimal		1	1	1	1	1	0	0	1	3	0
mild		0	0	0	1	0	0	1	0	0	2
LYMPHOID AGGREGATIONS		1	0	0	0	0	0	0	0	0	0
minimal		1	0	0	0	0	0	0	0	0	0
ATROPHY, EPITHELIAL		0	0	0	0	0	0	0	1	0	0
minimal		0	0	0	0	0	0	0	1	0	0

GROUP INCIDENCE: HISTOPATHOLOGY - ALL DATA

DAY 274

Kill type: TERMINAL

Tissue / Observation	Group:	1	2	3	4	5	1	2	3	4	5
	Sex:	M	M	M	M	M	F	F	F	F	F
	Number:	4	4	4	4	4	4	4	4	4	4
KIDNEY											
Number examined		4	4	4	4	4	4	4	4	4	4
DILATATION,TUBULAR		0	1	0	0	0	0	0	0	0	0
minimal		0	1	0	0	0	0	0	0	0	0
BASOPHILIA,TUBULAR		1	1	1	0	1	0	0	0	0	0
minimal		0	1	1	0	1	0	0	0	0	0
mild		1	0	0	0	0	0	0	0	0	0
CONGESTION		0	1	0	1	0	1	0	0	2	0
minimal		0	1	0	1	0	1	0	0	2	0
FIBROSIS		0	0	0	0	1	0	0	0	1	0
minimal		0	0	0	0	0	0	0	0	1	0
mild		0	0	0	0	1	0	0	0	0	0
CELL INFILTRATE, MONONUCLEAR		1	0	0	0	1	0	0	0	1	0
minimal		1	0	0	0	1	0	0	0	1	0
LARYNX											
Number examined		4	4	4	4	4	4	4	4	4	4
METAPLASIA, EPITHELIAL		0	1	3	1	2	3	3	1	0	3
minimal		0	0	2	1	1	3	2	0	0	1
mild		0	1	1	0	1	0	1	1	0	1
moderate		0	0	0	0	0	0	0	0	0	1
LYMPHOID AGGREGATIONS		0	1	0	0	1	0	1	1	0	1
minimal		0	1	0	0	1	0	1	1	0	1
LYMPH NODE, BRONCHIAL											
Number examined		4	4	4	4	4	4	4	4	4	4
HEMOSIDEROSIS		1	0	0	1	0	0	0	0	0	0
minimal		1	0	0	1	0	0	0	0	0	0
CONGESTION AND/OR HEMORRHAGE		2	1	0	1	0	0	0	2	1	1
minimal		1	0	0	1	0	0	0	2	1	1
mild		1	1	0	0	0	0	0	0	0	0
CELL INFILTRATE, NEUTROPHIL		1	0	0	1	3	0	0	0	1	1
minimal		1	0	0	1	1	0	0	0	1	0
mild		0	0	0	0	2	0	0	0	0	1
LACRIMAL GLAND											
Number examined		4	3	4	4	4	4	4	4	4	3
HEMORRHAGE		0	0	0	0	0	0	0	1	0	0
minimal		0	0	0	0	0	0	0	1	0	0
LIVER											
Number examined		4	4	4	4	4	4	4	4	4	4
HEMOSIDEROSIS		1	0	0	0	0	0	0	1	1	0
minimal		0	0	0	0	0	0	0	1	0	0
mild		1	0	0	0	0	0	0	0	1	0
EXTRAMEDULLARY HEMATOPOIESIS		0	0	0	0	1	0	0	0	0	0
mild		0	0	0	0	1	0	0	0	0	0
FIBROSIS,CAPSULAR		0	0	0	0	0	0	0	1	0	0
minimal		0	0	0	0	0	0	0	1	0	0

GROUP INCIDENCE: HISTOPATHOLOGY - ALL DATA

DAY 274

Kill type: TERMINAL

Tissue / Observation	Group:	1	2	3	4	5	1	2	3	4	5
	Sex:	M	M	M	M	M	F	F	F	F	F
	Number:	4	4	4	4	4	4	4	4	4	4
LYMPH NODE, MESENTERIC											
Number examined		4	4	4	4	4	4	4	4	4	4
CONGESTION AND/OR HEMORRHAGE		4	3	2	1	4	3	3	4	3	3
minimal		3	0	1	0	2	2	0	1	2	0
mild		0	1	0	1	2	1	2	3	0	3
moderate		1	2	1	0	0	0	1	0	1	0
LYMPH NODE, MANDIBULAR											
Number examined		4	4	4	4	4	4	4	4	4	4
HEMOSIDEROSIS		1	0	0	1	1	2	1	0	0	1
minimal		1	0	0	1	1	2	1	0	0	1
CONGESTION AND/OR HEMORRHAGE		0	0	0	0	0	1	1	1	0	0
minimal		0	0	0	0	0	1	0	1	0	0
mild		0	0	0	0	0	0	1	0	0	0
LYMPH NODE, RETROPHARYNGEAL											
Number examined		4	4	4	4	4	4	4	4	4	4
PIGMENT		1	0	0	0	1	0	0	0	1	1
mild		1	0	0	0	1	0	0	0	1	1
CONGESTION AND/OR HEMORRHAGE		0	3	3	2	3	0	2	3	3	4
minimal		0	0	2	1	2	0	0	0	0	0
mild		0	3	1	0	1	0	1	3	2	4
moderate		0	0	0	1	0	0	1	0	1	0
LUNG											
Number examined		4	4	4	4	4	4	4	4	4	4
HEMOSIDEROSIS		2	1	0	2	2	0	0	0	0	2
minimal		2	1	0	2	2	0	0	0	0	2
CONGESTION		1	0	0	1	0	0	0	0	0	1
minimal		0	0	0	1	0	0	0	0	0	0
mild		1	0	0	0	0	0	0	0	0	1
EMPHYSEMA		0	0	0	0	0	0	1	0	0	0
mild		0	0	0	0	0	0	1	0	0	0
MICROGRANULOMA		1	0	0	1	0	0	3	0	0	0
minimal		1	0	0	1	0	0	3	0	0	0
FIBROSIS,PLEURAL		0	1	0	0	0	0	1	0	0	1
minimal		0	1	0	0	0	0	1	0	0	1
PNEUMONIA,INTERSTITIAL		1	0	0	0	1	0	3	1	1	2
minimal		1	0	0	0	0	0	2	1	1	0
mild		0	0	0	0	1	0	1	0	0	2
CELL INFILTRATE, NEUTROPHIL		0	0	0	1	2	0	0	0	0	1
minimal		0	0	0	0	1	0	0	0	0	1
mild		0	0	0	1	1	0	0	0	0	0
CELL INFILTRATE, MIXED		0	1	0	0	2	0	1	0	0	0
minimal		0	0	0	0	0	0	1	0	0	0
mild		0	1	0	0	2	0	0	0	0	0
MACROPHAGES, ALVEOLAR		0	1	1	1	1	0	1	0	0	0
minimal		0	1	1	0	1	0	1	0	0	0
mild		0	0	0	1	0	0	0	0	0	0
CELL INFILTRATE, MONONUCLEAR		3	3	2	3	3	1	3	1	1	3

GROUP INCIDENCE: HISTOPATHOLOGY - ALL DATA

DAY 274

Kill type: TERMINAL

Tissue / Observation	Group:	1	2	3	4	5	1	2	3	4	5
	Sex:	M	M	M	M	M	F	F	F	F	F
	Number:	4	4	4	4	4	4	4	4	4	4
LUNG (continued)											
CELL INFILTRATE, MONONUCLEAR (continued)											
minimal		1	3	1	1	1	1	1	1	1	1
mild		2	0	1	2	1	0	2	0	0	2
moderate		0	0	0	0	1	0	0	0	0	0
METAPLASIA, EPITHELIAL		0	0	1	0	1	0	2	0	1	1
minimal		0	0	1	0	0	0	2	0	1	0
mild		0	0	0	0	1	0	0	0	0	1
MACROPHAGES, SUBPLEURAL		0	0	1	0	0	0	0	0	0	0
mild		0	0	1	0	0	0	0	0	0	0
HYPERPLASIA, EPITHELIAL		1	0	1	0	1	0	1	0	0	2
minimal		0	0	1	0	1	0	0	0	0	0
mild		1	0	0	0	0	0	1	0	0	2
UNDEREXPANSION		0	0	0	0	1	0	0	0	0	1
present		0	0	0	0	1	0	0	0	0	1
OBSTRUCTION, BRONCHIOLAR		0	0	0	0	0	0	1	0	0	0
minimal		0	0	0	0	0	0	1	0	0	0
LYMPH NODE											
Number examined		1	2	3	0	1	0	0	0	0	1
CONGESTION AND/OR HEMORRHAGE		1	2	3	0	1	0	0	0	0	1
minimal		0	0	1	0	0	0	0	0	0	0
mild		1	1	2	0	0	0	0	0	0	0
moderate		0	0	0	0	1	0	0	0	0	1
severe		0	1	0	0	0	0	0	0	0	0
CARINA											
Number examined		4	4	4	4	4	4	4	4	4	4
METAPLASIA, EPITHELIAL		1	0	0	1	2	2	2	1	2	2
minimal		1	0	0	0	0	2	2	1	2	0
mild		0	0	0	1	1	0	0	0	0	1
moderate		0	0	0	0	1	0	0	0	0	1
LYMPHOID AGGREGATIONS		1	0	0	2	0	0	0	1	0	0
minimal		1	0	0	2	0	0	0	1	0	0
NASAL CAVITY											
Number examined		4	4	4	4	4	4	4	4	4	4
EDEMA		1	1	0	1	0	0	0	0	0	0
minimal		0	1	0	1	0	0	0	0	0	0
mild		1	0	0	0	0	0	0	0	0	0
CELL INFILTRATE, MIXED		1	1	0	0	0	1	0	0	1	1
minimal		0	1	0	0	0	1	0	0	1	1
mild		1	0	0	0	0	0	0	0	0	0
CELL INFILTRATE, MONONUCLEAR		1	1	1	1	2	1	1	1	2	0
minimal		0	1	1	1	1	1	1	1	2	0
mild		1	0	0	0	1	0	0	0	0	0
METAPLASIA, EPITHELIAL		0	1	3	2	3	1	2	2	0	1
minimal		0	1	1	2	3	0	2	1	0	1
mild		0	0	2	0	0	1	0	1	0	0
LYMPHOID AGGREGATIONS		0	0	0	0	1	0	0	0	0	0

GROUP INCIDENCE: HISTOPATHOLOGY - ALL DATA

DAY 274

Kill type: TERMINAL

Tissue / Observation	Group:	1	2	3	4	5	1	2	3	4	5
	Sex:	M	M	M	M	M	F	F	F	F	F
	Number:	4	4	4	4	4	4	4	4	4	4
NASAL CAVITY (continued)											
LYMPHOID AGGREGATIONS (continued)											
minimal		0	0	0	0	1	0	0	0	0	0
KERATINIZATION, INCREASED		0	0	0	0	1	0	0	0	0	0
minimal		0	0	0	0	1	0	0	0	0	0
NASOPHARYNX											
Number examined		4	4	4	4	4	4	4	4	4	4
ATROPHY, EPITHELIAL		0	0	0	0	0	0	0	1	0	0
minimal		0	0	0	0	0	0	0	1	0	0
PHARYNX											
Number examined		4	4	4	4	4	4	4	4	4	4
HEMORRHAGE		0	1	0	0	0	0	0	0	0	0
minimal		0	1	0	0	0	0	0	0	0	0
CELL INFILTRATE, MONONUCLEAR		1	1	0	0	0	0	0	0	0	0
minimal		1	1	0	0	0	0	0	0	0	0
LYMPHOID AGGREGATIONS		0	0	3	1	0	1	3	0	0	1
minimal		0	0	0	1	0	0	2	0	0	1
mild		0	0	3	0	0	1	1	0	0	0
PITUITARY											
Number examined		4	4	4	4	4	4	4	4	4	4
CYST		0	0	0	0	1	2	0	0	1	1
minimal		0	0	0	0	1	0	0	0	0	0
mild		0	0	0	0	0	1	0	0	1	1
moderate		0	0	0	0	0	1	0	0	0	0
PROSTATE											
Number examined		4	4	4	4	4	-	-	-	-	-
ATROPHY		0	0	0	0	1	-	-	-	-	-
mild		0	0	0	0	1	-	-	-	-	-
NECROSIS,SINGLE CELL		0	1	0	0	0	-	-	-	-	-
minimal		0	1	0	0	0	-	-	-	-	-
CELL INFILTRATE, MIXED		0	1	0	0	0	-	-	-	-	-
minimal		0	1	0	0	0	-	-	-	-	-
CELL INFILTRATE, MONONUCLEAR		0	0	0	1	1	-	-	-	-	-
minimal		0	0	0	1	1	-	-	-	-	-
PARATHYROID											
Number examined		3	3	3	3	4	4	4	2	4	4
CYST		0	0	0	0	0	0	1	0	0	0
mild		0	0	0	0	0	0	1	0	0	0
SMALL INTESTINE, DUODENUM											
Number examined		4	4	4	4	4	4	4	4	4	4
CONGESTION AND/OR HEMORRHAGE		0	2	1	0	0	1	0	1	0	2
minimal		0	2	0	0	0	1	0	1	0	2
mild		0	0	1	0	0	0	0	0	0	0
DILATATION, CRYPTAL		1	0	0	0	0	0	0	0	1	0

GROUP INCIDENCE: HISTOPATHOLOGY - ALL DATA

DAY 274

Kill type: TERMINAL

Tissue / Observation	Group:	1	2	3	4	5	1	2	3	4	5
	Sex:	M	M	M	M	M	F	F	F	F	F
	Number:	4	4	4	4	4	4	4	4	4	4
SMALL INTESTINE, DUODENUM (continued)											
DILATATION, CRYPTAL (continued)											
minimal		1	0	0	0	0	0	0	0	1	0
ATROPHY, VILLOUS		0	0	0	0	0	0	0	0	0	1
moderate		0	0	0	0	0	0	0	0	0	1
SALIVARY GLAND											
Number examined		4	4	4	4	4	4	4	4	4	4
CELL INFILTRATE, MONONUCLEAR		0	0	0	1	0	0	0	0	0	1
minimal		0	0	0	1	0	0	0	0	0	1
SMALL INTESTINE, ILEUM											
Number examined		4	4	4	4	4	4	4	4	4	4
CONGESTION AND/OR HEMORRHAGE		1	0	0	1	0	0	0	1	0	0
minimal		0	0	0	1	0	0	0	1	0	0
mild		1	0	0	0	0	0	0	0	0	0
ATROPHY, VILLOUS		0	0	0	0	0	0	0	0	0	1
minimal		0	0	0	0	0	0	0	0	0	1
SMALL INTESTINE, JEJUNUM											
Number examined		4	4	4	4	4	4	4	4	4	4
CONGESTION AND/OR HEMORRHAGE		1	1	1	1	0	2	0	2	0	1
minimal		0	0	0	1	0	1	0	2	0	0
mild		1	0	1	0	0	0	0	0	0	1
moderate		0	1	0	0	0	1	0	0	0	0
ATROPHY, VILLOUS		0	0	0	0	0	0	0	0	0	1
minimal		0	0	0	0	0	0	0	0	0	1
SCIATIC NERVE											
Number examined		4	4	4	4	4	4	4	4	4	4
MYELINOPATHY, FIBRE		0	0	0	0	0	0	0	0	1	0
mild		0	0	0	0	0	0	0	0	1	0
SPLEEN											
Number examined		4	4	4	4	4	4	4	4	4	4
HEMOSIDEROSIS		2	1	2	1	1	2	1	2	3	2
minimal		1	0	2	0	0	2	1	1	2	1
mild		1	1	0	1	1	0	0	1	1	1
EXTRAMEDULLARY HEMATOPOIESIS		2	2	1	4	3	2	2	2	1	2
minimal		2	1	1	3	2	0	1	1	1	1
mild		0	1	0	1	1	2	1	1	0	1
FIBROSIS,CAPSULAR		0	1	0	0	0	0	0	0	0	1
minimal		0	0	0	0	0	0	0	0	0	1
mild		0	1	0	0	0	0	0	0	0	0
BLOOD VOLUME, DECREASED		3	4	4	0	3	4	2	4	4	4
minimal		0	0	1	0	0	0	1	0	0	1
mild		3	4	2	0	2	2	1	2	1	3
moderate		0	0	1	0	1	2	0	2	3	0

DAY 274

GROUP INCIDENCE: HISTOPATHOLOGY - ALL DATA

Kill type: TERMINAL

Tissue / Observation	Group:	1	2	3	4	5	1	2	3	4	5
	Sex:	M	M	M	M	M	F	F	F	F	F
	Number:	4	4	4	4	4	4	4	4	4	4
STERNUM & MARROW											
Number examined		4	4	4	4	4	4	4	4	4	4
OSSIFICATION		0	1	0	0	0	0	0	0	0	0
minimal		0	1	0	0	0	0	0	0	0	0
SKIN & SUBCUTIS											
Number examined		4	4	4	4	4	4	4	4	4	4
MICROGRANULOMA		1	0	0	1	0	0	0	0	0	0
minimal		1	0	0	1	0	0	0	0	0	0
CELL INFILTRATE, MONONUCLEAR		1	0	1	0	0	0	0	1	0	0
minimal		1	0	1	0	0	0	0	1	0	0
STOMACH											
Number examined		4	4	4	4	4	4	4	4	4	4
CONGESTION AND/OR HEMORRHAGE		0	1	0	1	0	1	0	0	0	1
minimal		0	1	0	1	0	1	0	0	0	1
THYMUS											
Number examined		4	4	4	4	4	4	4	4	4	4
ATROPHY		0	0	3	0	0	0	0	3	1	0
minimal		0	0	1	0	0	0	0	2	0	0
mild		0	0	2	0	0	0	0	1	1	0
CONGESTION AND/OR HEMORRHAGE		1	1	1	2	0	2	0	1	1	0
minimal		1	1	0	1	0	1	0	1	1	0
mild		0	0	1	1	0	1	0	0	0	0
TRACHEA											
Number examined		4	4	4	4	4	4	4	4	4	4
METAPLASIA, EPITHELIAL		0	1	0	0	0	0	0	0	0	0
mild		0	1	0	0	0	0	0	0	0	0
TESTIS											
Number examined		4	4	4	4	4	-	-	-	-	-
HYPOCELLULARITY		0	1	0	0	2	-	-	-	-	-
minimal		0	0	0	0	2	-	-	-	-	-
moderate		0	1	0	0	0	-	-	-	-	-
DEGENERATION, GERM CELLS		1	1	1	1	2	-	-	-	-	-
minimal		1	0	1	1	2	-	-	-	-	-
mild		0	1	0	0	0	-	-	-	-	-
THYROID LOBE											
Number examined		4	4	4	4	4	4	4	4	4	4
CYST		1	0	0	0	0	0	0	0	0	0
mild		1	0	0	0	0	0	0	0	0	0
C-CELL COMPLEXES		0	0	0	0	0	1	0	0	0	0
mild		0	0	0	0	0	1	0	0	0	0
URINARY BLADDER											
Number examined		4	4	4	4	4	4	4	4	4	4
CONGESTION		0	0	0	1	0	1	1	1	0	2

DAY 274

GROUP INCIDENCE: HISTOPATHOLOGY - ALL DATA

Kill type: TERMINAL

Tissue / Observation	Group:	1	2	3	4	5	1	2	3	4	5
	Sex:	M	M	M	M	M	F	F	F	F	F
	Number:	4	4	4	4	4	4	4	4	4	4
URINARY BLADDER (continued)											
CONGESTION (continued)											
minimal		0	0	0	1	0	1	1	1	0	2

GROUP INCIDENCE: HISTOPATHOLOGY - ALL DATA

Kill type: RECOVERY

Tissue / Observation	Group:	1	5	1	5
	Sex:	M	M	F	F
	Number:	2	2	2	2
CECUM					
Number examined		2	2	2	2
CONGESTION AND/OR HEMORRHAGE		1	0	0	0
minimal		1	0	0	0
COLON					
Number examined		2	2	2	2
CONGESTION AND/OR HEMORRHAGE		1	2	2	0
minimal		1	0	2	0
mild		0	2	0	0
BRONCHI					
Number examined		2	2	2	2
METAPLASIA, EPITHELIAL		1	1	0	1
minimal		1	1	0	1
LYMPHOID AGGREGATIONS		0	1	0	1
minimal		0	0	0	1
mild		0	1	0	0
KIDNEY					
Number examined		2	2	2	2
BASOPHILIA,TUBULAR		1	0	0	0
minimal		1	0	0	0
LARYNX					
Number examined		2	2	2	2
HEMORRHAGE		0	0	0	1
minimal		0	0	0	1
METAPLASIA, EPITHELIAL		2	1	1	1
minimal		2	1	0	1
mild		0	0	1	0

LYMPH NODE, BRONCHIAL				
Number examined	2	2	2	2
CONGESTION AND/OR HEMORRHAGE	0	2	1	1
minimal	0	2	0	0
mild	0	0	1	1
CELL INFILTRATE, NEUTROPHIL	0	1	0	0
mild	0	1	0	0
LIVER				
Number examined	2	2	2	2
FIBROSIS,CAPSULAR	1	0	0	0
mild	1	0	0	0
LYMPH NODE, MESENTERIC				
Number examined	2	2	2	2
CONGESTION AND/OR HEMORRHAGE	1	0	2	2
minimal	0	0	1	1
mild	0	0	1	0
moderate	1	0	0	1

GROUP INCIDENCE: HISTOPATHOLOGY - ALL DATA

Kill type: RECOVERY

Tissue / Observation	Group: / Sex: / Number:	1 / M / 2	5 / M / 2	1 / F / 2	5 / F / 2
LYMPH NODE, MANDIBULAR					
Number examined		2	2	2	2
CONGESTION AND/OR HEMORRHAGE		0	0	0	1
minimal		0	0	0	1
LYMPH NODE, RETROPHARYNGEAL					
Number examined		2	2	2	2
PIGMENT		1	0	1	0
minimal		1	0	0	0
mild		0	0	1	0
LUNG					
Number examined		2	2	2	2
HEMORRHAGE		0	1	0	0
mild		0	1	0	0
MICROGRANULOMA		1	0	1	1
minimal		1	0	1	1
FIBROSIS,PLEURAL		1	0	0	0
minimal		1	0	0	0
PNEUMONIA,INTERSTITIAL		1	0	0	0
minimal		1	0	0	0
CELL INFILTRATE, MIXED		1	0	1	1
minimal		1	0	0	1
mild		0	0	1	0
MACROPHAGES, ALVEOLAR		1	1	0	0
minimal		0	1	0	0
mild		1	0	0	0
CELL INFILTRATE, MONONUCLEAR		1	2	1	1
minimal		0	1	1	0
mild		1	1	0	1
METAPLASIA, EPITHELIAL		0	0	0	1
mild		0	0	0	1
HYPERPLASIA, EPITHELIAL		1	0	0	0
mild		1	0	0	0
UNDEREXPANSION		1	0	0	0
moderate		1	0	0	0

LYMPH NODE				
Number examined	1	0	0	0
CONGESTION AND/OR HEMORRHAGE	1	0	0	0
mild	1	0	0	0
FIBROSIS, INTERSTITIAL	1	0	0	0
mild	1	0	0	0
CARINA				
Number examined	2	2	2	2
LYMPHOID AGGREGATIONS	0	1	0	0
minimal	0	1	0	0

GROUP INCIDENCE: HISTOPATHOLOGY - ALL DATA

Kill type: RECOVERY

Tissue / Observation	Group: / Sex: / Number:	1 / M / 2	5 / M / 2	1 / F / 2	5 / F / 2
NASAL CAVITY					
Number examined		2	2	2	2
CELL INFILTRATE, MONONUCLEAR		0	2	0	1
minimal		0	1	0	1
mild		0	1	0	0
METAPLASIA, EPITHELIAL		0	0	1	0
mild		0	0	1	0
PITUITARY					
Number examined		2	2	2	2
CYST		2	1	1	1
mild		2	1	1	0
moderate		0	0	0	1
PARATHYROID					
Number examined		2	2	2	2
CYST		1	0	0	0
mild		1	0	0	0
SMALL INTESTINE, DUODENUM					
Number examined		2	2	2	2
DILATATION, CRYPTAL		1	0	0	0
mild		1	0	0	0
SMALL INTESTINE, JEJUNUM					
Number examined		2	2	2	2
CONGESTION AND/OR HEMORRHAGE		1	0	1	0
mild		1	0	0	0
moderate		0	0	1	0
SPLEEN					
Number examined		2	2	2	2
HEMOSIDEROSIS		1	1	0	0
minimal		1	1	0	0
EXTRAMEDULLARY HEMATOPOIESIS		0	0	0	1
mild		0	0	0	1
BLOOD VOLUME, DECREASED		2	2	2	2
minimal		1	0	1	0
mild		1	1	0	1
moderate		0	1	1	1
SKIN & SUBCUTIS					
Number examined		2	2	2	2
ULCER		0	1	0	0
mild		0	1	0	0
CELL INFILTRATE, MIXED		0	1	0	0
minimal		0	1	0	0
THYMUS					
Number examined		2	2	2	2
CYST		0	0	0	1

GROUP INCIDENCE: HISTOPATHOLOGY - ALL DATA

Kill type: RECOVERY

Tissue / Observation	Group:	1	5	1	5
	Sex:	M	M	F	F
	Number:	2	2	2	2
THYMUS (continued)					
CYST (continued)					
minimal		0	0	0	1
ATROPHY		1	1	0	0
minimal		0	1	0	0
moderate		1	0	0	0
CONGESTION AND/OR HEMORRHAGE		0	0	1	0
minimal		0	0	1	0
TRACHEA					
Number examined		2	2	2	2
METAPLASIA, EPITHELIAL		1	0	0	0
minimal		1	0	0	0
URINARY BLADDER					
Number examined		2	2	2	2
CONGESTION		0	0	0	1
minimal		0	0	0	1
VAGINA					
Number examined		-	-	2	2
CYST		-	-	0	1
mild		-	-	0	1

GROUP INCIDENCE: HISTOPATHOLOGY - ALL DATA

Kill type: FOUND DEAD

Tissue / Observation	Group:	1
	Sex:	M
	Number:	1
COLON		
Number examined		1
CONGESTION AND/OR HEMORRHAGE		1
mild		1
KIDNEY		
Number examined		1
CONGESTION		1
moderate		1
LYMPH NODE, BRONCHIAL		
Number examined		1
CONGESTION AND/OR HEMORRHAGE		1
mild		1
LIVER		
Number examined		1
DEGENERATION,VACUOLAR		1
mild		1
CONGESTION		1
mild		1
LUNG		
Number examined		1
HEMOSIDEROSIS		1
mild		1
CONGESTION		1
mild		1
HEMORRHAGE		1
mild		1
CELL INFILTRATE, MONONUCLEAR		1
mild		1
LYMPH NODE		
Number examined		1
HEMOSIDEROSIS		1
minimal		1
CONGESTION AND/OR HEMORRHAGE		1
moderate		1
CARINA		
Number examined		1
LYMPHOID AGGREGATIONS		1
minimal		1
SMALL INTESTINE, DUODENUM		
Number examined		1
CONGESTION AND/OR HEMORRHAGE		1
mild		1

GROUP INCIDENCE: HISTOPATHOLOGY - ALL DATA

Kill type: FOUND DEAD

Tissue / Observation	Group:	1
	Sex:	M
	Number:	1
SMALL INTESTINE, ILEUM		
Number examined		1
CONGESTION AND/OR HEMORRHAGE		1
moderate		1
SMALL INTESTINE, JEJUNUM		
Number examined		1
CONGESTION AND/OR HEMORRHAGE		1
moderate		1
SPLEEN		
Number examined		1
HEMOSIDEROSIS		1
mild		1
BLOOD VOLUME, DECREASED		1
moderate		1
THYMUS		
Number examined		1
CONGESTION		1
mild		1
HEMORRHAGE		1
moderate		1
CONGESTION AND/OR HEMORRHAGE		1
moderate		1
TESTIS		
Number examined		1
HYPOCELLULARITY		1
mild		1
THYROID LOBE		
Number examined		1
CONGESTION		1
mild		1

Toxicokinetics:

- Technosphere®: Exposure to Technosphere® particles was determined by measurement of its major component, FDKP.

The mean t_{max} values had a range of 7.5 to 37.5 min post end of dosing and were similar between days, sexes, and dose. Such t_{max} values were also similar between T and TI groups. The t ½ of FDKP ranged from 52.9 to 83.9 min. With the T groups (Groups 2 and 3), FDKP levels were relatively consistent throughout 39 weeks and between sexes. In addition, the mean AUC_{last} and AUC_{inf} were slightly less than dose proportional. For the high-dose T group (Group 3), the mean AUC_{last} values, obtained at the end of the study, were 395,370 (in males) and 493,440 (in females) ng•min/mL.

For the low-dose TI group (Group 4), female mean FDKP levels remained relatively consistent throughout the study, whereas its levels were lower in male dogs after Day 15. On Days 15 and 20 (in both males and females), and Week 20 (in females only), mean FDKP levels were similar the low dose T group (Group 2) and high dose TI group (Group 5), which received the same dose of T. In addition, On Week 20 (for males only) and Week 39 (both males and females), mean

FDKP levels were lower in the TI animals than in the corresponding low dose T group (Group 2). Such difference was unknown but the individual animal data showed a notable decrease in FDKP concentration was not uncommon in the high-dose TI animals (Group 5), with no consistency between animals.

TABLE 2. Mean Plasma FDKP Toxicokinetic Metrics Following 15 Minute Daily Inhalation of Technosphere® Particles or Technosphere®/Insulin to Beagle Dogs

	Group (mg/kg /day)	Day 15[a]		Day 22[a]		Week 20[a]		Week 39[b]	
		Male	Female	Male	Female	Male	Female	Male	Female
C_{max} (ng/mL)	2 (1.66)	857	1130	634	758	813	823	783	848
	3 (11.2)	3768	3679	4807	3559	2184	2882	3224	4524
	4 (0.33)	240	307	157	166	146	163	157	166
	5 (1.66)	1027	1129	554	775	553	771	692	806
t_{max} (min)	2	22.5	7.5	37.5	18.8	25.0	22.5	22.5	26.3
	3	15.0	30.0	11.3	18.8	11.3	33.8	15.0	15.0
	4	15.0	18.8	7.5	18.8	18.8	18.75	7.5	18.8
	5	16.5	16.5	7.5	15.0	22.5	22.5	20.0	17.5
AUC_{last} (ng*min /mL)	2	91304	96231	62147	84600	82860	90060	91367	103157
	3	468998	466596	433101	483218	272259	347559	395370	493440
	4	23099	23338	15216	19616	15022	20253	15216	19616
	5	98788	108215	56913	90293	56609	78197	68065	71525
AUC_{inf} (ng*min /mL)	2	102506	102130	66788	89670	87981	97230	102604	112946
	3	530619	534083	536084[3]	462019[3]	245681[3]	372225	395824[3]	527344
	4	24092	24958	16233	21373	15897	21893	16233	21373
	5	105142	115764	67888	98597	59681	83337	72512	76284
$t_{1/2}$ (min)	2	66.2	57.4	59.0	61.7	57.8	63.5	74.4	67.1
	3	75.0	75.9	83.9[3]	73.0[3]	74.3[3]	59.6	78.5[3]	61.0
	4	54.4	65.4	61.3	67.3	57.1	63.8	61.3	67.3
	5	58.6	59.3	66.4	69.8	54.4	57.6	56.8	60.0

a) n=4 for Groups 1-4 and n=10 for Group 5 b) n=4 for Groups 1-4 and n=6 for Groups 5
superscript indicates # animals used on calculation of mean, insufficient data to calculate terminal metrics in the rest of the animals

- Insulin:

There were no significant differences in exposure to insulin over the course of the study in the TI low dose group (Group 4), with the exception of low values of Week 20. However, insulin exposure could not be determined in TI high does group (Group 5) due to large variability in the data.

A number of samples were collected from T groups (Groups 2 and 3) that had quantifiable insulin levels. However, the insulin levels detected from those samples showed no relationship to dose. These insulin levels were also inconsistent in between time points and the presence of insulin in these samples were likely due to post exposure contact contamination, and were not related to the animal exposures. In addition, due to small number of samples involved and the amount of insulin detected, there was no interpretation of TK data.

In most cases, t_{max} was reached shortly after end of dosing although there were instances within the TI low dose group (Group 4) where the C_{max} occurred at later times. This was due to the overall low insulin levels, which resulted in no real difference in insulin levels over the duration of the sampling times. In addition, the t ½ of insulin ranged from a mean of 16.9 to 26.7 min in high dose TI group (Group 5).

TABLE 7. Mean Serum Insulin Toxicokinetic Metrics Following 15 Minute Daily Inhalation of Technosphere® Particles or Technosphere®/Insulin to Beagle Dogs

	Group	Day 15[a]		Day 22[a]		Week 20[a]		Week 39[b,d]	
		Male	Female	Male	Female	Male	Female	Male	Female
C_{max} (µU/mL)	4	47.9[2]	41.9	46.6	25.6	26.2	20.9	41.1[3]	23.2
	5	169	170	128	128	106	116	84.1	90.7
t_{max} (min)	4[d]	0.0[a]	15.0	0.0	15.0	0.0	7.5	30.0	7.5
	5	7.5	9.0	6.0	12.0	13.5	10.5	15.0	12.5
AUC_{last} (µU*min/mL)	4	2109[2]	1853	1427	1365	488	853	2682[3]	1379
	5	6730	5713	4635	4508	5478	5757	4278	3998
AUC_{inf} (µU*min/mL)	4	NR[c]	1608[1]	NR	2208[2]	736[1]	NR[c]	1570[1]	5078[1]
	5	7215[9]	6556[9]	5672[7]	5474[8]	6300[7]	6323	5039[4]	5838[3]
$t_{1/2}$ (min)	4	NR[c]	21.2[1]	NR[c]	14.9[2]	7.5[1]	NR[c]	13.2[1]	11.5[1]
	5	20.1[9]	16.9[9]	20.6[7]	18.8[5]	24.9[7]	25.6	25.0[4]	20.0[3]

a) n=4 for Group 4 and n=10 for Group 5 else superscript to indicate number of animals for the mean metrics.
b) n=4 for Group 4 and n=6 for Groups 5 else superscript to indicate number of animals for the mean metrics.
c) NR, not reportable, insufficient data to calculate the terminal metrics.
d) For Group 4 the median t_{max} value is reported due to heavy skewing of the mean by one animal.

Other:

- PCNA analyses in lung sections:

Cell proliferation activity was seen in the lungs of animals across all treatment groups and time points. Therefore, this was considered to be a reactive response to the inhalation procedure and unrelated to treatment with test or control article.

2.6.6.4 Genetic toxicology

4 studies were submitted by the sponsor:

- MKC-PC-2004-0002 (bacterial mutagenicity with T and TI)
- PDC-PC-2000-0006 (chromosome aberration with T)
- PDC-PC-2002-0003 (chromosome aberration with TI)
- PDC-PC-2002-0011- (mouse micronucleus with T)

MKC-PC-2004-002: AMES Technosphere and Technosphere/Insulin [By (b) (4) Study No. AA89DG-DH.503 (b) (4)]

Both Technosphere® (T) and Technosphere®/Insulin (TI) were each tested in the Bacterial Reverse Mutation Assay using *Salmonella typhimurium* tester strains (TA98, TA100, TA1535, and TA1537) and *Escherichia coli* tester strain (WP2 uvrA with or without S9). The first phase (initial toxicity-mutation assay) was used to establish the dose-range for the confirmatory

mutagenicity assay. The second phase (confirmatory mutagenicity assay) was used to evaluate and to confirm the mutagenic potential of T and TI.

Findings: For T, in the initial toxicity-mutation assay, the max dose tested was 5000 µg/plate. There were no positive mutagenic response and precipitate or appreciable toxicity were observed. Based on the findings, the max dose plated in the confirmatory mutagenicity assay was 5000 µg/plate. In the confirmatory mutagenicity assay, there were also no positive mutagenic response and precipitate or appreciable toxicity were observed. For TI, in the initial toxicity-mutation assay, the max dose tested was 5000 µg/plate. No positive mutagenic response was seen, and no precipitate nor appreciable toxicity was seen. Therefore, the max dose plated in the confirmatory mutagenicity assay was 5000 µg/plate. In the confirmatory mutagenicity assay, no positive mutagenic response was observed. Neither precipitate nor appreciable toxicity was observed. All in all, both T and TI were negative in the Bacterial Reverse Mutation Assay.

Test Article Id : Technosphere® formulation
Study Number : AA89DG.503.(b) (4) Experiment No : B1

Average Revertants Per Plate ± Standard Deviation

Liver Microsomes: None

Dose (µg/plate)	TA98	TA100	TA1535	TA1537	WP2 uvrA
Vehicle	36 ± 6	135 ± 30	20 ± 3	7 ± 4	25 ± 10
1.5	36 ± 1	164 ± 8	29 ± 5	8 ± 0	27 ± 0
5.0	32 ± 4	167 ± 22	20 ± 4	6 ± 3	23 ± 2
15	31 ± 4	152 ± 8	22 ± 6	7 ± 1	25 ± 6
50	32 ± 2	147 ± 21	24 ± 4	6 ± 1	23 ± 4
150	33 ± 4	165 ± 24	25 ± 4	5 ± 4	26 ± 7
500	27 ± 6	172 ± 6	28 ± 0	6 ± 3	24 ± 2
1500	31 ± 1	156 ± 17	28 ± 1	6 ± 1	26 ± 1
5000	30 ± 1	162 ± 8	26 ± 2	12 ± 1	24 ± 1
Positive	276 ± 56	661 ± 4	471 ± 117	1041 ± 73	130 ± 2

Liver Microsomes: Rat liver S9

Dose (µg/plate)	TA98	TA100	TA1535	TA1537	WP2 uvrA
Vehicle	31 ± 9	160 ± 36	26 ± 1	4 ± 1	18 ± 0
1.5	43 ± 1	193 ± 31	22 ± 4	11 ± 1	25 ± 1
5.0	45 ± 11	197 ± 8	20 ± 4	11 ± 2	28 ± 2
15	33 ± 3	182 ± 7	28 ± 2	10 ± 2	26 ± 4
50	30 ± 2	187 ± 8	22 ± 3	10 ± 4	21 ± 5
150	34 ± 2	162 ± 4	20 ± 4	13 ± 1	28 ± 4
500	33 ± 3	157 ± 17	31 ± 6	9 ± 2	26 ± 6
1500	33 ± 1	173 ± 8	24 ± 2	9 ± 4	18 ± 1
5000	38 ± 1	152 ± 26	28 ± 3	9 ± 5	25 ± 1
Positive	1499 ± 344	1505 ± 35	248 ± 16	234 ± 66	766 ± 171

Vehicle = Vehicle Control
Positive = Positive Control (50 µL plating aliquot)
Plating aliquot: 100 µL

Test Article Id : Technosphere® formulation
Study Number : AA89DG.503. (b) (4) Experiment No : B2

Average Revertants Per Plate ± Standard Deviation

Liver Microsomes: None

Dose (µg/plate)	TA98	TA100	TA1535	TA1537	WP2 uvrA
Vehicle	17 ± 2	140 ± 13	22 ± 4	6 ± 2	13 ± 2
50	17 ± 2	172 ± 12	23 ± 5	5 ± 3	19 ± 3
150	17 ± 2	154 ± 17	19 ± 3	6 ± 1	15 ± 3
500	14 ± 1	151 ± 12	24 ± 4	9 ± 3	17 ± 1
1500	17 ± 2	149 ± 3	21 ± 3	4 ± 3	16 ± 3
5000	18 ± 3	166 ± 13	22 ± 2	7 ± 4	16 ± 3
Positive	134 ± 25	554 ± 12	328 ± 46	402 ± 19	115 ± 7

Liver Microsomes: Rat liver S9

Dose (µg/plate)	TA98	TA100	TA1535	TA1537	WP2 uvrA
Vehicle	30 ± 8	161 ± 26	19 ± 4	5 ± 2	21 ± 3
50	25 ± 6	173 ± 10	18 ± 3	8 ± 2	20 ± 2
150	27 ± 3	175 ± 12	16 ± 5	7 ± 3	19 ± 4
500	32 ± 3	181 ± 29	20 ± 6	9 ± 2	19 ± 6
1500	24 ± 3	133 ± 13	19 ± 2	5 ± 2	18 ± 3
5000	27 ± 8	159 ± 5	21 ± 5	8 ± 3	21 ± 2
Positive	845 ± 196	928 ± 96	114 ± 6	125 ± 30	454 ± 160

Vehicle = Vehicle Control
Positive = Positive Control (50 µL plating aliquot)
Plating aliquot: 100 µL

Test Article Id : Technosphere®/Insulin formulation
Study Number : AA89DH.503 (b) (4) Experiment No : B1

Average Revertants Per Plate ± Standard Deviation

Liver Microsomes: None

Dose (µg/plate)	TA98	TA100	TA1535	TA1537	WP2 uvrA
Vehicle	19 ± 1	150 ± 6	17 ± 4	5 ± 3	14 ± 2
1.5	23 ± 5	177 ± 11	26 ± 5	6 ± 1	17 ± 7
5.0	18 ± 1	172 ± 11	27 ± 1	12 ± 1	19 ± 1
15	17 ± 1	154 ± 1	22 ± 4	8 ± 2	18 ± 0
50	19 ± 8	138 ± 10	21 ± 3	7 ± 1	20 ± 1
150	19 ± 1	159 ± 25	18 ± 1	10 ± 3	21 ± 6
500	21 ± 4	151 ± 18	20 ± 2	9 ± 1	19 ± 0
1500	15 ± 1	161 ± 14	20 ± 1	10 ± 2	14 ± 4
5000	20 ± 0	160 ± 9	21 ± 0	5 ± 1	17 ± 2
Positive	231 ± 23	780 ± 78	611 ± 2	862 ± 135	99 ± 1

Liver Microsomes: Rat liver S9

Dose (µg/plate)	TA98	TA100	TA1535	TA1537	WP2 uvrA
Vehicle	29 ± 0	183 ± 8	17 ± 0	9 ± 2	25 ± 1
1.5	25 ± 0	174 ± 3	18 ± 2	9 ± 1	21 ± 3
5.0	29 ± 0	180 ± 2	16 ± 1	7 ± 1	16 ± 1
15	31 ± 5	201 ± 27	22 ± 1	5 ± 1	24 ± 1
50	32 ± 3	154 ± 8	21 ± 4	11 ± 1	19 ± 3
150	29 ± 1	174 ± 31	21 ± 0	8 ± 1	23 ± 4
500	31 ± 4	183 ± 8	19 ± 1	8 ± 2	22 ± 1
1500	24 ± 1	167 ± 25	18 ± 1	5 ± 0	20 ± 1
5000	27 ± 1	169 ± 14	21 ± 1	9 ± 4	19 ± 1
Positive	1358 ± 268	1869 ± 38	256 ± 1	278 ± 18	841 ± 121

Vehicle = Vehicle Control
Positive = Positive Control (50 µL plating aliquot)
Plating aliquot: 100 µL

Test Article Id : Technosphere®/Insulin formulation
Study Number : AA89DH.503 (b) (4) Experiment No : B2

Average Revertants Per Plate ± Standard Deviation

Liver Microsomes: None

Dose (µg/plate)	TA98	TA100	TA1535	TA1537	WP2 uvrA
Vehicle	13 ± 2	166 ± 9	18 ± 1	7 ± 2	14 ± 1
50	13 ± 4	157 ± 30	17 ± 6	8 ± 6	18 ± 7
150	13 ± 4	138 ± 16	17 ± 1	8 ± 6	15 ± 4
500	14 ± 2	169 ± 16	23 ± 3	6 ± 3	18 ± 2
1500	13 ± 5	136 ± 4	23 ± 5	5 ± 2	13 ± 2
5000	10 ± 2	159 ± 5	23 ± 2	8 ± 2	15 ± 3
Positive	160 ± 42	553 ± 23	273 ± 15	567 ± 151	136 ± 17

Liver Microsomes: Rat liver S9

Dose (µg/plate)	TA98	TA100	TA1535	TA1537	WP2 uvrA
Vehicle	30 ± 1	181 ± 9	17 ± 1	7 ± 3	14 ± 3
50	28 ± 2	184 ± 9	21 ± 4	7 ± 3	15 ± 3
150	27 ± 3	152 ± 17	13 ± 3	8 ± 5	16 ± 3
500	31 ± 5	183 ± 4	18 ± 3	8 ± 1	20 ± 3
1500	26 ± 6	153 ± 14	20 ± 3	5 ± 3	15 ± 7
5000	30 ± 6	175 ± 5	18 ± 3	6 ± 1	14 ± 2
Positive	524 ± 117	690 ± 135	123 ± 20	75 ± 34	373 ± 134

Vehicle = Vehicle Control
Positive = Positive Control (50 µL plating aliquot)
Plating aliquot: 100 µL

PDC-PC-2000-0006: In Vitro Mammalian Chromosome Aberration Test [By (b) (4) Study No. AA37RS.341 (b) (4) reviewed by Herman Rhee and his review was dated 08/20/02 in DARRTS]

FDKP ((b) (4) was tested in the in vitro mammalian chromosome aberration test using human peripheral lymphocytes (HPBL) with or without S9 activation. This test was used to evaluate the clastogenic potential of FDKP. In the preliminary toxicity assay, the maximum dose was 4500 µg/ml. Based on that assay, the doses ranged from 35 to 4500 µg/ml were chosen for the chromosome aberration assay for non-activated and the S9 activated 4 hr exposure groups, and ranged from 17.5 to 2250 µg/ml for the non-activated 20 hr exposure group. In the chromosome aberration assay, the cells were treated with 4 and 20 hrs in the non-activated test system, and for 4 hrs in the S9 activated test system. All cells were harvested 20 hrs after treatment.

Findings: All cells were harvested at 20 hrs after treatment. Toxicity (mitotic inhibition) was approx. 61% and 55% at the highest dose levels evaluated from chromosome aberrations, 1125 and 140 µg/ml, in the non-activated 4 ht and 20 hr exposure groups, respectively. Toxicity (mitotic inhibition) was approx 34% at the highest dose levels evaluated for chromosome aberrations, 2250 µg/ml, in the S9 activated study. Due to excessive toxicity at 4500 µg/ml (78% reduction in mitotic index relative to the solvent DMSO control group), 2250 µg/ml was selected as high dose which was also the lowest precipitating dose level. In addition, the non-activated and activated 4 hr exposure groups showed that there were no significant increases in structural and numerical chromosome aberrations, relative to the solvent control groups, regardless of dose level. The non-activated 20 hr exposure groups also showed that there were no significant increases in structural and numerical chromosome aberrations when compared to the solvent control group.

Overall, these findings showed that (b) (4) FDPK was negative for the induction of structural and numerical chromosome aberrations in the in vitro mammalian chromosome aberration test using human peripheral lymphocytes.

SUMMARY

Treatment	S9 Activation	Treatment Time	Mean Mitotic Index	Cells Scored	Aberrations Per Cell (Mean +/- SD)	Cells With Aberrations Numerical (%)	Cells With Aberrations Structural (%)
DMSO	-	4	5.4	200	0.000 ±0.000	0.0	0.0
Fumaryl Technosphere™ (b) (4)							
281 ug/mL	-	4	3.7	200	0.000 ±0.000	0.0	0.0
562.5 ug/mL	-	4	3.2	200	0.000 ±0.000	0.0	0.0
1125 ug/mL	-	4	2.1	200	0.000 ±0.000	0.0	0.0
MMC 0.5 ug/mL	-	4	2.4	200	0.110 ±0.329	0.0	10.5**
DMSO	+	4	5.0	200	0.000 ±0.000	0.0	0.0
Fumaryl Technosphere™ (b) (4)							
562.5 ug/mL	+	4	4.3	200	0.000 ±0.000	0.0	0.0
1125 ug/mL	+	4	3.4	200	0.000 ±0.000	0.0	0.0
2250 ug/mL	+	4	3.3	200	0.000 ±0.000	0.0	0.0
CP 20 ug/mL	+	4	1.6	200	0.130 ±0.405	0.0	11.0**
DMSO	-	20	5.8	200	0.000 ±0.000	0.0	0.0
Fumaryl Technosphere™ (b) (4)							
35 ug/mL	-	20	3.6	200	0.000 ±0.000	0.0	0.0
70 ug/mL	-	20	3.9	200	0.000 ±0.000	0.0	0.0
140 ug/mL	-	20	2.6	200	0.005 ±0.071	0.0	0.5
MMC 0.25 ug/mL	-	20	1.9	200	0.095 ±0.396	0.0	7.5**

Treatment: Cells from all treatment conditions were harvested at 20 hours after the initiation of the treatments.
Aberrations per Cell: Severely damaged cells were counted as 10 aberrations.
Percent Aberrant Cells: *, $p \leq 0.05$; **, $p \leq 0.01$; using the Fisher's exact test.

Herman Rhee's review on this study:

SUMMARY: The sponsor performed standard chromosome aberration test with doses of 280, 562, 1125 and 2250 μg/ml of Fumaryl Technosphere. The procedures of experiments, criteria for positive results, and analysis methods were acceptable. Fumaryl Technosphere was not mutagenic in human peripheral blood lymphocytes.

PDC-PC-2002-0003: In Vitro Mammalian Chromosome Aberration Test [By (b) (4) Study No. AA60RE.341 (b) (4)

The Technosphere®/Insulin (TI) was tested in the in vitro mammalian chromosome aberration test using human peripheral lymphocytes (HPBL) with or without S9 activation. This test was used to evaluate the clastogenic potential of TI. In the preliminary toxicity assay, the maximum dose was 5000 µg/ml. Based on that assay, the doses ranged from 625 to 5000 µg/ml were chosen for the chromosome aberration assay for non-activated 4 hr and 20hr exposure groups, and ranged from 313 to 5000 µg/ml for the activated 4 hr exposure group. In the chromosome aberration assay, the cells were treated with 4 and 20 hrs in the non-activated test system, and for 4 hrs in the S9 activated test system. All cells were harvested 20 hrs after treatment.

Findings: There was no significant increase in the % of cells with structural aberrations in the TI-treated groups when compared to the solvent control at any dose level. Based on the findings of this study, TI was considered to be negative for the induction of structural and numerical chromosome aberrations in the in vitro mammalian chromosome aberration test using human peripheral lymphocytes.

Treatment (µg/mL)	S9 Activation	Treatment Time	Mean Mitotic Index	Cells Scored	Aberrations Per Cell (Mean +/- SD)	Cells With Aberrations Numerical (%)	Cells With Aberrations Structural (%)
PBS	-	4	9.2	200	0.000 ±0.000	0.0	0.0
Technosphere®/ Insulin							
1250	-	4	7.7	200	0.000 ±0.000	0.0	0.0
2500	-	4	8.2	200	0.000 ±0.000	0.0	0.0
5000	-	4	7.7	200	0.000 ±0.000	0.0	0.0
MMC 0.6	-	4	1.1	100	0.240 ±0.452	0.0‡	23.0**
PBS	+	4	8.1	200	0.000 ±0.000	0.0	0.0
Technosphere®/Insulin							
1250	+	4	7.4	200	0.000 ±0.000	0.0	0.0
2500	+	4	7.6	200	0.000 ±0.000	0.0	0.0
5000	+	4	6.2	200	0.005 ±0.071	0.0	0.5
CP, 20	+	4	0.9	200	0.080 ±0.272	0.5	8.0**
PBS	-	20	8.0	200	0.005 ±0.071	0.0	0.5
Technosphere®/ Insulin							
1250	-	20	7.9	200	0.005 ±0.071	0.0	0.5
2500	-	20	8.6	200	0.005 ±0.071	0.0	0.5
5000	-	20	8.8	200	0.000 ±0.000	0.0	0.0
MMC 0.3	-	20	3.6	100	0.300 ±0.577	0.0‡	25.0**

Treatment: Cells from all treatment conditions were harvested at 20 hours after the initiation of the treatments.
Aberrations per Cell: Severely damaged cells were counted as 10 aberrations.
Percent Aberrant Cells: *, $p \leq 0.05$; **, $p \leq 0.01$; using the Fisher's exact test.
‡ Numerical aberrations are out of 200 cells scored.

PDC-PC-2002-0011: Mammalian Erythrocyte Micronucleus Test [By (b) (4) Study No. AA68EH.123 (b) (4)

The Technosphere™ (T) was tested in the mouse micronucleus assay. During the first phase (dose range-finding) was designed to assess toxicity of T and set dose levels for the definitive study. The second phase (the definitive micronucleus study) was designed to evaluate the potential of T to increase in the incidence of micronucleated polychromatic erythrocytes in bone marrow of male and female ICR mice. This phase was consisted of a pilot toxicity study followed by a toxicity study. In the definitive phase, cyclophosphamide monohydrate (CP) was used as the positive control article at a dose of 50 mg/kg. In these two phases, test (T) and control (saline and CP) were administered at a dose volume of 20 ml/kg by a single IP injection.

Findings in the pilot study: 2 male mice each were exposed to T at 1, 10, 100, or 1000 mg/kg whereas 5 male and 5 female mice were exposed to T at 2000 mg/kg. Clinical signs including lethargy and piloerection were observed in males at 1000 mg/kg and in males and females at 2000 mg/kg. Finding In the toxicity study: 5 male and 5 female mice each were exposed to T at a dose of 1400, 1600, or 1800 mg/kg. Mortality was observed in 1/5 male mice at 1800 mg/kg. Clinical signs included ataxia and lethargy in males and females at 1400, 1600, and 1800 mg/kg and piloerection in males at all dose groups. The study results suggested the difference in toxicity between male and female mice, the high dose for the micronucleus test was therefore set at 1600 mg/kg for male mice and at 1800 mg/kg for female mice (as estimated maximum tolerated doses).

Finding in definitive study: 5 male and 5 female mice each were exposed to T at 400, 800, 1600 mg/kg (male mice) or 450, 900, and 1800 mg/kg (female mice). These animals were euthanized 24 hrs after treatment. Animals in negative control or T at 1600 mg/kg (males)/1800 mg/kg/ (females) were euthanized 48 hrs after treatment. Morality was observed in 1/15 female mice at 1800 mg/kg. Clinical signs included piloerection in males at 1600 mg/kg and in females at 1800 mg/kg. All other animals treated with the T or controls appeared normal following treatment. Bone marrow cells (polychromatic erythrocytes, PCEs) collected 24 or 48 hrs after treatment were examined microscopically for the presence of micronuclei (MPCEs). Reduction (up to 16%) in the ratio of polychromatic erythrocytes to total erythrocytes was observed in some T-treated groups when compared to the respective VH controls. There was no significant increase in micronucleated polychromatic erythrocytes in T-treated groups when compared to the respective VH controls in male or female mice at 24 or 48 hrs after treatment.

Overall, this study showed that a single IP injection of T at doses up to 1600 mg/kg (male mice) and 1800 mg/kg (female mice) did not induce a significant increase in micronucleated polychromatic erythrocytes in bone marrow. Therefore, Technosphere™ was considered negative in the mouse micronucleus assay.

Table 6: Summary of Bone Marrow Micronucleus Analysis Following Dose Administration of Technosphere™ in ICR Mice

Treatment (20 mL/kg)	Sex	Time (hr)	Number of Mice	PCE/Total Erythrocytes (Mean +/- SD)	Change from Control (%)	Micronucleated Polychromatic Erythrocytes: Number per 1000 PCEs (Mean +/- SD)	Micronucleated Polychromatic Erythrocytes: Number per PCEs Scored[1]
Saline	M	24	5	0.529 ± 0.03	---	0.6 ± 0.55	6 / 10000
	F	24	5	0.477 ± 0.06	---	0.5 ± 0.00	5 / 10000
Technosphere™							
400 mg/kg	M	24	5	0.484 ± 0.06	-9	0.8 ± 0.45	8 / 10000
450 mg/kg	F	24	5	0.480 ± 0.05	1	0.8 ± 0.67	8 / 10000
800 mg/kg	M	24	5	0.467 ± 0.04	-12	0.5 ± 0.35	5 / 10000
900 mg/kg	F	24	5	0.470 ± 0.04	-1	1.0 ± 1.12	10 / 10000
1600 mg/kg	M	24	5	0.442 ± 0.08	-16	0.4 ± 0.22	4 / 10000
1800 mg/kg	F	24	5	0.460 ± 0.05	-4	0.7 ± 0.76	7 / 10000
CP							
50 mg/kg	M	24	5	0.459 ± 0.08	-13	30.4 ± 4.63	*304 / 10000
	F	24	5	0.361 ± 0.07	-24	27.6 ± 5.85	*276 / 10000
Saline	M	48	5	0.504 ± 0.05	---	0.6 ± 0.22	6 / 10000
	F	48	5	0.517 ± 0.06	---	0.5 ± 0.35	5 / 10000
Technosphere™							
1600 mg/kg	M	48	5	0.507 ± 0.08	1	0.4 ± 0.42	4 / 10000
1800 mg/kg	F	48	5	0.506 ± 0.01	-2	0.4 ± 0.42	4 / 10000

[1]*Statistically significant, $p \leq 0.05$ (Kastenbaum-Bowman Tables)

2.6.6.5 Carcinogenicity

Pivotal Study #1

Study title: 104-Week Inhalation Carcinogenicity Study in Rats with Technosphere Insulin in Rats

Key study findings:

- Up to 1.23 mg/kg/day Technosphere® Insulin (TI) and 46 mg/kg/day Technosphere® particles (T) were well-tolerated in rats.
- Circulating insulin concentrations were measured in TI treatment groups. Variability prevented accurate TK analysis.
- Serum glucose concentrations following TI treatment generally decreased.
- FDKP exposure increased with increasing dose in all treated groups.
- There were no adverse events observed, and no indication of carcinogenic potential of either T or TI in males and females.
- The numbers of animal with masses were comparable across all groups. There were approx. 14-30% (males) and 64-76% (females) of masses present at the necropsy. The total number of masses were consistently higher in females than in males due to the presence of palpable mammary masses and was not an indication of any sex-related effects.
- Neoplastic histopathological findings showed that 1) adrenal cortical carcinoma was seen in 3/60 high-dose TI females, 2) malignant astrocytoma was observed in 4/60 low-dose T males, 4/60 high-dose T males, and 3/60 low-dose TI males, 3) malignant schwannoma in the nasal

cavity in 3/60 low-dose T males, and 4) fibroma in the skin/subcutis in 3/60 low-dose TI males and 2/60 low-dose TI females. The sponsor considered those findings as incidental.

- Non-neoplastic histopathological findings showed that lesions were observed in the nasal cavity in high-dose T males and females, which consisted of a higher incidence and severity of goblet cell hyperplasia of respiratory epithelium and accumulations of eosinophilic droplets in the olfactory and respiratory epithelia. These findings were considered non-specific protective or adaptive responses to chronic inhalation of T. These findings were seen with comparable frequency and severity in air control and TI treated groups.
- No differences were observed in the cell proliferation activity (PCNA) in the lung tissues, such as alveolar and bronchiolar cells, across all groups (0.5-0.6/1000 vs. 0/1000 between Group 1 vs. Groups 2-5).

Adequacy of the carcinogenicity study and appropriateness of the test model: Yes

Evaluation of tumor findings:
Study no.: MKC-PC-2004-0001
Volume #, and page #: eEDR, module 4.2.3.4.1
Conducting laboratory and location: (b) (4)
Date of study initiation: August 16th 2004
GLP compliance: Yes
QA report: yes (x), no ( )
Drug, lot #, and % purity:

- Technosphere® with ~ 98-99% purity: D03502008, D03502009, D035U03011, D035U03012, D035U04001 though D035U04009, D035U05001, D035U05004, D035UA04002, D232060001, D232060002,PM5069A, and PM5283A
- Technosphere® insulin with 11.3-12.8% insulin: D043RD04001 through D043RD04003, D043RD04005 through D043RD04008, and D043RD03001

CAC concurrence: Yes

Methods

Doses: See table below for achieved dosages.

Group	Group Designation	Sex	Estimated Mean Achieved Dose (mg/kg/day)	
			Technosphere particles or TI	Insulin
1	Air Control	M	-	-
		F	-	-
2	Technosphere® Control Low Dose	M	1.1041	-
		F	0.6784	-
3	Technosphere® Control High Dose	M	41	-
		F	46	-
4	Technosphere®/Insulin Low Dose	M	0.6572	0.0787
		F	0.4413	0.0529
5	Technosphere®/Insulin High Dose	M	1.2313	0.1457
		F	0.7608	0.0900

[a] Based on an estimated body weight of 0.250 kg

Abbreviations: F = Female; M = Male; TI = Technosphere® Insulin

- Targeted exposure concentrations and dose levels:

Group	Group Designation	Targeted Dose Level[1] (mg/kg/day)						Targeted Aerosol Concentration (mg/L)		
		Technosphere®		Insulin (IU)		Total		Technosphere®	Insulin	Total
		♂	♀	♂	♀	♂	♀			
1	Air Control	-	-	-	-	-	-	-	-	-
2	Technosphere® Control Low Dose	1.3259	0.7301	0	0	1.33	0.73	0.0419	0	0.0419
3	Technosphere® Control High Dose	50	50	0	0	50	50	1.58	0	1.58
4	Technosphere® Insulin Low Dose	0.7381	0.4429	0.0948 (2.5)	0.0569 (1.5)	0.83	0.50	0.0233	0.0030	0.0263
5	Technosphere® Insulin High Dose	1.3259	0.7301	0.1706 (4.5)	0.0948 (2.5)	1.50	0.82	0.0419	0.0054	0.0473

1 Based on an estimated body weight of 0.250 kg using the formula presented below:

- Duration of daily exposure:

Group Number	Group Designation	Exposure Duration (minutes)	
		♂	♀
1	Air Control	60	33
2	Technosphere® Control Low Dose	60	33
3	Technosphere® Control High Dose	60	60
4	Technosphere® Insulin Low Dose	60	36
5	Technosphere® Insulin High Dose	60	33

Basis of dose selection: FDKP: 25X AUC, insulin: MTD due to hypoglycemia

- Group 5 (high dose) was selected on minimal toxicity observed during a 13 week inhalation rat study (an interim group of an ongoing 26 week inhalation study). The high dose in that study was 5 IU/kg/day TI for males and 3 IU/kg/day TI for females.
- Group 4 (low dose) was selected based on a multiple of the anticipated human therapeutic dose.
- Group 2 (VH low dose) was exposed to VH (Technosphere® particles) at a mass concentration equal to that of Group 5.
- Croup 3 (VH high dose) was exposed to aerosols of VH 25X that of the anticipated human therapeutic does.

Species/strain: Spraque-Dawley rat [Crl:CD (SD) IGS BR]
Number/sex/group (main study): Main study: 60/sex/group
Route, formulation, volume: Inhalation as aerosolized powders

Test Article:	Identity:	Technosphere® Insulin
	Description:	White powder
	Purity:	11.4% insulin
	Storage Conditions:	Freezer (-10°C to -30°C)
	Handling Precautions:	Standard laboratory procedures during routine handling and the wearing of a cartridge respirator during dosing.
	Supplier:	MannKind BioPharmaceuticals 1 Casper Street, Danbury, CT 06810, USA
Control/Vehicle Article:	Identity:	Technosphere®
	Description:	White powder
	Storage Conditions:	Room temperature
	Handling Precautions:	Standard laboratory procedures during routine handling and the wearing of a cartridge respirator during dosing.
	Supplier:	MannKind BioPharmaceuticals One Casper Street, Danbury, CT 06810, USA
Air Control:	Description:	Medical Grade Air (NQ 5710-500/2000)
	Supplied By:	Kaeser SM-11 Air Compressor

Frequency of dosing: Once daily for minimum of 104 weeks (dosing phase)
Satellite groups used for toxicokinetics or special groups: TK study: 6/sex/group
Age: 6 weeks old
Animal housing: Individually housed after randomization
Restriction paradigm for dietary restriction studies: 5 (male) and 4 (female) food pellets per day for longer survival
Drug stability/homogeneity: The homogeneity of the exposure test/control aerosols was confirmed by collecting aerosol samples from the top, middle, and bottom of each exposure chamber. The concentration homogeneity of the aerosols within the exposure systems was acceptable if the CV and mean aerosol concentration at different ports was less than 20%.

TEST ATMOSPHERE HOMOGENEITY DATA

Sample Location	Test Atmosphere Concentration (mg Technosphere/L)	
	Group 2	Group 3
Top	0.0364	1.7852
Middle	0.0384	1.8027
Bottom	0.0371	2.0318
Mean	0.0373	1.8732
SD	0.00101	0.13760
CV (%)	2.7	7.3

Sample Location	Test Atmosphere Concentration (mg Insulin/L)	
	Group 4	Group 5
Top	0.0029	0.0042
Middle	0.0031	0.0050
Bottom	0.0030	0.0057
Mean	0.0030	0.0050
SD	0.00010	0.00075
CV (%)	3.3	15.1

Dual controls employed: Yes. Main study: vehicle control (T) and air control (air)
Interim sacrifices: Main-study animals were euthanized upon the completion of the 104-week treatment period. The necropsies were conducted over a 3-week period (Weeks 105-107) due to the larger number of animals.
Deviations from original study protocol: Minor deviations from the protocol, typically procedural.

Observation times
Mortality: Twice daily during all phases of the study
Clinical signs: Once in the morning (pre-dose) and before the end of the working day, following the end of dosing
Body weights: Day 1 (before dosing), weekly until Week 26, and every 4 weeks thereafter
Food consumption: A standard weight of food (pellets) was offered to each rate per day. To weight a sample of feed pellets once a month to confirm that the variability of this weight remained within acceptable limits throughout the study.
Histopathology: Upon completion of the 104-Week treatment period.
Peer review: yes (x), no ( )

ORGANS/TISSUES	Retained (•)	Examined (€)
Adrenals	•	€
Animal identification	•	
Aorta (thoracic)	•	€
Blood	•	€
Bone marrow smears (3)	•	
Brain	•	€
Cecum	•	€
Colon	•	€
Epididymides	•d	€
Esophagus	•	€
Eyes	•a	€
Femur + marrow	•	€
Heart	•	€
Kidneys	•	€
Liver (2 lobes)	•	€
Lungs + bronchi (2 lobes)	•bc	€
Lymph node, mandibular	•	€
Lymph node, mesenteric	•	€
Mammary gland (inguinal)	•	€
Optic nerves	•a	€
Ovaries	•	€
Pancreas	•	€
Pituitary	•	€
Prostate	•	€
Rectum	•	€
Salivary gland (mandibular)	•	€
Sciatic nerve	•	€
Seminal vesicles	•	€
Skeletal muscle	•	€
Skin + subcutis (inguinal)	•	€

a	Davidson's fluid (euthanised animals only)
b	Lungs were infused with 10% neutral buffered formalin (euthanised animal only) at a hydrostatic pressure of 30 cm of water. The lung was then tied off and placed in a jar of fixative.
c	Lungs weighed with trachea
d	Bouin's fluid (euthanised animals only)

ORGANS/TISSUES	Retained (•)	Examined (€)
Small intestine, duodenum	•	€
Small intestine, jejunum	•	€
Small intestine, ileum	•	€
Spinal cord (cervical)	•	€
Spleen	•	€
Sternum + marrow	•	€
Stomach	•	€
Testes	•d	€
Thymus	•	€
Thyroid lobes + parathyroids	•	€
Tongue	•	€
Trachea	•	€
Urinary bladder	•	€
Uterus	•	€
Vagina	•	€
Abnormal findings	•	€
Additional Tissues presented below		
Carina	•	€
Larynx (Transverse section)	•	€
Lymph node, bronchial	•	€
Nasal cavity (4 sections)	•	€
Nasopharynx	•	€

Notes

Parathyroids, mammary gland and optic nerves were only examined histologically if present in routine sections

- Peer review was conducted on-site at (b) (4) and consisted of:

The peer review was conducted on-site at (b) (4) and consisted of:

- Full tissue list for 10% of animals from Air Control (Group 1), Technosphere® Control Low Dose (Group 2), Technosphere® Control High Dose (Group 3), and Technosphere® / Insulin High Dose (Group 5)
- All tumors from Air Control (Group 1), Technosphere® Control Low Dose (Group 2), Technosphere® Control High Dose (Group 3), and Technosphere® / Insulin High Dose (Group 5)
- Any tissues in the remaining animals that warranted investigation, as identified by the pathologist

Toxicokinetics: Samples were collected from 3 rats/sex/group during Weeks 52, 78, and 104 at pre-dose and Tmax (10 min post end of exposure).

Results

Mortality: There were no differences in the number of mortalities across the groups. The cumulative survival at the end of the study was 65-73% for males and 62-72% for females. For

survival analysis the trend tests were not significant. The pairwise comparisons of the control groups were also not statistically significant.

Group Number	Group Designation	Sex	Mean Survival (Days)	Cumulative Survival (%)	
				Week 53	Week 104
1	Air Control	♂	674	95	68
		♀	679	93	72
2	Technosphere® Control Low Dose	♂	699	100	63
		♀	681	97	63
3	Technosphere® Control High Dose	♂	693	97	72
		♀	681	98	63
4	Technosphere® Insulin Low Dose	♂	690	98	68
		♀	672	97	62
5	Technosphere® Insulin High Dose	♂	692	98	73
		♀	689	98	67

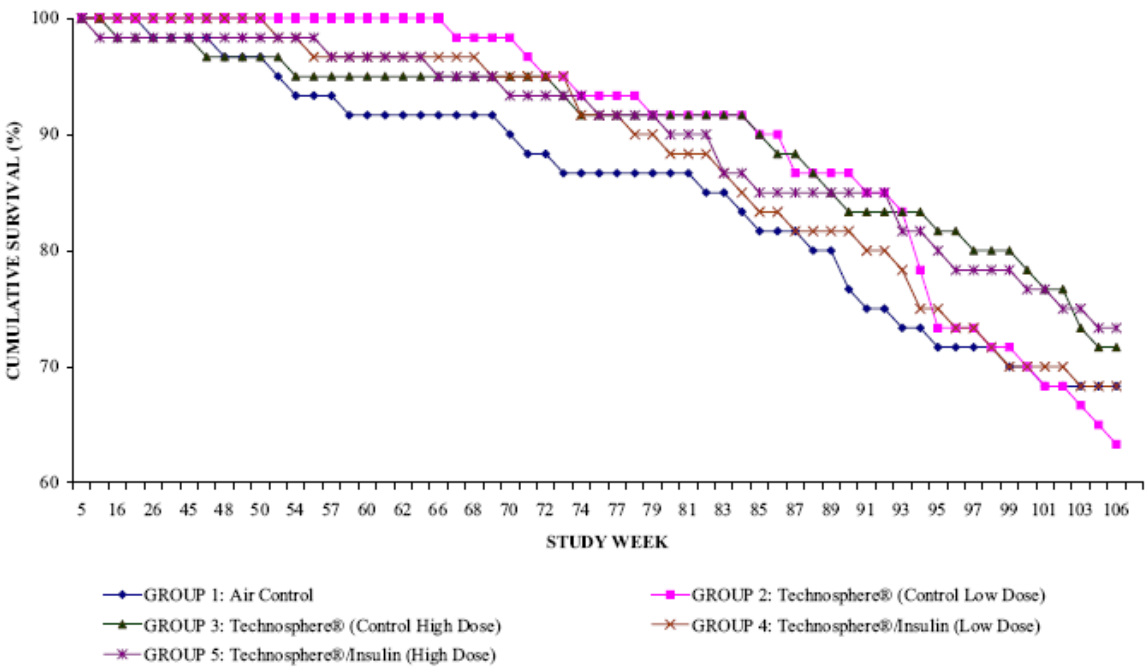
In addition, there were 5 deaths over the duration of the study which was unrelated to treatment. These deaths occurred during the exposure periods and were due to the stress associated with the restraint and handling.

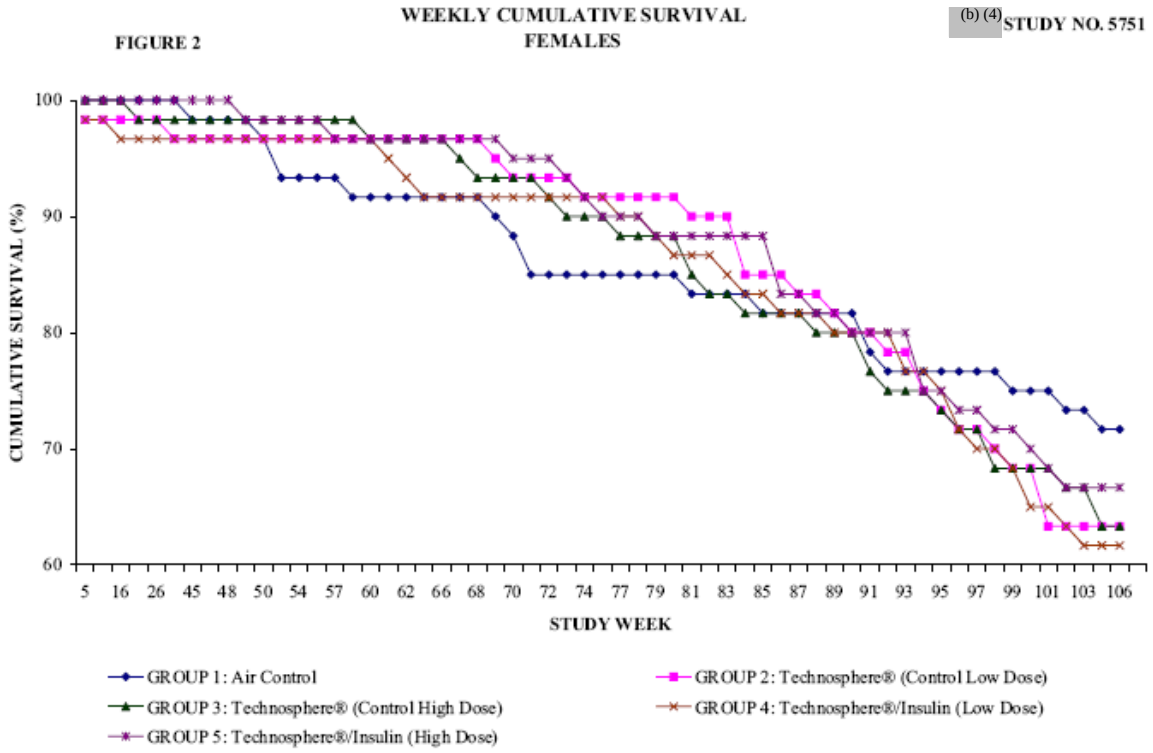
Group Number	Group Designation	Sex	Animal No.	Week of Death	Reason for Death
3	Technosphere® Control High Dose	♂	3031C	16	Procedure-related death due to tube restraint
		♀	3526C	81	Procedure-related death due to tube restraint
4	Technosphere® Insulin Low Dose	♀	4520D	83	Procedure-related death due to tube restraint
			4550D	5	Procedure-related death due to tube restraint
5	Technosphere® Insulin High Dose	♂	5004E	66	Procedure-related death due to tube restraint

FIGURE 1

WEEKLY CUMULATIVE SURVIVAL
MALES

(b) (4) STUDY NO. 5751





Clinical signs: There were no clinical signs related to treatment with T or TI. The animals seemed to be healthy over the duration of the study. Fur thinning, ungroomed/dull/matted/stained fur, and changes in feces were observed throughout the study. Since these signs were noted with a similar incidence across the treated and control groups, they were not considered test article-related. In addition, foot lesions (ulcerative pododermatitis) were observed in a number of animals across all groups, which were due to long-term housing. A course of topical application of iodine was used to present infection whereas Isoblocks® were placed to allow relief from the cage floor until the wounds were healed in these animals.

Group Number	Group Designation	Sex	Animal No.	Week of Death	Reason for Death
1	Air Control	♂	1048A	48	Killed *in extremis* due to a foot injury
		♀	1511A	45	Killed *in extremis* due to infection following the rupture of a mass
		♀	1549A	85	Killed *in extremis* due to a uterine prolapse
		♀	1556A	71	Killed *in extremis* due to infection following the rupture of a mass
2	Technosphere® Control Low Dose	♂	2007B	67	Killed *in extremis* due to a limb injury
		♂	2043B	91	Killed *in extremis* due to severity of ulcerative pododermatitis
		♀	2502B	74	Killed *in extremis* due to severity of restricted mobility
		♀	2510B	69	Killed *in extremis* due to excessive blood loss following the rupture of a mass
		♀	2534B	89	Killed *in extremis* due to infection following the rupture of a mass
		♀	2543B	44	Killed *in extremis* due to severity of restricted mobility
3	Technosphere® Control High Dose	♂	3028C	89	Killed *in extremis* due to severity of ulcerative pododermatitis
		♂	3050C	86	Killed *in extremis* due to excessive blood loss following the rupture of a mass
		♀	3502C	104	Killed *in extremis* due to excessive blood loss following an injury
4	Technosphere® Insulin Low Dose	♂	4046D	74	Killed *in extremis* due to a limb injury
		♀	4512D	86	Unrelated death due to necrotic atrophy following a tail injury
		♀	4532D	102	Killed *in extremis* due to infection following the rupture of a mass
		♀	4540D	97	Killed *in extremis* due to infection following the rupture of a mass
		♀	4542D	89	Unrelated death due to necrotic atrophy following a tail injury and rupture of a mass
		♀	4552D	96	Killed *in extremis* due to a vaginal prolapse

Body weights: There were no significant treatment-related effects on body weight in T- or TI-treated animals. Body weights were comparable across all groups, with only difference of 18 g (for males) and 31 g (for females) across groups at the end of the treatment period.

FIGURE 3 GROUP MEAN BODY WEIGHTS MALES - TREATMENT PERIOD (b) (4) STUDY NO. 5751

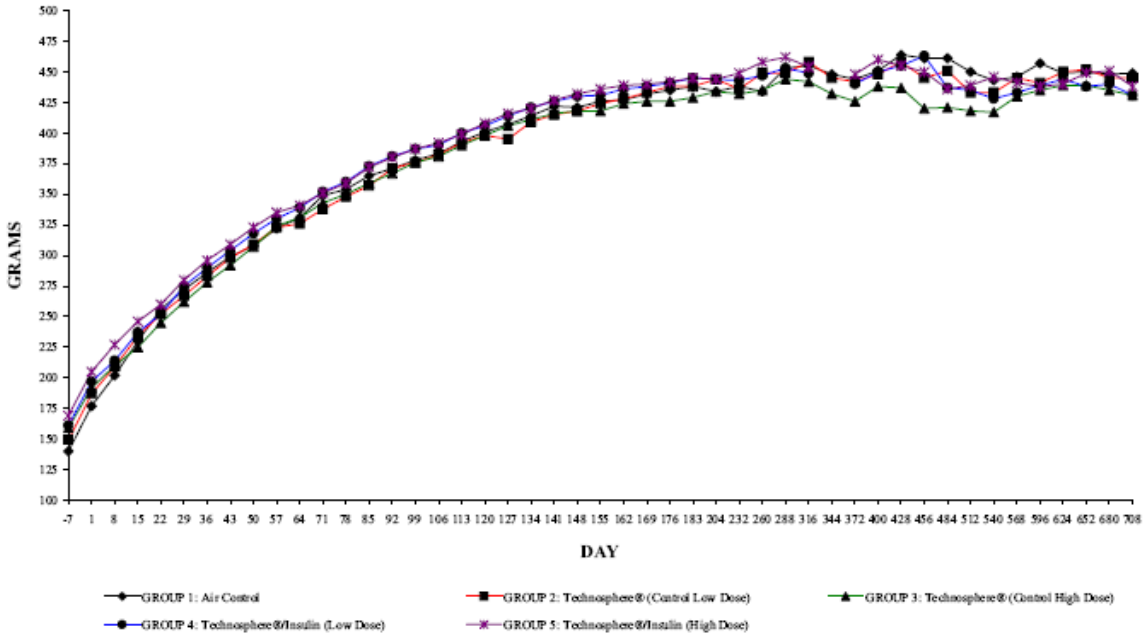
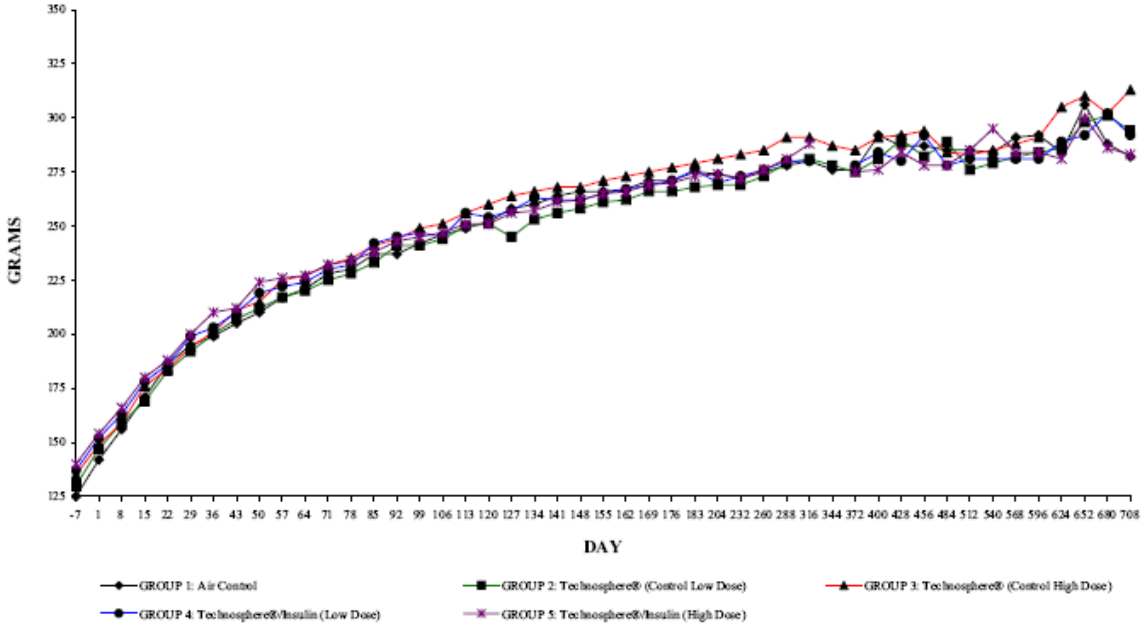


FIGURE 4 GROUP MEAN BODY WEIGHTS FEMALES - TREATMENT PERIOD (b) (4) STUDY NO. 5751



Food consumption: It was not measured in this study.

Ophthalmoscopy: There were no ocular changes caused by treatment with T or TI.

Hematology: No changes in hematological parameters were observed in animals treated with T or TI. Monocyte counts appeared higher than control values for males in Group 5 (high T) at Week 54 and Group 4 (low TI) at the end of the study. The sponsor justified such differences from control group values were due to values obtained from a single animal.

TABLE 2 HEMATOLOGY SUMMARY OF MEANS WEEK 54 (b) (4) STUDY NO. 5751

GROUP 1: Air Control
GROUP 2: Technosphere® (Control Low Dose)
GROUP 3: Technosphere® (Control High Dose)
GROUP 4: Technosphere®/Insulin (Low Dose)
GROUP 5: Technosphere®/Insulin (High Dose)

GROUP NO.		RBC $x10^{12}/L$	PLT $x10^9/L$	WBC $x10^9/L$	NEUT $x10^9/L$	LYMPH $x10^9/L$	MONO $x10^9/L$	EOS $x10^9/L$	BASO $x10^9/L$	LUC $x10^9/L$
	MALES									
1	MEAN	9.38	1195	4.85	1.09	3.48	0.13	0.09	0.05	0.02
	SD	0.238	221.3	1.130	0.409	0.700	0.022	0.045	0.011	0.015
	N	5	5	5	5	5	5	5	5	5
2	MEAN	8.72	1051	5.50	1.53	3.60	0.21	0.10	0.04	0.03
	SD	0.342	194.0	0.828	0.475	0.726	0.076	0.105	0.013	0.012
	N	6	6	6	6	6	6	6	6	6
3	MEAN	8.80	1155	6.11	2.04	3.60	0.29	0.11	0.05	0.03
	SD	0.375	135.4	0.925	0.530	0.720	0.079	0.038	0.009	0.023
	N	6	6	6	6	6	6	6	6	6
4	MEAN	8.99	1111	6.77	1.69	4.66	0.21	0.11	0.06	0.04
	SD	0.543	135.8	1.988	0.609	1.410	0.093	0.039	0.031	0.021
	N	6	6	6	6	6	6	6	6	6
5	MEAN	8.88	1021	6.13	1.29	4.29	0.40	0.09	0.05	0.02
	SD	0.521	95.8	1.955	0.940	1.428	0.481	0.066	0.017	0.012
	N	6	6	6	6	6	6	6	6	6
	FEMALES									
1	MEAN	7.76	1264	4.80	1.35	3.10	0.21	0.08	0.04	0.03
	SD	0.254	235.9	1.343	0.859	0.700	0.063	0.015	0.015	0.018
	N	6	6	6	6	6	6	6	6	6
2	MEAN	7.77	1060	5.80	1.13	4.23	0.22	0.12	0.05	0.04
	SD	0.286	119.0	1.309	0.623	1.006	0.076	0.031	0.019	0.017
	N	6	6	6	6	6	6	6	6	6
3	MEAN	8.06	1096	6.37	1.37	4.51	0.21	0.18	0.06	0.04
	SD	0.170	170.5	3.462	1.091	2.152	0.090	0.141	0.033	0.035
	N	5	5	5	5	5	5	5	5	5
4	MEAN	7.78	1163	4.14	0.90	2.91	0.18	0.09	0.04	0.02
	SD	0.845	106.2	0.691	0.293	0.364	0.053	0.027	0.008	0.006
	N	6	6	6	6	6	6	6	6	6
5	MEAN	7.82	1158	4.86	0.94	3.57	0.18	0.11	0.04	0.03
	SD	0.118	72.6	0.559	0.054	0.601	0.085	0.035	0.013	0.016
	N	5	5	5	5	5	5	5	5	5

TABLE 2 HEMATOLOGY SUMMARY OF MEANS END OF STUDY (b) (4) STUDY NO. 5751

GROUP 1: Air Control
GROUP 2: Technosphere® (Control Low Dose)
GROUP 3: Technosphere® (Control High Dose)
GROUP 4: Technosphere®/Insulin (Low Dose)
GROUP 5: Technosphere®/Insulin (High Dose)

GROUP NO.	RBC $x10^{12}/L$	PLT $x10^9/L$	WBC $x10^9/L$	NEUT $x10^9/L$	LYMPH $x10^9/L$	MONO $x10^9/L$	EOS $x10^9/L$	BASO $x10^9/L$	LUC $x10^9/L$

	MALES									
1	MEAN	7.43	1333	7.24	4.35	2.48	0.28	0.09	0.01	0.03
	SD	1.003	411.2	3.924	3.144	1.110	0.187	0.112	0.007	0.021
	N	10	10	10	10	10	10	10	10	10
2	MEAN	7.01	1144	6.30	3.17	2.79	0.23	0.08	0.01	0.02
	SD	1.449	302.1	1.925	1.482	0.604	0.096	0.079	0.006	0.012
	N	10	10	10	10	10	10	10	10	10
3	MEAN	6.99	1050	6.21	3.24	2.67	0.19	0.07	0.02	0.02
	SD	1.440	411.8	1.044	0.831	0.737	0.061	0.044	0.006	0.010
	N	10	10	10	10	10	10	10	10	10
4	MEAN	7.04	1195	5.29	2.72	1.90	0.53	0.04	0.01	0.09
	SD	1.382	296.0	1.868	2.194	0.791	0.626	0.028	0.006	0.154
	N	10	10	10	10	10	10	10	10	10
5	MEAN	7.29	1271	5.99	2.92	2.78	0.21	0.05	0.01	0.01
	SD	1.438	340.4	1.300	1.012	0.659	0.068	0.024	0.008	0.009
	N	10	10	10	10	10	10	10	10	10
	FEMALES									
1	MEAN	7.35	960	5.28	2.78	2.16	0.23	0.08	0.01	0.02
	SD	0.436	149.1	2.311	1.704	0.621	0.118	0.038	0.007	0.007
	N	9	9	9	9	9	9	9	9	9
2	MEAN	7.27	1071	4.17	1.92	1.99	0.18	0.07	0.01	0.01
	SD	0.796	108.2	1.528	1.172	0.803	0.088	0.045	0.005	0.009
	N	10	10	10	10	10	10	10	10	10
3	MEAN	7.28	1057	3.65	1.60	1.80	0.16	0.07	0.01	0.01
	SD	0.833	434.5	1.004	0.673	0.569	0.071	0.071	0.007	0.006
	N	10	10	10	10	10	10	10	10	10
4	MEAN	7.34	953	4.07	1.79	2.06	0.14	0.07	0.01	0.01
	SD	0.894	172.7	1.466	0.869	0.960	0.051	0.074	0.006	0.007
	N	10	10	10	10	10	10	10	10	10
5	MEAN	7.15	974	3.44	1.56	1.67	0.13	0.06	0.01	0.01
	SD	0.388	201.6	2.161	1.077	1.139	0.106	0.100	0.007	0.009
	N	10	10	10	10	10	10	10	10	10

Glucose: A definitive assessment of an effect was difficult due to the high variability of the data. However, post-dose glucose values were generally decreased in one or both TI groups (Groups 4 and 5) at Weeks 52, 78, and at the end of study, when compared to the corresponding pre-dose values. In contrast, glucose values in all other groups were increased post exposure, likely due to the stress associated with the dosing and/or restraint procedures.

TABLE 3 GLUCOSE DETERMINATION (mmol/L) WEEK 52 (b) (4) STUDY NO. 5751

GROUP 1: Air Control
GROUP 2: Technosphere® (Control Low Dose)
GROUP 3: Technosphere® (Control High Dose)
GROUP 4: Technosphere®/Insulin (Low Dose)
GROUP 5: Technosphere®/Insulin (High Dose)

GROUP NO.		Prior to Dosing	10 Minutes Post Dose		Prior to Dosing	10 Minutes Post Dose
	MALES			FEMALES		
1	MEAN	5.4	6.9	MEAN	5.8	7.4
	SD	0.42	0.45	SD	0.38	0.35
	N	2	3	N	3	3
2	MEAN	5.9	8.1	MEAN	6.0	7.5
	SD	0.21	0.40	SD	0.36	1.06
	N	3	3	N	3	3
3	MEAN	5.7	6.4&	MEAN	5.7	8.1
	SD	0.66	0.52	SD	0.21	0.07
	N	3	3	N	2	2
4	MEAN	5.2	4.2*&	MEAN	5.6	5.1*&
	SD	0.56	0.61	SD	0.35	0.38
	N	3	3	N	3	3
5	MEAN	5.9	6.9	MEAN	6.0	6.7
	SD	0.53	0.75	SD	0.26	1.32
	N	3	3	N	3	3

* = Statistically significant when compared with Group 1 at $p \leq 0.05$ (Dunnett's)
& = Statistically significant when compared with Group 2 at $p \leq 0.05$ (Dunnett's)

TABLE 3 GLUCOSE DETERMINATION (mmol/L) WEEK 78 (b) (4) STUDY NO. 5751

GROUP 1: Air Control
GROUP 2: Technosphere® (Control Low Dose)
GROUP 3: Technosphere® (Control High Dose)
GROUP 4: Technosphere®/Insulin (Low Dose)
GROUP 5: Technosphere®/Insulin (High Dose)

GROUP NO.		Prior to Dosing	10 Minutes Post Dose		Prior to Dosing	10 Minutes Post Dose
	MALES			FEMALES		
1	MEAN	4.5	8.4	MEAN	5.3	5.6
	SD	0.14	0.20	SD	0.30	1.59
	N	2	3	N	3	3
2	MEAN	5.4	7.3	MEAN	6.0	9.6*
	SD	1.05	1.22	SD	0.12	1.46
	N	3	3	N	3	3
3	MEAN	6.1	8.6	MEAN	6.2	9.1
	SD	0.47	0.90	SD	0.28	0.64
	N	3	3	N	2	2
4	MEAN	5.6	8.1	MEAN	5.6	6.6&
	SD	0.12	1.59	SD	0.26	1.37
	N	3	3	N	3	3
5	MEAN	5.1	4.5*&	MEAN	6.0	5.3&
	SD	0.14	0.38	SD	0.71	0.21
	N	2	3	N	2	2

* = Statistically significant when compared with Group 1 at $p \leq 0.05$ (Dunnett's)
& = Statistically significant when compared with Group 2 at $p \leq 0.05$ (Dunnett's)

TABLE 3 GLUCOSE DETERMINATION (mmol/L) WEEK 104 (b) (4) STUDY NO. 5751

GROUP 1: Air Control
GROUP 2: Technosphere® (Control Low Dose)
GROUP 3: Technosphere® (Control High Dose)
GROUP 4: Technosphere®/Insulin (Low Dose)
GROUP 5: Technosphere®/Insulin (High Dose)

GROUP NO.		Prior to Dosing	10 Minutes Post Dose		Prior to Dosing	10 Minutes Post Dose
	MALES			FEMALES		
1	MEAN	5.2	7.2	MEAN	4.1	6.2
	SD	0.07	0.49	SD	0.00	0.71
	N	2	2	N	2	2
2	MEAN	4.3	6.6	MEAN	5.4	7.3
	SD	0.64	2.15	SD	0.28	0.45
	N	2	3	N	2	3
3	MEAN	5.1	7.2	MEAN	5.8	8.4
	SD	1.06	2.84	SD	NA	0.07
	N	2	3	N	1	2
4	MEAN	5.6	4.9	MEAN	5.6	5.3
	SD	1.48	0.64	SD	0.35	NA
	N	2	2	N	2	1
5	MEAN	5.7	6.4	MEAN	4.8	7.0
	SD	0.99	2.76	SD	NA	0.85
	N	2	2	N	1	2

NA = Not applicable

Gross pathology: There was no evidence of treatment-related findings. All gross observations were noted as procedure-related or incidental since there was low incidence or occurred in control and treated animals.

Gross Pathology on Animals Survived at the End of the Main Study

GROUP INCIDENCE: NECROPSY - ALL DATA

Kill type: TERMINAL

Tissue / Observation	Group:	1	2	3	4	5	1	2	3	4	5
	Sex:	M	M	M	M	M	F	F	F	F	F
	Number:	41	38	43	41	44	43	38	38	37	40
ABDOMINAL CAVITY											
Mass		0	0	0	0	1	0	0	0	0	1
ADRENAL											
Not found		1	0	1	0	1	0	1	0	0	0
Dark area		0	0	0	0	0	2	1	1	1	0
Dark discoloration		0	0	1	0	0	0	0	0	0	1
Enlargement		1	0	1	0	0	4	5	7	4	7
Mass		1	0	0	0	0	1	0	0	0	2
Mottled		0	0	0	0	0	2	0	0	0	0
Pale discoloration		0	0	1	0	0	0	0	0	0	0
Raised area		0	0	0	0	0	0	0	0	1	0
Small		0	0	0	0	0	3	0	0	0	3
BONE											
Thickening		0	0	1	0	0	0	0	0	0	0
BRAIN											
Depressed area		1	4	5	6	5	14	9	9	3	10
Raised area		0	0	0	0	0	0	0	0	1	0
CECUM											
Nodule		0	0	1	0	0	0	0	0	0	0
CERVIX											
Enlargement		-	-	-	-	-	0	0	1	0	0
Mass		-	-	-	-	-	1	0	0	0	1
Nodule		-	-	-	-	-	0	0	0	1	0
Raised area		-	-	-	-	-	1	0	0	0	0
DIGESTIVE CONTENTS											
Dark material		1	0	0	0	0	0	0	0	0	0
EPIDIDYMIS											
Not found		0	0	0	0	1	-	-	-	-	-
Small		0	2	0	0	0	-	-	-	-	-
EYE											
Opacity		0	0	0	0	0	0	1	0	0	0
GUMS											
Mass		0	0	0	0	0	0	1	0	0	0
HEART											
Pale area		0	0	1	0	0	0	0	0	0	0
KIDNEY											
Adhesion		2	1	0	0	0	0	0	0	0	0
Calculus		0	0	0	0	0	0	1	0	0	0

Tissue / Observation	Group:	1	2	3	4	5	1	2	3	4	5
	Sex:	M	M	M	M	M	F	F	F	F	F
	Number:	41	38	43	41	44	43	38	38	37	40
KIDNEY (continued)											
Clear fluid		0	0	0	0	1	0	1	0	0	0
Cyst		1	1	2	1	1	2	0	1	0	0
Dark discoloration		0	0	0	0	0	0	0	0	0	1
Deformed		0	0	0	0	1	0	1	0	0	0
Depressed area		2	5	10	8	11	2	3	1	3	5
Dilatation		0	0	0	0	1	0	0	2	0	2
Enlargement		0	0	0	0	1	0	0	0	0	0
Mottled		1	0	0	0	0	1	0	0	0	0
Pale area		0	0	0	1	0	0	0	0	0	0
Small		0	0	0	0	0	1	0	0	0	0
LYMPH NODE, BRONCHIAL											
Not found		7	5	7	9	8	10	9	8	5	15
Enlargement		0	1	0	0	0	0	0	0	0	0
LIVER											
Adhesion		0	0	0	0	1	0	1	1	1	0
Cyst		0	0	0	0	1	0	0	1	0	0
Dark area		8	1	4	3	6	6	5	2	4	2
Dark focus		0	1	0	0	0	1	0	0	0	0
Depressed area		1	3	5	3	5	7	4	12	6	9
Enlargement		0	0	0	0	0	0	0	0	1	0
Mass		0	0	0	0	1	0	0	0	0	0
Mottled		0	0	1	0	0	0	0	0	0	0
Pale area		0	1	0	1	0	0	0	0	0	0
Raised area		2	0	0	0	0	0	2	0	3	0
LYMPH NODE, MESENTERIC											
Enlargement		0	0	0	0	0	0	1	0	0	0
Mottled		0	0	1	0	0	0	0	0	0	0
LYMPH NODE, MANDIBULAR											
Enlargement		0	1	0	0	0	1	0	0	0	0
Mass		0	0	0	0	1	0	0	0	0	0
LUNG											
Cyst		0	0	0	0	1	0	0	0	0	0
Dark area		1	0	0	0	1	0	0	0	1	0
Depressed area		0	0	0	0	1	0	0	0	0	0
Pale area		5	2	2	3	2	5	2	2	2	3
Pale discoloration		0	0	0	0	0	0	0	0	1	0
Pale focus		0	0	0	0	0	1	0	0	0	1
Raised area		1	0	0	0	1	0	0	0	0	0
LYMPH NODE											
Cyst		4	2	3	2	2	0	0	0	0	0
Dark discoloration		0	0	0	1	0	0	0	0	0	0
Dark material		0	0	1	0	0	0	0	0	0	0

Tissue / Observation	Group:	1	2	3	4	5	1	2	3	4	5
	Sex:	M	M	M	M	M	F	F	F	F	F
	Number:	41	39	43	41	44	43	39	38	37	40
LYMPH NODE (continued)											
Deformed		0	0	0	0	0	0	0	0	0	1
Enlargement		4	5	6	4	6	3	1	0	3	1
Mottled		1	0	0	0	0	0	0	0	0	0
MESENTERY											
Mass		0	0	0	0	1	0	0	0	0	0
NASAL CAVITY											
Deformed		0	0	0	0	1	0	0	0	0	0
OVARY											
Clear fluid		-	-	-	-	-	0	1	0	0	0
Cyst		-	-	-	-	-	4	6	5	5	6
Enlargement		-	-	-	-	-	1	0	0	0	0
Mass		-	-	-	-	-	1	1	0	0	0
PANCREAS											
Mass		0	0	0	0	0	0	1	0	0	0
PITUITARY											
Cyst		3	2	1	2	1	1	2	1	3	5
Dark area		2	3	2	1	2	2	2	4	5	5
Dark discoloration		0	0	0	0	0	0	0	0	1	1
Dark focus		1	4	1	1	1	1	2	2	2	1
Enlargement		1	0	0	0	0	1	0	0	3	3
Mass		5	6	9	12	6	14	11	11	4	10
Pale discoloration		1	0	0	0	0	0	0	0	1	0
Raised area		2	5	3	4	6	6	3	2	0	4
PROSTATE											
Enlargement		0	0	0	1	0	-	-	-	-	-
Gelatinous material		0	0	0	1	1	-	-	-	-	-
Small		0	1	0	0	0	-	-	-	-	-
RECTUM											
Dark material		0	0	1	0	0	0	0	0	0	0
Raised area		0	0	0	0	1	0	0	0	0	0
SMALL INTESTINE, DUODENUM											
Dark area		1	0	0	0	0	0	0	0	0	0
Dark discoloration		0	0	0	0	0	0	0	0	1	0
Dark focus		0	0	0	0	0	0	0	0	1	0
SALIVARY GLAND											
Gelatinous material		0	0	0	1	0	0	0	0	0	0

Tissue / Observation	Group:	1	2	3	4	5	1	2	3	4	5
	Sex:	M	M	M	M	M	F	F	F	F	F
	Number:	41	38	43	41	44	43	38	38	37	40
SPLEEN											
Adhesion		3	0	1	1	0	0	1	0	1	0
Deformed		0	0	1	0	0	0	0	0	0	0
Depressed area		1	0	0	0	0	0	0	0	0	0
Enlargement		1	0	0	2	2	0	0	0	0	0
Pale area		1	0	0	2	0	0	0	0	0	0
Pale discoloration		0	0	1	0	1	0	0	0	0	0
Pale focus		0	0	0	0	1	0	0	0	0	0
Small		0	0	1	0	0	0	0	1	0	0
SKIN & SUBCUTIS											
Crust		1	0	0	0	1	1	0	0	0	0
Dark discoloration		0	0	0	0	0	0	0	0	1	0
Fur thin		0	0	0	0	0	0	0	1	0	0
Gelatinous material		0	0	1	0	1	0	0	0	0	0
Mass 1		11	15	8	11	11	11	12	6	17	11
Mass 2		2	3	2	5	0	4	2	0	3	4
Mass 3		0	0	0	0	0	4	1	0	2	1
Mass 4		0	0	0	0	0	3	1	0	1	1
Mass 5		0	0	0	0	0	2	0	0	0	1
Mass 6		0	0	0	0	0	1	0	0	0	1
Mass A		0	2	1	1	1	1	3	2	2	3
Mass B		0	0	1	0	1	1	0	0	0	0
Mass C		0	0	1	0	1	0	0	0	0	0
Scab		0	0	0	2	0	0	0	0	0	0
Thickening		1	0	0	0	0	0	0	0	0	0
Wound		0	0	0	0	0	0	0	0	1	0
STOMACH											
Cyst		0	0	0	1	0	0	0	0	0	0
Dark area		0	0	0	0	0	0	0	0	1	0
Depressed area		0	0	0	1	0	0	0	1	0	3
Mass		0	0	0	1	0	0	0	0	0	0
Raised area		1	0	0	0	0	0	0	0	0	1
Thickening		1	0	0	0	0	0	0	0	0	0
SEMINAL VESICLE											
Small		0	2	0	0	0	-	-	-	-	-
THORACIC CAVITY											
Mass		0	0	1	1	0	0	0	0	2	0
THYMUS											
Not found		6	13	14	11	14	9	5	5	8	10
Gelatinous material		1	2	2	0	2	1	0	0	1	0
Mass		0	0	0	0	1	0	0	0	0	0

Tissue / Observation	Group:	1	2	3	4	5	1	2	3	4	5
	Sex:	M	M	M	M	M	F	F	F	F	F
	Number:	41	38	43	41	44	43	38	38	37	40
TONGUE											
Mass		0	0	0	0	0	0	1	0	0	0
Raised area		0	0	0	0	1	0	0	0	0	0
TRACHEA											
Mass		0	0	0	0	0	0	0	0	0	1
Nodule		0	0	0	0	0	1	0	0	0	0
TESTIS											
Not found		0	0	0	0	1	-	-	-	-	-
Dark discoloration		0	0	0	1	0	-	-	-	-	-
Enlargement		1	1	0	2	0	-	-	-	-	-
Mottled		2	1	0	2	0	-	-	-	-	-
Small		7	6	4	6	3	-	-	-	-	-
Soft		2	0	2	2	0	-	-	-	-	-
THYROID LOBE											
Mass		1	0	1	0	1	0	1	0	0	0
Pale area		0	0	0	0	0	1	0	0	0	0
URINARY BLADDER											
Calculus		1	0	0	0	0	0	0	0	0	0
Dark discoloration		1	0	0	0	0	0	0	0	0	0
Thickening		1	0	0	0	0	0	0	0	0	0
URETER											
Calculus		0	0	0	0	0	0	1	0	0	0
Dilatation		0	0	0	0	1	0	1	0	0	0
UTERUS											
Clear fluid		-	-	-	-	-	0	0	1	0	1
Cyst		-	-	-	-	-	1	0	0	0	0
Dark area		-	-	-	-	-	0	0	0	1	0
Dark material		-	-	-	-	-	1	0	0	0	0
Deformed		-	-	-	-	-	1	0	0	1	0
Dilatation		-	-	-	-	-	1	0	1	2	1
Enlargement		-	-	-	-	-	1	0	0	0	0
Mass		-	-	-	-	-	1	0	1	2	0
Thickening		-	-	-	-	-	1	0	1	0	0

Gross Pathology on Animals Found Dead prior to the End of the Study

Tissue / Observation	Group:	1	2	3	4	5	1	2	3	4	5
	Sex:	M	M	M	M	M	F	F	F	F	F
	Number:	19	22	17	19	16	17	22	22	23	20
ABDOMINAL CAVITY											
Clear fluid		1	0	0	0	0	0	0	1	1	0
Irregular surface		1	0	0	0	0	0	0	1	0	0
Mass		0	1	0	1	1	0	1	1	0	0
Nodule		1	0	0	0	0	0	0	0	0	0
Pale material		1	0	0	0	0	0	0	0	0	0
ADRENAL											
Not found		0	1	0	0	1	0	1	1	0	0
Dark area		0	1	2	2	1	2	2	2	4	2
Dark discoloration		3	2	1	0	3	2	4	1	2	3
Dark focus		0	1	0	1	0	2	0	0	0	2
Enlargement		2	4	2	2	1	5	8	6	6	6
Mass		0	0	0	0	0	0	1	0	0	0
Mottled		1	0	0	0	1	4	4	3	2	2
Pale discoloration		1	4	0	1	0	2	1	3	0	0
Pale focus		0	1	1	1	0	2	1	4	6	4
Raised area		0	0	0	0	0	0	0	0	0	1
Small		0	1	0	1	1	0	1	0	0	0
Soft		0	1	0	0	0	4	2	3	1	0
AORTA											
Adhesion		1	0	0	0	0	0	0	0	0	0
Dark discoloration		0	1	0	0	0	0	0	0	0	0
Dilatation		0	0	0	0	2	0	0	0	0	0
BONE											
Deformed		0	1	0	0	0	0	0	0	0	0
Firm		0	0	1	0	0	0	0	0	0	0
Thickening		0	1	1	0	0	0	0	0	0	0
BRAIN											
Adhesion		1	2	0	0	0	1	1	2	0	0
Dark area		0	1	1	1	0	0	0	0	0	0
Depressed area		2	3	4	9	4	12	13	13	9	10
Dilatation		0	0	0	0	0	0	1	0	0	0
Mass		0	0	0	0	1	0	0	0	0	0
Pale area		0	0	1	0	0	0	0	0	0	0
Pale discoloration		0	0	0	0	0	1	0	1	1	0
Pale focus		0	0	0	0	0	0	0	0	1	0
Pale material		0	0	1	0	0	0	0	0	0	0
Soft		1	2	2	2	0	1	3	4	0	0
Thickening		0	1	0	0	0	0	0	0	0	0
BONE MARROW											
Pale discoloration		0	0	0	0	0	1	0	1	1	1

Tissue / Observation	Group:	1	2	3	4	5	1	2	3	4	5
	Sex:	M	M	M	M	M	F	F	F	F	F
	Number:	19	22	17	19	16	17	22	22	23	20
CRANIAL CAVITY											
Mass		0	0	0	0	0	0	0	0	1	0
Dark fluid		0	0	0	0	0	1	0	0	0	0
Mottled		1	0	0	0	0	0	0	0	0	0
Soft		1	0	0	0	0	0	0	0	0	0
Thickening		1	0	0	0	0	0	0	0	0	0
CECUM											
Dark discoloration		0	0	0	1	1	0	0	0	0	0
Dark focus		0	0	1	0	0	0	0	0	0	0
Dilatation		2	0	1	1	1	1	0	1	0	0
Gaseous material		0	0	1	0	0	0	0	0	0	0
CERVIX											
Dilatation		-	-	-	-	-	0	0	1	0	0
Thickening		-	-	-	-	-	0	1	0	0	0
COLON											
Dark discoloration		0	0	0	1	0	0	0	0	0	0
Dilatation		0	1	1	1	0	0	0	0	0	0
Gaseous material		0	0	1	0	0	0	0	0	0	0
Pale discoloration		0	0	0	0	0	0	0	0	0	1
DIGESTIVE CONTENTS											
Clear fluid		1	0	0	0	0	0	1	0	0	0
Dark material		3	2	2	3	2	1	3	5	3	3
Gaseous material		0	1	0	1	0	1	1	1	0	0
DEFERENT DUCT											
Dark discoloration		0	0	0	1	0	-	-	-	-	-
DIAPHRAGM											
Adhesion		0	0	0	0	0	0	0	1	0	0
Irregular surface		0	0	0	0	0	0	0	1	0	0
Nodule		1	0	0	0	0	0	0	0	0	0
Thickening		0	0	0	0	0	0	0	1	0	0
EAR											
Firm		0	1	0	0	0	0	0	0	0	0
Mass		0	0	0	1	0	0	0	0	0	0
Thickening		0	1	0	0	0	0	0	0	0	1
EPIDIDYMIS											
Dark area		0	0	0	1	0	-	-	-	-	-
Dark discoloration		2	1	0	0	0	-	-	-	-	-
Nodule		1	0	0	0	0	-	-	-	-	-
Pale discoloration		2	2	0	1	1	-	-	-	-	-
Small		4	1	5	5	3	-	-	-	-	-
Soft		1	2	1	2	2	-	-	-	-	-

Tissue / Observation	Group:	1	2	3	4	5	1	2	3	4	5
	Sex:	M	M	M	M	M	F	F	F	F	F
	Number:	19	22	17	19	16	17	22	22	23	20
ESOPHAGUS											
Clear fluid		0	0	0	0	1	0	0	0	0	0
Dark material		0	0	1	1	0	0	0	0	0	1
Dilatation		0	0	2	0	2	0	0	0	0	1
Gaseous material		0	0	1	0	0	0	0	0	0	0
Pale material		1	1	1	0	0	0	1	1	0	0
EYE											
Dark area		0	0	0	0	0	0	0	0	1	0
Dark discoloration		0	0	0	0	0	0	0	0	1	0
Enlargement		0	0	0	0	0	1	0	0	0	0
Irregular surface		0	0	1	0	0	0	0	0	0	0
Pale area		0	0	0	0	0	1	0	0	0	0
Protruding		0	0	0	0	0	1	0	0	0	0
Scab		0	0	0	0	0	0	0	0	1	0
Soft		0	0	1	0	0	0	0	0	0	0
FAT											
Dark discoloration		0	1	0	0	0	0	0	0	0	0
Mass		0	1	0	1	0	0	0	0	0	0
HEART											
Enlargement		2	0	0	1	0	0	0	0	0	1
Firm		0	0	0	0	0	0	0	1	0	0
Pale discoloration		0	0	0	0	0	1	1	1	0	0
Small		0	0	0	0	0	0	0	0	1	0
Soft		1	0	0	0	0	0	0	0	0	0
HARDERIAN GLAND											
Thickening		0	0	0	0	0	0	0	1	0	0
JOINT											
Deformed		0	0	0	0	0	0	0	0	0	1
Firm		0	2	0	0	0	0	0	0	0	1
Thickening		0	1	0	0	0	0	0	0	0	0
KIDNEY											
Adhesion		1	1	1	0	1	0	0	2	1	0
Clear fluid		1	0	0	1	0	0	1	0	0	0
Cyst		0	0	0	0	0	1	0	0	0	0
Dark area		0	1	0	0	0	0	0	0	0	0
Dark discoloration		4	2	1	1	1	4	2	1	2	0
Dark focus		0	0	0	1	0	0	0	0	0	0
Depressed area		1	2	0	1	5	0	2	2	0	0
Dilatation		2	0	0	2	0	3	0	0	1	1
Displacement		0	0	0	0	0	0	0	1	0	0
Enlargement		2	2	2	2	0	0	0	0	0	0
Firm		1	0	0	0	0	0	0	1	0	0
Irregular surface		0	0	0	0	0	0	0	0	0	1

Tissue / Observation	Group:	1	2	3	4	5	1	2	3	4	5
	Sex:	M	M	M	M	M	F	F	F	F	F
	Number:	19	22	17	19	16	17	22	22	23	20
KIDNEY (continued)											
Mottled		1	0	1	1	0	0	0	0	0	0
Nodule		1	0	0	0	0	0	0	0	0	0
Pale area		1	0	0	0	0	0	0	1	1	0
Pale discoloration		3	1	0	0	0	1	0	2	1	0
Pale material		0	0	0	1	0	0	0	0	0	0
Raised area		0	0	0	1	0	0	0	0	0	1
Soft		1	0	0	0	0	1	2	0	0	0
LYMPH NODE, BRONCHIAL											
Not found		4	12	4	7	5	4	9	6	12	5
Dark discoloration		0	0	1	0	1	0	1	1	0	1
Enlargement		0	0	1	1	0	0	0	0	0	1
Mottled		1	1	0	1	0	0	1	0	0	0
LACRIMAL GLAND											
Dark discoloration		0	0	0	0	0	0	1	0	1	0
LIVER											
Not found		0	0	0	0	0	0	0	1	0	0
Adhesion		1	1	0	0	0	0	0	1	1	0
Dark area		1	2	1	1	0	1	1	0	0	0
Dark discoloration		1	0	1	0	2	0	0	1	0	0
Dark focus		2	1	0	0	0	0	0	0	1	0
Depressed area		0	0	0	1	1	0	1	0	0	0
Enlargement		4	2	5	3	4	1	2	3	3	2
Firm		2	0	1	1	2	0	1	1	0	0
Irregular surface		1	1	0	0	0	0	0	0	0	0
Mottled		4	2	1	1	0	0	2	0	0	1
Pale area		0	0	1	1	1	1	2	2	0	0
Pale discoloration		6	5	4	3	0	2	2	2	1	2
Prominent lobular architecture		0	1	1	0	0	0	0	1	0	0
Raised area		0	2	0	0	0	0	1	0	0	0
Rupture		0	0	0	0	0	0	0	0	1	0
Soft		1	0	0	0	0	0	1	0	1	1
LYMPH NODE, MESENTERIC											
Not found		1	0	0	0	0	0	0	0	0	0
Dark discoloration		1	3	0	1	0	0	1	1	0	1
Enlargement		2	0	0	1	0	0	1	1	1	0
Gelatinous material		0	0	0	0	0	0	1	0	0	0
Mass		0	0	0	0	0	0	0	0	0	1
Mottled		1	0	0	2	0	0	0	0	1	0
Pale discoloration		0	0	0	0	0	0	2	0	0	0
LYMPH NODE, MANDIBULAR											
Dark area		0	0	0	0	0	1	0	0	0	1
Dark discoloration		0	0	0	1	1	1	4	4	2	1
Dark focus		1	0	0	0	0	0	1	2	2	0

Tissue / Observation	Group:	1	2	3	4	5	1	2	3	4	5
	Sex:	M	M	M	M	M	F	F	F	F	F
	Number:	19	22	17	19	16	17	22	22	23	20
LYMPH NODE, MANDIBULAR (continued)											
Enlargement		1	0	0	1	1	0	1	2	2	0
Firm		0	0	0	0	1	0	0	0	0	0
Mottled		1	0	1	0	0	0	0	1	0	0
LUNG											
Adhesion		0	1	1	1	0	0	0	1	0	1
Cyst		0	0	0	0	0	0	1	0	0	0
Dark area		0	1	1	1	1	1	1	3	2	1
Dark discoloration		2	1	1	0	0	0	0	0	0	0
Dark focus		1	0	0	0	1	0	0	0	2	0
Deformed		0	0	0	0	0	0	0	1	0	0
Enlargement		1	0	0	1	0	1	1	0	0	1
Firm		0	0	0	0	0	0	1	0	0	0
Mass		0	1	0	0	0	0	0	0	0	0
Mottled		4	1	3	3	5	1	4	3	2	2
Nodule		1	0	0	0	0	0	1	0	0	0
Non-collapsing		11	9	10	11	10	5	6	6	9	10
Pale area		1	0	0	0	1	0	0	1	1	3
Pale discoloration		0	0	1	1	0	0	0	1	3	0
Raised area		0	1	0	1	0	0	3	0	0	0
LYMPH NODE											
Dark area		0	1	0	0	1	0	0	0	0	0
Dark discoloration		3	2	2	6	1	2	7	1	2	4
Dark focus		0	0	0	1	0	0	0	0	1	1
Enlargement		6	4	3	3	3	2	8	3	3	7
Firm		0	0	0	0	0	0	0	0	0	1
Mottled		2	2	1	1	0	0	2	1	0	1
Pale discoloration		0	0	0	0	1	0	1	0	0	0
MESENTERY											
Adhesion		0	0	0	0	0	0	0	0	0	1
Firm		1	0	0	0	0	0	0	0	0	0
Mass		1	0	0	0	0	0	0	0	0	0
Mottled		1	0	0	0	0	0	0	0	0	0
Nodule		1	0	0	0	0	0	1	0	0	0
Pale material		0	0	0	0	0	0	0	1	0	0
Thickening		1	0	0	0	0	0	0	1	0	0
MAMMARY GLAND											
Dark discoloration		0	0	0	0	0	0	0	0	1	0
Nodule		0	0	0	1	0	0	0	0	0	0
ORAL CAVITY											
Dark material		1	1	1	1	0	0	0	0	0	2
Pale material		1	1	2	1	0	1	1	2	0	1

Tissue / Observation	Group:	1	2	3	4	5	1	2	3	4	5
	Sex:	M	M	M	M	M	F	F	F	F	F
	Number:	19	22	17	19	16	17	22	22	23	20
OVARY											
Not found		-	-	-	-	-	0	0	0	1	0
Adhesion		-	-	-	-	-	0	0	1	0	0
Cyst		-	-	-	-	-	1	1	3	4	2
Dark discoloration		-	-	-	-	-	0	0	1	0	0
Enlargement		-	-	-	-	-	0	0	0	1	0
Gelatinous material		-	-	-	-	-	0	0	0	1	0
Pale discoloration		-	-	-	-	-	3	1	2	1	0
Pale material		-	-	-	-	-	1	0	0	0	0
Small		-	-	-	-	-	3	3	2	5	4
Soft		-	-	-	-	-	0	0	1	0	0
PANCREAS											
Not found		0	0	0	0	0	0	0	1	0	0
Dark discoloration		1	2	0	0	0	0	0	0	0	0
Mass		0	1	0	0	0	0	0	0	0	0
Nodule		1	0	0	0	0	0	0	0	0	0
Pale discoloration		1	0	0	2	0	0	1	1	1	0
Small		0	0	0	0	0	0	0	0	0	1
Soft		0	0	0	0	0	0	1	0	0	0
PELVIC CAVITY											
Mass		0	0	0	1	0	0	0	0	0	0
PHARYNX											
Dark material		0	0	1	0	0	0	0	0	0	0
PITUITARY											
Cyst		3	2	0	1	0	3	0	2	0	2
Dark area		0	0	1	2	0	0	0	0	3	1
Dark discoloration		1	1	1	0	0	1	1	0	1	0
Enlargement		1	3	0	0	0	4	0	1	4	2
Gelatinous material		1	0	0	0	0	0	0	0	0	0
Mass		2	3	4	8	3	10	14	13	9	10
Mottled		0	0	0	0	1	1	0	0	0	0
Pale discoloration		1	1	0	0	1	0	1	0	1	0
Raised area		1	1	1	1	1	0	0	0	0	1
Soft		1	1	0	1	1	3	1	0	4	2
PREPUTIAL GLAND											
Enlargement		1	0	0	0	0	-	-	-	-	-
PEYER'S PATCH											
Enlargement		0	0	0	0	0	0	0	0	1	0
Thickening		0	0	0	0	0	0	0	0	1	0
PROSTATE											
Dark area		0	1	0	0	0	-	-	-	-	-
Dark discoloration		3	1	0	2	1	-	-	-	-	-

Tissue / Observation	Group:	1	2	3	4	5	1	2	3	4	5
	Sex:	M	M	M	M	M	F	F	F	F	F
	Number:	19	22	17	19	16	17	22	22	23	20
PROSTATE (continued)											
Enlargement		2	0	0	0	0	-	-	-	-	-
Firm		1	0	1	1	0	-	-	-	-	-
Gelatinous material		1	0	0	1	0	-	-	-	-	-
Mass		1	0	0	0	0	-	-	-	-	-
Mottled		0	0	1	2	0	-	-	-	-	-
Pale area		0	0	0	1	0	-	-	-	-	-
Pale discoloration		0	2	0	1	0	-	-	-	-	-
Small		5	4	2	5	4	-	-	-	-	-
Soft		1	0	0	1	0	-	-	-	-	-
RECTUM											
Pale discoloration		0	0	0	0	0	0	0	1	1	1
SMALL INTESTINE, DUODENUM											
Dark area		1	0	1	0	0	0	0	0	0	0
Dark discoloration		0	0	0	0	1	0	0	0	0	1
Dark focus		1	1	1	0	0	0	0	0	0	0
Dilatation		1	0	1	0	0	0	0	0	0	0
Gaseous material		0	0	1	0	0	0	0	0	0	0
Pale discoloration		1	0	0	0	0	0	0	0	0	0
Pale focus		0	0	0	0	0	0	1	0	0	1
Thickening		0	1	2	0	0	0	0	0	0	0
SALIVARY GLAND											
Dark discoloration		0	0	0	0	1	0	0	0	0	0
Gelatinous material		1	0	1	0	0	0	0	0	0	0
Mass		0	0	0	0	0	0	0	1	0	0
Pale discoloration		1	0	0	0	0	0	1	0	0	0
Small		0	0	0	0	1	0	0	0	0	0
SMALL INTESTINE, ILEUM											
Dark discoloration		0	0	0	1	0	0	0	0	0	0
Dilatation		1	0	1	0	1	0	0	1	0	0
Gaseous material		1	0	1	0	0	0	0	0	0	0
SMALL INTESTINE, JEJUNUM											
Dark area		1	0	0	0	0	0	0	0	0	0
Dark discoloration		1	0	0	1	0	0	0	0	0	0
Dark focus		0	0	0	0	0	0	0	0	0	1
Dark material		0	0	0	0	0	0	0	0	0	1
Dilatation		1	0	1	0	1	0	0	1	0	1
Gaseous material		0	0	1	0	0	0	0	0	0	0
Mass		0	0	0	0	0	0	0	1	0	0
Thickening		0	0	0	0	0	0	0	1	0	0
SKELETAL MUSCLE											
Firm		0	0	0	0	0	0	0	0	0	1
Mass B		0	1	0	0	0	0	0	0	0	0

Tissue / Observation	Group:	1	2	3	4	5	1	2	3	4	5
	Sex:	M	M	M	M	M	F	F	F	F	F
	Number:	19	22	17	19	16	17	22	22	23	20
SKELETAL MUSCLE (continued)											
Thickening		0	0	0	0	0	0	0	0	0	1
SCIATIC NERVE											
Thickening		0	0	0	1	0	0	0	0	0	0
SPLEEN											
Not found		0	0	0	0	0	0	0	1	0	0
Adhesion		0	0	0	1	2	0	0	0	0	1
Cyst		0	0	1	0	0	0	0	0	0	0
Enlargement		2	1	0	2	0	1	3	1	4	3
Mass		0	0	0	1	0	0	0	0	0	0
Pale area		1	1	0	1	1	1	0	0	0	0
Pale discoloration		2	0	2	1	1	0	2	0	1	1
Pale focus		0	0	0	0	0	0	0	0	1	0
Small		5	3	3	2	0	3	2	1	2	6
SKIN & SUBCUTIS											
Not found		0	0	0	0	0	0	0	0	0	1
Cyst		0	0	0	0	0	1	0	0	0	0
Dark area		1	0	0	0	0	0	0	0	1	0
Dark discoloration		1	1	0	0	0	0	1	1	4	1
Dark focus		0	0	1	0	0	0	0	0	0	0
Dark material		0	0	0	0	0	0	0	1	0	0
Firm		0	0	0	0	0	0	0	0	1	1
Fur thin		1	0	0	0	1	0	0	0	0	0
Fur loss		0	0	0	0	1	0	0	0	0	0
Irregular surface		0	0	0	0	0	0	0	0	1	1
Gelatinous material		0	0	1	1	0	0	0	0	0	0
Mass 1		2	3	1	11	4	3	12	2	12	6
Mass 2		2	0	1	3	1	0	5	0	5	1
Mass 3		1	0	0	0	0	0	1	0	3	0
Mass 4		0	0	0	0	0	0	0	0	1	0
Mass 5		0	0	0	0	0	0	0	0	1	0
Mass 6		0	0	0	0	0	0	0	0	1	0
Mass 7		0	0	0	0	0	0	0	0	1	0
Mass 8		0	0	0	0	0	0	0	0	1	0
Mass 9		0	0	0	0	0	0	0	0	1	0
Mass A		2	1	2	0	1	1	3	3	1	0
Mass B		0	0	0	0	1	0	3	1	1	0
Mass C		0	0	0	0	0	0	2	0	1	0
Mottled		0	0	1	0	0	0	0	0	0	0
Nodule		0	0	1	0	0	0	0	0	0	0
Pale material		1	0	0	0	0	0	0	1	0	0
Raised area		1	2	0	1	1	0	4	1	0	2
Rupture		1	0	0	0	0	1	1	0	2	0
Scab		1	2	1	3	1	2	0	2	1	2
Thickening		0	3	1	0	1	0	0	1	0	1
Wound		1	3	0	2	1	0	1	1	2	1

Tissue / Observation	Group:	1	2	3	4	5	1	2	3	4	5
	Sex:	M	M	M	M	M	F	F	F	F	F
	Number:	19	22	17	19	16	17	22	22	23	20
SKIN & SUBCUTIS (continued)											
Mass 10		0	0	0	0	0	0	0	0	1	0
Mass D		0	0	0	0	0	0	1	0	0	0
STOMACH											
Not found		0	0	0	0	0	0	0	1	0	0
Dark area		4	2	3	1	2	0	1	0	1	5
Dark discoloration		2	0	0	0	1	0	0	0	0	0
Dark focus		0	1	0	1	0	0	1	1	0	2
Dark material		3	2	0	2	0	0	1	0	0	1
Depressed area		0	0	2	1	0	0	2	1	2	1
Dilatation		5	2	1	4	0	2	0	0	2	2
Gaseous material		0	1	1	0	0	0	1	0	0	0
Mottled		0	1	0	0	0	0	0	0	0	0
Pale area		0	0	0	0	0	0	1	0	0	0
Pale discoloration		1	1	0	1	0	0	0	0	1	0
Raised area		1	1	1	1	0	0	0	1	1	1
Thickening		1	1	3	2	1	1	0	0	0	1
SEMINAL VESICLE											
Adhesion		1	0	0	0	0	-	-	-	-	-
Dark discoloration		2	0	0	1	0	-	-	-	-	-
Enlargement		3	1	0	0	0	-	-	-	-	-
Gelatinous material		3	0	0	2	0	-	-	-	-	-
Mottled		2	2	0	0	0	-	-	-	-	-
Small		4	6	4	7	3	-	-	-	-	-
THORACIC CAVITY											
Dark area		0	0	0	0	0	0	0	0	1	0
Dark focus		0	0	0	0	0	0	0	0	1	0
Dark material		1	1	0	0	0	0	0	0	0	0
Mass		3	1	1	1	2	0	0	1	1	1
Dark fluid		2	0	0	0	1	0	0	0	0	0
Pale material		0	0	0	0	0	0	0	0	1	0
THYMUS											
Not found		6	7	5	6	3	5	7	6	6	8
Dark area		0	1	0	1	0	0	0	0	0	0
Dark discoloration		2	0	0	1	0	0	1	0	1	1
Dark focus		2	0	1	2	2	0	1	0	1	1
Deformed		0	0	1	0	0	0	0	0	0	0
Gelatinous material		3	0	1	2	1	0	1	1	1	1
Mottled		0	1	1	1	2	0	0	2	1	0
Pale discoloration		2	0	0	0	0	0	0	0	0	0
Small		9	9	7	13	9	11	10	14	13	10
Soft		0	0	0	0	0	2	1	0	1	0

Tissue / Observation	Group:	1	2	3	4	5	1	2	3	4	5
	Sex:	M	M	M	M	M	F	F	F	F	F
	Number:	19	22	17	19	16	17	22	22	23	20
TONGUE											
Cyst		0	0	0	0	1	0	0	0	0	0
Pale material		0	0	1	0	0	0	0	0	0	0
Thickening		0	0	0	0	1	0	0	0	0	0
TRACHEA											
Deformed		0	0	0	0	0	0	0	0	1	0
Frothy material		2	0	0	0	0	1	1	0	0	2
TESTIS											
Not found		0	1	0	0	0	-	-	-	-	-
Dark area		0	0	0	1	0	-	-	-	-	-
Dark discoloration		3	2	1	3	1	-	-	-	-	-
Enlargement		1	0	0	0	0	-	-	-	-	-
Nodule		1	0	0	0	0	-	-	-	-	-
Pale area		0	1	0	0	0	-	-	-	-	-
Pale discoloration		2	1	0	1	1	-	-	-	-	-
Small		6	4	5	6	4	-	-	-	-	-
Soft		8	4	6	9	4	-	-	-	-	-
THYROID LOBE											
Not found		1	0	0	0	0	0	0	0	0	0
Cyst		0	0	0	0	0	0	1	0	0	0
Dark discoloration		2	0	0	1	1	0	0	0	0	0
Enlargement		1	0	0	1	1	1	0	1	1	0
Pale discoloration		0	0	0	0	0	2	1	1	1	0
Small		1	0	0	0	0	0	0	1	0	0
Soft		0	0	0	0	0	1	1	0	0	0
URINARY BLADDER											
Adhesion		1	0	0	0	0	0	0	1	0	0
Dark area		0	0	0	1	1	0	0	1	0	0
Dark discoloration		3	0	0	1	0	0	0	0	0	1
Dark focus		0	0	1	0	0	0	0	0	0	0
Dark material		1	1	0	0	0	0	0	0	0	1
Dilatation		4	1	1	3	1	0	0	0	0	2
Dark fluid		3	0	0	1	0	0	0	0	0	0
Mottled		0	1	0	1	0	0	0	0	0	0
Opaque fluid		0	1	0	1	0	0	0	0	0	0
Pale material		0	0	0	1	0	0	0	0	0	0
Thickening		2	0	0	0	0	0	0	1	0	0
UTERUS											
Adhesion		-	-	-	-	-	0	0	1	0	0
Dark discoloration		-	-	-	-	-	0	1	1	1	0
Dilatation		-	-	-	-	-	1	0	0	0	0
Mass		-	-	-	-	-	0	1	1	0	1
Mass A		-	-	-	-	-	1	0	0	0	0
Mass B		-	-	-	-	-	1	0	0	0	0

Tissue / Observation	Group:	1	2	3	4	5	1	2	3	4	5
	Sex:	M	M	M	M	M	F	F	F	F	F
	Number:	19	22	17	19	16	17	22	22	23	20
UTERUS (continued)											
Mass C		-	-	-	-	-	1	0	0	0	0
Opaque fluid		-	-	-	-	-	1	0	0	0	0
Raised area		-	-	-	-	-	1	0	1	0	0
Thickening		-	-	-	-	-	1	0	0	0	0
VAGINA											
Dark area		-	-	-	-	-	1	0	0	0	0
Dark discoloration		-	-	-	-	-	1	0	0	0	0
Dark material		-	-	-	-	-	0	0	1	0	0
Gelatinous material		-	-	-	-	-	0	0	1	0	0
Mass		-	-	-	-	-	0	0	1	1	0
Mottled		-	-	-	-	-	0	0	0	0	1
Pale discoloration		-	-	-	-	-	0	0	0	1	0
Pale material		-	-	-	-	-	1	2	0	0	1
Protruding		-	-	-	-	-	0	0	0	1	0
Raised area		-	-	-	-	-	1	0	0	0	0
Thickening		-	-	-	-	-	0	0	0	1	1

Histopathology:

Non-neoplastic:

Nonproliferative lesions were noted in the nasal cavity in high-dose T males and females (Group 3), which consisted of a higher incidence and severity of goblet cell hyperplasia of respiratory epithelium and accumulations of eosinophilic droplets in the olfactory and respiratory epithelia. Both of these lesions were considered non-specific protective or adaptive responses to chronic inhalation of Technosphere particles. These findings were seen with comparable frequency and severity in air control and TI treated groups.

Male					
Group Designation	Air Control	Technosphere® Low Dose	Technosphere® High Dose	Technosphere® Insulin Low Dose	Technosphere® Insulin High Dose
Number Examined	60	60	60	60	60
Hyperplasia, goblet cell					
Nasal cavity #1	19 (1.1)[a]	30* (1.1)	22 (1.3)	22 (1.0)	16 (1.0)
Nasal cavity #2	2 (1.0)	2 (1.0)	2 (1.0)	2 (1.0)	1 (1.0)
Nasal cavity #3	3 (1.3)	8 (1.5)	18** (1.6)	4 (1.3)	1 (1.0)
Nasal cavity #4	2 (1.5)	7 (1.3)	20** (1.6)	3 (1.0)	2 (1.5)
Droplets, eosinophilic					
Nasal cavity #1	0	0	2 (1.0)	1 (1.0)	0
Nasal cavity #2	23 (1.0)	22 (1.0)	39** (1.0)	20 (1.0)	18 (1.0)
Nasal cavity #3	59 (1.0)	60 (1.3)	60 (1.6)	57 (1.0)	59 (1.1)
Nasal cavity #4	57 (1.0)	59 (1.1)	59 (1.5)	56 (1.1)	57 (1.0)

Female					
Group Designation	Air Control	Technosphere® Low Dose	Technosphere® High Dose	Technosphere® Insulin Low Dose	Technosphere® Insulin High Dose
Number Examined	60	60	60	60	60
Hyperplasia, goblet cell					
Nasal cavity #1	16 (1.1)	28* (1.2)	29* (1.2)	19 (1.1)	19 (1.1)
Nasal cavity #2	2 (1.0)	3 (1.0)	5 (1.0)	3 (1.0)	3 (1.0)
Nasal cavity #3	2 (1.0)	7 (1.1)	20** (1.4)	4 (1.3)	4 (1.0)
Nasal cavity #4	1 (1.5)	6 (1.3)	23** (1.6)	3 (1.0)	4 (1.5)
Droplets, eosinophilic					
Nasal cavity #1	0	2 (1.0)	3 (1.3)	2 (1.0)	2 (1.0)
Nasal cavity #2	24 (1.0)	25 (1.0)	46** (1.1)	25 (1.0)	30 (1.0)
Nasal cavity #3	58 (1.0)	57 (1.1)	60 (1.4)	58 (1.0)	60 (1.0)
Nasal cavity #4	55 (1.0)	58 (1.0)	59 (1.1)	54 (1.0)	59 (1.0)

* Significantly different ($p \leq 0.05$) from the air control group by the Fisher exact test.
** Significantly different ($p \leq 0.01$) from the air control group by the Fisher exact test.
[a] Average severity grade of lesions in affected animals: 1=minimal, 2=mild, 3=moderate, 4=severe.
Note: Nasal cavity #1 - 4 refers to the serial transverse sections obtained across the nasal cavity.

Neoplastic:

The numbers of animal with masses were comparable across all groups. These masses were transient with 14-30% (in males) and 64-76% (in females) of masses present at the necropsy. The total number of masses was consistently higher in females than in males due to the presence of palpable mammary masses and was not an indication of any sex-related effects.

Group Number	Group Designation	Sex	Total No. of Animals with Palpable Masses	Total No. of Palpable Masses	Mean Time of Onset (Week No.)	Total No. of Palpable Masses Present at Necropsy
1	Air Control	♂	15	20	71	6
		♀	14	28	82	18
2	Technosphere® Control Low Dose	♂	18	21	68	5
		♀	24	35	77	24
3	Technosphere® Control High Dose	♂	9	12	66	3
		♀	9	10	83	7
4	Technosphere® Insulin Low Dose	♂	24	34	66	10
		♀	29	50	78	38
5	Technosphere® Insulin High Dose	♂	19	22	61	3
		♀	18	27	71	19

Adrenal cortical carcinoma was seen in 3 high-dose TI females (Group 5), with no evidence of an effect in males or in females from any other dose group. The incidence rate of 5% was statistically significant for the trend test but not by the pairwise comparison. It was slightly exceeded air control and historical incidence of 1%. In addition, there was no indication of increased incidences of preneoplastic lesions and other benign and malignant tumors in the adrenal cortex in this group, which cortical adenoma observed in 1 out 60 controls (Group 1) and 2 out of 60 high-dose T males (Group 3). The sponsor indicated that the occurrence of 3 tumors at this dose alone was incidental due to the absence of an effect of treatment on the overall

incidence of focal proliferative lesions and the limited statistical significance of tumors in high-dose TI females.

Male					
Group Designation	Air Control	Technosphere® Low Dose	Technosphere® High Dose	Technosphere® Insulin Low Dose	Technosphere® Insulin High Dose
Number Examined	60	60	60	60	60
Adenoma, cortical	1	0	2	0	0
Carcinoma, cortical	0	0	0	0	1

Female					
Group Designation	Air Control	Technosphere® Low Dose	Technosphere® High Dose	Technosphere® Insulin Low Dose	Technosphere® Insulin High Dose
Number Examined	60	60	60	60	60
Adenoma, cortical	0	0	0	0	0
Ganglioneuroma, benign	1	0	0	0	0
Pheochromocytoma, benign	0	1	1	0	0
Carcinoma, cortical	0	0	0	0	3
Overall Rate	0/60 (0%)	0/60 (0%)	0/60 (0%)	0/60 (0%)	3/60 (5.0%)
Terminal Rate	0/43 (0%)	0/38 (0%)	0/38 (0%)	0/37 (0%)	3/40 (7.5%)
Tests with Group 1 as control					
Trend test				p=1.000	p=0.015
Pairwise comparisons		p=1.000		p=1.000	p=0.108
Tests with Group 2 as control					
Trend test					p=0.040
Pairwise comparisons				p=1.000	p=0.130
Pheochromocytoma, malignant	0	2	0	0	0

Incidence of malignant astrocytoma was marginally higher in low and high-dose T males (Groups 2 and 3) and in the low-dose TI group (Group 4) when compared with the air control. The sponsor considered such as incidental.

Male					
Group Designation	Air Control	Technosphere® Low Dose	Technosphere® High Dose	Technosphere® Insulin Low Dose	Technosphere® Insulin High Dose
Number Examined	60	60	60	60	60
Oligodendroglioma, benign	0	0	0	1	0
Granular cell tumor, benign	0	0	0	0	1
Astrocytoma, malignant	1	4	4	3	1
Overall Rate	1/60 (1.7%)	4/60 (6.7%)	4/60 (6.7%)	3/60 (5.0%)	1/60 (1.7%)
Terminal Rate	1/41 (2.4%)	2/38 (5.3%)	1/43 (2.4%)	3/41 (7.3%)	1/44 (2.3%)
Tests with Group 1 as control					
Trend test					p=0.643
Pairwise comparisons		p=0.193		p=0.308	p=0.770
Tests with Group 2 as control					
Trend test					p=0.947
Pairwise comparisons				p=0.786	p=0.972
Oligodendroglioma, malignant	1	1	0	0	0

Female					
Group Designation	Air Control	Technosphere® Low Dose	Technosphere® High Dose	Technosphere® Insulin Low Dose	Technosphere® Insulin High Dose
Number Examined	60	60	60	60	60
Granular cell tumor, benign	0	0	0	1	0
Astrocytoma, malignant	1	0	1	0	0
Reticulosis, malignant	0	0	0	1	0

There was a slight increase in the incidence of malignant Schwannoma (neurilemmoma) in the nasal cavity in low does T males (Group 2). On the other hand, there was no dose-dependent PCNA activity in respiratory tissue in animals sacrificed at the end of the study. Therefore, the sponsor considered such as incidental. In addition, there was a slight increase in the incidence of fibroma in the skin/subcutis in low-dose TI males (Group 4).

Male					
Group Designation	Air Control	Technosphere® Low Dose	Technosphere® High Dose	Technosphere® Insulin Low Dose	Technosphere® Insulin High Dose
Number Examined	60	60	60	60	60
Schwannoma, malignant					
Nasal cavity #1	0	0	0	0	0
Nasal cavity #2	0	0	0	1	0
Nasal cavity #3	0	0	0	0	1
Nasal cavity #4	0	3	0	0	0
Skin and subcutis, fibroma	0	1	1	3	0

Female					
Group Designation	Air Control	Technosphere® Low Dose	Technosphere® High Dose	Technosphere® Insulin Low Dose	Technosphere® Insulin High Dose
Number Examined	60	60	60	60	60
Schwannoma, malignant					
Nasal cavity #1	0	0	0	0	0
Nasal cavity #2	0	0	0	0	0
Nasal cavity #3	0	0	0	0	0
Nasal cavity #4	0	0	0	0	0
Skin and subcutis, fibroma	1	0	0	2	0

Note: Nasal cavity#1 - 4 refers to the serial transverse sections obtained across the nasal cavity.

PCNA activity data:

Group Number	Group Designation	Sex	Mean No. of Positive Cells/Total No. Investigated
1	Air Control	♂ ♀	0.5/1000 0.6/1000
2	Technosphere® Control Low Dose	♂ ♀	0/1000 0/1000
3	Technosphere® Control High Dose	♂ ♀	0/1000 0/1000
4	Technosphere® Insulin Low Dose	♂ ♀	0/1000 0/1000
5	Technosphere® Insulin High Dose	♂ ♀	0/1000 0/1000

There was a slight decrease in the incidence of pars distalis (in anterior pituitary) adenoma in low-dose TI females (Group 4). This was not in dose-dependent manner and was considered incidental by the sponsor.

Tumor Histopathology on Animal Survived at the End of the Study

GROUP INCIDENCE: HISTOPATHOLOGY - TUMOUR DATA

Kill type: TERMINAL

Tissue / Observation	Group:	1	2	3	4	5	1	2	3	4	5
	Sex:	M	M	M	M	M	F	F	F	F	F
	Number:	41	38	43	41	44	43	38	38	37	40
ADRENAL											
Number examined		41	38	43	41	44	43	38	38	37	40
ADENOMA, CORTICAL		1	0	1	0	0	0	0	0	0	0
PHEOCHROMOCYTOMA, BENIGN		2	3	2	1	0	0	1	1	0	0
CARCINOMA, CORTICAL		0	0	0	0	0	0	0	0	0	3
PHEOCHROMOCYTOMA, MALIGNANT		1	0	0	0	1	0	0	0	0	0
BRAIN											
Number examined		41	38	43	41	44	43	38	38	37	40
OLIGODENDROGLIOMA, BENIGN		0	0	0	1	0	0	0	0	0	0
TUMOR, GRANULAR CELL, BENIGN		0	0	0	0	1	0	0	0	1	0
ASTROCYTOMA, MALIGNANT		1	2	1	3	1	1	0	1	0	0
HEART											
Number examined		41	38	43	41	44	43	38	38	37	40
SCHWANNOMA, BENIGN		0	0	0	0	1	0	0	0	1	0
HEMOLYMPHORETICULAR(ALL SITES)											
Number examined		0	0	0	0	0	0	1	0	2	0
SARCOMA, HISTIOCYTIC		0	0	0	0	0	0	1	0	2	0
LIVER											
Number examined		41	38	43	41	44	43	38	38	37	40
ADENOMA, HEPATOCELLULAR		2	0	0	0	2	0	0	0	0	1
LYMPH NODE, MESENTERIC											
Number examined		41	38	43	41	44	43	38	38	37	40
HEMANGIOMA		0	0	1	0	0	0	0	0	0	0
LUNG											
Number examined		41	38	43	41	44	43	38	38	37	40
ADENOMA, BRONCHIOLO-ALVEOLAR		1	0	0	0	0	0	0	0	0	0
CARCINOMA, BRONCHIOLO-ALVEOLAR		0	0	0	0	1	0	0	0	0	0
LYMPH NODE											
Number examined		8	9	10	7	9	11	10	3	14	10
LEIOMYOSARCOMA		0	0	0	0	1	0	0	0	0	0
MAMMARY GLAND											
Number examined		39	37	37	37	42	42	35	38	37	38
ADENOMA		0	0	0	0	0	5	0	1	3	2
FIBROADENOMA		0	0	0	0	0	9	8	4	9	9
ADENOCARCINOMA		0	0	0	0	0	3	3	2	6	3
NASAL CAVITY (section 1)											
Number examined		41	38	43	41	44	43	38	38	37	40
FIBROMA		0	0	0	0	0	1	0	0	0	0

Tissue / Observation	Group:	1	2	3	4	5	1	2	3	4	5
	Sex:	M	M	M	M	M	F	F	F	F	F
	Number:	41	38	43	41	44	43	38	38	37	40
ORAL CAVITY											
Number examined		2	0	2	0	1	4	1	0	0	1
CARCINOMA, SQUAMOUS CELL		0	0	0	0	1	0	0	0	0	0
FIBROSARCOMA		0	0	0	0	0	0	1	0	0	0
OPTIC NERVE											
Number examined		33	27	28	27	36	29	22	24	20	27
SCHWANNOMA, MALIGNANT		0	1	0	0	0	0	0	0	0	0
OVARY											
Number examined		-	-	-	-	-	43	38	38	36	40
THECOMA, BENIGN		-	-	-	-	-	2	1	0	0	0
TUMOR, SEX CORD STROMAL, MIXED, BENIGN		-	-	-	-	-	1	0	0	0	0
PANCREAS											
Number examined		41	38	43	41	43	43	37	38	37	40
ADENOMA, ACINAR-ISLET CELL		1	0	0	2	0	0	0	0	0	0
ADENOMA, ISLET CELL		4	1	1	4	3	1	2	0	3	0
CARCINOMA, ISLET CELL		0	0	0	0	0	0	0	0	0	1
PITUITARY											
Number examined		41	37	42	41	44	42	38	37	37	39
ADENOMA, PARS DISTALIS		14	18	19	20	16	27	17	18	15	22
ADENOMA, PARS INTERMEDIA		1	1	0	0	1	0	0	1	0	0
CARCINOMA, PARS DISTALIS		0	0	1	0	0	0	0	0	1	0
PROSTATE											
Number examined		41	38	43	41	44	-	-	-	-	-
ADENOMA		1	0	1	1	0	-	-	-	-	-
PARATHYROID											
Number examined		37	31	29	34	39	33	28	30	29	35
ADENOMA		1	1	1	1	2	0	1	0	0	0
SCIATIC NERVE											
Number examined		41	38	43	41	44	43	38	38	37	40
SCHWANNOMA, BENIGN		0	0	0	0	0	0	1	0	0	0
SKIN & SUBCUTIS											
Number examined		41	38	43	41	44	43	38	38	37	40
FIBROMA		0	1	1	3	0	1	0	0	2	0
KERATOACANTHOMA, BENIGN		1	0	0	1	2	0	0	0	0	0
LIPOMA		1	0	0	0	0	0	0	0	0	0
PAPILLOMA, SQUAMOUS CELL		0	0	0	1	0	0	0	0	0	0
CARCINOMA, SQUAMOUS CELL		0	0	0	0	1	0	0	0	0	0
FIBROSARCOMA		0	0	1	0	1	0	0	0	0	0
HIBERNOMA, MALIGNANT		0	0	0	0	0	0	0	1	0	0
SCHWANNOMA, MALIGNANT		1	0	0	0	0	0	0	0	0	0

Tissue / Observation	Group:	1	2	3	4	5	1	2	3	4	5
	Sex:	M	M	M	M	M	F	F	F	F	F
	Number:	41	38	43	41	44	43	38	38	37	40
STOMACH											
Number examined		41	38	43	41	44	43	38	38	37	40
ADENOMA		0	0	0	1	0	0	0	0	0	0
LEIOMYOSARCOMA		0	0	0	1	0	0	0	0	0	0
THORACIC CAVITY											
Number examined		0	0	1	1	0	0	0	0	2	0
HIBERNOMA,MALIGNANT		0	0	1	1	0	0	0	0	2	0
TONGUE											
Number examined		41	38	43	41	44	43	38	38	37	40
PAPILLOMA,SQUAMOUS CELL		0	0	0	0	1	0	1	0	0	0
TESTIS											
Number examined		41	38	43	41	44	-	-	-	-	-
ADENOMA,INTERSTITIAL(LEYDIG) CELL		2	3	0	3	0	-	-	-	-	-
MESOTHELIOMA,MALIGNANT		0	0	0	1	0	-	-	-	-	-
THYROID LOBE											
Number examined		41	38	43	41	44	43	38	38	37	40
ADENOMA,C-CELL		6	2	7	5	2	2	2	0	0	1
ADENOMA,FOLLICULAR CELL		0	1	0	1	0	1	1	1	0	1
CARCINOMA,C-CELL		0	0	1	0	0	1	0	0	0	0
CARCINOMA,FOLLICULAR CELL		0	0	0	0	0	1	1	0	0	0
URINARY BLADDER											
Number examined		41	38	43	41	44	43	38	37	37	40
PAPILLOMA,TRANSITIONAL CELL		0	0	0	0	0	0	0	1	0	0
UTERUS											
Number examined		-	-	-	-	-	43	38	38	37	39
POLYP,ENDOMETRIAL STROMAL		-	-	-	-	-	5	1	3	8	2
LEIOMYOSARCOMA		-	-	-	-	-	1	0	0	0	0
SCHWANNOMA,MALIGNANT		-	-	-	-	-	0	0	0	1	0
NASAL CAVITY (section 3)											
Number examined		41	38	43	41	44	43	38	38	37	40
ADENOMA		0	0	0	0	1	0	0	0	0	0
NASAL CAVITY (section 4)											
Number examined		41	38	43	41	44	43	38	38	37	40
SCHWANNOMA,MALIGNANT		0	1	0	0	0	0	0	0	0	0
ZYMBAL'S GLAND											
Number examined		1	0	0	0	0	0	0	0	0	0
ADENOMA		1	0	0	0	0	0	0	0	0	0

Tumor Histopathology on Animal Found Dead prior to the End of the Study

GROUP INCIDENCE: HISTOPATHOLOGY - TUMOUR DATA

Kill type: PRETERMINAL SACRIFICE FOUND DEAD

Tissue / Observation	Group:	1	2	3	4	5	1	2	3	4	5
	Sex:	M	M	M	M	M	F	F	F	F	F
	Number:	19	22	17	19	16	17	22	22	23	20
ABDOMINAL CAVITY											
Number examined		1	1	0	1	0	0	0	1	0	0
HIBERNOMA,MALIGNANT		0	1	0	0	0	0	0	0	0	0
ADRENAL											
Number examined		19	22	17	19	16	17	22	22	23	20
ADENOMA,CORTICAL		0	0	1	0	0	0	0	0	0	0
GANGLIONEUROMA,BENIGN		0	0	0	0	0	1	0	0	0	0
PHEOCHROMOCYTOMA,BENIGN		0	1	0	0	0	0	0	0	0	0
CARCINOMA,CORTICAL		0	0	0	0	1	0	0	0	0	0
PHEOCHROMOCYTOMA,MALIGNANT		0	0	0	0	0	0	2	0	0	0
BONE											
Number examined		0	0	0	1	0	0	0	0	0	1
OSTEOSARCOMA		0	0	0	1	0	0	0	0	0	1
BRAIN											
Number examined		19	22	17	19	16	17	22	22	23	20
ASTROCYTOMA,MALIGNANT		0	2	3	0	0	0	0	0	0	0
OLIGODENDROGLIOMA,MALIGNANT		1	1	0	0	0	0	0	0	0	0
RETICULOSIS,MALIGNANT		0	0	0	0	0	0	0	0	1	0
HEART											
Number examined		19	22	17	19	16	17	22	22	23	20
SCHWANNOMA,BENIGN		0	1	0	0	0	0	0	0	0	0
SCHWANNOMA,ENDOCARDIAL,MALIGNANT		0	0	0	1	1	0	0	0	0	0
HEMOLYMPHORETICULAR(ALL SITES)											
Number examined		1	1	1	2	0	0	0	2	2	1
LYMPHOMA,MALIGNANT		1	0	0	1	0	0	0	0	2	0
SARCOMA,HISTIOCYTIC		0	1	1	1	0	0	0	2	0	1
LIVER											
Number examined		19	22	17	19	16	17	22	22	23	20
ADENOMA,HEPATOCELLULAR		0	0	0	0	0	0	1	0	0	0
MAMMARY GLAND											
Number examined		16	20	16	16	14	17	20	22	22	19
ADENOMA		0	0	0	0	0	0	0	1	0	0
FIBROADENOMA		0	0	0	0	0	0	3	3	6	3
ADENOCARCINOMA		0	0	0	0	0	4	8	0	4	1
TUMOR,MIXED,MALIGNANT		1	0	0	0	0	0	0	0	0	0
ORAL CAVITY											
Number examined		0	1	1	0	0	1	1	1	2	0
CARCINOMA,SQUAMOUS CELL		0	1	1	0	0	0	0	0	2	0

Tissue / Observation	Group:	1	2	3	4	5	1	2	3	4	5
	Sex:	M	M	M	M	M	F	F	F	F	F
	Number:	19	22	17	19	16	17	22	22	23	20
OVARY											
Number examined		-	-	-	-	-	17	22	22	23	20
THECOMA, BENIGN		-	-	-	-	-	1	0	0	0	0
PANCREAS											
Number examined		19	22	17	19	15	17	22	21	23	20
ADENOMA, ISLET CELL		1	5	0	2	0	0	0	0	0	0
PITUITARY											
Number examined		19	21	17	18	16	16	21	21	22	19
ADENOMA, PARS DISTALIS		8	9	7	10	5	12	15	13	12	11
ADENOMA, PARS INTERMEDIA		0	1	0	0	0	0	0	0	0	0
CARCINOMA, PARS DISTALIS		0	0	0	0	0	1	0	0	0	0
PROSTATE											
Number examined		19	22	17	19	16	-	-	-	-	-
ADENOCARCINOMA		1	0	0	0	0	-	-	-	-	-
PARATHYROID											
Number examined		17	12	16	14	12	14	16	17	13	17
ADENOMA		0	1	0	0	0	0	0	0	0	0
SPINAL CORD											
Number examined		19	22	17	19	16	17	21	22	23	20
SCHWANNOMA, BENIGN		0	0	0	0	0	1	0	0	0	0
SKULL											
Number examined		0	0	0	0	1	0	0	0	0	0
SCHWANNOMA, MALIGNANT		0	0	0	0	1	0	0	0	0	0
SKIN & SUBCUTIS											
Number examined		19	22	17	19	16	17	22	22	23	20
FIBROSARCOMA		0	0	1	1	0	0	0	0	0	2
TUMOR, BASAL CELL, MALIGNANT		0	0	0	0	0	0	1	0	0	0
STOMACH											
Number examined		19	21	17	19	16	17	22	22	22	20
ADENOCARCINOMA		1	0	0	0	0	0	0	0	0	0
TAIL											
Number examined		2	3	1	1	2	2	4	2	3	6
SCHWANNOMA, MALIGNANT		0	0	0	0	0	0	0	0	1	0
THORACIC CAVITY											
Number examined		3	3	1	1	1	0	0	1	1	1
HIBERNOMA, MALIGNANT		3	2	1	1	1	0	0	1	0	1
LIPOSARCOMA		0	1	0	0	0	0	0	0	0	0

Tissue / Observation	Group:	1	2	3	4	5	1	2	3	4	5
	Sex:	M	M	M	M	M	F	F	F	F	F
	Number:	19	22	17	19	16	17	22	22	23	20
TONGUE											
Number examined		19	22	17	19	16	17	22	22	23	20
RHABDOMYOSARCOMA		0	0	0	0	1	0	0	0	0	0
THYROID LOBE											
Number examined		18	22	17	19	16	17	20	22	23	20
ADENOMA, C-CELL		0	0	0	0	0	1	0	0	0	1
ADENOMA, FOLLICULAR CELL		0	0	0	0	0	0	0	0	1	0
URINARY BLADDER											
Number examined		19	22	17	19	16	17	21	22	23	19
CARCINOMA, TRANSITIONAL CELL		0	0	0	0	0	0	1	0	0	0
UTERUS											
Number examined		-	-	-	-	-	17	22	22	23	19
POLYP, ENDOMETRIAL STROMAL		-	-	-	-	-	0	2	1	0	0
SCHWANNOMA, MALIGNANT		-	-	-	-	-	1	0	0	0	1
VAGINA											
Number examined		-	-	-	-	-	17	21	22	23	19
POLYP, VAGINAL		-	-	-	-	-	0	0	0	1	0
SCHWANNOMA, MALIGNANT		-	-	-	-	-	0	0	1	0	1
NASAL CAVITY (section 2)											
Number examined		19	22	17	19	16	17	22	22	23	20
SCHWANNOMA, MALIGNANT		0	0	0	1	0	0	0	0	0	0
NASAL CAVITY (section 3)											
Number examined		19	22	17	19	16	17	22	22	23	20
SCHWANNOMA, MALIGNANT		0	0	0	0	1	0	0	0	0	0
NASAL CAVITY (section 4)											
Number examined		19	22	17	19	16	17	22	22	23	20
SCHWANNOMA, MALIGNANT		0	2	0	0	0	0	0	0	0	0
ZYMBAL'S GLAND											
Number examined		0	0	0	2	0	0	0	0	0	0
ADENOMA		0	0	0	2	0	0	0	0	0	0

Non-Tumor Histopathology on Animals Survived at the End of the Study

GROUP INCIDENCE: HISTOPATHOLOGY - NON-TUMOUR DATA

Kill type: TERMINAL

Tissue / Observation	Group:	1	2	3	4	5	1	2	3	4	5
	Sex:	M	M	M	M	M	F	F	F	F	F
	Number:	41	38	43	41	44	43	38	38	37	40
ABDOMINAL CAVITY											
Number examined		0	0	0	0	1	0	0	0	0	1
Tumor infiltrate/metastasis		0	0	0	0	1	0	0	0	0	0
severe		0	0	0	0	1	0	0	0	0	0
ADRENAL											
Number examined		41	38	43	41	44	43	38	38	37	40
Angiectasis		12	5	7	9	6	35	34	33	35	34
minimal		12	5	7	9	6	32	30	31	32	33
mild		0	0	0	0	0	3	4	2	3	1
Atrophy,cortical		0	0	0	0	0	1	0	0	0	2
mild		0	0	0	0	0	0	0	0	0	1
severe		0	0	0	0	0	1	0	0	0	1
Vacuolation,cortical,diffuse		1	0	0	0	0	0	0	0	0	0
minimal		1	0	0	0	0	0	0	0	0	0
Degeneration,cystic		1	0	3	1	1	22	12	19	14	23
minimal		0	0	2	1	1	12	6	14	13	11
mild		1	0	1	0	0	5	4	5	1	7
moderate		0	0	0	0	0	5	2	0	0	4
severe		0	0	0	0	0	0	0	0	0	1
Necrosis,focal		0	0	0	0	0	0	0	1	0	0
minimal		0	0	0	0	0	0	0	1	0	0
Mineralization		0	0	0	0	0	0	0	1	0	1
minimal		0	0	0	0	0	0	0	0	0	1
mild		0	0	0	0	0	0	0	1	0	0
Hematopoiesis,extramedullary		1	0	0	1	0	0	0	1	1	0
minimal		1	0	0	1	0	0	0	1	1	0
Hypertrophy,zona glomerulosa,focal		40	36	39	38	40	32	35	35	35	36
minimal		40	36	39	38	40	32	35	35	34	36
mild		0	0	0	0	0	0	0	0	1	0
Vacuolation,zona fasciculata,focal		2	2	1	1	5	2	0	0	1	0
minimal		2	2	1	1	4	2	0	0	1	0
mild		0	0	0	0	1	0	0	0	0	0
Pigment,corticomedullary		37	34	38	37	44	40	33	37	34	38
minimal		37	34	38	37	44	40	33	37	34	38
Vacuolation,zona reticularis,focal		0	0	0	1	1	0	0	0	0	0
minimal		0	0	0	1	1	0	0	0	0	0
Congestion/hemorrhage,agonal		1	0	0	0	0	0	0	0	0	0
present		1	0	0	0	0	0	0	0	0	0
Hypertrophy,cortical,focal		20	11	18	23	23	28	17	26	22	25
minimal		20	10	17	23	22	25	17	24	22	17
mild		0	1	1	0	1	3	0	2	0	8
Cell infiltrate,pericapsular		0	0	1	0	0	0	0	0	0	0
minimal		0	0	1	0	0	0	0	0	0	0
Cell infiltrate,medullary		0	1	0	2	1	0	0	0	0	0
minimal		0	1	0	2	1	0	0	0	0	0
Hyperplasia,cortical,focal		3	1	4	1	0	1	3	2	2	1
present		3	1	4	1	0	1	3	2	2	1
Hyperplasia,medullary,focal		8	7	8	7	6	0	2	0	0	2

Tissue / Observation	Group:	1	2	3	4	5	1	2	3	4	5
	Sex:	M	M	M	M	M	F	F	F	F	F
	Number:	41	38	43	41	44	43	38	38	37	40
ADRENAL (continued)											
Hyperplasia,medullary,focal (continued)											
present		8	7	8	7	6	0	2	0	0	2
AORTA											
Number examined		41	38	43	41	44	43	38	38	37	40
Hemorrhage,focal		0	0	0	0	1	0	0	0	0	0
minimal		0	0	0	0	1	0	0	0	0	0
BONE											
Number examined		0	0	1	0	0	0	0	0	0	0
Tumor infiltrate/metastasis		0	0	1	0	0	0	0	0	0	0
minimal		0	0	1	0	0	0	0	0	0	0
BRAIN											
Number examined		41	38	43	41	44	43	38	38	37	40
Dilatation,ventricular		0	3	2	3	4	10	2	5	1	9
minimal		0	3	1	1	4	10	2	4	1	7
mild		0	0	1	2	0	0	0	1	0	2
Mineralization		3	1	5	1	3	0	2	4	3	2
minimal		3	1	5	1	3	0	2	4	3	2
Hemorrhage,focal		1	0	0	0	0	0	0	1	0	0
minimal		1	0	0	0	0	0	0	1	0	0
Cell infiltrate,perivascular		0	0	0	0	1	0	0	0	1	0
minimal		0	0	0	0	1	0	0	0	1	0
Indentation		2	3	4	6	5	12	10	9	3	9
present		2	3	4	6	5	12	10	9	3	9
Cell infiltrate,meningeal		0	0	0	0	1	1	0	0	0	0
minimal		0	0	0	0	1	1	0	0	0	0
Tumor infiltrate/metastasis		0	0	0	0	0	0	0	1	1	0
minimal		0	0	0	0	0	0	0	1	1	0
BLOOD VESSEL											
Number examined		0	0	0	0	0	0	0	1	0	0
Thrombus		0	0	0	0	0	0	0	1	0	0
minimal		0	0	0	0	0	0	0	1	0	0
CECUM											
Number examined		41	38	43	41	44	43	38	38	37	40
Hemorrhage,focal		0	0	0	0	0	1	0	0	0	0
minimal		0	0	0	0	0	1	0	0	0	0
Cell infiltrate,mucosal/submucosal		0	0	0	1	2	0	0	0	0	0
minimal		0	0	0	1	2	0	0	0	0	0
Inflammation		0	0	0	1	0	0	0	0	0	0
minimal		0	0	0	1	0	0	0	0	0	0
Dilatation,crypt		1	0	0	0	0	0	0	0	0	0
minimal		1	0	0	0	0	0	0	0	0	0

Tissue / Observation	Group:	1	2	3	4	5	1	2	3	4	5
	Sex:	M	M	M	M	M	F	F	F	F	F
	Number:	41	38	43	41	44	43	38	38	37	40
CLITORAL GLAND											
Number examined		-	-	-	-	-	0	0	0	0	1
Ectasia,duct		-	-	-	-	-	0	0	0	0	1
mild		-	-	-	-	-	0	0	0	0	1
CERVIX											
Number examined		-	-	-	-	-	2	0	1	1	1
Hypertrophy,stromal		-	-	-	-	-	1	0	1	0	1
mild		-	-	-	-	-	1	0	1	0	0
moderate		-	-	-	-	-	0	0	0	0	1
COLON											
Number examined		41	38	43	41	44	43	38	38	37	40
Cell infiltrate,serosal		0	0	0	0	0	0	0	0	1	0
minimal		0	0	0	0	0	0	0	0	1	0
Inflammation		0	0	0	1	0	0	0	0	0	0
minimal		0	0	0	1	0	0	0	0	0	0
Arteritis		1	0	0	0	0	0	0	0	0	0
mild		1	0	0	0	0	0	0	0	0	0
EPIDIDYMIS											
Number examined		41	38	43	41	44	-	-	-	-	-
Spermatocele		1	0	0	1	0	-	-	-	-	-
minimal		1	0	0	1	0	-	-	-	-	-
Degeneration/cribriform change		12	14	10	11	14	-	-	-	-	-
minimal		9	11	7	10	8	-	-	-	-	-
mild		3	3	3	1	6	-	-	-	-	-
Cell infiltrate,perivascular		26	25	19	27	19	-	-	-	-	-
minimal		26	25	19	27	19	-	-	-	-	-
Fibrosis,efferent duct		1	0	0	0	0	-	-	-	-	-
moderate		1	0	0	0	0	-	-	-	-	-
Oligospermia/aspermia		12	9	8	12	12	-	-	-	-	-
minimal		1	0	0	1	2	-	-	-	-	-
mild		1	1	1	1	0	-	-	-	-	-
moderate		1	4	7	7	4	-	-	-	-	-
severe		9	4	0	3	6	-	-	-	-	-
Inflammation,granulomatous		1	0	0	0	0	-	-	-	-	-
mild		1	0	0	0	0	-	-	-	-	-
Arteritis		0	0	0	1	0	-	-	-	-	-
minimal		0	0	0	1	0	-	-	-	-	-
Cellular debris,ductal		4	4	2	3	9	-	-	-	-	-
minimal		3	1	1	1	8	-	-	-	-	-
mild		1	3	1	2	1	-	-	-	-	-
Tumor infiltrate/metastasis		0	0	0	0	1	-	-	-	-	-
mild		0	0	0	0	1	-	-	-	-	-
ESOPHAGUS											
Number examined		41	38	43	41	44	43	38	38	37	40
Cell infiltrate,muscular		0	0	0	1	0	0	0	0	0	0

Tissue / Observation	Group:	1	2	3	4	5	1	2	3	4	5
	Sex:	M	M	M	M	M	F	F	F	F	F
	Number:	41	38	43	41	44	43	38	38	37	40
ESOPHAGUS (continued)											
Cell infiltrate,muscular (continued)											
minimal		0	0	0	1	0	0	0	0	0	0
EYE											
Number examined		41	38	43	41	44	43	38	38	37	40
Atrophy,retinal		31	32	33	31	29	35	28	36	34	36
minimal		30	32	32	28	25	35	26	33	33	33
mild		1	0	1	3	3	0	1	3	1	3
moderate		0	0	0	0	1	0	0	0	0	0
severe		0	0	0	0	0	0	1	0	0	0
Degeneration,lenticular		12	6	11	11	11	8	7	8	4	5
minimal		11	5	10	7	7	6	5	4	3	4
mild		1	1	1	3	3	2	1	4	0	0
moderate		0	0	0	1	1	0	1	0	1	1
Mineralization,corneal		1	0	0	0	1	0	0	0	0	0
minimal		1	0	0	0	1	0	0	0	0	0
Cell infiltrate,focal		1	0	0	0	0	0	0	0	0	0
minimal		1	0	0	0	0	0	0	0	0	0
Mucoid material,corneal		6	5	6	7	5	6	6	5	8	6
minimal		6	5	6	7	5	6	6	5	8	6
Mucoid material,conjuctival		0	0	0	1	0	0	0	0	1	0
minimal		0	0	0	1	0	0	0	0	1	0
Dysplasia,retinal		0	0	0	1	2	0	0	0	1	0
minimal		0	0	0	0	1	0	0	0	1	0
mild		0	0	0	1	0	0	0	0	0	0
moderate		0	0	0	0	1	0	0	0	0	0
FAT											
Number examined		0	0	0	0	1	0	0	0	0	0
Tumor infiltrate/metastasis		0	0	0	0	1	0	0	0	0	0
moderate		0	0	0	0	1	0	0	0	0	0
FEMUR & MARROW											
Number examined		41	38	43	41	44	43	38	38	37	39
Fat infiltration		6	4	7	7	6	8	8	7	4	9
minimal		5	4	5	7	6	7	8	6	4	7
mild		1	0	2	0	0	1	0	1	0	2
Degeneration,chondromucinous		0	0	0	0	0	0	0	0	0	1
minimal		0	0	0	0	0	0	0	0	0	1
Osteosclerosis		0	0	0	0	0	0	0	0	1	0
minimal		0	0	0	0	0	0	0	0	1	0
Hyperostosis		0	0	0	1	0	0	0	0	0	0
minimal		0	0	0	1	0	0	0	0	0	0
Hypercellularity,marrow		18	17	19	14	20	7	8	9	5	6
minimal		9	10	7	5	13	5	6	6	4	4
mild		9	7	12	9	7	2	2	3	1	2
Thrombus		0	0	1	0	0	0	0	0	0	0
minimal		0	0	1	0	0	0	0	0	0	0

Tissue / Observation	Group:	1	2	3	4	5	1	2	3	4	5
	Sex:	M	M	M	M	M	F	F	F	F	F
	Number:	41	38	43	41	44	43	38	38	37	40
FEMUR & MARROW (continued)											
Granuloma		0	0	0	0	1	1	0	1	0	0
minimal		0	0	0	0	1	1	0	1	0	0
Fibrosis,focal		0	0	1	3	0	0	0	0	0	0
minimal		0	0	1	3	0	0	0	0	0	0
Tumor infiltrate/metastasis		1	0	0	0	0	0	1	0	1	0
minimal		0	0	0	0	0	0	1	0	0	0
mild		1	0	0	0	0	0	0	0	1	0
HEART											
Number examined		41	38	43	41	44	43	38	38	37	40
Adhesion		0	0	1	0	0	0	0	0	0	0
minimal		0	0	1	0	0	0	0	0	0	0
Cardiomyopathy		13	9	6	9	12	1	2	1	2	2
minimal		13	9	6	9	12	1	2	1	2	2
Mineralization,vascular		3	0	0	4	1	0	1	1	1	0
minimal		3	0	0	4	1	0	1	1	1	0
Mineralization,myocardial		0	0	0	0	1	0	0	0	0	0
minimal		0	0	0	0	1	0	0	0	0	0
Cell infiltrate,epicardial		0	0	0	0	0	0	0	0	1	0
minimal		0	0	0	0	0	0	0	0	1	0
Hematocyst,valular		1	0	0	0	0	0	0	0	0	0
present		1	0	0	0	0	0	0	0	0	0
Hyperplasia,endocardial		0	0	1	0	0	2	0	0	2	0
minimal		0	0	0	0	0	0	0	0	2	0
mild		0	0	1	0	0	2	0	0	0	0
KIDNEY											
Number examined		41	38	43	41	44	43	38	38	37	40
Adhesion		0	0	0	0	0	0	0	0	0	1
present		0	0	0	0	0	0	0	0	0	1
Calculus		3	2	5	6	2	11	11	7	4	14
minimal		3	2	5	5	1	11	8	6	4	12
mild		0	0	0	1	1	0	2	1	0	2
severe		0	0	0	0	0	0	1	0	0	0
Cyst		2	0	1	1	1	2	1	1	0	0
present		2	0	1	1	1	2	1	1	0	0
Dilatation,pelvic		0	0	1	0	1	1	2	4	1	5
minimal		0	0	1	0	0	1	1	2	0	2
mild		0	0	0	0	0	0	0	2	1	3
moderate		0	0	0	0	1	0	1	0	0	0
Dilatation,tubular		0	0	1	1	1	1	0	2	1	2
minimal		0	0	1	1	1	1	0	2	1	1
mild		0	0	0	0	0	0	0	0	0	1
Dilatation,tubular,cystic		1	1	0	0	0	0	0	0	0	0
minimal		1	0	0	0	0	0	0	0	0	0
mild		0	1	0	0	0	0	0	0	0	0
Necrosis,papillary		0	1	0	0	0	0	0	0	0	0
minimal		0	1	0	0	0	0	0	0	0	0

Tissue / Observation	Group:	1	2	3	4	5	1	2	3	4	5
	Sex:	M	M	M	M	M	F	F	F	F	F
	Number:	41	38	43	41	44	43	38	38	37	40
KIDNEY (continued)											
Chronic progressive nephropathy		9	3	2	4	6	1	0	0	0	1
minimal		7	3	2	3	3	1	0	0	0	1
mild		2	0	0	1	2	0	0	0	0	0
severe		0	0	0	0	1	0	0	0	0	0
Basophilia,tubular		23	24	31	20	25	20	14	17	15	16
minimal		22	24	31	20	25	20	14	16	15	16
mild		1	0	0	0	0	0	0	1	0	0
Hyaline droplet,tubular cell		0	0	0	1	1	0	0	0	1	0
minimal		0	0	0	1	0	0	0	0	0	0
mild		0	0	0	0	0	0	0	0	1	0
moderate		0	0	0	0	1	0	0	0	0	0
Pigment,tubular		0	0	0	0	0	0	1	0	0	0
minimal		0	0	0	0	0	0	1	0	0	0
Cast		4	3	4	0	2	3	0	0	0	0
minimal		4	3	4	0	2	3	0	0	0	0
Mineralization,tubular cell		2	1	1	1	0	1	1	0	1	0
minimal		2	1	1	1	0	1	1	0	1	0
Mineralization,papillary		0	0	0	0	1	0	2	1	0	1
minimal		0	0	0	0	1	0	2	1	0	1
Thrombus		0	0	0	0	0	0	1	0	0	0
minimal		0	0	0	0	0	0	1	0	0	0
Congestion/hemorrhage		0	0	1	0	0	0	1	0	0	0
minimal		0	0	1	0	0	0	1	0	0	0
Cell infiltrate,interstitial		2	3	1	0	2	0	1	2	0	0
minimal		1	3	1	0	2	0	1	2	0	0
mild		1	0	0	0	0	0	0	0	0	0
Fibrosis/scarring,focal		8	6	10	9	4	3	3	4	2	3
minimal		7	5	8	9	2	3	3	3	2	3
mild		1	1	2	0	2	0	0	1	0	0
Nephropathy,tubular		0	0	0	0	0	1	0	0	0	0
moderate		0	0	0	0	0	1	0	0	0	0
Arteritis		1	0	0	0	0	0	0	0	0	0
mild		1	0	0	0	0	0	0	0	0	0
Pyelitis		6	4	4	6	8	6	2	8	5	6
minimal		6	3	3	6	6	5	1	4	2	4
mild		0	1	1	0	2	1	1	4	3	2
Pyelonephritis		0	0	1	1	1	0	1	1	0	0
minimal		0	0	1	1	1	0	0	0	0	0
moderate		0	0	0	0	0	0	0	1	0	0
severe		0	0	0	0	0	0	1	0	0	0
Developmental anomaly		0	0	0	0	0	1	0	0	0	0
present		0	0	0	0	0	1	0	0	0	0
Deformity		0	0	0	0	0	0	0	0	0	1
present		0	0	0	0	0	0	0	0	0	1
Tumor infiltrate/metastasis		0	0	0	0	0	0	0	0	1	0
minimal		0	0	0	0	0	0	0	0	1	0

Tissue / Observation	Group:	1	2	3	4	5	1	2	3	4	5
	Sex:	M	M	M	M	M	F	F	F	F	F
	Number:	41	38	43	41	44	43	38	38	37	40
LARYNX											
Number examined		40	38	43	41	44	43	38	38	37	40
Hemorrhage, focal		0	0	0	0	0	1	0	0	0	0
minimal		0	0	0	0	0	1	0	0	0	0
Cell infiltrate, mucosal/submucosal		1	0	0	1	1	1	2	0	1	0
minimal		1	0	0	1	1	1	2	0	1	0
Inflammation		0	1	0	0	0	0	0	0	0	0
mild		0	1	0	0	0	0	0	0	0	0
Dilatation, submucosal gland		21	24	26	20	18	17	15	14	15	13
minimal		21	24	26	20	18	17	15	14	15	13
Foreign material		0	0	0	1	1	1	1	0	0	0
present		0	0	0	1	1	1	1	0	0	0
Exudate, inflammatory		0	0	0	0	1	0	0	0	0	0
minimal		0	0	0	0	1	0	0	0	0	0
Metaplasia, squamous cell		0	0	1	0	1	1	0	0	0	2
minimal		0	0	1	0	1	1	0	0	0	2
LYMPH NODE, BRONCHIAL											
Number examined		25	24	26	26	24	27	22	27	25	22
Pigmentation		0	0	0	0	1	0	0	0	0	0
minimal		0	0	0	0	1	0	0	0	0	0
Erythrocytosis, sinus		0	0	0	0	0	1	1	1	1	0
minimal		0	0	0	0	0	1	1	1	1	0
Ectasia, lymphatic		0	0	0	1	2	0	1	0	1	0
minimal		0	0	0	1	2	0	1	0	1	0
LIVER											
Number examined		41	38	43	41	44	43	38	38	37	40
Cyst		1	1	0	0	0	1	0	1	0	0
present		1	1	0	0	0	1	0	1	0	0
Cyst, biliary		0	0	2	0	1	1	0	1	0	2
present		0	0	2	0	1	1	0	1	0	2
Dilatation, sinusoidal		5	3	7	8	7	8	13	13	8	9
minimal		5	3	5	8	7	8	13	12	8	9
mild		0	0	2	0	0	0	0	1	0	0
Angiectasis		2	0	0	0	2	3	2	0	0	0
minimal		2	0	0	0	2	3	1	0	0	0
mild		0	0	0	0	0	0	1	0	0	0
Vacuolation, hepatocellular		0	0	0	1	1	0	3	1	0	0
minimal		0	0	0	1	1	0	3	1	0	0
Necrosis, focal		1	3	0	0	3	1	0	1	0	3
minimal		1	2	0	0	3	1	0	1	0	3
mild		0	1	0	0	0	0	0	0	0	0
Necrosis, single cell		0	0	1	0	1	0	0	0	0	0
minimal		0	0	0	0	1	0	0	0	0	0
mild		0	0	1	0	0	0	0	0	0	0
Pigment		0	0	0	0	1	0	1	0	0	0
minimal		0	0	0	0	1	0	1	0	0	0
Hematopoiesis, extramedullary		3	2	2	4	3	1	1	4	3	1

Tissue / Observation	Group:	1	2	3	4	5	1	2	3	4	5
	Sex:	M	M	M	M	M	F	F	F	F	F
	Number:	41	38	43	41	44	43	38	38	37	40
LIVER (continued)											
Hematopoiesis,extramedullary (continued)											
minimal		3	2	2	4	3	1	1	4	3	1
Microgranuloma		0	1	1	1	1	1	0	2	0	1
minimal		0	1	1	1	1	1	0	2	0	1
Fibrosis,capsular		0	0	0	0	1	0	0	0	1	0
minimal		0	0	0	0	1	0	0	0	0	0
mild		0	0	0	0	0	0	0	0	1	0
Fibrosis,focal		0	1	1	1	1	1	3	1	0	0
minimal		0	1	1	1	1	1	3	1	0	0
Cell infiltrate,peribiliary		4	0	0	0	2	0	0	0	0	0
minimal		4	0	0	0	2	0	0	0	0	0
Focus,vacuolated		0	1	0	1	1	0	0	1	2	0
minimal		0	1	0	1	1	0	0	1	2	0
Fibrosis,peribiliary		1	2	5	0	3	1	1	1	1	3
minimal		1	2	5	0	3	1	1	1	1	3
Hypertrophy,hepatocellular		0	0	0	0	1	0	0	1	0	1
minimal		0	0	0	0	1	0	0	1	0	1
Hyperplasia,bile ductular		17	9	10	19	22	10	11	10	6	14
minimal		15	9	10	19	21	9	11	10	6	13
mild		2	0	0	0	1	1	0	0	0	1
Hyperplasia,oval cell		0	0	0	1	0	0	0	0	0	0
minimal		0	0	0	1	0	0	0	0	0	0
Focus,basophilic cell		1	0	1	0	1	0	0	3	0	0
minimal		1	0	1	0	1	0	0	3	0	0
Focus,clear cell		1	1	3	0	2	1	0	0	0	0
minimal		1	1	3	0	2	1	0	0	0	0
Focus,eosinophilic cell		7	5	8	13	4	8	7	11	15	11
minimal		7	5	8	13	4	5	6	7	13	8
mild		0	0	0	0	0	2	1	4	1	3
moderate		0	0	0	0	0	1	0	0	0	0
severe		0	0	0	0	0	0	0	0	1	0
Focus,mixed cell		0	1	2	2	1	0	0	0	0	1
minimal		0	0	2	2	0	0	0	0	0	1
mild		0	1	0	0	1	0	0	0	0	0
Focus,basophilic,tigroid cell		10	9	7	7	14	11	8	9	9	16
present		0	0	0	0	0	0	1	0	0	0
minimal		10	9	7	7	14	11	7	8	9	15
mild		0	0	0	0	0	0	0	1	0	1
Tumor infiltrate/metastasis		1	0	0	0	0	0	1	0	2	0
minimal		1	0	0	0	0	0	0	0	0	0
mild		0	0	0	0	0	0	0	0	1	0
moderate		0	0	0	0	0	0	1	0	1	0
LYMPH NODE, MESENTERIC											
Number examined		41	38	43	41	44	43	38	38	37	40
Atrophy,lymphoid		6	4	4	8	9	5	4	5	5	7
minimal		2	4	3	3	3	3	3	2	2	3
mild		3	0	1	3	6	1	0	3	2	4

Tissue / Observation	Group:	1	2	3	4	5	1	2	3	4	5
	Sex:	M	M	M	M	M	F	F	F	F	F
	Number:	41	38	43	41	44	43	38	38	37	40
LYMPH NODE, MESENTERIC (continued)											
Atrophy,lymphoid (continued)											
moderate		1	0	0	2	0	1	1	0	1	0
Histiocytosis		39	38	36	40	40	40	33	36	33	36
minimal		39	38	36	39	40	39	33	35	33	36
mild		0	0	0	1	0	1	0	1	0	0
Fibrosis		0	0	0	0	0	0	0	0	1	1
minimal		0	0	0	0	0	0	0	0	1	1
Erythrocytosis,sinus		0	2	3	1	0	6	2	2	2	1
minimal		0	2	3	1	0	5	2	2	2	1
mild		0	0	0	0	0	1	0	0	0	0
Ectasia,lymphatic		1	0	1	1	0	0	0	0	0	1
minimal		1	0	1	1	0	0	0	0	0	0
mild		0	0	0	0	0	0	0	0	0	1
Hyperplasia,angiomatous		0	0	2	0	0	0	0	0	0	0
minimal		0	0	2	0	0	0	0	0	0	0
Hyperplasia,lymphoid		0	0	1	0	1	0	2	2	0	0
minimal		0	0	0	0	1	0	0	2	0	0
mild		0	0	1	0	0	0	1	0	0	0
moderate		0	0	0	0	0	0	1	0	0	0
LYMPH NODE, MANDIBULAR											
Number examined		32	38	40	40	41	37	36	37	34	40
Plasmacytosis		0	0	0	0	0	1	0	0	0	0
mild		0	0	0	0	0	1	0	0	0	0
Erythrocytosis,sinus		0	0	1	0	0	0	0	0	0	1
minimal		0	0	1	0	0	0	0	0	0	1
Ectasia,lymphatic		1	0	2	4	2	0	0	3	0	1
minimal		1	0	2	4	2	0	0	3	0	1
Tumor infiltrate/metastasis		0	0	0	0	1	0	0	0	0	0
moderate		0	0	0	0	1	0	0	0	0	0
LUNG											
Number examined		41	38	43	41	44	43	38	38	37	40
Mineralization,vascular		40	38	42	41	42	33	34	36	32	34
minimal		40	38	42	41	42	33	34	35	32	34
severe		0	0	0	0	0	0	0	1	0	0
Hemorrhage,focal		1	0	3	1	0	0	1	2	0	0
minimal		1	0	3	1	0	0	1	2	0	0
Thrombus		0	0	0	0	0	0	0	0	0	1
minimal		0	0	0	0	0	0	0	0	0	1
Embolus		0	0	0	0	1	0	0	0	0	0
mild		0	0	0	0	1	0	0	0	0	0
Histiocytosis,alveolar		21	18	28	23	26	20	20	22	17	16
minimal		20	18	28	21	25	20	19	22	17	14
mild		1	0	0	2	1	0	1	0	0	2
Granuloma		1	3	0	2	1	1	1	1	3	0
minimal		1	3	0	2	1	1	1	1	3	0
Granuloma,foreign body		0	0	0	0	0	0	0	1	0	0

Tissue / Observation	Group:	1	2	3	4	5	1	2	3	4	5
	Sex:	M	M	M	M	M	F	F	F	F	F
	Number:	41	38	43	41	44	43	38	38	37	40
LUNG (continued)											
Granuloma,foreign body (continued)											
minimal		0	0	0	0	0	0	0	1	0	0
Fibrosis,focal		0	0	0	0	2	0	0	0	1	0
minimal		0	0	0	0	2	0	0	0	1	0
Arteritis		0	0	1	0	0	0	0	0	0	0
minimal		0	0	1	0	0	0	0	0	0	0
Granuloma,cholesterol		2	0	2	1	2	1	1	2	0	0
minimal		2	0	2	1	2	1	1	2	0	0
Fibrosis,pleural		0	0	0	0	2	0	0	0	0	0
minimal		0	0	0	0	2	0	0	0	0	0
Mineralization,alveolar wall		1	2	3	4	4	3	7	0	0	3
minimal		1	2	3	4	4	3	7	0	0	3
Cell infiltrate,alveolar		0	0	0	1	0	0	0	0	1	0
minimal		0	0	0	1	0	0	0	0	1	0
Metaplasia,osseous		2	0	0	0	1	2	0	0	1	0
present		2	0	0	0	1	2	0	0	1	0
Hyperplasia,bronchiolo-alveolar		0	0	0	0	0	1	0	3	0	1
present		0	0	0	0	0	1	0	3	0	1
Hyperplasia,neuroendocrine,focal		0	0	0	0	1	0	0	0	0	0
present		0	0	0	0	1	0	0	0	0	0
Tumor infiltrate/metastasis		0	0	1	0	2	0	0	0	1	0
minimal		0	0	1	0	0	0	0	0	0	0
mild		0	0	0	0	2	0	0	0	1	0
LYMPH NODE											
Number examined		8	9	10	7	9	11	10	3	14	10
Plasmacytosis		2	3	6	2	5	1	1	0	2	0
minimal		0	0	0	0	0	0	0	0	1	0
mild		2	3	5	2	5	1	1	0	1	0
moderate		0	0	1	0	0	0	0	0	0	0
Erythrocytosis,sinus		1	0	0	0	0	1	0	0	3	2
minimal		1	0	0	0	0	1	0	0	3	2
Cell infiltrate,pericapsular		0	0	1	0	0	0	0	0	0	0
mild		0	0	1	0	0	0	0	0	0	0
Ectasia,lymphatic		6	7	4	4	5	3	2	0	2	2
minimal		1	2	1	1	3	1	2	0	2	2
mild		5	5	2	2	2	2	0	0	0	0
moderate		0	0	1	1	0	0	0	0	0	0
Cell infiltrate,sinus		1	0	0	0	0	0	0	0	0	0
minimal		1	0	0	0	0	0	0	0	0	0
Tumor infiltrate/metastasis		0	0	0	0	1	0	1	0	0	0
severe		0	0	0	0	1	0	1	0	0	0
MESENTERY											
Number examined		0	0	0	0	1	0	0	0	0	0
Necrosis,fat		0	0	0	0	1	0	0	0	0	0
mild		0	0	0	0	1	0	0	0	0	0

Tissue / Observation	Group:	1	2	3	4	5	1	2	3	4	5
	Sex:	M	M	M	M	M	F	F	F	F	F
	Number:	41	38	43	41	44	43	38	38	37	40
MAMMARY GLAND											
Number examined		39	37	37	37	42	42	35	38	37	38
Fibrosis,interstitial		0	0	1	0	0	0	0	0	0	0
moderate		0	0	1	0	0	0	0	0	0	0
Inflammation,chronic		0	1	0	0	0	0	0	0	0	0
mild		0	1	0	0	0	0	0	0	0	0
Cyst,ductal/alveolar		8	10	10	12	7	26	16	19	17	19
minimal		3	7	3	7	3	18	11	13	15	17
mild		5	3	7	5	4	7	5	6	2	2
moderate		0	0	0	0	0	1	0	0	0	0
Hyperplasia,lobular		0	0	0	0	0	7	6	10	5	4
present		0	0	0	0	0	0	0	0	1	0
minimal		0	0	0	0	0	5	4	5	3	3
mild		0	0	0	0	0	2	2	4	1	1
moderate		0	0	0	0	0	0	0	1	0	0
Hyperplasia,focal		0	0	1	0	0	5	3	1	6	4
present		0	0	0	0	0	5	3	1	6	4
minimal		0	0	1	0	0	0	0	0	0	0
Tumor infiltrate/metastasis		0	0	0	0	0	0	0	0	2	0
minimal		0	0	0	0	0	0	0	0	2	0
NASAL CAVITY (section 1)											
Number examined		41	38	43	41	44	43	38	38	37	40
Cell infiltrate,mucosal/submucosal		1	0	0	1	0	1	0	0	0	1
minimal		1	0	0	1	0	1	0	0	0	1
Inflammation,nasal cavity		2	0	1	1	3	1	2	0	0	1
minimal		2	0	1	1	1	0	1	0	0	0
mild		0	0	0	0	1	1	1	0	0	1
moderate		0	0	0	0	1	0	0	0	0	0
Inflammation,nasolacrimal duct		0	0	0	0	2	0	1	1	0	0
minimal		0	0	0	0	0	0	1	0	0	0
mild		0	0	0	0	1	0	0	1	0	0
moderate		0	0	0	0	1	0	0	0	0	0
Dilatation,submucosal gland		2	1	3	0	2	0	0	0	2	0
minimal		2	1	3	0	2	0	0	0	2	0
Droplets,eosinophilic		0	0	2	1	0	0	1	1	1	1
minimal		0	0	2	1	0	0	1	0	1	1
mild		0	0	0	0	0	0	0	1	0	0
Dilatation,diverticula		0	0	0	0	1	0	0	0	0	0
minimal		0	0	0	0	1	0	0	0	0	0
Exudate,inflammatory		0	1	0	0	3	0	0	0	1	1
minimal		0	1	0	0	3	0	0	0	1	1
Deformity		1	0	0	0	1	1	1	0	0	0
present		1	0	0	0	1	1	1	0	0	0
Exudate,proteinaceous		1	0	0	0	2	1	0	0	1	2
minimal		1	0	0	0	2	1	0	0	1	2
Hyperplasia,respiratory		0	0	0	0	0	0	1	0	0	0
minimal		0	0	0	0	0	0	1	0	0	0
Hyperplasia,goblet cell		16	22	16	17	13	9	22	18	12	14

Tissue / Observation	Group: 1	2	3	4	5	1	2	3	4	5
Sex:	M	M	M	M	M	F	F	F	F	F
Number:	41	38	43	41	44	43	38	38	37	40
NASAL CAVITY (section 1) (continued)										
Hyperplasia, goblet cell (continued)										
minimal	15	20	11	16	13	9	16	13	11	12
mild	1	2	5	1	0	0	6	5	1	2
Tumor infiltrate/metastasis	0	0	0	0	1	0	0	0	0	0
severe	0	0	0	0	1	0	0	0	0	0
NASOPHARYNX										
Number examined	41	38	43	41	44	43	38	38	37	40
Cell infiltrate, submucosal	0	0	0	1	1	0	0	0	0	1
minimal	0	0	0	1	1	0	0	0	0	1
Hyperplasia, goblet cell	0	0	0	0	0	0	0	1	0	0
minimal	0	0	0	0	0	0	0	1	0	0
ORAL CAVITY										
Number examined	2	0	2	0	1	4	1	0	0	1
Cyst, squamous	0	0	0	0	0	1	0	0	0	0
present	0	0	0	0	0	1	0	0	0	0
Inflammation	2	0	0	0	0	2	0	0	0	1
minimal	1	0	0	0	0	1	0	0	0	0
mild	1	0	0	0	0	1	0	0	0	1
Gingivitis	0	0	2	0	0	1	0	0	0	0
minimal	0	0	2	0	0	1	0	0	0	0
OVARY										
Number examined	-	-	-	-	-	43	38	38	36	40
Cyst	-	-	-	-	-	7	8	10	9	6
present	-	-	-	-	-	7	8	10	9	6
Cystic ovarian bursa	-	-	-	-	-	0	2	0	0	1
present	-	-	-	-	-	0	2	0	0	1
Atrophy	-	-	-	-	-	37	35	35	36	38
minimal	-	-	-	-	-	11	8	11	12	8
mild	-	-	-	-	-	12	17	14	14	10
moderate	-	-	-	-	-	14	10	10	10	19
severe	-	-	-	-	-	0	0	0	0	1
Hyperplasia, sex cord stromal	-	-	-	-	-	24	22	23	23	27
minimal	-	-	-	-	-	18	13	15	12	14
mild	-	-	-	-	-	5	6	7	7	11
moderate	-	-	-	-	-	1	3	1	4	2
Tumor infiltrate/metastasis	-	-	-	-	-	0	0	0	1	0
mild	-	-	-	-	-	0	0	0	1	0
PANCREAS										
Number examined	41	38	43	41	43	43	37	38	37	40
Dilatation, ductal	0	0	0	0	1	0	0	0	0	0
minimal	0	0	0	0	1	0	0	0	0	0
Fat infiltration	0	1	1	1	1	0	0	0	1	0
minimal	0	1	1	1	1	0	0	0	1	0
Atrophy, acinar, focal	14	5	4	13	4	8	10	4	7	6

Tissue / Observation	Group:	1	2	3	4	5	1	2	3	4	5
	Sex:	M	M	M	M	M	F	F	F	F	F
	Number:	41	38	43	41	44	43	38	38	37	40
PANCREAS (continued)											
Atrophy,acinar,focal (continued)											
minimal		13	5	4	13	4	8	9	3	6	6
mild		1	0	0	0	0	0	0	1	1	0
moderate		0	0	0	0	0	0	1	0	0	0
Mineralization,vascular		0	0	0	0	1	0	0	0	0	0
minimal		0	0	0	0	1	0	0	0	0	0
Cell infiltrate,interstitial		9	7	11	14	11	10	7	7	9	8
minimal		9	7	11	14	11	10	7	7	9	8
Cell infiltrate,perivascular		0	0	0	0	0	0	1	0	0	0
minimal		0	0	0	0	0	0	1	0	0	0
Inflammation		2	0	1	0	0	0	0	0	3	0
minimal		1	0	1	0	0	0	0	0	2	0
mild		1	0	0	0	0	0	0	0	1	0
Arteritis		1	0	0	0	1	0	0	0	0	0
mild		1	0	0	0	1	0	0	0	0	0
Zymogen granules,decreased		0	0	1	1	0	0	0	2	0	1
minimal		0	0	1	1	0	0	0	2	0	0
mild		0	0	0	0	0	0	0	0	0	1
Focus,hypertrophic,basophilic		2	1	6	4	4	6	1	3	3	2
minimal		2	1	6	4	4	6	1	3	3	2
Hyperplasia,intimal		0	0	0	1	0	0	0	0	0	0
mild		0	0	0	1	0	0	0	0	0	0
Hyperplasia,islet cell		0	3	1	2	2	0	0	0	0	1
present		0	3	1	2	2	0	0	0	0	1
Tumor infiltrate/metastasis		0	0	0	0	0	0	0	0	1	0
mild		0	0	0	0	0	0	0	0	1	0
PITUITARY											
Number examined		41	37	42	41	44	42	38	37	37	39
Cyst		0	1	1	0	2	0	1	0	1	1
present		0	1	1	0	2	0	1	0	1	1
Aberrant craniopharyngeal tissue		0	0	0	0	1	0	0	0	1	1
minimal		0	0	0	0	1	0	0	0	1	1
Gliosis		1	0	0	0	0	0	0	0	0	0
minimal		1	0	0	0	0	0	0	0	0	0
Accumulation,pigmented,macrophage		0	0	0	0	0	0	0	0	1	0
minimal		0	0	0	0	0	0	0	0	1	0
Hypertrophy,cellular		2	0	0	0	0	0	0	0	0	0
minimal		2	0	0	0	0	0	0	0	0	0
Hyperplasia,pars distalis,diffuse		0	0	0	0	2	0	2	3	0	1
minimal		0	0	0	0	2	0	2	2	0	0
mild		0	0	0	0	0	0	0	1	0	1
Hyperplasia,pars distalis,focal		22	19	20	18	22	10	11	12	17	16
present		22	19	20	18	22	10	11	12	17	16
Hyperplasia,pars intermedia,focal		0	0	0	0	0	0	1	0	0	0
present		0	0	0	0	0	0	1	0	0	0

Tissue / Observation	Group:	1	2	3	4	5	1	2	3	4	5
	Sex:	M	M	M	M	M	F	F	F	F	F
	Number:	41	38	43	41	44	43	38	38	37	40
PREPUTIAL GLAND											
Number examined		0	1	1	1	1	-	-	-	-	-
Inflammation		0	0	1	0	0	-	-	-	-	-
minimal		0	0	1	0	0	-	-	-	-	-
Ectasia, duct		0	1	1	1	1	-	-	-	-	-
minimal		0	0	0	1	0	-	-	-	-	-
mild		0	0	1	0	0	-	-	-	-	-
moderate		0	1	0	0	1	-	-	-	-	-
PROSTATE											
Number examined		41	38	43	41	44	-	-	-	-	-
Atrophy		1	1	1	0	4	-	-	-	-	-
minimal		0	0	1	0	2	-	-	-	-	-
mild		1	0	0	0	2	-	-	-	-	-
severe		0	1	0	0	0	-	-	-	-	-
Cell infiltrate, interstitial		3	2	4	3	5	-	-	-	-	-
minimal		3	2	4	3	5	-	-	-	-	-
Prostatitis		17	21	24	22	22	-	-	-	-	-
minimal		13	14	15	14	12	-	-	-	-	-
mild		4	3	8	6	8	-	-	-	-	-
moderate		0	4	1	2	2	-	-	-	-	-
Hyperplasia, acinar cell, focal		1	0	1	0	1	-	-	-	-	-
present		1	0	1	0	1	-	-	-	-	-
Tumor infiltrate/metastasis		0	0	0	0	1	-	-	-	-	-
minimal		0	0	0	0	1	-	-	-	-	-
PARATHYROID											
Number examined		37	31	29	34	39	33	28	30	29	35
Hyperplasia, focal		2	4	0	7	6	1	0	0	1	2
present		2	4	0	7	6	1	0	0	1	2
RECTUM											
Number examined		41	38	43	41	43	43	38	38	37	40
Inflammation		0	0	0	1	0	0	0	0	0	0
mild		0	0	0	1	0	0	0	0	0	0
Arteritis		0	0	0	1	0	0	0	0	0	0
mild		0	0	0	1	0	0	0	0	0	0
Erosion/ulcer, mucosal		0	0	0	0	1	0	0	0	0	0
moderate		0	0	0	0	1	0	0	0	0	0
Inflammation, serosal		0	0	1	1	0	0	0	0	0	0
minimal		0	0	0	1	0	0	0	0	0	0
mild		0	0	1	0	0	0	0	0	0	0
SPINAL CORD											
Number examined		41	38	43	41	44	43	38	38	37	40
Necrosis		0	0	0	0	0	1	0	0	0	0
mild		0	0	0	0	0	1	0	0	0	0
Gliosis		0	0	0	2	0	0	0	0	0	0
minimal		0	0	0	2	0	0	0	0	0	0

Tissue / Observation	Group:	1	2	3	4	5	1	2	3	4	5
	Sex:	M	M	M	M	M	F	F	F	F	F
	Number:	41	38	43	41	44	43	38	38	37	40
SPINAL CORD (continued)											
Cell infiltrate, focal		0	0	0	1	0	0	0	0	0	0
minimal		0	0	0	1	0	0	0	0	0	0
Radiculoneuropathy		0	0	0	2	0	0	1	0	0	1
minimal		0	0	0	2	0	0	1	0	0	1
Tumor infiltrate/metastasis		0	0	0	1	1	0	0	0	0	0
minimal		0	0	0	1	1	0	0	0	0	0
SMALL INTESTINE, DUODENUM											
Number examined		41	38	43	41	44	43	38	38	37	40
Congestion/hemorrhage, agonal		0	0	0	0	0	0	0	0	1	0
present		0	0	0	0	0	0	0	0	1	0
Hyperplasia, regenerative		0	0	0	0	0	0	0	0	1	0
minimal		0	0	0	0	0	0	0	0	1	0
SALIVARY GLAND											
Number examined		41	38	43	41	44	42	38	38	37	40
Atrophy, acinar, diffuse		0	0	0	0	0	0	1	0	0	0
mild		0	0	0	0	0	0	1	0	0	0
SMALL INTESTINE, ILEUM											
Number examined		41	38	43	41	44	42	38	38	37	40
Inflammation		0	0	1	1	0	0	0	0	0	0
minimal		0	0	0	1	0	0	0	0	0	0
mild		0	0	1	0	0	0	0	0	0	0
SMALL INTESTINE, JEJUNUM											
Number examined		41	38	43	41	44	43	38	38	37	40
Cell infiltrate, serosal		0	0	0	1	0	0	0	0	0	0
minimal		0	0	0	1	0	0	0	0	0	0
SKELETAL MUSCLE											
Number examined		41	38	43	41	44	43	38	38	37	40
Mineralization, muscular		0	0	0	0	0	0	1	0	0	0
minimal		0	0	0	0	0	0	1	0	0	0
Cell infiltrate, muscular		0	1	1	0	0	2	0	2	1	2
minimal		0	1	1	0	0	2	0	2	1	2
Degeneration/atrophy, muscle fiber		9	6	4	5	11	13	7	7	8	4
minimal		9	5	4	4	11	13	7	7	8	4
mild		0	1	0	1	0	0	0	0	0	0
Tumor infiltrate/metastasis		0	0	0	0	0	0	0	0	2	0
minimal		0	0	0	0	0	0	0	0	1	0
mild		0	0	0	0	0	0	0	0	1	0
SCIATIC NERVE											
Number examined		41	38	43	41	44	43	38	38	37	40
Mineralization, vascular		0	0	1	0	1	1	0	0	1	1
minimal		0	0	1	0	1	1	0	0	1	1
Cell infiltrate, perivascular		0	0	0	0	0	0	0	0	1	0

Tissue / Observation	Group:	1	2	3	4	5	1	2	3	4	5
	Sex:	M	M	M	M	M	F	F	F	F	F
	Number:	41	38	43	41	44	43	38	38	37	40
SCIATIC NERVE (continued)											
Cell infiltrate,perivascular (continued)											
minimal		0	0	0	0	0	0	0	0	1	0
Neuropathy		2	0	2	0	1	2	1	1	1	1
minimal		2	0	1	0	0	2	1	1	1	1
mild		0	0	1	0	1	0	0	0	0	0
Tumor infiltrate/metastasis		0	0	0	0	0	0	0	0	1	0
minimal		0	0	0	0	0	0	0	0	1	0
SPLEEN											
Number examined		41	38	43	41	44	43	37	38	37	40
Atrophy		0	0	0	0	0	0	0	1	0	0
minimal		0	0	0	0	0	0	0	1	0	0
Atrophy,lymphoid		0	0	1	1	2	0	0	0	1	0
minimal		0	0	0	1	0	0	0	0	0	0
mild		0	0	1	0	2	0	0	0	1	0
Hematopoiesis,increased		17	15	17	13	16	6	3	6	7	4
minimal		14	13	16	10	12	5	3	3	6	4
mild		2	2	1	2	4	1	0	3	0	0
moderate		1	0	0	1	0	0	0	0	1	0
Granuloma		0	0	1	0	0	0	0	0	0	0
minimal		0	0	1	0	0	0	0	0	0	0
Fibrosis,capsular		2	2	2	4	1	0	1	0	4	0
minimal		2	2	2	4	1	0	1	0	4	0
Inflammation,chronic		1	0	0	1	0	0	0	0	0	0
mild		1	0	0	1	0	0	0	0	0	0
Pigmentation,increased		2	7	5	5	4	12	11	6	11	9
minimal		2	7	5	4	4	11	11	6	11	9
mild		0	0	0	1	0	1	0	0	0	0
Inflammation,capsular		0	0	0	0	0	0	0	0	1	0
minimal		0	0	0	0	0	0	0	0	1	0
Tumor infiltrate/metastasis		0	0	0	0	0	0	1	0	0	0
minimal		0	0	0	0	0	0	1	0	0	0
STERNUM & MARROW											
Number examined		41	37	43	41	44	43	37	38	37	40
Cyst		1	0	0	0	0	0	0	0	0	0
present		1	0	0	0	0	0	0	0	0	0
Fat infiltration		2	0	4	0	2	3	0	2	0	2
minimal		2	0	4	0	2	3	0	2	0	2
Degeneration,chondromucinous		12	7	9	13	9	16	14	19	13	17
minimal		12	7	9	13	8	16	14	19	13	17
mild		0	0	0	0	1	0	0	0	0	0
Hypercellularity,marrow		21	18	23	19	22	7	4	7	7	6
minimal		12	10	11	8	7	5	2	5	7	5
mild		9	8	12	10	14	2	2	2	0	1
moderate		0	0	0	1	1	0	0	0	0	0
Granuloma		0	0	1	0	0	0	0	0	0	0
minimal		0	0	1	0	0	0	0	0	0	0

Tissue / Observation	Group:	1	2	3	4	5	1	2	3	4	5
	Sex:	M	M	M	M	M	F	F	F	F	F
	Number:	41	38	43	41	44	43	38	38	37	40
STERNUM & MARROW (continued)											
Tumor infiltrate/metastasis		1	0	0	0	0	0	0	0	1	0
minimal		0	0	0	0	0	0	0	0	1	0
mild		1	0	0	0	0	0	0	0	0	0
SKIN & SUBCUTIS											
Number examined		41	38	43	41	44	43	38	38	37	40
Cyst,epidermal		0	1	0	0	0	0	0	0	0	0
non-fatal		0	1	0	0	0	0	0	0	0	0
Atrophy,follicular		0	0	0	0	0	0	0	1	0	0
mild		0	0	0	0	0	0	0	1	0	0
Abscess		1	0	0	0	0	0	0	0	0	0
mild		1	0	0	0	0	0	0	0	0	0
Ulcer		1	0	0	0	0	0	0	0	0	0
mild		1	0	0	0	0	0	0	0	0	0
Inflammation		0	0	1	0	0	0	0	0	1	1
minimal		0	0	1	0	0	0	0	0	0	1
mild		0	0	0	0	0	0	0	0	1	0
Polliculitis		1	0	0	0	0	0	0	0	0	0
minimal		1	0	0	0	0	0	0	0	0	0
Acanthosis		0	1	0	0	0	0	0	0	0	0
minimal		0	1	0	0	0	0	0	0	0	0
Tumor infiltrate/metastasis		0	0	0	0	1	0	1	0	2	0
mild		0	0	0	0	1	0	0	0	2	0
moderate		0	0	0	0	0	0	1	0	0	0
STOMACH											
Number examined		41	38	43	41	44	43	38	38	37	40
Cyst,squamous		0	1	0	1	0	0	0	0	0	0
present		0	1	0	1	0	0	0	0	0	0
Dilatation,glandular		23	15	19	19	19	27	21	19	19	19
minimal		23	15	19	19	19	27	21	19	19	19
Abscess,crypt		0	0	0	0	0	0	0	0	1	0
minimal		0	0	0	0	0	0	0	0	1	0
Hyperplasia,regenerative,focal		1	3	2	4	3	1	2	5	0	0
minimal		1	3	2	4	3	1	2	5	0	0
Edema,submucosal		0	0	1	0	0	0	0	0	1	0
minimal		0	0	1	0	0	0	0	0	0	0
mild		0	0	0	0	0	0	0	0	1	0
Cell infiltrate,mucosal/submucosal		1	0	0	0	1	0	0	1	2	0
minimal		0	0	0	0	1	0	0	1	2	0
severe		1	0	0	0	0	0	0	0	0	0
Cell infiltrate,serosal		0	0	0	1	0	0	0	0	1	0
minimal		0	0	0	1	0	0	0	0	1	0
Fibrosis,focal		0	0	0	0	1	0	0	0	1	0
minimal		0	0	0	0	1	0	0	0	1	0
Congestion/hemorrhage,agonal		0	0	0	0	0	0	0	0	1	0
present		0	0	0	0	0	0	0	0	1	0
Erosion/Ulcer,glandular stomach		2	0	1	1	1	1	1	3	0	2

Tissue / Observation	Group:	1	2	3	4	5	1	2	3	4	5
	Sex:	M	M	M	M	M	F	F	F	F	F
	Number:	41	38	43	41	44	43	38	38	37	40
STOMACH (continued)											
Erosion/Ulcer,glandular stomach (continued)											
minimal		2	0	1	1	1	1	1	2	0	2
mild		0	0	0	0	0	0	0	1	0	0
Hyperplasia,limiting ridge		0	2	1	2	0	1	0	1	1	3
minimal		0	2	1	1	0	1	0	0	1	3
mild		0	0	0	1	0	0	0	1	0	0
Fibrosis,lamina propria		14	10	13	9	10	9	12	7	6	9
minimal		14	10	13	9	10	9	12	7	6	9
Inflammation,non-glandular		0	0	0	0	0	0	0	0	0	1
minimal		0	0	0	0	0	0	0	0	0	1
Hyperplasia,basal cell,focal		0	0	0	1	0	0	0	0	0	0
minimal		0	0	0	1	0	0	0	0	0	0
Tumor infiltrate/metastasis		0	0	0	0	0	0	0	0	1	0
minimal		0	0	0	0	0	0	0	0	1	0
SEMINAL VESICLE											
Number examined		41	38	43	41	44	-	-	-	-	-
Atrophy		0	2	0	1	0	-	-	-	-	-
minimal		0	0	0	1	0	-	-	-	-	-
mild		0	1	0	0	0	-	-	-	-	-
severe		0	1	0	0	0	-	-	-	-	-
Vesiculitis		0	5	0	2	1	-	-	-	-	-
minimal		0	3	0	1	0	-	-	-	-	-
mild		0	2	0	1	1	-	-	-	-	-
Hyperplasia,focal		0	1	0	0	0	-	-	-	-	-
present		0	1	0	0	0	-	-	-	-	-
Tumor infiltrate/metastasis		0	0	0	0	1	-	-	-	-	-
minimal		0	0	0	0	1	-	-	-	-	-
TAIL											
Number examined		1	1	0	0	0	1	0	0	0	0
Abscess		0	0	0	0	0	1	0	0	0	0
minimal		0	0	0	0	0	1	0	0	0	0
Inflammation		1	0	0	0	0	1	0	0	0	0
minimal		0	0	0	0	0	1	0	0	0	0
mild		1	0	0	0	0	0	0	0	0	0
Acanthosis		0	1	0	0	0	0	0	0	0	0
minimal		0	1	0	0	0	0	0	0	0	0
THYMUS											
Number examined		32	25	28	28	31	33	33	33	29	28
Cyst		0	0	0	0	0	4	0	2	0	0
present		0	0	0	0	0	4	0	2	0	0
Involution		32	25	28	28	30	33	31	33	28	27
minimal		0	0	0	0	1	0	0	0	1	0
mild		3	1	4	0	1	8	7	3	8	5
moderate		29	24	24	24	26	25	24	30	19	22
severe		0	0	0	4	2	0	0	0	0	0

Tissue / Observation	Group:	1	2	3	4	5	1	2	3	4	5
	Sex:	M	M	M	M	M	F	F	F	F	F
	Number:	41	38	43	41	44	43	38	38	37	40
THYMUS (continued)											
Hyperplasia,epithelial		1	3	4	1	4	14	13	15	12	12
minimal		1	3	4	1	4	10	12	12	10	10
mild		0	0	0	0	0	4	1	3	2	2
Tumor infiltrate/metastasis		0	0	0	0	1	0	0	0	0	0
severe		0	0	0	0	1	0	0	0	0	0
TONGUE											
Number examined		41	38	43	41	44	43	38	38	37	40
Mineralization,vascular		1	0	0	0	0	0	0	0	0	0
minimal		1	0	0	0	0	0	0	0	0	0
Erosion/ulcer		0	0	0	0	0	0	1	0	0	0
minimal		0	0	0	0	0	0	1	0	0	0
Arteritis		0	0	0	0	0	0	0	0	1	0
minimal		0	0	0	0	0	0	0	0	1	0
TRACHEA											
Number examined		41	38	43	41	44	43	37	38	37	40
Tumor infiltrate/metastasis		0	0	0	0	1	0	0	0	0	1
minimal		0	0	0	0	1	0	0	0	0	0
mild		0	0	0	0	0	0	0	0	0	1
TESTIS											
Number examined		41	38	43	41	44	-	-	-	-	-
Dilatation,rete testis		1	0	0	0	0	-	-	-	-	-
minimal		1	0	0	0	0	-	-	-	-	-
Atrophy,seminiferous tubular		16	11	11	10	11	-	-	-	-	-
minimal		5	3	4	2	0	-	-	-	-	-
mild		1	2	2	1	3	-	-	-	-	-
moderate		7	2	4	4	8	-	-	-	-	-
severe		3	4	1	3	0	-	-	-	-	-
Mineralization,vascular		1	0	0	0	0	-	-	-	-	-
minimal		1	0	0	0	0	-	-	-	-	-
Arteritis		0	0	1	1	0	-	-	-	-	-
minimal		0	0	1	1	0	-	-	-	-	-
Stasis,sperm		5	8	4	4	5	-	-	-	-	-
minimal		5	8	4	4	4	-	-	-	-	-
mild		0	0	0	0	1	-	-	-	-	-
Hyperplasia,Leydig cell,focal		9	5	7	9	3	-	-	-	-	-
present		9	5	7	9	3	-	-	-	-	-
Tumor infiltrate/metastasis		0	0	0	0	1	-	-	-	-	-
mild		0	0	0	0	1	-	-	-	-	-
TOOTH											
Number examined		3	7	5	5	8	9	7	10	6	2
Cell infiltrate,neutrophilic		0	0	0	0	0	0	0	0	1	0
minimal		0	0	0	0	0	0	0	0	1	0
Periodontitis		2	7	3	5	6	7	6	9	5	1
minimal		1	6	2	5	3	6	4	8	4	0

Tissue / Observation	Group:	1	2	3	4	5	1	2	3	4	5
	Sex:	M	M	M	M	M	F	F	F	F	F
	Number:	41	38	43	41	44	43	38	38	37	40
TOOTH (continued)											
Periodontitis (continued)											
mild		1	1	1	0	0	1	1	1	1	0
moderate		0	0	0	0	2	0	0	0	0	1
severe		0	0	0	0	1	0	1	0	0	0
Dysplasia,dental		1	0	4	0	3	2	2	1	2	2
minimal		0	0	3	0	1	1	2	0	2	1
mild		1	0	1	0	2	1	0	1	0	1
THYROID LOBE											
Number examined		41	38	43	41	44	43	38	38	37	40
Cystic follicle		0	0	0	0	0	0	2	0	0	0
minimal		0	0	0	0	0	0	2	0	0	0
Cell infiltrate,interstitial		0	0	1	0	0	0	0	0	0	0
minimal		0	0	1	0	0	0	0	0	0	0
Dilated follicle		1	2	2	0	1	1	1	0	0	0
minimal		1	2	2	0	1	1	1	0	0	0
Hyperplasia,C-cell,diffuse		20	22	23	19	24	24	21	25	23	22
minimal		20	21	23	19	24	23	21	25	23	22
mild		0	1	0	0	0	1	0	0	0	0
Hyperplasia,C-cell,focal		4	2	5	3	6	2	1	4	3	3
present		4	2	5	3	6	2	1	4	3	3
Hyperplasia,follicular cell,focal		0	0	0	0	2	0	0	0	0	2
present		0	0	0	0	2	0	0	0	0	2
URINARY BLADDER											
Number examined		41	38	43	41	44	43	38	37	37	40
Calculus		0	1	0	0	1	0	0	0	0	0
present		0	1	0	0	0	0	0	0	0	0
minimal		0	0	0	0	1	0	0	0	0	0
Dilatation,luminal		2	1	4	5	1	4	2	3	0	5
minimal		1	0	1	3	0	2	2	2	0	4
mild		1	0	3	2	1	2	0	1	0	1
moderate		0	1	0	0	0	0	0	0	0	0
Edema,submucosal		0	0	0	0	0	1	0	0	0	1
minimal		0	0	0	0	0	1	0	0	0	1
Cell infiltrate,mucosal/submucosal		1	2	1	2	1	1	0	1	0	5
minimal		1	2	1	2	1	1	0	1	0	5
Cystitis		0	2	1	0	1	3	3	8	4	2
minimal		0	0	1	0	1	2	0	1	2	1
mild		0	0	0	0	0	0	2	2	0	1
moderate		0	2	0	0	0	1	1	5	2	0
Erosion,mucosal		0	0	0	0	1	0	0	0	0	0
minimal		0	0	0	0	1	0	0	0	0	0
Cell infiltrate,adventitial		0	0	0	1	0	0	0	0	0	0
mild		0	0	0	1	0	0	0	0	0	0
Hyperplasia,urothelial		1	0	1	0	1	0	0	0	0	1
minimal		0	0	0	0	0	0	0	0	0	1
mild		1	0	1	0	1	0	0	0	0	0

Tissue / Observation	Group:	1	2	3	4	5	1	2	3	4	5
	Sex:	M	M	M	M	M	F	F	F	F	F
	Number:	41	38	43	41	44	43	38	38	37	40
URINARY BLADDER (continued)											
Tumor infiltrate/metastasis		0	0	0	0	0	0	0	0	2	0
minimal		0	0	0	0	0	0	0	0	1	0
mild		0	0	0	0	0	0	0	0	1	0
URETER											
Number examined		0	0	0	0	0	0	1	0	0	0
Urethritis		0	0	0	0	0	0	1	0	0	0
minimal		0	0	0	0	0	0	1	0	0	0
UTERUS											
Number examined		-	-	-	-	-	43	38	38	37	39
Dilatation,luminal		-	-	-	-	-	3	3	2	2	4
minimal		-	-	-	-	-	3	2	1	2	2
mild		-	-	-	-	-	0	1	0	0	2
moderate		-	-	-	-	-	0	0	1	0	0
Atrophy		-	-	-	-	-	0	0	1	0	0
mild		-	-	-	-	-	0	0	1	0	0
Cell infiltrate,muscular		-	-	-	-	-	0	1	0	2	1
minimal		-	-	-	-	-	0	1	0	2	1
Endometritis		-	-	-	-	-	0	1	1	1	0
minimal		-	-	-	-	-	0	0	0	1	0
mild		-	-	-	-	-	0	1	0	0	0
moderate		-	-	-	-	-	0	0	1	0	0
Cystic gland		-	-	-	-	-	3	0	1	5	6
minimal		-	-	-	-	-	3	0	1	4	4
mild		-	-	-	-	-	0	0	0	1	2
Hyperplasia,glandular		-	-	-	-	-	0	0	0	1	0
mild		-	-	-	-	-	0	0	0	1	0
Hypertrophy,myometrial		-	-	-	-	-	0	1	0	0	0
mild		-	-	-	-	-	0	1	0	0	0
Hyperplasia,endometrial,cystic		-	-	-	-	-	0	0	1	1	0
minimal		-	-	-	-	-	0	0	1	1	0
VAGINA											
Number examined		-	-	-	-	-	43	38	38	37	39
Mucification		-	-	-	-	-	13	12	15	15	14
minimal		-	-	-	-	-	6	10	11	13	11
mild		-	-	-	-	-	7	2	4	2	3
Atrophy		-	-	-	-	-	18	12	8	8	11
minimal		-	-	-	-	-	4	3	2	0	0
mild		-	-	-	-	-	4	4	1	5	4
moderate		-	-	-	-	-	8	2	5	2	4
severe		-	-	-	-	-	2	3	0	1	3
NASAL CAVITY (section 2)											
Number examined		41	38	43	41	44	43	38	38	37	40
Atrophy,olfactory		1	1	0	0	0	0	0	0	0	0
minimal		1	0	0	0	0	0	0	0	0	0

Tissue / Observation	Group:	1	2	3	4	5	1	2	3	4	5
	Sex:	M	M	M	M	M	F	F	F	F	F
	Number:	41	38	43	41	44	43	38	38	37	40
NASAL CAVITY (section 2) (continued)											
Atrophy,olfactory (continued)											
mild		0	1	0	0	0	0	0	0	0	0
Cell infiltrate,mucosal/submucosal		1	0	0	0	0	0	0	0	0	0
minimal		1	0	0	0	0	0	0	0	0	0
Inflammation,nasal cavity		0	0	0	1	2	1	0	0	0	0
minimal		0	0	0	1	0	0	0	0	0	0
mild		0	0	0	0	0	1	0	0	0	0
moderate		0	0	0	0	2	0	0	0	0	0
Inflammation,submucosal gland		0	0	0	0	0	0	0	1	0	0
minimal		0	0	0	0	0	0	0	1	0	0
Inflammation,nasolacrimal duct		0	0	0	0	2	0	2	1	1	0
minimal		0	0	0	0	0	0	1	1	0	0
mild		0	0	0	0	1	0	1	0	0	0
moderate		0	0	0	0	1	0	0	0	1	0
Dilatation,submucosal gland		16	15	14	21	16	14	11	8	11	10
minimal		16	15	14	21	16	14	11	8	11	10
Droplets,eosinophilic		16	16	29	15	12	16	16	32	19	21
minimal		16	16	28	15	12	15	16	29	19	21
mild		0	0	1	0	0	1	0	3	0	0
Dilatation,diverticula		1	0	0	0	0	0	0	0	0	1
minimal		0	0	0	0	0	0	0	0	0	1
mild		1	0	0	0	0	0	0	0	0	0
Exudate,inflammatory		3	5	8	1	4	3	1	1	3	4
minimal		3	5	8	1	4	3	1	1	3	4
Deformity		1	0	0	0	1	1	1	0	0	0
present		1	0	0	0	1	1	1	0	0	0
Exudate,proteinaceous		3	1	1	1	2	0	1	2	0	1
minimal		3	1	1	1	2	0	1	2	0	1
Hyperplasia,respiratory		0	0	0	0	0	0	1	0	0	0
minimal		0	0	0	0	0	0	1	0	0	0
Hyperplasia,goblet cell		1	2	2	1	0	1	3	3	1	2
minimal		1	2	2	1	0	1	3	2	1	2
mild		0	0	0	0	0	0	0	1	0	0
Tumor infiltrate/metastasis		0	0	0	0	1	0	0	0	0	0
severe		0	0	0	0	1	0	0	0	0	0
NASAL CAVITY (section 3)											
Number examined		41	38	43	41	44	43	38	38	37	40
Atrophy,olfactory		0	1	0	0	0	0	0	0	0	0
mild		0	1	0	0	0	0	0	0	0	0
Inflammation,nasal cavity		0	0	0	0	0	1	0	0	0	0
minimal		0	0	0	0	0	1	0	0	0	0
Inflammation,nasolacrimal duct		0	0	0	0	1	0	0	0	0	0
mild		0	0	0	0	1	0	0	0	0	0
Cellular debris,ductal		1	0	0	0	0	0	0	0	0	0
minimal		1	0	0	0	0	0	0	0	0	0
Dilatation,submucosal gland		28	23	33	28	29	27	22	21	24	19
minimal		28	23	33	28	29	27	22	21	24	19

Tissue / Observation	Group:	1	2	3	4	5	1	2	3	4	5
	Sex:	M	M	M	M	M	F	F	F	F	F
	Number:	41	38	43	41	44	43	38	38	37	40
NASAL CAVITY (section 3) (continued)											
Neuropathy		0	1	0	0	0	0	0	0	0	0
minimal		0	1	0	0	0	0	0	0	0	0
Droplets, eosinophilic		41	38	43	40	43	42	37	38	37	40
minimal		39	25	12	39	41	42	32	23	36	39
mild		2	13	30	1	2	0	5	15	1	1
moderate		0	0	1	0	0	0	0	0	0	0
Exudate, inflammatory		0	0	0	0	0	2	0	0	0	0
minimal		0	0	0	0	0	2	0	0	0	0
Exudate, proteinaceous		1	0	0	0	0	0	0	1	0	0
minimal		1	0	0	0	0	0	0	1	0	0
Hyperplasia, goblet cell		2	6	16	4	1	2	6	16	3	3
minimal		2	4	6	3	0	2	6	10	3	3
mild		0	2	10	1	1	0	0	6	0	0
Tumor infiltrate/metastasis		0	0	0	0	1	0	0	0	0	0
mild		0	0	0	0	1	0	0	0	0	0
NASAL CAVITY (section 4)											
Number examined		41	38	43	41	44	43	38	38	37	40
Atrophy, olfactory		0	1	0	0	0	0	0	0	0	0
mild		0	1	0	0	0	0	0	0	0	0
Dilatation, submucosal gland		0	1	0	0	0	0	0	0	0	0
minimal		0	1	0	0	0	0	0	0	0	0
Droplets, eosinophilic		40	37	43	41	44	40	38	37	35	39
minimal		40	34	22	38	44	40	37	32	35	38
mild		0	3	20	3	0	0	1	5	0	1
moderate		0	0	1	0	0	0	0	0	0	0
Exudate, proteinaceous		0	0	0	0	0	1	0	0	0	0
minimal		0	0	0	0	0	1	0	0	0	0
Hyperplasia, goblet cell		1	5	17	3	2	1	5	17	3	3
minimal		1	5	6	3	1	1	5	13	3	3
mild		0	0	11	0	1	0	0	4	0	0
Tumor infiltrate/metastasis		0	0	0	0	1	0	0	0	0	0
minimal		0	0	0	0	1	0	0	0	0	0

Non-Tumor Histopathology on Animals Found Dead Prior to the End of the Study

GROUP INCIDENCE: HISTOPATHOLOGY - NON-TUMOUR DATA

Kill type: PRETERMINAL SACRIFICE FOUND DEAD

Tissue / Observation	Group:	1	2	3	4	5	1	2	3	4	5
	Sex:	M	M	M	M	M	F	F	F	F	F
	Number:	19	22	17	19	16	17	22	22	23	20
ABDOMINAL CAVITY											
Number examined		1	1	0	1	0	0	0	1	0	0
Necrosis,fat		0	0	0	1	0	0	0	0	0	0
moderate		0	0	0	1	0	0	0	0	0	0
Tumor infiltrate/metastasis		1	0	0	0	0	0	0	1	0	0
moderate		1	0	0	0	0	0	0	1	0	0
ADRENAL											
Number examined		19	22	17	19	16	17	22	22	23	20
Angiectasis		4	7	3	4	2	14	15	17	16	14
minimal		3	6	3	4	2	14	15	13	11	10
mild		1	1	0	0	0	0	0	4	5	4
Atrophy,cortical		0	0	0	0	1	0	0	0	0	0
severe		0	0	0	0	1	0	0	0	0	0
Vacuolation,cortical,diffuse		0	1	0	1	0	0	0	0	0	0
minimal		0	1	0	1	0	0	0	0	0	0
Degeneration,cystic		1	1	1	0	1	7	12	9	10	7
minimal		0	0	1	0	1	3	5	9	6	5
mild		0	1	0	0	0	3	5	0	4	2
moderate		1	0	0	0	0	0	2	0	0	0
severe		0	0	0	0	0	1	0	0	0	0
Necrosis,focal		0	0	0	0	0	0	1	1	0	0
minimal		0	0	0	0	0	0	1	1	0	0
Mineralization		0	0	0	0	0	0	1	0	0	1
minimal		0	0	0	0	0	0	1	0	0	0
mild		0	0	0	0	0	0	0	0	0	1
Hematopoiesis,extramedullary		0	0	1	1	0	1	4	2	0	2
minimal		0	0	1	1	0	1	3	1	0	1
mild		0	0	0	0	0	0	1	1	0	1
Hypertrophy,zona glomerulosa,focal		9	13	12	7	5	5	8	11	14	7
minimal		9	13	12	7	5	5	8	11	14	7
Vacuolation,zona fasciculata,focal		5	0	1	0	0	0	2	1	1	0
minimal		5	0	1	0	0	0	2	1	1	0
Pigment,corticomedullary		11	17	7	9	7	8	16	11	9	5
minimal		11	17	7	9	7	8	15	11	9	5
mild		0	0	0	0	0	0	1	0	0	0
Congestion/hemorrhage,agonal		3	2	0	2	2	0	2	0	0	0
present		3	2	0	2	2	0	2	0	0	0
Hypertrophy,cortical,focal		2	5	4	3	3	5	4	9	11	9
minimal		2	5	4	3	3	5	4	5	10	7
mild		0	0	0	0	0	0	0	4	1	2
Hyperplasia,cortical,focal		1	0	1	0	1	1	1	0	0	0
present		1	0	1	0	1	1	1	0	0	0
Hyperplasia,medullary,focal		1	1	1	0	0	0	0	1	0	0
present		1	1	1	0	0	0	0	1	0	0
Tumor infiltrate/metastasis		1	0	0	1	0	0	1	0	2	0
minimal		1	0	0	1	0	0	0	0	1	0
mild		0	0	0	0	0	0	1	0	0	0
moderate		0	0	0	0	0	0	0	0	1	0

Tissue / Observation	Group:	1	2	3	4	5	1	2	3	4	5
	Sex:	M	M	M	M	M	F	F	F	F	F
	Number:	19	22	17	19	16	17	22	22	23	20
AORTA											
Number examined		19	22	17	19	16	17	22	22	23	20
Mineralization		2	0	1	0	1	0	1	0	0	0
minimal		1	0	0	0	0	0	1	0	0	0
mild		1	0	1	0	0	0	0	0	0	0
severe		0	0	0	0	1	0	0	0	0	0
Tumor infiltrate/metastasis		0	0	1	0	0	0	0	0	0	0
minimal		0	0	1	0	0	0	0	0	0	0
BRAIN											
Number examined		19	22	17	19	16	17	22	22	23	20
Dilatation,ventricular		2	2	3	6	3	8	7	9	7	6
minimal		1	1	1	4	2	6	6	5	2	1
mild		1	1	2	2	1	2	1	4	5	5
Gliosis		0	0	0	0	0	1	0	0	0	0
minimal		0	0	0	0	0	1	0	0	0	0
Mineralization		1	1	0	1	1	0	0	0	1	0
minimal		1	1	0	1	1	0	0	0	1	0
Hemorrhage,focal		0	0	0	1	0	2	0	4	0	0
minimal		0	0	0	0	0	1	0	4	0	0
mild		0	0	0	0	0	1	0	0	0	0
moderate		0	0	0	1	0	0	0	0	0	0
Fibrosis,meningeal		0	1	0	0	0	0	0	0	0	0
minimal		0	1	0	0	0	0	0	0	0	0
Inflammation		0	0	0	1	0	0	0	0	0	0
mild		0	0	0	1	0	0	0	0	0	0
Malacia/glial scar		0	1	0	0	0	0	0	0	0	0
minimal		0	1	0	0	0	0	0	0	0	0
Indentation		2	3	4	8	4	12	12	13	10	10
present		2	3	4	8	4	12	12	13	10	10
Tumor infiltrate/metastasis		1	0	0	0	0	1	0	0	1	0
minimal		0	0	0	0	0	1	0	0	1	0
mild		1	0	0	0	0	0	0	0	0	0
BLOOD VESSEL											
Number examined		0	0	0	0	0	1	0	0	0	0
Arteritis		0	0	0	0	0	1	0	0	0	0
mild		0	0	0	0	0	1	0	0	0	0
CRANIAL CAVITY											
Number examined		1	0	0	0	0	0	0	0	1	0
Tumor infiltrate/metastasis		1	0	0	0	0	0	0	0	1	0
minimal		0	0	0	0	0	0	0	0	1	0
severe		1	0	0	0	0	0	0	0	0	0
CECUM											
Number examined		15	19	16	13	11	16	17	20	19	17
Edema,submucosal		0	0	0	0	1	0	0	0	0	0
mild		0	0	0	0	1	0	0	0	0	0

Tissue / Observation	Group:	1	2	3	4	5	1	2	3	4	5
	Sex:	M	M	M	M	M	F	F	F	F	F
	Number:	19	22	17	19	16	17	22	22	23	20
CECUM (continued)											
Cell infiltrate, mucosal/submucosal		0	0	0	0	1	0	0	0	0	0
minimal		0	0	0	0	1	0	0	0	0	0
Arteritis		0	1	0	0	0	0	0	0	0	0
mild		0	1	0	0	0	0	0	0	0	0
Tumor infiltrate/metastasis		0	0	1	0	0	0	0	1	1	0
minimal		0	0	1	0	0	0	0	1	0	0
mild		0	0	0	0	0	0	0	0	1	0
CLITORAL GLAND											
Number examined		-	-	-	-	-	0	0	0	1	0
Ectasia, duct		-	-	-	-	-	0	0	0	1	0
mild		-	-	-	-	-	0	0	0	1	0
CERVIX											
Number examined		-	-	-	-	-	1	1	1	0	0
Hypertrophy, stromal		-	-	-	-	-	0	0	1	0	0
mild		-	-	-	-	-	0	0	1	0	0
Tumor infiltrate/metastasis		-	-	-	-	-	1	0	0	0	0
mild		-	-	-	-	-	1	0	0	0	0
COLON											
Number examined		17	20	17	17	13	16	17	21	20	18
Hyperplasia, lymphoid		0	0	0	0	0	0	1	0	0	0
mild		0	0	0	0	0	0	1	0	0	0
Tumor infiltrate/metastasis		0	0	0	0	0	0	0	1	0	0
minimal		0	0	0	0	0	0	0	1	0	0
CARINA											
Number examined		19	22	17	19	16	17	22	22	23	20
Mineralization, muscular		1	0	0	0	0	0	0	0	0	0
minimal		1	0	0	0	0	0	0	0	0	0
Tumor infiltrate/metastasis		0	0	1	0	0	0	0	0	0	0
minimal		0	0	1	0	0	0	0	0	0	0
DEFERENT DUCT											
Number examined		0	0	0	1	0	-	-	-	-	-
Hemorrhage		0	0	0	1	0	-	-	-	-	-
minimal		0	0	0	1	0	-	-	-	-	-
DIAPHRAGM											
Number examined		1	0	0	0	0	0	0	1	0	0
Tumor infiltrate/metastasis		1	0	0	0	0	0	0	1	0	0
mild		1	0	0	0	0	0	0	0	0	0
moderate		0	0	0	0	0	0	0	1	0	0
EAR											
Number examined		0	1	0	1	0	0	0	0	0	1
Abscess		0	0	0	0	0	0	0	0	0	1

Tissue / Observation	Group:	1	2	3	4	5	1	2	3	4	5
	Sex:	M	M	M	M	M	F	F	F	F	F
	Number:	19	22	17	19	16	17	22	22	23	20
EAR (continued)											
Abscess (continued)											
mild		0	0	0	0	0	0	0	0	0	1
Inflammation		0	0	0	1	0	0	0	0	0	0
moderate		0	0	0	1	0	0	0	0	0	0
Hyperplasia,cartilage		0	1	0	0	0	0	0	0	0	0
mild		0	1	0	0	0	0	0	0	0	0
EPIDIDYMIS											
Number examined		18	22	17	19	16	-	-	-	-	-
Spermatocele		0	0	0	1	0	-	-	-	-	-
mild		0	0	0	1	0	-	-	-	-	-
Degeneration/cribriform change		7	9	5	6	4	-	-	-	-	-
minimal		2	7	2	4	3	-	-	-	-	-
mild		5	2	3	2	1	-	-	-	-	-
Cell infiltrate,perivascular		2	8	5	2	7	-	-	-	-	-
minimal		2	8	5	2	7	-	-	-	-	-
Granuloma		0	0	1	0	0	-	-	-	-	-
minimal		0	0	1	0	0	-	-	-	-	-
Oligospermia/aspermia		4	7	4	7	3	-	-	-	-	-
minimal		0	1	0	1	1	-	-	-	-	-
mild		0	3	0	0	0	-	-	-	-	-
moderate		2	2	2	4	1	-	-	-	-	-
severe		2	1	2	2	1	-	-	-	-	-
Cellular debris,ductal		6	7	8	9	3	-	-	-	-	-
minimal		4	5	7	2	3	-	-	-	-	-
mild		2	2	1	7	0	-	-	-	-	-
Tumor infiltrate/metastasis		2	0	0	0	0	-	-	-	-	-
moderate		1	0	0	0	0	-	-	-	-	-
severe		1	0	0	0	0	-	-	-	-	-
ESOPHAGUS											
Number examined		19	22	17	19	16	17	22	22	23	20
Dilatation,luminal		0	0	2	1	2	0	1	1	0	1
minimal		0	0	1	1	2	0	1	0	0	1
mild		0	0	1	0	0	0	0	1	0	0
Foreign material		1	2	0	0	0	1	0	1	0	0
present		1	2	0	0	0	1	0	1	0	0
Exudate,inflammatory		0	1	0	0	0	0	0	0	0	0
minimal		0	1	0	0	0	0	0	0	0	0
Hyperkeratosis		0	0	0	0	1	0	0	0	0	0
minimal		0	0	0	0	1	0	0	0	0	0
Tumor infiltrate/metastasis		0	0	1	0	0	0	0	0	0	0
minimal		0	0	1	0	0	0	0	0	0	0
EYE											
Number examined		19	22	16	19	16	17	22	22	23	20
Atrophy,retinal		2	6	3	5	1	5	3	8	9	2
minimal		2	6	2	5	1	4	2	8	8	2

Tissue / Observation	Group:	1	2	3	4	5	1	2	3	4	5
	Sex:	M	M	M	M	M	F	F	F	F	F
	Number:	19	22	17	19	16	17	22	22	23	20
EYE (continued)											
Atrophy,retinal (continued)											
mild		0	0	0	0	0	1	1	0	0	0
moderate		0	0	1	0	0	0	0	0	1	0
Degeneration,lenticular		0	2	0	1	0	1	1	2	1	0
minimal		0	1	0	1	0	1	1	2	0	0
mild		0	1	0	0	0	0	0	0	0	0
moderate		0	0	0	0	0	0	0	0	1	0
Mineralization,corneal		4	0	2	1	0	1	2	0	0	0
minimal		4	0	2	1	0	1	2	0	0	0
Conjunctivitis		2	0	0	0	0	0	0	0	0	0
minimal		2	0	0	0	0	0	0	0	0	0
Keratitis		0	0	0	1	0	0	0	0	1	0
mild		0	0	0	1	0	0	0	0	0	0
severe		0	0	0	0	0	0	0	0	1	0
Panophthalmitis		0	1	0	0	0	0	0	0	0	0
mild		0	1	0	0	0	0	0	0	0	0
Mucoid material,corneal		1	2	3	1	1	0	1	1	0	0
minimal		1	2	3	1	1	0	1	1	0	0
Dysplasia,retinal		0	0	0	0	0	0	0	1	1	0
minimal		0	0	0	0	0	0	0	1	1	0
Tumor infiltrate/metastasis		1	1	0	0	0	0	0	0	1	0
minimal		0	1	0	0	0	0	0	0	0	0
mild		0	0	0	0	0	0	0	0	1	0
moderate		1	0	0	0	0	0	0	0	0	0
FAT											
Number examined		0	0	0	0	0	0	0	0	0	1
Necrosis,fat		0	0	0	0	0	0	0	0	0	1
minimal		0	0	0	0	0	0	0	0	0	1
FEMUR & MARROW											
Number examined		19	21	16	19	16	17	21	21	22	20
Fat infiltration		5	7	2	1	4	4	2	4	6	2
minimal		1	6	1	1	3	2	1	2	6	2
mild		4	1	1	0	1	2	1	2	0	0
Hypercellularity,marrow		3	3	3	7	1	4	8	3	4	8
minimal		1	2	2	4	1	2	4	3	3	2
mild		0	1	0	2	0	1	4	0	1	4
moderate		2	0	1	1	0	1	0	0	0	1
severe		0	0	0	0	0	0	0	0	0	1
Tumor infiltrate/metastasis		1	0	0	2	0	0	0	1	2	0
minimal		0	0	0	0	0	0	0	1	0	0
moderate		0	0	0	1	0	0	0	0	0	0
severe		1	0	0	1	0	0	0	0	2	0
FOOT											
Number examined		1	3	1	2	1	2	0	1	0	0
Ulcer		0	0	0	1	0	0	0	0	0	0

Tissue / Observation	Group:	1	2	3	4	5	1	2	3	4	5
	Sex:	M	M	M	M	M	F	F	F	F	F
	Number:	19	22	17	19	16	17	22	22	23	20
FOOT (continued)											
Ulcer (continued)											
mild		0	0	0	1	0	0	0	0	0	0
Ulcer,decubitus		1	3	1	1	1	1	0	1	0	0
minimal		0	0	1	0	0	0	0	0	0	0
mild		1	2	0	1	0	1	0	1	0	0
moderate		0	0	0	0	1	0	0	0	0	0
severe		0	1	0	0	0	0	0	0	0	0
Hyperkeratosis		0	0	0	0	0	1	0	0	0	0
minimal		0	0	0	0	0	1	0	0	0	0
HEART											
Number examined		19	22	17	19	16	17	22	22	23	20
Cyst		0	0	0	0	0	1	0	0	0	0
present		0	0	0	0	0	1	0	0	0	0
Cardiomyopathy		5	9	5	4	7	1	2	3	2	2
minimal		5	8	4	4	6	1	2	3	2	2
mild		0	1	1	0	1	0	0	0	0	0
Mineralization,vascular		1	0	0	0	1	0	0	0	0	0
minimal		1	0	0	0	0	0	0	0	0	0
severe		0	0	0	0	1	0	0	0	0	0
Mineralization,myocardial		0	0	1	0	0	0	0	0	0	0
mild		0	0	1	0	0	0	0	0	0	0
Myocarditis,focal		0	0	0	0	1	0	0	0	0	0
minimal		0	0	0	0	1	0	0	0	0	0
Congestion/hemorrhage,agonal		0	0	0	1	0	0	0	0	0	0
present		0	0	0	1	0	0	0	0	0	0
Proliferation,valvular		0	1	0	0	0	0	0	0	0	0
mild		0	1	0	0	0	0	0	0	0	0
Hypertrophy,myocardial		0	1	0	0	1	0	0	0	0	0
minimal		0	1	0	0	1	0	0	0	0	0
Hyperplasia,endocardial		0	0	0	0	0	0	1	0	0	0
mild		0	0	0	0	0	0	1	0	0	0
Hyperplasia,mesothelial		0	0	0	0	0	0	0	0	1	0
minimal		0	0	0	0	0	0	0	0	1	0
Tumor infiltrate/metastasis		0	0	0	1	0	0	1	0	2	0
minimal		0	0	0	1	0	0	1	0	2	0
HARDERIAN GLAND											
Number examined		1	0	0	0	0	0	0	1	0	0
Hyperplasia,acinar cell,focal		1	0	0	0	0	0	0	0	0	0
present		1	0	0	0	0	0	0	0	0	0
JOINT											
Number examined		0	1	1	0	0	0	0	0	0	1
Synovitis		0	0	1	0	0	0	0	0	0	0
minimal		0	0	1	0	0	0	0	0	0	0

Tissue / Observation	Group:	1	2	3	4	5	1	2	3	4	5
	Sex:	M	M	M	M	M	F	F	F	F	F
	Number:	19	22	17	19	16	17	22	22	23	20
KIDNEY											
Number examined		19	22	17	19	16	17	22	22	23	20
Adhesion		0	0	0	1	0	0	0	0	1	0
present		0	0	0	1	0	0	0	0	1	0
Calculus		2	2	1	3	3	4	4	3	8	4
minimal		2	2	1	2	2	4	4	3	8	4
mild		0	0	0	1	1	0	0	0	0	0
Cyst		1	2	0	0	0	1	0	0	0	0
present		1	2	0	0	0	1	0	0	0	0
Dilatation,pelvic		4	2	0	1	1	3	2	1	2	2
minimal		1	2	0	0	0	2	1	0	1	2
mild		2	0	0	1	1	1	1	1	1	0
moderate		1	0	0	0	0	0	0	0	0	0
Dilatation,tubular		4	1	0	0	0	0	0	0	0	2
minimal		0	0	0	0	0	0	0	0	0	2
mild		4	1	0	0	0	0	0	0	0	0
Vacuolation,tubular cell		0	2	0	1	0	0	0	1	0	0
minimal		0	2	0	1	0	0	0	1	0	0
Necrosis,single cell		0	1	0	0	0	0	0	0	0	0
minimal		0	1	0	0	0	0	0	0	0	0
Chronic progressive nephropathy		2	1	4	5	2	0	1	0	0	0
minimal		0	0	2	2	2	0	0	0	0	0
mild		1	1	1	3	0	0	1	0	0	0
moderate		1	0	1	0	0	0	0	0	0	0
Basophilia,tubular		10	9	2	5	5	4	4	8	5	6
minimal		8	9	2	5	5	4	4	8	5	6
mild		2	0	0	0	0	0	0	0	0	0
Hyaline droplet,tubular cell		0	0	1	2	0	0	0	1	0	1
minimal		0	0	0	1	0	0	0	0	0	0
mild		0	0	1	0	0	0	0	1	0	1
moderate		0	0	0	1	0	0	0	0	0	0
Pigment,tubular		0	0	0	0	0	0	0	0	1	0
minimal		0	0	0	0	0	0	0	0	1	0
Cast		1	1	1	0	0	0	1	0	0	1
minimal		1	1	1	0	0	0	1	0	0	1
Mineralization,vascular		3	0	0	0	1	0	0	0	0	0
minimal		2	0	0	0	0	0	0	0	0	0
mild		1	0	0	0	1	0	0	0	0	0
Mineralization,glomerular		1	0	1	0	0	0	0	0	0	0
minimal		1	0	1	0	0	0	0	0	0	0
Mineralization,tubular cell		2	1	1	2	0	0	0	0	0	1
minimal		0	1	0	1	0	0	0	0	0	0
mild		2	0	0	1	0	0	0	0	0	1
moderate		0	0	1	0	0	0	0	0	0	0
Mineralization,papillary		0	0	0	1	0	0	1	0	0	0
minimal		0	0	0	1	0	0	1	0	0	0
Fibrosis/scarring,focal		3	2	3	1	4	0	2	1	1	2
minimal		3	1	3	0	4	0	1	0	1	2
mild		0	1	0	1	0	0	1	1	0	0

Tissue / Observation	Group:	1	2	3	4	5	1	2	3	4	5
	Sex:	M	M	M	M	M	F	F	F	F	F
	Number:	19	22	17	19	16	17	22	22	23	20
KIDNEY (continued)											
Nephropathy,tubular		0	0	0	1	1	0	0	0	0	0
moderate		0	0	0	1	1	0	0	0	0	0
Pyelitis		6	5	2	2	4	1	6	1	3	1
minimal		3	5	1	1	4	1	3	1	3	1
mild		3	0	1	0	0	0	3	0	0	0
moderate		0	0	0	1	0	0	0	0	0	0
Pyelonephritis		0	0	0	1	1	0	0	0	1	1
minimal		0	0	0	1	0	0	0	0	0	0
mild		0	0	0	0	1	0	0	0	0	0
severe		0	0	0	0	0	0	0	0	1	1
Congestion/hemorrhage,agonal		2	2	2	0	1	1	0	1	0	0
present		2	2	2	0	1	1	0	1	0	0
Necrosis,tubular,acute		2	2	0	2	0	1	4	5	4	2
minimal		1	0	0	1	0	1	0	1	2	0
mild		1	1	0	0	0	0	4	4	1	2
moderate		0	1	0	1	0	0	0	0	1	0
Tumor infiltrate/metastasis		2	0	0	2	0	0	0	1	1	0
minimal		0	0	0	1	0	0	0	1	0	0
mild		2	0	0	1	0	0	0	0	1	0
LARYNX											
Number examined		18	22	17	19	16	17	22	22	23	20
Dilatation,luminal		0	0	0	1	0	0	0	0	0	1
mild		0	0	0	1	0	0	0	0	0	1
Cell infiltrate,mucosal/submucosal		0	1	1	0	0	1	0	1	1	0
minimal		0	1	1	0	0	1	0	1	1	0
Cell infiltrate,perivascular		1	0	0	1	0	0	0	0	0	0
minimal		1	0	0	1	0	0	0	0	0	0
Inflammation		1	0	1	0	0	0	1	0	1	1
minimal		0	0	0	0	0	0	1	0	1	0
mild		1	0	1	0	0	0	0	0	0	1
Dilatation,submucosal gland		7	8	1	4	1	1	3	5	5	3
minimal		7	8	1	4	1	1	3	5	5	3
Foreign material		2	1	0	2	0	1	1	0	2	1
present		2	1	0	2	0	1	1	0	2	1
Exudate,inflammatory		0	0	0	0	1	0	0	0	1	0
minimal		0	0	0	0	1	0	0	0	1	0
Hyperplasia,transitional		1	0	0	0	0	0	1	0	1	0
minimal		0	0	0	0	0	0	0	0	1	0
mild		1	0	0	0	0	0	1	0	0	0
Metaplasia,squamous cell		1	1	1	1	1	0	1	0	0	0
minimal		1	1	1	1	1	0	1	0	0	0
Tumor infiltrate/metastasis		1	1	0	0	0	0	0	0	0	0
minimal		1	0	0	0	0	0	0	0	0	0
mild		0	1	0	0	0	0	0	0	0	0

Tissue / Observation	Group:	1	2	3	4	5	1	2	3	4	5
	Sex:	M	M	M	M	M	F	F	F	F	F
	Number:	19	22	17	19	16	17	22	22	23	20
LYMPH NODE, BRONCHIAL											
Number examined		11	10	9	8	10	6	12	13	6	9
Atrophy,lymphoid		0	0	0	0	1	0	0	0	0	0
mild		0	0	0	0	1	0	0	0	0	0
Pigmentation		0	0	0	0	0	0	0	0	0	1
minimal		0	0	0	0	0	0	0	0	0	1
Inflammation		1	0	0	0	0	0	0	0	0	0
mild		1	0	0	0	0	0	0	0	0	0
Congestion/hemorrhage,agonal		1	1	0	0	1	0	1	1	0	0
present		1	1	0	0	1	0	1	1	0	0
Erythrocytosis,sinus		0	0	0	1	0	0	0	0	0	0
minimal		0	0	0	1	0	0	0	0	0	0
Ectasia,lymphatic		0	0	0	0	0	0	0	0	0	1
minimal		0	0	0	0	0	0	0	0	0	1
Tumor infiltrate/metastasis		1	0	1	1	0	0	1	0	0	0
minimal		0	0	1	0	0	0	0	0	0	0
moderate		0	0	0	1	0	0	1	0	0	0
severe		1	0	0	0	0	0	0	0	0	0
LIVER											
Number examined		19	22	17	19	16	17	22	22	23	20
Adhesion		1	1	0	0	0	0	0	0	0	0
present		1	1	0	0	0	0	0	0	0	0
Cyst,biliary		1	0	0	0	0	0	0	0	0	0
present		1	0	0	0	0	0	0	0	0	0
Dilatation,sinusoidal		0	2	0	0	1	1	1	0	1	1
minimal		0	2	0	0	1	1	0	0	0	1
mild		0	0	0	0	0	0	1	0	1	0
Angiectasis		0	1	0	1	0	0	0	0	0	0
minimal		0	1	0	1	0	0	0	0	0	0
Vacuolation,hepatocellular		1	1	2	0	1	1	0	1	2	0
minimal		0	1	2	0	1	1	0	1	1	0
mild		1	0	0	0	0	0	0	0	0	0
moderate		0	0	0	0	0	0	0	0	1	0
Degeneration,cystic		0	0	0	0	0	0	1	0	0	0
minimal		0	0	0	0	0	0	1	0	0	0
Necrosis,centrilobular		0	0	0	0	0	0	2	0	1	1
mild		0	0	0	0	0	0	2	0	1	1
Necrosis,focal		1	1	0	2	3	0	2	1	2	3
minimal		0	1	0	2	2	0	1	1	2	1
mild		1	0	0	0	1	0	1	0	0	2
Necrosis,massive		0	2	1	0	0	1	0	0	0	0
minimal		0	0	1	0	0	0	0	0	0	0
mild		0	1	0	0	0	1	0	0	0	0
moderate		0	1	0	0	0	0	0	0	0	0
Necrosis,single cell		1	0	0	0	0	0	0	0	1	1
minimal		1	0	0	0	0	0	0	0	1	0
mild		0	0	0	0	0	0	0	0	0	1
Pigment		0	0	1	0	0	0	1	0	0	0

Tissue / Observation	Group:	1	2	3	4	5	1	2	3	4	5
	Sex:	M	M	M	M	M	F	F	F	F	F
	Number:	19	22	17	19	16	17	22	22	23	20
LIVER (continued)											
Pigment (continued)											
minimal		0	0	1	0	0	0	1	0	0	0
Hematopoiesis,extramedullary		1	0	1	2	0	2	6	0	2	6
minimal		0	0	1	2	0	2	4	0	2	5
mild		1	0	0	0	0	0	2	0	0	1
Hemorrhage,focal		0	0	0	0	0	0	1	0	0	0
minimal		0	0	0	0	0	0	1	0	0	0
Microgranuloma		0	0	1	1	3	3	0	1	1	0
minimal		0	0	1	1	3	3	0	1	1	0
Fibrosis,capsular		1	0	0	0	0	0	2	0	1	0
minimal		1	0	0	0	0	0	2	0	0	0
mild		0	0	0	0	0	0	0	0	1	0
Fibrosis,focal		1	0	0	0	0	0	1	1	0	1
minimal		1	0	0	0	0	0	1	1	0	1
Congestion/hemorrhage,agonal		4	2	5	0	4	1	0	1	0	1
present		2	2	5	0	4	1	0	1	0	1
present		2	0	0	0	0	0	0	0	0	0
Change,clear cell		0	0	0	0	0	1	0	0	0	0
minimal		0	0	0	0	0	1	0	0	0	0
Fibrosis,peribiliary		0	0	0	1	0	0	0	1	1	1
minimal		0	0	0	1	0	0	0	1	0	1
mild		0	0	0	0	0	0	0	0	1	0
Hypertrophy,hepatocellular		0	0	0	0	0	0	0	0	1	0
minimal		0	0	0	0	0	0	0	0	1	0
Hyperplasia,bile ductular		2	2	2	1	2	0	0	2	1	2
minimal		2	2	2	1	2	0	0	2	1	2
Hyperplasia,oval cell		1	0	0	0	0	0	0	0	0	0
minimal		1	0	0	0	0	0	0	0	0	0
Focus,basophilic cell		0	0	0	0	0	0	1	1	0	0
minimal		0	0	0	0	0	0	1	1	0	0
Focus,clear cell		0	1	0	0	0	0	0	0	0	0
minimal		0	1	0	0	0	0	0	0	0	0
Focus,eosinophilic cell		0	0	1	1	1	0	6	2	1	0
minimal		0	0	1	1	1	0	6	1	1	0
moderate		0	0	0	0	0	0	0	1	0	0
Focus,basophilic,tigroid cell		0	1	2	1	1	2	2	4	0	0
minimal		0	1	2	1	1	2	2	4	0	0
Tumor infiltrate/metastasis		2	2	1	2	0	0	1	2	2	0
minimal		1	1	0	0	0	0	0	0	0	0
mild		0	0	1	0	0	0	0	1	1	0
moderate		1	0	0	1	0	0	1	1	0	0
severe		0	1	0	1	0	0	0	0	1	0
LYMPH NODE, MESENTERIC											
Number examined		18	22	17	18	16	17	22	22	23	20
Atrophy,lymphoid		14	12	8	8	8	7	11	7	10	9
minimal		1	2	3	2	2	2	0	1	3	1
mild		3	4	2	0	2	4	6	4	4	3

Tissue / Observation	Group:	1	2	3	4	5	1	2	3	4	5
	Sex:	M	M	M	M	M	F	F	F	F	F
	Number:	19	22	17	19	16	17	22	22	23	20
LYMPH NODE, MESENTERIC (continued)											
Atrophy,lymphoid (continued)											
moderate		5	5	3	5	2	1	5	2	2	3
severe		5	1	0	1	2	0	0	0	1	2
Histiocytosis		17	20	17	14	13	16	19	20	14	16
minimal		17	20	16	14	13	15	19	20	14	16
mild		0	0	1	0	0	1	0	0	0	0
Plasmacytosis		0	0	0	0	0	0	0	1	0	0
mild		0	0	0	0	0	0	0	1	0	0
Inflammation		0	0	0	1	0	0	0	1	0	0
minimal		0	0	0	1	0	0	0	0	0	0
mild		0	0	0	0	0	0	0	1	0	0
Congestion/hemorrhage,agonal		1	1	0	1	0	0	0	1	1	0
present		1	1	0	1	0	0	0	1	1	0
Erythrocytosis,sinus		2	1	0	1	1	0	0	4	0	0
minimal		2	1	0	1	1	0	0	3	0	0
mild		0	0	0	0	0	0	0	1	0	0
Cell infiltrate,pericapsular		0	0	0	1	0	0	0	0	0	0
mild		0	0	0	1	0	0	0	0	0	0
Ectasia,lymphatic		0	1	0	0	0	0	0	0	0	0
minimal		0	1	0	0	0	0	0	0	0	0
Hyperplasia,angiomatous		0	1	0	0	0	0	0	0	0	0
minimal		0	1	0	0	0	0	0	0	0	0
Hyperplasia,lymphoid		0	0	0	0	0	0	1	0	0	1
minimal		0	0	0	0	0	0	0	0	0	1
mild		0	0	0	0	0	0	1	0	0	0
Tumor infiltrate/metastasis		1	0	0	1	0	0	0	0	1	1
mild		0	0	0	1	0	0	0	0	0	0
severe		1	0	0	0	0	0	0	0	1	1
LYMPH NODE, MANDIBULAR											
Number examined		13	20	17	16	16	16	22	22	22	20
Histiocytosis,sinus		1	0	0	0	0	0	0	0	0	0
minimal		1	0	0	0	0	0	0	0	0	0
Plasmacytosis		0	0	1	0	0	0	1	2	1	0
minimal		0	0	1	0	0	0	0	0	0	0
mild		0	0	0	0	0	0	1	2	1	0
Congestion/hemorrhage,agonal		0	0	0	1	1	1	1	3	2	1
present		0	0	0	1	1	1	1	3	2	1
Erythrocytosis,sinus		1	0	1	0	0	1	1	2	0	1
minimal		1	0	1	0	0	1	1	2	0	1
Ectasia,lymphatic		0	1	0	0	2	0	0	0	0	0
minimal		0	1	0	0	1	0	0	0	0	0
mild		0	0	0	0	1	0	0	0	0	0
Tumor infiltrate/metastasis		1	1	1	1	1	0	1	0	1	0
minimal		0	1	1	0	0	0	0	0	0	0
mild		0	0	0	1	0	0	0	0	0	0
moderate		0	0	0	0	0	0	1	0	0	0
severe		1	0	0	0	1	0	0	0	1	0

Tissue / Observation	Group:	1	2	3	4	5	1	2	3	4	5
	Sex:	M	M	M	M	M	F	F	F	F	F
	Number:	19	22	17	19	16	17	22	22	23	20
LUNG											
Number examined		19	22	17	19	16	17	22	22	23	20
Adhesion		0	0	0	0	1	0	0	0	0	1
present		0	0	0	0	1	0	0	0	0	1
Pigment		0	1	0	0	0	0	0	0	0	0
minimal		0	1	0	0	0	0	0	0	0	0
Mineralization,vascular		17	19	16	19	16	15	17	17	16	16
minimal		17	19	16	19	16	15	17	17	16	16
Edema		2	2	1	1	1	1	0	1	2	0
mild		2	1	1	1	1	1	0	1	2	0
moderate		0	1	0	0	0	0	0	0	0	0
Hemorrhage,focal		1	1	1	0	0	0	0	1	1	0
minimal		1	0	1	0	0	0	0	1	0	0
mild		0	1	0	0	0	0	0	0	1	0
Thrombus		1	0	0	0	0	0	0	0	0	0
minimal		1	0	0	0	0	0	0	0	0	0
Embolus		1	0	0	0	0	0	0	0	0	0
minimal		1	0	0	0	0	0	0	0	0	0
Congestion/hemorrhage		1	0	0	0	0	0	0	0	0	1
mild		1	0	0	0	0	0	0	0	0	1
Cell infiltrate,subpleural		0	0	0	0	0	0	0	1	0	0
minimal		0	0	0	0	0	0	0	1	0	0
Cell infiltrate,perivascular		0	1	0	1	1	0	0	0	0	1
minimal		0	1	0	1	1	0	0	0	0	1
Histiocytosis,alveolar		10	10	8	10	7	10	9	11	10	10
minimal		8	10	8	9	6	9	9	11	10	9
mild		2	0	0	1	1	1	0	0	0	1
Granuloma,foreign body		1	0	0	1	0	1	0	1	0	0
minimal		1	0	0	1	0	1	0	1	0	0
Inflammation,acute		0	1	0	2	1	1	0	0	2	3
minimal		0	1	0	1	1	0	0	0	1	1
mild		0	0	0	1	0	1	0	0	1	2
Inflammation,subacute		2	0	1	0	0	0	0	0	0	0
mild		2	0	1	0	0	0	0	0	0	0
Inflammation,chronic		0	1	1	1	0	0	0	0	0	0
minimal		0	1	0	1	0	0	0	0	0	0
mild		0	0	1	0	0	0	0	0	0	0
Inflammation,granulomatous		0	0	0	0	0	0	1	0	1	0
minimal		0	0	0	0	0	0	0	0	1	0
mild		0	0	0	0	0	0	1	0	0	0
Congestion/hemorrhage,agonal		11	8	9	10	10	4	7	8	8	8
present		11	8	9	10	10	4	7	8	8	8
Partial underinflation		5	2	2	1	2	1	0	1	0	2
present		5	2	2	1	2	1	0	1	0	2
Granuloma,cholesterol		0	1	2	2	0	1	0	2	1	1
minimal		0	1	2	2	0	1	0	2	1	1
Fibrosis,pleural		0	0	0	0	1	0	0	0	0	0
minimal		0	0	0	0	1	0	0	0	0	0
Mineralization,alveolar wall		1	1	2	2	1	0	1	0	0	2

Tissue / Observation	Group:	1	2	3	4	5	1	2	3	4	5
	Sex:	M	M	M	M	M	F	F	F	F	F
	Number:	19	22	17	19	16	17	22	22	23	20
LUNG (continued)											
Mineralization,alveolar wall (continued)											
minimal		1	1	2	2	0	0	1	0	0	2
mild		0	0	0	0	1	0	0	0	0	0
Metaplasia,osseous		0	0	0	1	2	0	0	0	0	0
present		0	0	0	1	2	0	0	0	0	0
Hypertrophy,medial		0	1	0	0	1	0	0	0	0	0
minimal		0	1	0	0	0	0	0	0	0	0
mild		0	0	0	0	1	0	0	0	0	0
Hyperplasia,bronchiolo-alveolar		0	0	0	0	0	0	0	0	1	0
present		0	0	0	0	0	0	0	0	1	0
Hyperplasia,bronchiolar,reactive		0	1	0	0	0	0	0	1	0	0
minimal		0	1	0	0	0	0	0	1	0	0
Tumor infiltrate/metastasis		4	2	1	1	2	0	3	2	2	1
minimal		1	0	0	0	1	0	0	1	0	0
mild		2	1	1	1	1	0	1	1	0	1
moderate		1	1	0	0	0	0	2	0	2	0
LYMPH NODE											
Number examined		6	3	5	7	5	4	14	3	9	8
Pigment		0	0	0	0	0	0	1	0	0	0
minimal		0	0	0	0	0	0	1	0	0	0
Plasmacytosis		0	0	3	2	1	1	5	1	1	4
minimal		0	0	1	0	0	0	1	1	1	2
mild		0	0	2	2	1	0	4	0	0	2
moderate		0	0	0	0	0	1	0	0	0	0
Inflammation		0	0	1	1	0	0	0	0	0	0
minimal		0	0	1	1	0	0	0	0	0	0
Congestion/hemorrhage,agonal		2	0	0	2	1	0	1	1	0	0
present		2	0	0	2	1	0	1	1	0	0
Erythrocytosis,sinus		2	0	0	3	0	0	1	0	1	1
minimal		1	0	0	3	0	0	1	0	1	1
mild		1	0	0	0	0	0	0	0	0	0
Ectasia,lymphatic		0	2	0	0	2	0	0	0	0	2
minimal		0	1	0	0	2	0	0	0	0	1
mild		0	1	0	0	0	0	0	0	0	1
Tumor infiltrate/metastasis		1	1	1	1	1	0	3	1	2	0
mild		0	0	1	0	0	0	1	0	0	0
moderate		0	0	0	1	0	0	1	0	0	0
severe		1	1	0	0	1	0	1	1	2	0
MESENTERY											
Number examined		1	0	0	0	0	0	1	1	0	0
Tumor infiltrate/metastasis		1	0	0	0	0	0	0	1	0	0
mild		0	0	0	0	0	0	0	1	0	0
moderate		1	0	0	0	0	0	0	0	0	0

Tissue / Observation	Group:	1	2	3	4	5	1	2	3	4	5
	Sex:	M	M	M	M	M	F	F	F	F	F
	Number:	19	22	17	19	16	17	22	22	23	20
MAMMARY GLAND											
Number examined		16	20	16	16	14	17	20	22	22	19
Atrophy		0	0	0	0	0	0	0	0	0	2
mild		0	0	0	0	0	0	0	0	0	1
moderate		0	0	0	0	0	0	0	0	0	1
Fibrosis,interstitial		0	0	0	0	0	0	1	0	0	0
moderate		0	0	0	0	0	0	1	0	0	0
Cyst,ductal/alveolar		4	5	1	6	3	10	9	8	8	8
minimal		2	2	0	2	1	8	5	7	4	4
mild		2	3	1	2	1	2	4	1	4	4
moderate		0	0	0	2	1	0	0	0	0	0
Hyperplasia,lobular		0	0	0	0	0	4	6	8	4	5
minimal		0	0	0	0	0	4	6	5	0	4
mild		0	0	0	0	0	0	0	2	4	0
moderate		0	0	0	0	0	0	0	1	0	0
severe		0	0	0	0	0	0	0	0	0	1
Hyperplasia,focal		0	0	0	0	0	0	1	3	2	0
present		0	0	0	0	0	0	1	3	2	0
Tumor infiltrate/metastasis		0	0	0	0	0	0	0	1	0	0
minimal		0	0	0	0	0	0	0	1	0	0
NASAL CAVITY (section 1)											
Number examined		19	22	17	19	16	17	22	22	23	20
Hemorrhage,focal		0	0	0	0	0	0	0	1	0	0
minimal		0	0	0	0	0	0	0	1	0	0
Cell infiltrate,mucosal/submucosal		3	0	1	0	2	1	0	1	0	0
minimal		3	0	1	0	2	1	0	1	0	0
Inflammation,nasal cavity		2	2	0	0	0	1	2	2	1	0
minimal		1	1	0	0	0	1	1	1	0	0
mild		1	0	0	0	0	0	1	1	1	0
severe		0	1	0	0	0	0	0	0	0	0
Inflammation,nasolacrimal duct		0	0	2	0	0	0	1	1	0	0
minimal		0	0	2	0	0	0	1	1	0	0
Dilatation,submucosal gland		1	1	0	0	1	2	0	0	1	0
minimal		1	1	0	0	1	2	0	0	1	0
Fracture		0	0	0	0	0	0	0	1	0	0
present		0	0	0	0	0	0	0	1	0	0
Droplets,eosinophilic		0	0	0	0	0	0	1	2	1	1
minimal		0	0	0	0	0	0	1	2	1	1
Exudate,inflammatory		3	0	2	1	2	0	0	1	1	1
minimal		3	0	2	1	2	0	0	1	1	1
Hyperplasia,transitional		0	0	0	1	0	0	0	0	0	0
minimal		0	0	0	1	0	0	0	0	0	0
Exudate,proteinaceous		0	0	0	0	1	0	0	0	0	1
minimal		0	0	0	0	1	0	0	0	0	1
Degeneration/regeneration,olfactory		0	0	0	0	0	0	0	0	1	0
mild		0	0	0	0	0	0	0	0	1	0
Hyperplasia,respiratory		0	0	0	0	0	0	2	0	0	0
minimal		0	0	0	0	0	0	1	0	0	0

Tissue / Observation	Group:	1	2	3	4	5	1	2	3	4	5
	Sex:	M	M	M	M	M	F	F	F	F	F
	Number:	19	22	17	19	16	17	22	22	23	20
NASAL CAVITY (section 1) (continued)											
Hyperplasia,respiratory (continued)											
mild		0	0	0	0	0	0	1	0	0	0
Metaplasia,squamous		0	0	0	0	1	0	0	0	0	0
minimal		0	0	0	0	1	0	0	0	0	0
Hyperplasia,goblet cell		3	8	6	5	3	7	6	11	7	5
minimal		2	6	5	5	3	6	6	10	6	5
mild		1	2	1	0	0	1	0	1	1	0
Tumor infiltrate/metastasis		1	0	0	0	0	0	0	0	1	0
moderate		0	0	0	0	0	0	0	0	1	0
severe		1	0	0	0	0	0	0	0	0	0
NASOPHARYNX											
Number examined		19	22	17	19	16	17	22	22	23	20
Hyperplasia,epithelial		0	1	0	0	1	0	1	0	0	0
minimal		0	1	0	0	1	0	0	0	0	0
mild		0	0	0	0	0	0	1	0	0	0
Mineralization,mucosal		0	0	0	0	1	0	0	0	0	0
mild		0	0	0	0	1	0	0	0	0	0
Hyperplasia,goblet cell		0	0	0	0	1	0	0	0	0	0
minimal		0	0	0	0	1	0	0	0	0	0
Tumor infiltrate/metastasis		1	1	0	1	1	0	0	0	1	0
minimal		0	0	0	0	1	0	0	0	0	0
mild		1	0	0	1	0	0	0	0	1	0
moderate		0	1	0	0	0	0	0	0	0	0
ORAL CAVITY											
Number examined		0	1	1	0	0	1	1	1	2	0
Cyst,squamous		0	0	0	0	0	1	0	1	0	0
present		0	0	0	0	0	1	0	1	0	0
Inflammation		0	0	0	0	0	0	1	0	0	0
mild		0	0	0	0	0	0	1	0	0	0
OVARY											
Number examined		-	-	-	-	-	17	22	22	23	20
Cyst		-	-	-	-	-	5	2	4	4	2
present		-	-	-	-	-	5	2	4	4	2
Atrophy		-	-	-	-	-	16	21	20	19	19
minimal		-	-	-	-	-	4	3	9	3	7
mild		-	-	-	-	-	6	5	6	3	1
moderate		-	-	-	-	-	6	13	5	13	11
Hyperplasia,sex cord stromal		-	-	-	-	-	7	6	6	8	5
minimal		-	-	-	-	-	5	4	3	6	3
mild		-	-	-	-	-	2	1	2	1	2
moderate		-	-	-	-	-	0	1	1	1	0
Tumor infiltrate/metastasis		-	-	-	-	-	0	0	1	1	0
minimal		-	-	-	-	-	0	0	1	0	0
severe		-	-	-	-	-	0	0	0	1	0

Tissue / Observation	Group:	1	2	3	4	5	1	2	3	4	5
	Sex:	M	M	M	M	M	F	F	F	F	F
	Number:	19	22	17	19	16	17	22	22	23	20
PANCREAS											
Number examined		19	22	17	19	15	17	22	21	23	20
Cyst		0	0	0	0	0	0	0	1	0	0
minimal		0	0	0	0	0	0	0	1	0	0
Fat infiltration		2	0	0	0	0	0	0	0	0	0
minimal		1	0	0	0	0	0	0	0	0	0
mild		1	0	0	0	0	0	0	0	0	0
Atrophy,acinar		0	0	0	0	0	0	0	0	0	1
severe		0	0	0	0	0	0	0	0	0	1
Atrophy,acinar,focal		1	4	4	2	2	0	4	3	2	2
minimal		1	4	4	1	2	0	4	3	1	2
mild		0	0	0	1	0	0	0	0	1	0
Cell infiltrate,interstitial		1	1	0	1	1	0	0	3	1	0
minimal		1	1	0	1	1	0	0	3	1	0
Zymogen granules,decreased		6	5	3	2	2	3	2	1	1	5
minimal		0	1	0	2	0	0	1	0	1	2
mild		2	2	2	0	2	3	1	1	0	2
moderate		4	2	1	0	0	0	0	0	0	0
severe		0	0	0	0	0	0	0	0	0	1
Focus,hypertrophic,basophilic		0	2	2	2	0	0	4	2	0	2
minimal		0	2	2	2	0	0	4	2	0	2
Hyperplasia,ductal		0	0	1	0	0	0	0	0	0	0
minimal		0	0	1	0	0	0	0	0	0	0
Hyperplasia,islet cell		1	0	0	1	0	0	0	0	0	0
present		1	0	0	1	0	0	0	0	0	0
Tumor infiltrate/metastasis		1	0	1	0	0	0	1	0	0	0
minimal		0	0	0	0	0	0	1	0	0	0
mild		0	0	1	0	0	0	0	0	0	0
moderate		1	0	0	0	0	0	0	0	0	0
PENIS											
Number examined		1	0	0	1	0	-	-	-	-	-
Mineralization		0	0	0	1	0	-	-	-	-	-
mild		0	0	0	1	0	-	-	-	-	-
Balanoposthitis		1	0	0	0	0	-	-	-	-	-
moderate		1	0	0	0	0	-	-	-	-	-
PITUITARY											
Number examined		19	21	17	18	16	16	21	21	22	19
Cyst		0	0	0	0	0	0	0	1	0	0
present		0	0	0	0	0	0	0	1	0	0
Congestion/hemorrhage,agonal		1	0	1	1	1	0	0	0	0	0
present		1	0	1	1	1	0	0	0	0	0
Hypertrophy,cellular		2	1	0	1	0	0	0	0	0	0
minimal		2	1	0	1	0	0	0	0	0	0
Hyperplasia,pars distalis,focal		1	7	7	3	3	2	3	1	3	3
present		1	7	7	3	3	2	3	1	3	3
Tumor infiltrate/metastasis		1	0	0	0	0	0	0	0	2	0
minimal		0	0	0	0	0	0	0	0	1	0

Tissue / Observation	Group:	1	2	3	4	5	1	2	3	4	5
	Sex:	M	M	M	M	M	F	F	F	F	F
	Number:	19	22	17	19	16	17	22	22	23	20
PITUITARY (continued)											
Tumor infiltrate/metastasis (continued)											
moderate		1	0	0	0	0	0	0	0	1	0
PREPUTIAL GLAND											
Number examined		1	0	0	2	0	-	-	-	-	-
Abscess		1	0	0	0	0	-	-	-	-	-
mild		1	0	0	0	0	-	-	-	-	-
Inflammation		0	0	0	1	0	-	-	-	-	-
mild		0	0	0	1	0	-	-	-	-	-
Ectasia,duct		0	0	0	1	0	-	-	-	-	-
mild		0	0	0	1	0	-	-	-	-	-
PEYER'S PATCH											
Number examined		0	0	0	0	0	0	0	0	1	0
Tumor infiltrate/metastasis		0	0	0	0	0	0	0	0	1	0
severe		0	0	0	0	0	0	0	0	1	0
PROSTATE											
Number examined		19	22	17	19	16	-	-	-	-	-
Atrophy		5	3	2	3	4	-	-	-	-	-
minimal		2	0	1	1	2	-	-	-	-	-
mild		3	3	0	2	2	-	-	-	-	-
moderate		0	0	1	0	0	-	-	-	-	-
Hemorrhage		0	1	0	0	0	-	-	-	-	-
mild		0	1	0	0	0	-	-	-	-	-
Cell infiltrate,interstitial		1	1	0	3	1	-	-	-	-	-
minimal		1	0	0	3	1	-	-	-	-	-
mild		0	1	0	0	0	-	-	-	-	-
Prostatitis		10	12	7	14	7	-	-	-	-	-
minimal		5	5	2	8	4	-	-	-	-	-
mild		2	6	4	4	2	-	-	-	-	-
moderate		2	1	0	2	0	-	-	-	-	-
severe		1	0	1	0	1	-	-	-	-	-
Tumor infiltrate/metastasis		1	0	1	0	0	-	-	-	-	-
minimal		0	0	1	0	0	-	-	-	-	-
moderate		1	0	0	0	0	-	-	-	-	-
PARATHYROID											
Number examined		17	12	16	14	12	14	16	17	13	17
Hyperplasia,focal		2	0	2	2	0	0	0	0	0	2
present		2	0	2	2	0	0	0	0	0	2
RECTUM											
Number examined		19	21	17	18	15	16	20	22	21	19
Cell infiltrate,serosal		2	0	0	0	0	0	0	0	0	0
minimal		1	0	0	0	0	0	0	0	0	0
mild		1	0	0	0	0	0	0	0	0	0
Tumor infiltrate/metastasis		0	0	0	0	0	0	0	0	0	1

Tissue / Observation	Group:	1	2	3	4	5	1	2	3	4	5
	Sex:	M	M	M	M	M	F	F	F	F	F
	Number:	19	22	17	19	16	17	22	22	23	20
RECTUM (continued)											
Tumor infiltrate/metastasis (continued)											
minimal		0	0	0	0	0	0	0	0	0	1
SPINAL CORD											
Number examined		19	22	17	19	16	17	21	22	23	20
Necrosis		0	0	0	0	0	0	0	0	1	0
mild		0	0	0	0	0	0	0	0	1	0
Hemorrhage,focal		0	0	0	0	0	0	0	1	0	0
mild		0	0	0	0	0	0	0	1	0	0
Radiculoneuropathy		0	1	0	0	0	0	0	0	0	0
minimal		0	1	0	0	0	0	0	0	0	0
SMALL INTESTINE, DUODENUM											
Number examined		18	19	16	19	14	16	19	21	19	18
Ectopic pancreas		0	0	0	0	0	0	0	1	0	0
present		0	0	0	0	0	0	0	1	0	0
Dilatation,glandular		0	0	0	0	0	0	0	1	0	0
minimal		0	0	0	0	0	0	0	1	0	0
Congestion/hemorrhage,agonal		0	0	0	0	1	0	0	0	0	0
present		0	0	0	0	1	0	0	0	0	0
Erosion/ulcer,mucosal		0	1	1	0	0	0	0	0	0	0
mild		0	1	1	0	0	0	0	0	0	0
Tumor infiltrate/metastasis		1	0	0	0	0	0	0	1	0	0
minimal		1	0	0	0	0	0	0	1	0	0
SALIVARY GLAND											
Number examined		19	21	17	19	16	17	21	22	23	20
Congestion/hemorrhage,agonal		0	0	0	0	1	0	0	0	0	0
present		0	0	0	0	1	0	0	0	0	0
Atrophy,acinar,diffuse		0	1	0	0	1	0	0	0	0	0
minimal		0	1	0	0	0	0	0	0	0	0
moderate		0	0	0	0	1	0	0	0	0	0
SMALL INTESTINE, ILEUM											
Number examined		15	18	15	12	10	16	16	18	18	13
Cell infiltrate,mucosal/submucosal		0	0	0	0	0	0	0	0	0	1
minimal		0	0	0	0	0	0	0	0	0	1
Tumor infiltrate/metastasis		0	0	0	0	0	0	0	1	1	0
minimal		0	0	0	0	0	0	0	1	0	0
severe		0	0	0	0	0	0	0	0	1	0
SMALL INTESTINE, JEJUNUM											
Number examined		16	17	15	13	12	16	18	19	19	17
Erosion/ulcer,mucosal		0	0	0	0	0	0	0	1	0	1
minimal		0	0	0	0	0	0	0	0	0	1
moderate		0	0	0	0	0	0	0	1	0	0
Tumor infiltrate/metastasis		0	0	0	0	0	0	0	1	0	0
minimal		0	0	0	0	0	0	0	1	0	0

Tissue / Observation	Group:	1	2	3	4	5	1	2	3	4	5
	Sex:	M	M	M	M	M	F	F	F	F	F
	Number:	19	22	17	19	16	17	22	22	23	20
SKELETAL MUSCLE											
Number examined		19	22	17	19	16	17	22	22	23	20
Cell infiltrate, muscular		1	1	0	0	0	0	0	1	0	2
minimal		1	1	0	0	0	0	0	1	0	2
Degeneration/atrophy, muscle fiber		3	9	6	5	5	2	7	4	4	4
minimal		3	7	5	5	3	2	5	3	3	3
mild		0	1	1	0	2	0	2	1	1	1
moderate		0	1	0	0	0	0	0	0	0	0
Tumor infiltrate/metastasis		1	1	0	0	0	0	0	0	0	1
minimal		1	0	0	0	0	0	0	0	0	0
mild		0	1	0	0	0	0	0	0	0	0
moderate		0	0	0	0	0	0	0	0	0	1
SCIATIC NERVE											
Number examined		19	22	16	19	16	17	22	22	23	20
Necrosis, focal		0	0	0	0	1	0	0	0	0	0
minimal		0	0	0	0	1	0	0	0	0	0
Neuropathy		0	2	1	1	1	1	0	1	0	2
minimal		0	1	0	1	1	1	0	1	0	2
mild		0	1	1	0	0	0	0	0	0	0
SPLEEN											
Number examined		19	22	17	19	16	17	22	22	23	20
Cyst		0	0	1	0	0	0	0	0	0	0
present		0	0	1	0	0	0	0	0	0	0
Atrophy		4	2	0	2	1	1	1	1	3	2
minimal		0	0	0	0	0	0	0	1	1	0
mild		2	1	0	2	1	1	1	0	2	2
moderate		2	1	0	0	0	0	0	0	0	0
Atrophy, lymphoid		7	4	7	5	5	5	6	3	5	8
minimal		1	2	2	0	2	3	2	2	1	0
mild		4	2	3	5	1	2	1	1	3	6
moderate		2	0	2	0	2	0	2	0	1	2
severe		0	0	0	0	0	0	1	0	0	0
Hematopoiesis, increased		4	5	3	5	2	3	9	2	6	5
minimal		4	4	2	3	2	1	6	0	1	0
mild		0	1	1	2	0	1	0	1	3	2
moderate		0	0	0	0	0	1	3	1	2	3
Fibrosis, capsular		0	2	1	0	1	0	0	1	0	1
minimal		0	2	0	0	1	0	0	1	0	1
mild		0	0	1	0	0	0	0	0	0	0
Congestion/hemorrhage, agonal		1	0	0	0	0	0	0	0	0	0
present		1	0	0	0	0	0	0	0	0	0
Pigmentation, increased		9	5	3	6	4	6	6	10	9	10
minimal		7	5	3	5	3	5	6	9	8	6
mild		2	0	0	1	1	1	0	1	1	4
Inflammation, capsular		1	0	0	0	0	0	0	0	0	1
minimal		1	0	0	0	0	0	0	0	0	0
mild		0	0	0	0	0	0	0	0	0	1

Tissue / Observation	Group:	1	2	3	4	5	1	2	3	4	5
	Sex:	M	M	M	M	M	F	F	F	F	F
	Number:	19	22	17	19	16	17	22	22	23	20
SPLEEN (continued)											
Tumor infiltrate/metastasis		1	0	0	2	0	0	1	1	3	0
minimal		0	0	0	0	0	0	0	1	0	0
mild		0	0	0	0	0	0	0	0	1	0
moderate		0	0	0	0	0	0	1	0	0	0
severe		1	0	0	2	0	0	0	0	2	0
STERNUM & MARROW											
Number examined		19	22	17	19	16	16	22	22	23	20
Fat infiltration		1	2	0	1	2	2	2	3	3	2
minimal		1	2	0	1	2	2	2	3	3	2
Degeneration,chondromucinous		3	6	2	2	2	4	5	4	7	5
minimal		3	6	2	2	2	4	5	4	7	5
Hypercellularity,marrow		3	5	3	7	3	4	9	2	4	8
minimal		1	3	2	2	1	2	7	1	4	7
mild		0	2	0	4	2	1	2	1	0	1
moderate		2	0	1	1	0	1	0	0	0	0
Tumor infiltrate/metastasis		1	0	0	2	0	0	1	0	2	0
minimal		0	0	0	0	0	0	1	0	0	0
moderate		0	0	0	2	0	0	0	0	0	0
severe		1	0	0	0	0	0	0	0	2	0
SKIN & SUBCUTIS											
Number examined		19	22	17	19	16	17	22	22	23	20
Cyst,epidermal		1	0	0	0	0	0	0	0	0	1
present		1	0	0	0	0	0	0	0	0	1
Atrophy,follicular		1	0	0	0	1	0	0	0	0	0
moderate		1	0	0	0	1	0	0	0	0	0
Mineralization		0	0	0	0	1	0	0	0	0	0
minimal		0	0	0	0	1	0	0	0	0	0
Edema,subcutaneous		0	0	1	1	0	0	1	0	0	0
mild		0	0	1	1	0	0	1	0	0	0
Erosion/ulcer		0	0	0	1	0	0	0	0	0	0
mild		0	0	0	1	0	0	0	0	0	0
Ulcer		0	0	0	0	1	0	0	0	0	0
minimal		0	0	0	0	1	0	0	0	0	0
Inflammation		0	0	0	1	0	0	0	0	0	0
minimal		0	0	0	1	0	0	0	0	0	0
Folliculitis		0	0	0	0	0	0	0	1	0	0
minimal		0	0	0	0	0	0	0	1	0	0
Cell infiltrate,subcutaneous		0	0	1	0	0	0	0	0	0	0
minimal		0	0	1	0	0	0	0	0	0	0
Acanthosis		0	0	0	2	1	0	0	0	0	0
minimal		0	0	0	2	1	0	0	0	0	0
Hyperkeratosis		0	0	0	2	1	0	0	0	0	0
minimal		0	0	0	1	1	0	0	0	0	0
mild		0	0	0	1	0	0	0	0	0	0
Tumor infiltrate/metastasis		1	1	1	0	0	0	0	2	0	0
mild		0	1	0	0	0	0	0	1	0	0

Tissue / Observation	Group:	1	2	3	4	5	1	2	3	4	5
	Sex:	M	M	M	M	M	F	F	F	F	F
	Number:	19	22	17	19	16	17	22	22	23	20
SKIN & SUBCUTIS (continued)											
Tumor infiltrate/metastasis (continued)											
moderate		1	0	1	0	0	0	0	0	0	0
severe		0	0	0	0	0	0	0	1	0	0
STOMACH											
Number examined		19	21	17	19	16	17	22	22	22	20
Ectopic forestomach mucosa		0	0	0	0	0	0	0	1	0	0
present		0	0	0	0	0	0	0	1	0	0
Cyst,squamous		0	0	0	0	1	0	0	0	1	0
present		0	0	0	0	1	0	0	0	1	0
Dilatation,glandular		3	5	4	3	4	5	3	7	4	8
minimal		3	4	4	3	4	5	3	7	4	8
mild		0	1	0	0	0	0	0	0	0	0
Hyperplasia,regenerative,focal		2	0	0	0	3	1	0	0	0	0
minimal		2	0	0	0	2	1	0	0	0	0
mild		0	0	0	0	1	0	0	0	0	0
Mineralization,vascular		1	0	1	0	0	0	0	0	0	0
minimal		1	0	1	0	0	0	0	0	0	0
Mineralization,muscular		2	0	0	0	0	0	0	0	0	0
minimal		2	0	0	0	0	0	0	0	0	0
Edema,submucosal		2	1	0	0	0	0	0	0	0	0
minimal		0	1	0	0	0	0	0	0	0	0
mild		2	0	0	0	0	0	0	0	0	0
Congestion/hemorrhage		0	0	0	0	0	0	0	0	0	1
minimal		0	0	0	0	0	0	0	0	0	1
Cell infiltrate,mucosal/submucosal		1	1	0	0	0	0	0	0	0	1
minimal		1	0	0	0	0	0	0	0	0	0
mild		0	1	0	0	0	0	0	0	0	1
Erosion/ulcer,forestomach		0	1	0	0	0	0	0	1	0	0
minimal		0	1	0	0	0	0	0	0	0	0
mild		0	0	0	0	0	0	0	1	0	0
Congestion/hemorrhage,agonal		0	0	0	1	1	0	0	0	0	1
present		0	0	0	1	1	0	0	0	0	1
Erosion/Ulcer,glandular stomach		6	2	2	7	2	0	4	1	2	8
minimal		4	2	2	4	2	0	2	1	2	6
mild		2	0	0	2	0	0	2	0	0	2
moderate		0	0	0	1	0	0	0	0	0	0
Hyperplasia,limiting ridge		2	0	0	0	1	0	1	2	0	0
minimal		0	0	0	0	1	0	1	2	0	0
mild		2	0	0	0	0	0	0	0	0	0
Hyperplasia,squamous cell,reactive		0	1	0	1	0	0	0	1	0	0
minimal		0	0	0	1	0	0	0	0	0	0
mild		0	1	0	0	0	0	0	1	0	0
Vacuolation,perietal cell		0	1	0	0	0	0	0	0	0	0
minimal		0	1	0	0	0	0	0	0	0	0
Fibrosis,lamina propria		1	2	0	2	1	0	0	1	1	1
minimal		1	2	0	2	1	0	0	1	1	1
Hypertrophy,surface mucous cell		0	0	0	0	0	1	0	0	0	0

Tissue / Observation	Group:	1	2	3	4	5	1	2	3	4	5
	Sex:	M	M	M	M	M	F	F	F	F	F
	Number:	19	22	17	19	16	17	22	22	23	20
STOMACH (continued)											
Hypertrophy,surface mucous cell (continued)											
mild		0	0	0	0	0	1	0	0	0	0
Mineralization,mucosal		2	0	1	0	1	0	0	0	0	0
minimal		1	0	1	0	0	0	0	0	0	0
mild		1	0	0	0	1	0	0	0	0	0
Hyperkeratosis		1	0	0	0	0	0	0	1	0	0
minimal		1	0	0	0	0	0	0	1	0	0
Tumor infiltrate/metastasis		1	0	0	0	0	0	0	1	1	0
mild		1	0	0	0	0	0	0	0	1	0
severe		0	0	0	0	0	0	0	1	0	0
SEMINAL VESICLE											
Number examined		19	22	17	19	16	-	-	-	-	-
Atrophy		4	5	4	7	3	-	-	-	-	-
minimal		2	0	1	5	1	-	-	-	-	-
mild		2	5	2	2	2	-	-	-	-	-
moderate		0	0	1	0	0	-	-	-	-	-
Vesiculitis		4	2	1	0	1	-	-	-	-	-
minimal		1	0	1	0	0	-	-	-	-	-
mild		3	1	0	0	0	-	-	-	-	-
moderate		0	1	0	0	0	-	-	-	-	-
severe		0	0	0	0	1	-	-	-	-	-
Tumor infiltrate/metastasis		2	0	0	0	0	-	-	-	-	-
mild		1	0	0	0	0	-	-	-	-	-
severe		1	0	0	0	0	-	-	-	-	-
TAIL											
Number examined		2	3	1	1	2	2	4	2	3	6
Necrosis		1	0	0	0	0	0	0	0	2	3
mild		0	0	0	0	0	0	0	0	1	2
moderate		1	0	0	0	0	0	0	0	1	1
Abscess		1	3	0	0	2	0	1	0	1	1
minimal		1	0	0	0	1	0	1	0	1	1
mild		0	3	0	0	1	0	0	0	0	0
Ulcer		0	0	0	0	1	0	0	0	1	1
minimal		0	0	0	0	1	0	0	0	0	0
mild		0	0	0	0	0	0	0	0	1	1
Inflammation		0	1	1	1	1	2	0	2	1	1
minimal		0	1	0	1	1	0	0	1	1	1
mild		0	0	1	0	0	2	0	0	0	0
moderate		0	0	0	0	0	0	0	1	0	0
Folliculitis		0	0	0	0	0	1	2	1	1	1
minimal		0	0	0	0	0	0	2	1	1	1
mild		0	0	0	0	0	1	0	0	0	0
Acanthosis		1	2	1	0	1	1	1	1	1	2
minimal		1	1	1	0	1	0	1	1	0	1
mild		0	1	0	0	0	1	0	0	1	1
Hyperkeratosis		0	0	0	1	0	0	1	0	0	0

Tissue / Observation	Group:	1	2	3	4	5	1	2	3	4	5
	Sex:	M	M	M	M	M	F	F	F	F	F
	Number:	19	22	17	19	16	17	22	22	23	20
TAIL (continued)											
Hyperkeratosis (continued)											
minimal		0	0	0	1	0	0	1	0	0	0
THORACIC CAVITY											
Number examined		3	3	1	1	1	0	0	1	1	1
Tumor infiltrate/metastasis		0	0	0	0	0	0	0	0	1	0
severe		0	0	0	0	0	0	0	0	1	0
THYMUS											
Number examined		13	13	12	11	10	12	14	15	17	11
Cyst		0	0	0	0	0	1	1	0	0	2
present		0	0	0	0	0	1	1	0	0	2
Involution		12	13	11	11	8	12	13	14	15	11
minimal		2	0	1	0	0	1	1	0	0	0
mild		2	3	1	1	2	4	1	5	3	4
moderate		6	7	9	8	6	7	11	9	11	6
severe		2	3	0	2	0	0	0	0	1	1
Mineralization,vascular		0	0	0	1	0	0	0	0	0	0
minimal		0	0	0	1	0	0	0	0	0	0
Congestion/hemorrhage,agonal		3	0	2	2	2	0	1	2	2	1
present		3	0	2	2	2	0	1	2	2	1
Hyperplasia,epithelial		0	1	0	1	0	1	4	3	6	4
minimal		0	1	0	1	0	1	4	3	6	4
Tumor infiltrate/metastasis		1	0	1	0	0	0	0	0	1	0
moderate		1	0	1	0	0	0	0	0	0	0
severe		0	0	0	0	0	0	0	0	1	0
TONGUE											
Number examined		19	22	17	19	16	17	22	22	23	20
Cell infiltrate,muscular		0	0	0	0	0	0	0	1	0	1
minimal		0	0	0	0	0	0	0	1	0	1
Erosion/ulcer		0	1	0	0	0	0	0	0	0	0
minimal		0	1	0	0	0	0	0	0	0	0
TRACHEA											
Number examined		19	22	17	19	16	17	22	22	23	20
Cell infiltrate,submucosal		0	0	0	0	1	0	0	0	0	0
minimal		0	0	0	0	1	0	0	0	0	0
Inflammation,acute		0	0	1	0	0	0	0	0	1	0
minimal		0	0	0	0	0	0	0	0	1	0
mild		0	0	1	0	0	0	0	0	0	0
Inflammation,chronic		0	0	0	1	0	0	0	0	0	0
minimal		0	0	0	1	0	0	0	0	0	0
Hyperplasia,epithelial		0	0	1	0	0	0	0	0	0	0
minimal		0	0	1	0	0	0	0	0	0	0
Tumor infiltrate/metastasis		0	0	1	0	0	0	0	0	1	0
minimal		0	0	1	0	0	0	0	0	0	0
mild		0	0	0	0	0	0	0	0	1	0

Tissue / Observation	Group:	1	2	3	4	5	1	2	3	4	5
	Sex:	M	M	M	M	M	F	F	F	F	F
	Number:	19	22	17	19	16	17	22	22	23	20
TESTIS											
Number examined		18	22	17	19	16	-	-	-	-	-
Atrophy,seminiferous tubular		8	8	7	8	4	-	-	-	-	-
minimal		1	2	3	1	0	-	-	-	-	-
mild		2	4	1	3	1	-	-	-	-	-
moderate		3	1	1	3	2	-	-	-	-	-
severe		2	1	2	1	1	-	-	-	-	-
Mineralization,vascular		0	2	2	0	2	-	-	-	-	-
minimal		0	2	2	0	2	-	-	-	-	-
Arteritis		0	0	1	0	1	-	-	-	-	-
minimal		0	0	1	0	1	-	-	-	-	-
Congestion/hemorrhage,agonal		0	1	0	1	0	-	-	-	-	-
present		0	1	0	1	0	-	-	-	-	-
Necrosis,germ cell		2	0	1	0	0	-	-	-	-	-
minimal		0	0	1	0	0	-	-	-	-	-
mild		2	0	0	0	0	-	-	-	-	-
Stasis,sperm		2	0	1	2	0	-	-	-	-	-
minimal		2	0	1	2	0	-	-	-	-	-
Hyperplasia,Leydig cell,focal		0	1	2	0	1	-	-	-	-	-
present		0	1	2	0	1	-	-	-	-	-
Tumor infiltrate/metastasis		1	0	0	0	0	-	-	-	-	-
minimal		1	0	0	0	0	-	-	-	-	-
TOOTH											
Number examined		5	3	1	2	3	2	2	3	1	2
Periodontitis		3	2	1	2	2	1	1	3	1	2
minimal		1	2	1	1	2	0	0	3	0	1
mild		1	0	0	1	0	1	1	0	1	0
moderate		1	0	0	0	0	0	0	0	0	1
Dysplasia,dental		3	2	0	0	1	1	1	0	0	0
minimal		2	2	0	0	1	1	1	0	0	0
moderate		1	0	0	0	0	0	0	0	0	0
THYROID LOBE											
Number examined		18	22	17	19	16	17	20	22	23	20
Cystic follicle		0	0	0	0	0	1	0	0	0	0
present		0	0	0	0	0	1	0	0	0	0
Arteritis		0	0	0	0	1	0	0	0	0	0
minimal		0	0	0	0	1	0	0	0	0	0
Dilated follicle		0	0	0	0	0	0	1	0	1	1
minimal		0	0	0	0	0	0	1	0	1	1
Hyperplasia,C-cell,diffuse		6	8	4	5	2	4	2	6	6	4
minimal		6	8	4	5	1	4	2	5	6	4
mild		0	0	0	0	1	0	0	1	0	0
Hyperplasia,C-cell,focal		0	0	1	0	1	0	1	1	0	1
present		0	0	1	0	1	0	1	1	0	1
Hyperplasia,follicular cell,focal		0	0	0	0	0	0	0	0	0	1
present		0	0	0	0	0	0	0	0	0	1
Tumor infiltrate/metastasis		0	1	0	0	0	0	0	0	0	0

Tissue / Observation	Group:	1	2	3	4	5	1	2	3	4	5
	Sex:	M	M	M	M	M	F	F	F	F	F
	Number:	19	22	17	19	16	17	22	22	23	20
THYROID LOBE (continued)											
Tumor infiltrate/metastasis (continued)											
minimal		0	1	0	0	0	0	0	0	0	0
URINARY BLADDER											
Number examined		19	22	17	19	16	17	21	22	23	19
Calculus		0	0	0	0	0	0	1	0	0	0
present		0	0	0	0	0	0	1	0	0	0
Dilatation,luminal		7	1	3	5	3	0	3	1	2	3
minimal		1	0	2	0	1	0	1	0	2	1
mild		5	1	1	3	2	0	2	1	0	1
moderate		1	0	0	1	0	0	0	0	0	1
severe		0	0	0	1	0	0	0	0	0	0
Edema,submucosal		0	0	0	0	0	1	0	0	0	0
minimal		0	0	0	0	0	1	0	0	0	0
Hemorrhage		0	1	0	1	0	0	0	0	0	0
mild		0	0	0	1	0	0	0	0	0	0
moderate		0	1	0	0	0	0	0	0	0	0
Cell infiltrate,mucosal/submucosal		0	0	0	0	1	0	0	0	2	0
minimal		0	0	0	0	1	0	0	0	2	0
Cystitis		3	2	0	2	1	0	5	0	1	2
minimal		0	0	0	0	0	0	0	0	1	0
mild		0	1	0	0	0	0	4	0	0	2
moderate		0	1	0	1	1	0	1	0	0	0
severe		3	0	0	1	0	0	0	0	0	0
Exudate,inflammatory		0	1	0	0	0	0	0	0	0	0
present		0	1	0	0	0	0	0	0	0	0
Cell infiltrate,adventitial		0	1	0	0	0	0	0	0	0	0
minimal		0	1	0	0	0	0	0	0	0	0
Metaplasia,squamous cell		0	0	0	0	0	0	1	0	0	0
mild		0	0	0	0	0	0	1	0	0	0
Hyperplasia,urothelial		0	0	0	0	0	1	0	1	1	0
minimal		0	0	0	0	0	0	0	1	1	0
mild		0	0	0	0	0	1	0	0	0	0
Tumor infiltrate/metastasis		2	0	1	0	0	0	0	1	0	0
minimal		1	0	1	0	0	0	0	0	0	0
mild		0	0	0	0	0	0	0	1	0	0
severe		1	0	0	0	0	0	0	0	0	0
URETHRA											
Number examined		0	0	0	0	0	0	1	0	0	0
Angiectasis		0	0	0	0	0	0	1	0	0	0
minimal		0	0	0	0	0	0	1	0	0	0
UTERUS											
Number examined		-	-	-	-	-	17	22	22	23	19
Dilatation,luminal		-	-	-	-	-	2	1	1	0	1
minimal		-	-	-	-	-	1	0	0	0	1
mild		-	-	-	-	-	0	1	1	0	0

Tissue / Observation	Group:	1	2	3	4	5	1	2	3	4	5
	Sex:	M	M	M	M	M	F	F	F	F	F
	Number:	19	22	17	19	16	17	22	22	23	20
UTERUS (continued)											
Dilatation,luminal (continued)											
moderate		-	-	-	-	-	1	0	0	0	0
Atrophy		-	-	-	-	-	0	1	0	0	1
mild		-	-	-	-	-	0	1	0	0	0
moderate		-	-	-	-	-	0	0	0	0	1
Cystic gland		-	-	-	-	-	1	1	0	2	1
minimal		-	-	-	-	-	1	1	0	2	1
Hyperplasia,glandular		-	-	-	-	-	0	0	1	0	0
present		-	-	-	-	-	0	0	1	0	0
Metaplasia,squamous,glands		-	-	-	-	-	1	0	0	0	0
minimal		-	-	-	-	-	1	0	0	0	0
Tumor infiltrate/metastasis		-	-	-	-	-	0	0	1	0	1
mild		-	-	-	-	-	0	0	1	0	1
VAGINA											
Number examined		-	-	-	-	-	17	21	22	23	19
Mucification		-	-	-	-	-	4	4	7	2	3
minimal		-	-	-	-	-	2	3	2	2	3
mild		-	-	-	-	-	2	1	5	0	0
Atrophy		-	-	-	-	-	6	11	9	13	12
minimal		-	-	-	-	-	0	0	1	0	1
mild		-	-	-	-	-	2	1	3	2	1
moderate		-	-	-	-	-	2	6	3	7	3
severe		-	-	-	-	-	2	4	2	4	7
Tumor infiltrate/metastasis		-	-	-	-	-	1	0	0	0	0
moderate		-	-	-	-	-	1	0	0	0	0
NASAL CAVITY (section 2)											
Number examined		19	22	17	19	16	17	22	22	23	20
Hemorrhage,focal		0	0	0	0	1	1	0	0	0	0
minimal		0	0	0	0	1	1	0	0	0	0
Cell infiltrate,mucosal/submucosal		3	0	0	0	0	0	0	0	0	0
minimal		3	0	0	0	0	0	0	0	0	0
Inflammation,nasal cavity		1	2	0	0	0	0	1	1	1	0
minimal		0	1	0	0	0	0	0	0	0	0
mild		1	0	0	0	0	0	0	1	1	0
moderate		0	0	0	0	0	0	1	0	0	0
severe		0	1	0	0	0	0	0	0	0	0
Inflammation,nasolacrimal duct		2	1	1	1	0	0	2	1	1	0
minimal		1	1	1	0	0	0	1	1	0	0
mild		1	0	0	1	0	0	1	0	1	0
Dilatation,submucosal gland		2	5	3	3	2	2	2	5	4	2
minimal		2	5	3	3	2	2	2	5	4	2
Fracture		0	0	0	0	0	0	0	1	0	0
present		0	0	0	0	0	0	0	1	0	0
Droplets,eosinophilic		7	6	10	5	6	8	9	14	6	9
minimal		7	6	10	5	6	8	9	12	6	9
mild		0	0	0	0	0	0	0	2	0	0

Tissue / Observation	Group:	1	2	3	4	5	1	2	3	4	5
	Sex:	M	M	M	M	M	F	F	F	F	F
	Number:	19	22	17	19	16	17	22	22	23	20
NASAL CAVITY (section 2) (continued)											
Exudate,inflammatory		3	2	3	3	1	0	2	1	1	0
minimal		3	2	3	3	1	0	2	1	1	0
Deformity		0	0	0	1	0	0	1	0	0	1
present		0	0	0	1	0	0	1	0	0	1
Exudate,proteinaceous		0	1	1	0	0	0	0	2	0	0
minimal		0	1	1	0	0	0	0	2	0	0
Degeneration/regeneration,olfactory		0	0	0	0	0	0	0	0	1	0
mild		0	0	0	0	0	0	0	0	1	0
Hyperplasia,respiratory		0	0	0	0	0	0	2	0	0	0
mild		0	0	0	0	0	0	2	0	0	0
Hyperplasia,goblet cell		1	0	0	1	1	1	0	2	2	1
minimal		1	0	0	1	1	1	0	2	1	1
mild		0	0	0	0	0	0	0	0	1	0
Tumor infiltrate/metastasis		1	0	0	0	0	0	0	0	2	0
minimal		0	0	0	0	0	0	0	0	1	0
severe		1	0	0	0	0	0	0	0	1	0
NASAL CAVITY (section 3)											
Number examined		19	22	17	19	16	17	22	22	23	20
Hemorrhage,focal		0	2	0	0	1	1	0	1	0	0
minimal		0	2	0	0	1	1	0	1	0	0
Cell infiltrate,mucosal/submucosal		3	0	0	0	0	0	0	0	0	0
minimal		3	0	0	0	0	0	0	0	0	0
Inflammation,nasal cavity		0	1	1	0	0	0	1	0	1	0
minimal		0	0	1	0	0	0	1	0	0	0
mild		0	0	0	0	0	0	0	0	1	0
severe		0	1	0	0	0	0	0	0	0	0
Inflammation,maxillary sinus		0	1	1	0	0	0	1	0	0	0
minimal		0	0	1	0	0	0	0	0	0	0
mild		0	0	0	0	0	0	1	0	0	0
severe		0	1	0	0	0	0	0	0	0	0
Dilatation,submucosal gland		9	15	5	9	7	8	11	10	11	9
minimal		9	15	5	9	7	8	11	10	11	9
Droplets,eosinophilic		18	22	17	17	16	16	20	22	21	20
minimal		18	19	11	16	15	16	19	16	20	20
mild		0	3	6	1	1	0	1	6	1	0
Exudate,inflammatory		0	0	1	0	0	1	0	0	0	0
minimal		0	0	1	0	0	1	0	0	0	0
Deformity		0	0	0	0	0	0	0	1	2	1
present		0	0	0	0	0	0	0	1	2	1
Exudate,proteinaceous		0	0	0	0	0	0	0	0	1	0
minimal		0	0	0	0	0	0	0	0	1	0
Degeneration/regeneration,olfactory		1	0	0	0	0	0	0	0	1	0
minimal		1	0	0	0	0	0	0	0	0	0
mild		0	0	0	0	0	0	0	0	1	0
Hyperplasia,respiratory		0	0	1	0	0	0	0	0	0	0
mild		0	0	1	0	0	0	0	0	0	0
Hyperplasia,goblet cell		1	2	2	0	0	0	1	4	1	1

Tissue / Observation	Group:	1	2	3	4	5	1	2	3	4	5
	Sex:	M	M	M	M	M	F	F	F	F	F
	Number:	19	22	17	19	16	17	22	22	23	20
NASAL CAVITY (section 3) (continued)											
Hyperplasia,goblet cell (continued)											
minimal		0	0	1	0	0	0	0	3	0	1
mild		1	2	1	0	0	0	1	1	1	0
Tumor infiltrate/metastasis		1	0	0	0	0	0	0	0	2	1
mild		0	0	0	0	0	0	0	0	2	1
severe		1	0	0	0	0	0	0	0	0	0
NASAL CAVITY (section 4)											
Number examined		19	22	17	19	16	17	22	22	23	20
Hemorrhage,focal		0	1	0	0	1	1	1	0	0	0
minimal		0	1	0	0	1	1	1	0	0	0
Cell infiltrate,mucosal/submucosal		2	0	0	0	0	0	0	0	0	0
minimal		2	0	0	0	0	0	0	0	0	0
Inflammation,nasal cavity		0	1	2	0	0	0	0	0	1	0
minimal		0	0	2	0	0	0	0	0	0	0
mild		0	0	0	0	0	0	0	0	1	0
severe		0	1	0	0	0	0	0	0	0	0
Malacia/gliosis,olfactory bulb		0	0	1	0	0	0	0	0	0	0
mild		0	0	1	0	0	0	0	0	0	0
Droplets,eosinophilic		17	22	16	15	13	15	20	22	19	20
minimal		17	19	10	14	13	15	20	19	19	20
mild		0	3	6	1	0	0	0	3	0	0
Exudate,inflammatory		0	0	0	0	0	0	0	0	1	0
minimal		0	0	0	0	0	0	0	0	1	0
Deformity		0	0	1	1	0	1	0	0	1	1
present		0	0	1	1	0	1	0	0	1	1
Exudate,proteinaceous		0	0	0	1	0	0	0	0	0	0
minimal		0	0	0	1	0	0	0	0	0	0
Degeneration/regeneration,olfactory		0	0	0	0	0	0	0	0	1	0
mild		0	0	0	0	0	0	0	0	1	0
Metaplasia,respiratory		0	0	1	0	0	0	0	0	0	0
minimal		0	0	1	0	0	0	0	0	0	0
Hyperplasia,goblet cell		1	2	3	0	0	0	1	6	0	1
minimal		0	0	2	0	0	0	0	5	0	1
mild		1	2	1	0	0	0	1	1	0	0
Tumor infiltrate/metastasis		1	0	0	0	0	0	0	0	3	1
mild		0	0	0	0	0	0	0	0	1	1
moderate		0	0	0	0	0	0	0	0	1	0
severe		1	0	0	0	0	0	0	0	1	0

Toxicokinetics:

FDKP was quantifiable in all T or TI groups at concentrations which increased with the corresponding increase in dose of T. C_{max} concentrations were generally comparable between each sampling time point (Weeks 52, 78, and 104). FDPK was also quantifiable in most pre-dose samples but at concentrations notably less than post-exposure samples.

Moreover, a number of samples collected from control animals (Group1) had quantifiable concentrations of FDKP. They were generally close to the limit of detection and were inconsistent in between time points. The presence of FDKP in these samples was likely due to post exposure contact contamination of samples and not relate to the animal exposures.

Group Number	Group Designation	Sex	C_{max} (FDKP ng/mL)		
			Week 52	Week 78	Week 104
1	Air Control	♂ ♀	- -	- -	- -
2	Technosphere® Control Low Dose	♂ ♀	388 203	175 135	465 262
3	Technosphere® Control High Dose	♂ ♀	6309 7458	4577 3062	4488 6260
4	Technosphere® Insulin Low Dose	♂ ♀	686 223	94 102	285 133
5	Technosphere® Insulin High Dose	♂ ♀	813 509	186 84	545 160

Insulin was only quantifiable in TI-treated animals (Groups 4 and 5) at the post exposure sampling. Due to high variability of the data, a definitive comparison of circulating insulin levels between groups and sexes was not possible.

Group Number	Group Designation	Sex	C_{max} (Insulin µU/mL)		
			Week 52	Week 78	Week 104
1	Air Control	♂ ♀	BLQ BLQ	BLQ BLQ	BLQ BLQ
2	Technosphere® Control Low Dose	♂ ♀	BLQ BLQ	BLQ BLQ	BLQ BLQ
3	Technosphere® Control High Dose	♂ ♀	BLQ BLQ	BLQ BLQ	BLQ BLQ
4	Technosphere® Insulin Low Dose	♂ ♀	53.080 4.483	7.037 8.986	20.094 15.352
5	Technosphere® Insulin High Dose	♂ ♀	44.734 27.881	18.713 8.316	36.550 13.511

BLQ = all samples were below the limit of quantification

Pivotal Study #2

Study title: 26-Week Subcutaneous Injection Oncogenicity Study With Technosphere Particles and Technosphere Insulin in Transgenic (Model 001178-T (hemizygous rasH2)) Mice

Key study findings:

- Due to Technosphere® Insulin (TI) tolerability issues in female mice, doses were lowered from 2.5 to 0.6 mg/kg/day (Group 5) and from 5.0 to 1.25 mg/kg/day (Group 6). The dose adjustment on Day 77 to 0.6 and 1.25 mg/kg/day was well-tolerated. There were no comparable dose limitations in males.
- Technosphere® particles (T) alone were well-tolerated at doses of 25 and 75 mg/kg/day.
- Exposure to FDKP, the primary component of T, was generally dose proportional. There were no sex differences or evidence of accumulation.
- Exposure to insulin was generally dose-proportional for males, but greater than dose-proportional for females, with no evidence of accumulation.
- There was no evidence of increased oncogenicity, or other clinical, gross or microscopic pathological effects related to treatment with T alone up to 75 mg/kg/day, or with TI up to 5.0 mg/kg/day in males, or up to 5.0/1.25 mg/kg/day in females after 26 weeks of treatment in transgenic mice.

Adequacy of the carcinogenicity study and appropriateness of the test model: Yes

Evaluation of tumor findings:
Study no.: MKC-PC-2007-0003
Volume # and page #: eEDR, module 4.2.3.4.2
Conducting laboratory and location: (b) (4)
Date of study initiation: June 26th 2007
GLP compliance: Yes
QA report: yes (x), no ( )
Drug, lot #, and % purity: See table below

Test Articles	Lot No.	Storage[a]	Purity	Expiration Date	Reserve (Archive) Sample
Technosphere® Particles	D-232-06-0004	15 to 30°C, protected from light	98.8 %	31 May 2009	1 g
Technosphere®/ Insulin	D-231-07-0001	-10 to -30°C, protected from light	99 %	30 Sept 2009	1 g

a See Protocol Deviations.

CAC concurrence: Yes

Methods

Doses: Technosphere Particles: 0, 25, 75 mg/kg/day (male and female)
TI: 0, 2.5, 5 mg/kg/day (male) and 0, 2.5/0.6, 5/1.25 mg/kg/day (female)

Group Designation and Dose Levels

Group[ab]	No. of Animals		Dose Level[f] (mg/kg/day)		Dose Concentration (mg/mL)	
	Male	Female	Male	Female	Male	Female
Toxicity Animals						
1 (Control - Sham)	25	25	0	0	0	0
2 (Vehicle Control - PBS)[g]	25	25	0	0	0	0
3 (Technosphere® Particles – Low)	25	25	25	25	2.5	2.5
4 (Technosphere® Particles – High)	25	25	75	75	7.5	7.5
5 (Technosphere®/Insulin – Low)	25	25	2.5	2.5/0.6[e]	0.25	0.25/0.06[e]
6 (Technosphere®/Insulin – High)	25	25	5	5/1.25[e]	0.5	0.5/0.125[e]
7 (Positive Control - MNU)	25	25	75	75	7.5	7.5
Toxicokinetic Animals[cd]						
8 (Vehicle Control - PBS)[g]	18	18	0	0	0	0
9 (Technosphere® Particles - Low)	38	38	25	25	2.5	2.5
10 (Technosphere® Particles - High)	38	38	75	75	7.5	7.5
11 (Technosphere®/Insulin – Low)	38	38	2.5	2.5/0.6[e]	0.25	0.25/0.06[e]
12 (Technosphere®/Insulin – High)	38	38	5	5/1.25[e]	0.5	0.5/0.125[e]

a Group 1 received a sham dose only by subcutaneous injection with an empty syringe needle.
b Group 7 animals were dosed with one intraperitoneal dose of MNU on Day 1 of study at a dose volume of 10 mL/kg.
c Toxicokinetic animals were wild-type mice (Model 001178-W, CB6F1/Jic-TgrasH2@Tac) and were included solely for the purpose of blood sample collections; three/sex/group were bled for each collection timepoint, then discarded without necropsy.
d Two extra animals/test article group were added as potential replacements.
e Due to a high incidence of unscheduled deaths in females, beginning Day 77 of the dosing phase the dose level for females in Groups 5 and 11 was lowered to 0.6 mg/kg/day and to 1.25 mg/kg/day for females in Groups 6 and 12.
f The dose volume administered to all groups except sham control was 10 mL/kg/dose.
g Vehicle control groups received 10 mM phosphate buffered saline prepared with sterile water for injection.

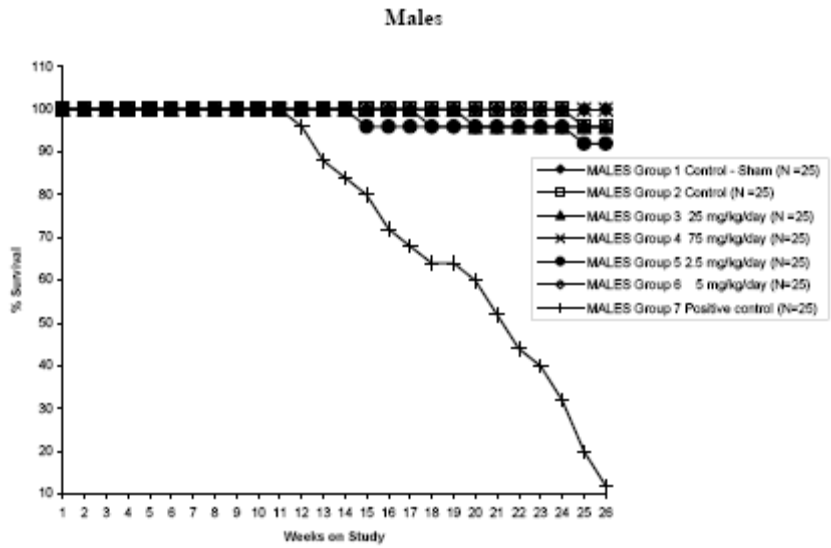
Basis of dose selection (MTD, MFD, AUC etc.): MFD for T and MTD for TI
Species/strain: Model 001178-T transgenic mice
Number/sex/group (main study): Main study: 25/sex/group
Route, formulation, volume: SC injection at the volume of 10 ml/kg
Frequency of dosing: Once daily for at least 26 weeks (dosing phase)
Satellite groups used for toxicokinetics or special groups: Model 001178-W (wt)
Age: 8 weeks old
Animal housing: Individually housed after randomization
Restriction paradigm for dietary restriction studies: None
Drug stability/homogeneity: Tested. 2 weeks when stored refrigerated (MKC-PC-2006-018)
Dual controls employed: Yes. Main study: sham, PBS (VH), and MNU (positive)
Interim sacrifices: No, all surviving animals were euthanized at the end of study.
Deviations from original study protocol: Minor deviations from the protocol, typically procedural.

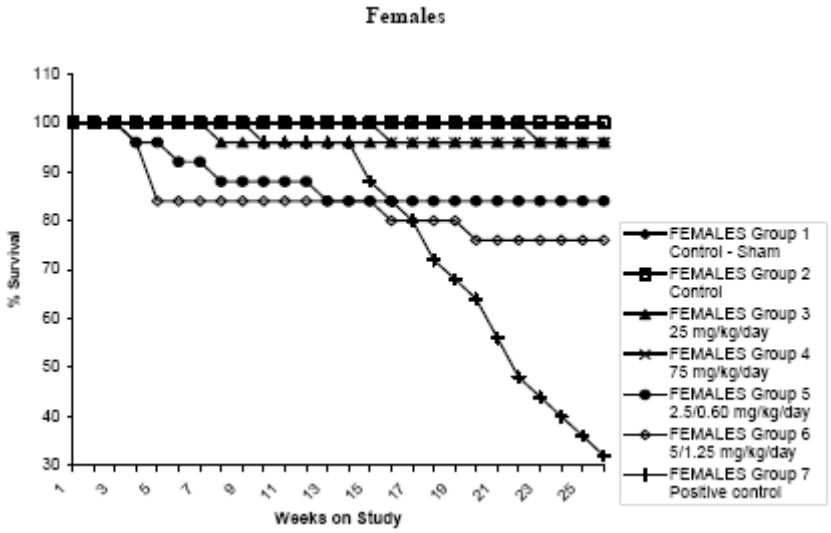
Observation times
Mortality: Daily
Clinical signs: Twice daily (a.m. and p.m.)
Body weights: Once during the pre-dose phase, before dosing on Day 1 of the dosing phase, and weekly thereafter

Food consumption: Weekly during the dosing phase
Histopathology: All tissues from animals in the control and high dose groups (Groups 1,2,4,6, and 7) and from animals that died or were sacrificed at an unscheduled interval
Peer review: yes (x), no ( )
Toxicokinetics: Samples were collected from 3 mice/sex/group (Group 9 through 12) on Day 1 and 178 (Week 26) predose and at 15, 30, 45, and 120 min postdose (± 2 min)

Results
Mortality:

- Males: 1 death in Group 1, 3, and 6, 2 deaths in Group 5, and 22 deaths in Group 7.
- Females: 1 death in Group 1, 3, and 4, 4 deaths in Group 4, 6 deaths in Group 6, and 17 deaths in Group 7.





Unscheduled deaths of undetermined cause in females in the 2.5 and 5 mg/kg/day TI groups prior to Day 77 were attributed to the pharmacologic activity of insulin. Only 1 death in female Group 5 and Group 6 occurred after the Day 77 when the doses were lowered. Therefore, for other

unscheduled deaths in the TI-treated groups where cause of death was undetermined histologically, an association with insulin pharmacology is plausible. The sponsor provided the summary table (see below) of unscheduled death.

Animal	Death Day	Clinical Signs Prior to Death	Cause of Death Determined by Histopathology
Sham Control			
Female A98838 (found dead)	159	Hunched posture; tremors (head); hypoactivity; labored respiration; rough haircoat	Myeloproliferative neoplasia
Vehicle Control			
Male A98522 (found dead)	172	No clinical signs prior to death	Hemangiosarcoma
Technosphere® (low; 25 mg/kg/day)			
Male A98541 (found dead)	140	Hypoactivity; irregular respiration	Hemangiosarcoma
Female A98894 (euthanized)	53	Limited use of hind limbs; swollen rectum; alopecia; rough haircoat; sore/scab; general debilitation	Skin, ulceration/inflammation
Technosphere® (high; 75 mg/kg/day)			
Female A98909 (found dead)	107	Swollen abdomen; rough haircoat	Squamous cell sarcoma
Technosphere®/Insulin (low; 2.5 mg/kg/day)			
Male A98591 (found dead)	101	Hunched posture; hypoactivity; irregular respiration; rough haircoat	Undetermined
Male A98595 (found dead)	175	Hunched posture; rough haircoat; thin appearance; mass	Salivary gland sarcoma
Female A98937 (found dead)	41	No clinical signs prior to death	Undetermined
Female A98939 (found dead)	49	Hypoactivity; irregular respiration; rough haircoat	Heart, valvular thrombosis and atrial dilatation
Female A98940 (found dead)	27	No clinical signs prior to death	Undetermined
Technosphere®/Insulin (low; 2.5/0.6 mg/kg/day)			
Female A98954 (found dead)	89	No clinical signs prior to death	Undetermined
Technosphere®/Insulin (high; 5 mg/kg/day)			
Male A98625(found dead)	125	No clinical signs prior to death	Hemangiosarcoma
Female A98968 (found dead)	28	No clinical signs prior to death	Undetermined
Female A98974 (found dead)	32	No clinical signs prior to death	Undetermined
Female A98972 (euthanized)	30	Ataxia; hypoactivity; cold to the touch; general debilitation	Undetermined
Female A98979 (found dead)	32	No clinical signs prior to death	Undetermined
Technosphere®/Insulin (high; 5/1.25 mg/kg/day)			
Female A98976 (found dead)	109	Malocclusion; rough haircoat	Undetermined
Female A98960 (euthanized)	137	Hypoactivity; recumbency; labored respiration; cold to the touch; pale tail; general debilitation	Hemangiosarcoma.

Clinical signs: An increased incidence of rough haircoat was observed in animals (that survived to scheduled termination) given 75 mg/kg/day T and 2.5 and 5 mg/kg/day TI, when compared to VH controls. In addition, hypoactivity was seen in two males given 5 mg/kg/day TI. Convulsions and hunched posture were seen in 1 male given 75 mg/kg/day T. Moreover, hypoactivity and irregular respiration were seen in two 2.5 mg/kg/day TI-treated females.

Summary of Clinical Signs

Category / Sign	Sex:	Males			
	Group:	1	2	3	4
	Dose Level:	0.00	0.00	25.00	75.00
	Dose Units:	mg/kg/day	mg/kg/day	mg/kg/day	mg/kg/day
	Number in Group:	25	25	25	25
		N	N	N	N
Appearance					
Bent Tail		0	0	0	1
Bent Tail, Distal Tail		0	0	0	1
Bent Tail, Mid Tail		0	0	0	0
Convulsions, Mild		0	0	0	0
Convulsions, Moderate		0	0	0	0
Hunched		0	0	0	0
Limited Use, Hind Legs		0	0	0	0
Malocclusion		0	0	0	0
Missing, Partial Ears		1	0	0	0
Missing, Partial Left Ear		1	0	0	0
Missing, Partial Right Ear		1	0	0	0
Missing, Teeth		0	0	0	0
Protruding Penis		0	0	0	0
Swollen, Distal Tail		0	0	0	0
Swollen, Left Lateral Abdomen		0	0	0	1
Swollen, Midline Ventral Abdomen		0	0	0	0
Swollen, Nose		0	1	0	0
Swollen, Perineal Area		0	0	0	0
Swollen, Proximal Tail		0	0	1	0
Swollen, Rectum		0	0	0	0
Swollen, Right Front Digit(s)		0	0	0	0
Swollen, Testes		0	0	0	0
Thin		0	0	0	0
Tremors Body		0	0	0	0

N = Number of animals with observed sign

SUMMARY OF CLINICAL SIGNS

Category / Sign	Sex:	Males		
	Group:	5	6	7
	Dose Level:	2.50	5.00	75.00
	Dose Units:	mg/kg/day	mg/kg/day	mg/kg/day
	Number in Group:	25	25	25
		N	N	N
Appearance				
Bent Tail		0	0	0
Bent Tail, Distal Tail		0	0	0
Bent Tail, Mid Tail		0	0	0
Convulsions, Mild		0	0	0
Convulsions, Moderate		0	0	0
Hunched		2	0	9
Limited Use, Hind Legs		0	1	0
Malocclusion		0	0	0
Missing, Partial Ears		0	0	0
Missing, Partial Left Ear		0	0	0
Missing, Partial Right Ear		0	0	0
Missing, Teeth		0	1	0
Protruding Penis		0	0	1
Swollen, Distal Tail		0	0	0
Swollen, Left Lateral Abdomen		0	0	0
Swollen, Midline Ventral Abdomen		0	0	5
Swollen, Nose		0	0	0
Swollen, Perineal Area		0	0	0
Swollen, Proximal Tail		0	0	0
Swollen, Rectum		0	0	0
Swollen, Right Front Digit(s)		1	0	0
Swollen, Testes		0	1	0
Thin		1	0	1
Tremors Body		0	0	0

N = Number of animals with observed sign

SUMMARY OF CLINICAL SIGNS

Category / Sign	Sex:	Males			
	Group:	1	2	3	4
	Dose Level:	0.00	0.00	25.00	75.00
	Dose Units:	mg/kg/day	mg/kg/day	mg/kg/day	mg/kg/day
	Number in Group:	25	25	25	25
		N	N	N	N
Appearance					
Tremors Head		0	0	0	0
Behavior					
Ataxic		0	0	0	0
Hypoactive		0	0	1	0
Recumbent, Lateral		0	0	0	0
Recumbent, Midline Ventral Abdomen		0	0	0	0
Discharge					
Red Oral		0	0	0	0
Other, Red in Color		0	0	0	0
Other, Red in Color, Left Ear		0	0	0	0
Excretion(s)					
Few Feces		0	0	0	0
Eye(s)					
Clear Discharge-Right Eye		0	0	0	0
Squinted-Left Eye		0	0	0	0
Mass					
Mass		0	0	0	0
Mass, Right Front Paw		0	0	0	0
Respiration					
Irregular		0	0	1	0

N = Number of animals with observed sign

SUMMARY OF CLINICAL SIGNS

Category / Sign	Sex:	Males		
	Group:	5	6	7
	Dose Level:	2.50	5.00	75.00
	Dose Units:	mg/kg/day	mg/kg/day	mg/kg/day
	Number in Group:	25	25	25
		N	N	N
Appearance				
Tremors Head		0	0	0
Behavior				
Ataxic		0	0	0
Hypoactive		1	2	8
Recumbent, Lateral		0	0	0
Recumbent, Midline Ventral Abdomen		0	1	0
Discharge				
Red Oral		0	0	0
Other, Red in Color		0	0	0
Other, Red in Color, Left Ear		0	0	0
Excretion(s)				
Few Feces		0	0	0
Eye(s)				
Clear Discharge-Right Eye		0	1	0
Squinted-Left Eye		0	0	0
Mass				
Mass		3	0	8
Mass, Right Front Paw		1	0	0
Respiration				
Irregular		1	0	7

N = Number of animals with observed sign

SUMMARY OF CLINICAL SIGNS

Category / Sign	Sex:	Males			
	Group:	1	2	3	4
	Dose Level:	0.00	0.00	25.00	75.00
	Dose Units:	mg/kg/day	mg/kg/day	mg/kg/day	mg/kg/day
	Number in Group:	25	25	25	25
		N	N	N	N
Respiration					
Labored		0	0	0	0
Skin & Pelage					
Alopecia, Dorsal		0	0	0	0
Alopecia, Front Paws		0	0	0	0
Alopecia, Left Dorsal Abdomen (Back)		0	0	0	0
Alopecia, Perineal Area		0	0	0	0
Cold To Touch		0	0	0	0
Cold To Touch, Entire Body		0	0	0	0
Pale		0	0	0	0
Pale, Distal Tail		0	0	0	0
Pale, Mid Tail		0	0	0	0
Rough Haircoat		7	9	7	18
Sore/Scab, Distal Tail		0	0	0	0
Sore/Scab, Dorsal Abdomen (Back)		0	0	0	0
Sore/Scab, Left Dorsal Abdomen (Back)		0	0	0	0
Sore/Scab, Left Dorsal Thorax		0	0	0	0
Sore/Scab, Left Ear		0	0	0	0
Sore/Scab, Mid Tail		1	0	0	0
Sore/Scab, Penis		0	0	0	0
Sore/Scab, Proximal Tail		1	1	0	0
Sore/Scab, Rectum		0	0	0	0
Yellow Haircoat, Perineal Area		0	0	0	0

N = Number of animals with observed sign

SUMMARY OF CLINICAL SIGNS

Category / Sign	Sex:	Males		
	Group:	5	6	7
	Dose Level:	2.50	5.00	75.00
	Dose Units:	mg/kg/day	mg/kg/day	mg/kg/day
	Number in Group:	25	25	25
		N	N	N
Respiration				
Labored		0	1	4
Skin & Pelage				
Alopecia, Dorsal		0	0	0
Alopecia, Front Paws		0	1	0
Alopecia, Left Dorsal Abdomen (Back)		1	0	0
Alopecia, Perineal Area		0	1	0
Cold To Touch		0	0	0
Cold To Touch, Entire Body		0	0	0
Pale		0	0	0
Pale, Distal Tail		0	6	2
Pale, Mid Tail		0	0	0
Rough Haircoat		20	18	19
Sore/Scab, Distal Tail		0	0	0
Sore/Scab, Dorsal Abdomen (Back)		0	0	0
Sore/Scab, Left Dorsal Abdomen (Back)		1	0	0
Sore/Scab, Left Dorsal Thorax		0	0	0
Sore/Scab, Left Ear		0	0	0
Sore/Scab, Mid Tail		0	0	0
Sore/Scab, Penis		1	0	0
Sore/Scab, Proximal Tail		0	0	0
Sore/Scab, Rectum		0	0	0
Yellow Haircoat, Perineal Area		0	0	1

N = Number of animals with observed sign

SUMMARY OF CLINICAL SIGNS

Category / Sign	Sex:	Females			
	Group:	1	2	3	4
	Dose Level:	0.00	0.00	25.00	75.00
	Dose Units:	mg/kg/day	mg/kg/day	mg/kg/day	mg/kg/day
	Number in Group:	25	25	25	25
		N	N	N	N
Appearance					
Bent Tail		0	0	0	0
Bent Tail, Distal Tail		0	0	0	0
Bent Tail, Mid Tail		0	0	0	0
Convulsions, Mild		0	0	0	1
Convulsions, Moderate		0	0	0	1
Hunched		1	0	0	1
Limited Use, Hind Legs		0	0	1	0
Malocclusion		0	0	0	0
Missing, Partial Ears		0	0	0	0
Missing, Partial Left Ear		0	0	0	0
Missing, Partial Right Ear		0	0	0	0
Missing, Teeth		0	0	0	0
Protruding Penis		0	0	0	0
Swollen, Distal Tail		0	1	0	0
Swollen, Left Lateral Abdomen		0	0	0	0
Swollen, Midline Ventral Abdomen		0	0	0	1
Swollen, Nose		0	0	0	0
Swollen, Perineal Area		1	0	1	0
Swollen, Proximal Tail		0	0	0	0
Swollen, Rectum		0	0	1	0
Swollen, Right Front Digit(s)		0	0	0	0
Swollen, Testes		0	0	0	0
Thin		0	0	0	0
Tremors Body		0	0	0	0

N = Number of animals with observed sign

SUMMARY OF CLINICAL SIGNS

Category / Sign	Sex:	Females		
	Group:	5	6	7
	Dose Level:	2.50/0.60	5.00/1.25	75.00
	Dose Units:	mg/kg/day	mg/kg/day	mg/kg/day
	Number in Group:	25	25	25
		N	N	N
Appearance				
Bent Tail		0	0	0
Bent Tail, Distal Tail		0	0	0
Bent Tail, Mid Tail		0	1	0
Convulsions, Mild		0	0	0
Convulsions, Moderate		0	0	0
Hunched		0	0	8
Limited Use, Hind Legs		0	0	0
Malocclusion		0	1	0
Missing, Partial Ears		0	0	0
Missing, Partial Left Ear		0	0	0
Missing, Partial Right Ear		0	0	0
Missing, Teeth		0	0	0
Protruding Penis		0	0	0
Swollen, Distal Tail		0	0	0
Swollen, Left Lateral Abdomen		0	0	0
Swollen, Midline Ventral Abdomen		0	0	3
Swollen, Nose		0	0	0
Swollen, Perineal Area		0	0	0
Swollen, Proximal Tail		0	0	0
Swollen, Rectum		0	0	0
Swollen, Right Front Digit(s)		0	0	0
Swollen, Testes		0	0	0
Thin		0	0	5
Tremors Body		0	0	1

N = Number of animals with observed sign

SUMMARY OF CLINICAL SIGNS

Category / Sign	Sex:	Females			
	Group:	1	2	3	4
	Dose Level:	0.00	0.00	25.00	75.00
	Dose Units:	mg/kg/day	mg/kg/day	mg/kg/day	mg/kg/day
	Number in Group:	25	25	25	25
		N	N	N	N
Appearance					
Tremors Head		1	0	0	0
Behavior					
Ataxic		0	0	0	0
Hypoactive		1	0	0	0
Recumbent, Lateral		0	0	0	0
Recumbent, Midline Ventral Abdomen		0	0	0	0
Discharge					
Red Oral		0	0	0	0
Other, Red in Color		0	0	0	0
Other, Red in Color, Left Ear		0	0	0	0
Excretion(s)					
Few Feces		0	0	0	0
Eye(s)					
Clear Discharge-Right Eye		0	0	0	0
Squinted-Left Eye		0	0	0	0
Mass					
Mass		1	1	0	0
Mass, Right Front Paw		0	0	0	0
Respiration					
Irregular		0	0	0	0

N = Number of animals with observed sign

SUMMARY OF CLINICAL SIGNS

Category / Sign	Sex:	Females		
	Group:	5	6	7
	Dose Level:	2.50/0.60	5.00/1.25	75.00
	Dose Units:	mg/kg/day	mg/kg/day	mg/kg/day
	Number in Group:	25	25	25
		N	N	N
Appearance				
Tremors Head		0	0	1
Behavior				
Ataxic		0	2	0
Hypoactive		2	4	8
Recumbent, Lateral		0	1	0
Recumbent, Midline Ventral Abdomen		0	0	0
Discharge				
Red Oral		0	0	1
Other, Red in Color[a]		0	0	1
Other, Red in Color, Left Ear		0	0	1
Excretion(s)				
Few Feces		0	0	1
Eye(s)				
Clear Discharge-Right Eye		0	0	0
Squinted-Left Eye		0	0	1
Mass				
Mass		0	1	21
Mass, Right Front Paw		0	0	0
Respiration				
Irregular		2	2	4

N = Number of animals with observed sign

[a] Comments: anal discharge for Animal A98995

SUMMARY OF CLINICAL SIGNS

Category / Sign	Sex:	Females			
	Group:	1	2	3	4
	Dose Level:	0.00	0.00	25.00	75.00
	Dose Units:	mg/kg/day	mg/kg/day	mg/kg/day	mg/kg/day
	Number in Group:	25	25	25	25
		N	N	N	N
Respiration					
Labored		1	0	0	0
Skin & Pelage					
Alopecia, Dorsal		0	0	1	0
Alopecia, Front Paws		0	0	0	0
Alopecia, Left Dorsal Abdomen (Back)		0	0	0	0
Alopecia, Perineal Area		0	0	0	0
Cold To Touch		0	0	0	0
Cold To Touch, Entire Body		0	0	0	0
Pale		0	0	0	0
Pale, Distal Tail		0	0	0	0
Pale, Mid Tail		0	0	0	0
Rough Haircoat		2	0	1	2
Sore/Scab, Distal Tail		0	1	0	0
Sore/Scab, Dorsal Abdomen (Back)		0	0	1	0
Sore/Scab, Left Dorsal Abdomen (Back)		0	0	0	0
Sore/Scab, Left Dorsal Thorax		0	0	0	0
Sore/Scab, Left Ear		0	0	0	0
Sore/Scab, Mid Tail		0	0	0	0
Sore/Scab, Penis		0	0	0	0
Sore/Scab, Proximal Tail		0	0	0	0
Sore/Scab, Rectum		0	0	1	0
Yellow Haircoat, Perineal Area		0	0	0	0

N = Number of animals with observed sign

SUMMARY OF CLINICAL SIGNS

Category / Sign	Sex:	Females		
	Group:	5	6	7
	Dose Level:	2.50/0.60	5.00/1.25	75.00
	Dose Units:	mg/kg/day	mg/kg/day	mg/kg/day
	Number in Group:	25	25	25
		N	N	N
Respiration				
Labored		0	2	3
Skin & Pelage				
Alopecia, Dorsal		0	0	0
Alopecia, Front Paws		0	0	0
Alopecia, Left Dorsal Abdomen (Back)		0	0	0
Alopecia, Perineal Area		0	0	0
Cold To Touch		0	2	2
Cold To Touch, Entire Body		0	0	1
Pale		0	0	1
Pale, Distal Tail		0	6	4
Pale, Mid Tail		0	1	1
Rough Haircoat		2	1	14
Sore/Scab, Distal Tail		0	0	0
Sore/Scab, Dorsal Abdomen (Back)		0	0	0
Sore/Scab, Left Dorsal Abdomen (Back)		0	1	0
Sore/Scab, Left Dorsal Thorax		0	0	1
Sore/Scab, Left Ear		0	0	1
Sore/Scab, Mid Tail		0	0	0
Sore/Scab, Penis		0	0	0
Sore/Scab, Proximal Tail		0	0	0
Sore/Scab, Rectum		0	0	0
Yellow Haircoat, Perineal Area		0	0	0

N = Number of animals with observed sign

Hematology: There were no treatment-related abnormalities in the hematology data from the unscheduled-sacrifice females when compared to either the sham or VH control group. In addition, there was no conclusive evidence in the clinical pathology data of an effect from the administration of either T or TI in the control and treated groups during the scheduled intervals. Moreover, the hematology data were, in general, similar among control, and T or TI-treated groups.

WBC – 3.98±0.813 E3/µl (T75 female) vs. 5.67±1.293 E3/µl (VH control female)

Clinical chemistry data/glucose level: There were no treatment-related effects on clinical chemistry parameters. There was no difference on mean blood glucose level.

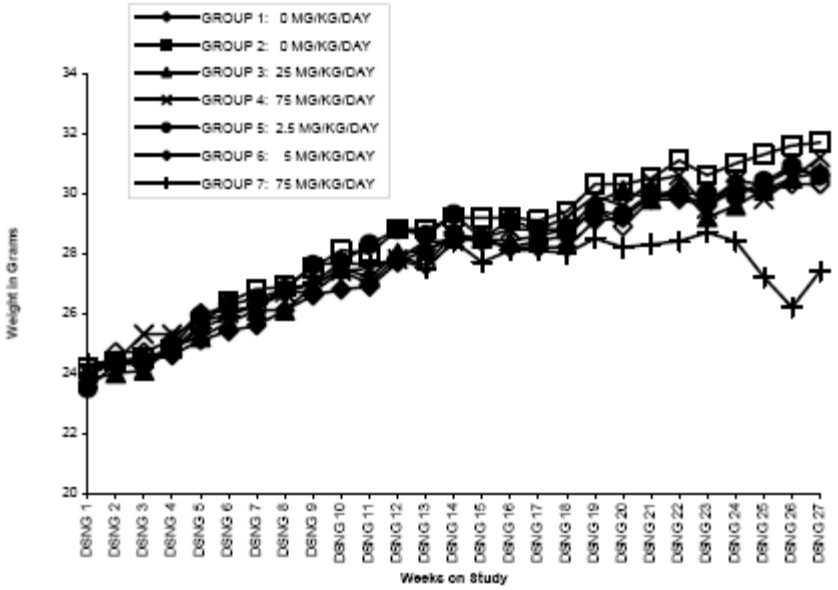
TRIG - 86±24.3 (TI5 male) vs. 131±44.7 (VH control male)
ALP - 103±7.8 U/L (TI5/1.25 female) vs. 120±12.6 (VH control female)

Body weights: There were no significant treatment-related effects on mean body weight or body weight gain in T or TI-treated animals when compared with VH controls.

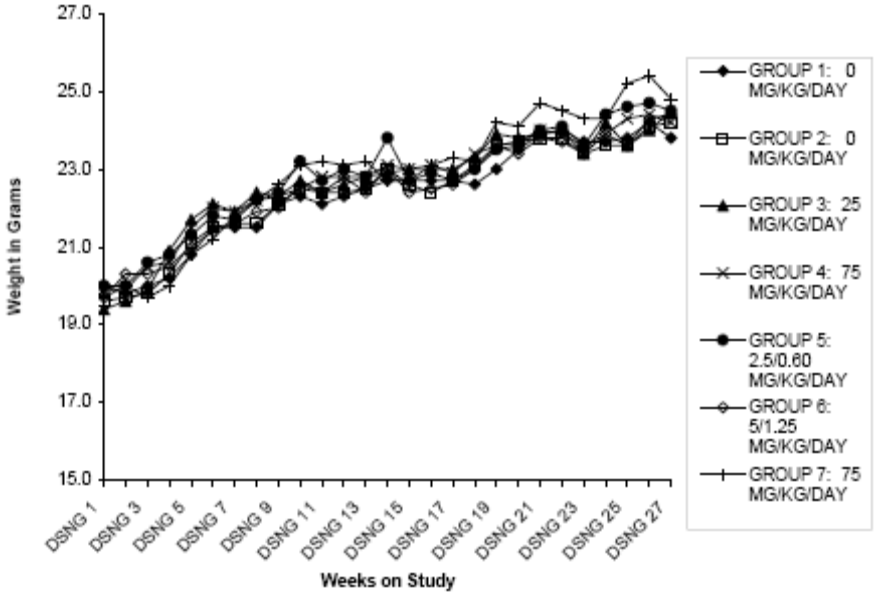
Differences observed included:

- Less than 5% decreases in mean body weight were observed at 25 and 75 mg/kg/day of T and at 2.5 and 5 mg/kg/day of TI, when compared to VH control males during Week 27.
- A decrease in mean body weight gain was observed in 2.5 (4%) and 5 (15%) TI males during Week 1 to 26 in relation to VH control males.
- Slight decreases in mean body weight gain were observed in 75 mg/kg/day (6.5%) T females and 0.6 (6.5%) and 1.25 (2%) mg/kg/day TI females in relation to VH controls.

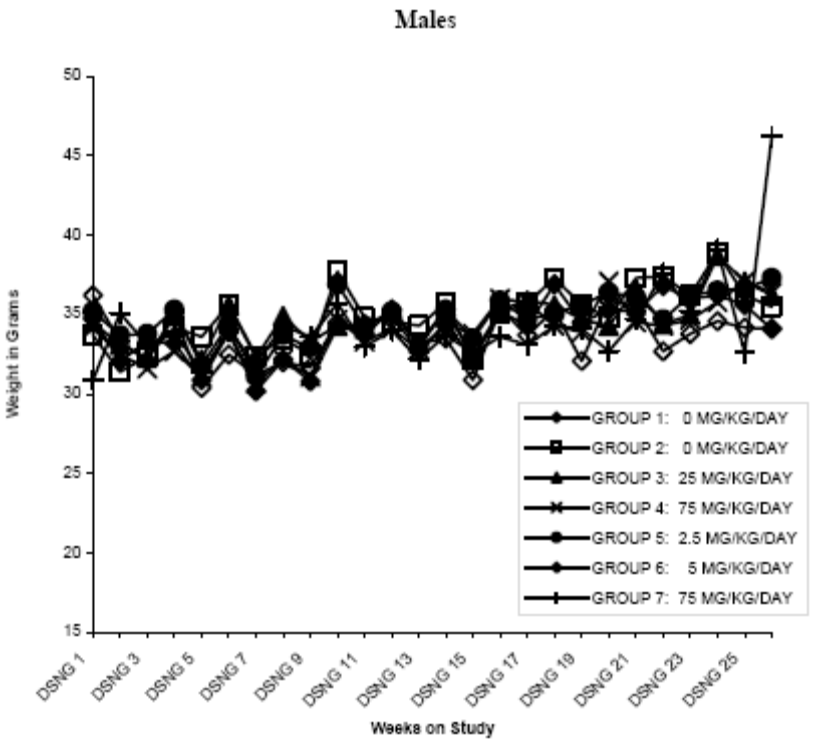
Males



Females



Food consumption: There were no treatment-related effects on mean food consumption in T or TI-treated animals when compared with VH controls. Weekly mean food consumption values for T or TI groups were comparable to controls. Even though there were occasional significant differences, these differences were not toxicologically meaningful on the basis of magnitude and in the absence of effects on body weight or body weight gain.



Gross pathology: There were few in incidence in the controls and treated groups in the unscheduled deaths and dosing phase final sacrifice. No evidence of treatment-related (both T and TI) macroscopic findings were noted. The most frequent findings were observed in the spleen, such as discolored, large, mess, or mottled. The sponsor indicated these was correlated to pigment or vascular neoplasm in many affected control and treated groups and occurred unrelated to test article administration.

Summary of Macroscopic Observations – Unscheduled Deaths

	-- Males --							-- Females --						
Group:	1	2	3	4	5	6	7	1	2	3	4	5	6	7
Number in group:	0	1	1	0	2	1	23	1	0	1	1	4	6	17
Examined/No remarkable findings ...	0	0	0	0	0	0	0	1	0	0	0	3	4	0
Brain														
Soft	0	0	0	0	2	0	1	0	0	0	0	0	0	0
Esophagus														
Perforation	0	0	0	0	0	0	1	0	0	0	0	0	0	0
Liver														
Discolored	0	0	0	0	0	0	3	0	0	0	0	0	0	1
Mass	0	0	0	0	0	0	1	0	0	0	0	0	0	0
Raised Area	0	0	0	0	0	0	1	0	0	0	0	0	0	0
Spleen														
Discolored	0	0	0	0	0	0	3	0	0	0	0	0	0	2
Large	0	0	0	0	0	0	6	0	0	0	0	0	0	7
Mass	0	1	1	0	0	1	1	0	0	0	0	0	1	0
Lung														
Discolored	0	0	0	0	1	0	1	0	0	0	0	0	1	0
Mottled	0	0	0	0	0	0	1	0	0	0	0	0	0	0
Thymus														
Discolored	0	0	0	0	0	0	0	0	0	0	0	0	0	1
Firm	0	0	0	0	0	0	1	0	0	0	0	0	0	0
Large	0	0	0	0	0	0	9	0	0	0	0	0	0	7
Mass	0	0	0	0	0	0	2	0	0	0	0	0	0	2
Kidney														
Discolored	0	0	0	0	0	0	2	0	0	0	0	0	0	1
Stomach, Gl														
Mass	0	0	0	0	0	0	5	0	0	0	1	0	0	3
Raised Area	0	0	0	0	0	0	1	0	0	0	0	0	0	0
Stomach, Nongl														
Mass	0	0	0	0	0	0	11	0	0	0	0	0	0	7
Raised Area	0	0	0	0	0	0	0	0	0	0	0	0	0	1
Rough Surface	0	0	0	0	0	0	1	0	0	0	0	0	0	1
Thickened	0	0	0	0	0	0	2	0	0	0	0	0	0	0
Duodenum														
Distended	0	0	0	0	0	0	1	0	0	0	0	0	0	0

	-- Males --							-- Females --						
Group:	1	2	3	4	5	6	7	1	2	3	4	5	6	7
Number in group:	0	1	1	0	2	1	23	1	0	1	1	4	6	17
Jejunum														
Discolored	0	0	0	0	0	0	1	0	0	0	0	0	0	0
Distended	0	0	0	0	0	0	1	0	0	0	0	0	0	0
Intussusception	0	0	0	0	0	0	1	0	0	0	0	0	0	0
Mass	0	0	0	0	0	0	0	0	0	0	0	0	0	1
Rectum														
Abrasion	0	0	0	0	0	0	0	0	0	1	0	0	0	0
LN, Mesenteric														
Discolored	0	0	0	0	0	0	1	0	0	0	0	0	0	0
Large	0	0	0	0	0	0	1	0	0	0	0	0	0	1
LN, Mandibular														
Large	0	0	0	0	0	0	0	0	0	0	0	0	0	1
Gl, Mandib Saliv														
Confirmed mass	0	0	0	0	1	0	0	0	0	0	0	0	0	1
Testis														
Discolored	0	0	0	0	0	0	2	0	0	0	0	0	0	0
Mammary, Female														
Confirmed mass	0	0	0	0	0	0	0	0	0	0	0	0	0	1
Vagina														
Confirmed mass	0	0	0	0	0	0	0	0	0	0	0	0	0	1
Untreat Skin/SQ														
Confirmed mass	0	0	0	0	0	0	2	0	0	0	0	0	0	1
LN, Other														
Large	0	0	0	0	0	0	1	0	0	0	0	0	0	1
Cavity, Abdomin														
Contains Fluid	0	0	0	0	0	0	1	0	0	0	0	0	0	0
Penis														
Protruding	0	0	0	0	0	0	1	0	0	0	0	0	0	0
Skin/Subcutis														
Confirmed mass	0	0	0	0	0	0	0	0	0	0	0	0	0	4

	-- Males --							-- Females --						
Group:	1	2	3	4	5	6	7	1	2	3	4	5	6	7
Number in group:	0	1	1	0	2	1	23	1	0	1	1	4	6	17
Cavity, Thoracic														
Contains Fluid	0	0	0	0	0	0	0	0	0	0	0	1	0	0
Tongue														
Mass	0	0	0	0	0	0	1	0	0	0	0	0	0	0
Mammary, Male														
Confirmed mass	0	0	0	0	0	0	1	0	0	0	0	0	0	0

Summary of Macroscopic Observations – Dosing Phase – Final Phase Sacrifice

	-- Males --							-- Females --						
Group:	1	2	3	4	5	6	7	1	2	3	4	5	6	7
Number in group:	25	24	24	25	23	24	2	24	25	24	24	21	19	8
Examined/No remarkable findings ...	23	20	23	24	21	22	0	19	20	22	19	19	16	0
Spleen														
Discolored	1	2	0	1	1	0	1	1	1	1	0	1	0	0
Large	0	0	0	0	0	0	1	0	1	0	1	0	0	3
Mass	0	0	0	0	0	1	0	0	0	0	1	0	0	1
Mottled	0	0	0	0	0	0	0	1	0	0	0	0	0	0
Thymus														
Large	0	0	0	0	0	0	1	0	0	0	0	0	0	0
Mass	0	0	0	0	0	0	0	0	0	0	0	0	0	1
Kidney														
Cyst	0	0	0	0	0	0	0	0	0	0	0	0	0	1
Discolored	0	0	0	0	0	0	0	0	0	0	0	0	0	1
Urinary Bladder														
Distended	0	0	1	0	0	0	0	0	0	0	0	0	0	0
Stomach, Gl														
Mass	0	0	0	0	0	0	0	0	0	0	0	0	0	1
Rough Surface	0	0	0	0	0	0	0	0	0	1	0	0	0	0
Stomach, Nongl														
Mass	0	0	0	0	1	0	2	0	0	0	0	0	0	7
Testis														
Soft	0	0	0	0	0	1	0	0	0	0	0	0	0	0
Epididymis														
Abnormal Shape	0	0	0	0	0	1	0	0	0	0	0	0	0	0
Discolored	0	1	0	0	0	0	0	0	0	0	0	0	0	0
Ovary														
Cyst	0	0	0	0	0	0	0	0	0	0	0	1	0	1
Uterus														
Distended	0	0	0	0	0	0	0	2	1	0	0	0	1	0
Mass	0	0	0	0	0	0	0	0	0	0	0	0	0	1
Thickened	0	0	0	0	0	0	0	0	1	0	3	1	0	0
Vagina														
Confirmed mass	0	0	0	0	0	0	0	1	0	0	0	0	0	3

	-- Males --							-- Females --						
Group:	1	2	3	4	5	6	7	1	2	3	4	5	6	7
Number in group:	25	24	24	25	23	24	2	24	25	24	24	21	19	8
Subcutan Site														
Abrasion	0	0	0	0	0	0	0	0	0	0	0	0	1	0
Skin/Subcutis														
Abrasion	1	1	0	0	0	0	0	0	0	0	0	0	0	0
Confirmed mass	0	0	0	0	0	0	1	0	0	0	0	0	0	5
Tail														
Swollen	0	0	0	0	0	0	0	0	1	0	0	0	0	0
Foot/Foot Pad														
Confirmed mass	0	0	0	0	0	0	0	0	0	0	0	0	1	0

Organ weight: There was no evidence of treatment-related organ weight changes in mice given T or TI. Organ weight changes included an increase in kidney weight parameters in males and females given 25 mg/kg/day and/or 75 mg/kg/day T, and a decrease in heart weight in females given 25 mg/kg/day. The sponsor considered incidental and unrelated to treatment due to lack of a dose response and/or absence of microscopic correlation. The significant findings with regards to organ weights were listed in the table below.

Organ wt	Sex	Sham	VH control	T 25	T 75	TI 2.5 or 2.5/0.6	TI 5 5/1.25	Pos. Control
↑ Kidney (g)	M		0.6530 ±0.06612		0.7219 ±0.05552			
↑ Kidney (% body wt)	M		2.0637 ±0.11726	2.2555 ±0.09864	2.3763 ±0.13508			
↑ Kidney (% brain wt)	M		131.2970 ±12.00312	139.4080 ±12.15421	146.7065 ±10.95531			
↓ Liver/gall bladder (g)	M		1.6548 ±0.13885			1.5043 ±0.12323	1.4693 ±0 19300	
↓ Liver/gall bladder (% body wt)	M		5.2420 ±0.38156				4.9046 ±0.44002	
↓ Liver/gall bladder (% brain wt)	M		333.2344 ±30.32631			307.2913 ±24.39981	296.4446 ±37.49246	
↑ Kidney (g)	F		0.4259 ±0.03710		0.4548 ±0.03626			

↑ Kidney (% body wt)	F		1.7523 ±0.10426		1.8639 ±0.13189			
↑ Kidney (% brain wt)	F		83.3688 ±6.32287		89.3992 ±5.79941			
↓ Heart (g)	F		0.1711 ±0.01768			0.1485 ±0.04514		
↓ Heart (% brain wt)	F		33.5085 ±3.30551			29.0125 ±9.04082		

*generated by the reviewer.

Histopathology:

Non-neoplastic: The incidence of non-neoplastic findings was comparable between the control and treated groups.

Minimal to slight inflammation (i.e. a mixed macrophage and neutrophil infiltrates, a focal lesion in the subcutis) was seen in the sc injection sites of most mice among all groups (sham, VH control, T_{high} and TI_{high}). There was no microscopic evidence of pre-neoplasia or neoplasia at sc injection sites.

In addition, minimal to slight degenerative myopathy in the skeletal muscle and pigment in the spleen were observed in mice from all groups. This was considered common findings for this mouse strain. The sponsor marked these microscopic changes as typical incidental findings with relation to treatment.

Neoplastic: There was no evidence of increased oncogenicity associated with the sc administration of the test articles (T or TI). The overall incidence of neoplasia was low for all groups (compared to positive controls).

The sponsor provided a summary table noted the incidence of the more frequent neoplasms (see below). Main neoplastic findings, presented in the control sham, VH control, high T, and high TI groups included: bronchiolar-alveolar adenoma/carcinoma in the lung, hemangioma/hemangiosarcoma (primarily spleen), Harderian glad adenoma/carcinoma, squamous cell neoplasms (multiple sites), and myeloproliferative neoplasia. The sponsor suggested that these neoplastic findings were typical neoplasms commonly seen in this mouse strain and noted as no relation to treatment.

Incidence of Common Neoplasms in Control Groups 1, 2, and 7 (Sham, Vehicle and Positive Controls) and Treated Groups 4 and 6 (Technosphere® Particles-high and Technosphere®/Insulin-high)

Sex	Males					Females				
Group	1	2	4	6	7	1	2	4	6	7
Number Examined	25	25	25	25	25	25	25	25	25	25
LUNG										
Adenoma, Bronchiolar-Alveolar	3	2	2	1	3	1	2	0	1	11
Carcinoma, Bronchiolar-Alveolar	1	0	0	1	0	0	0	0	0	0
STOMACH, NON GLANDULAR										
Carcinoma, Squamous Cell	0	0	0	0	6	0	0	1	0	7
Papilloma, Squamous Cell	0	0	0	0	12	0	0	0	0	15
HARDERIAN GLAND										
Adenoma	1	0	0	1	0	0	1	0	1	1
Carcinoma	0	0	0	0	2	0	1	0	0	0

VAGINA										
Carcinoma, Squamous Cell	-	-	-	-	-	0	0	0	0	2
Papilloma, Squamous Cell	-	-	-	-	-	0	0	0	0	3
URETHRA										
Papilloma, Transitional Cell	0	0	0	0	2	0	0	0	0	9
BODY, WHOLE/CAV										
Hemangioma	2	0	0	0	2	1	1	0	1	1
Hemangiosarcoma	2	1	1	4	4	1	1	1	3	3
Lymphosarcoma	0	0	0	0	17	0	0	0	0	14
Myeloproliferative Neoplasia	0	0	0	0	2	1	1	0	0	3
SKIN/SUBCUTIS										
Papilloma. Squamous Cell	0	1	0	0	0	0	0	0	0	6
Carcinoma, Squamous Cell	0	0	0	0	1	0	0	0	0	3

Incidence of microscopic observations – dosing phase – final phase sacrifice:

Note: Findings with Group 7 (positive control, MNU) was not included.

Incidence of Microscopic Observations – Dosing Phase – Final Phase Sacrifice

Controls from group(s): 1	Animal sex:	-- Animals Affected -- Males --						-- Females --					
Tissues With Diagnoses	Dosage group:	Ctls	2	3	4	5	6	Ctls	2	3	4	5	6
	No. in group:	25	24	24	25	23	24	24	25	24	24	21	19
Brain	Number examined:	25	24	0	25	0	24	24	25	0	24	0	19
	Unremarkable:	25	24	0	25	0	24	24	25	0	24	0	19
Inflammation		0	0	0	0	0	0	0	0	0	0	0	0
C-Hematopoietic Neoplasm, see Body, Whole for type		0	0	0	0	0	0	0	0	0	0	0	0
Spinal Cord	Number examined:	25	24	0	25	0	24	24	25	0	24	0	18
	Unremarkable:	25	24	0	25	0	24	24	25	0	23	0	18
Cyst, Keratin		0	0	0	0	0	0	0	0	0	1	0	0
Adrenal, Cortex	Number examined:	25	24	0	25	0	24	24	25	0	24	0	19
	Unremarkable:	20	18	0	19	0	15	4	9	0	5	0	2
Hyperplasia, Subcapsular Cell		5	6	0	6	0	9	20	16	0	19	0	17
C-Hematopoietic Neoplasm, see Body, Whole for type		0	0	0	0	0	0	0	0	0	0	0	0
Adrenal, Medulla	Number examined:	25	24	0	22	0	24	24	25	0	24	0	19
	Unremarkable:	25	24	0	22	0	24	24	25	0	24	0	19
Pituitary	Number examined:	25	24	0	25	0	24	24	25	0	24	0	19
	Unremarkable:	24	24	0	25	0	24	24	25	0	24	0	19
Hyperplasia		1	0	0	0	0	0	0	0	0	0	0	0
C-Hematopoietic Neoplasm, see Body, Whole for type		0	0	0	0	0	0	0	0	0	0	0	0
Nerve, Sciatic	Number examined:	25	24	0	25	0	24	23	25	0	24	0	19
	Unremarkable:	25	23	0	25	0	23	22	21	0	20	0	17
Inflammation, perineural		0	1	0	0	0	0	1	4	0	3	0	2
Inflammation		0	0	0	0	0	1	0	0	0	1	0	0
Trachea	Number examined:	25	24	0	25	0	24	24	25	0	24	0	19
	Unremarkable:	25	24	0	25	0	24	24	25	0	24	0	19
Esophagus	Number examined:	25	24	0	25	0	24	24	25	0	24	0	19
	Unremarkable:	25	24	0	25	0	24	24	25	0	24	0	19
B-Papilloma, Squamous Cell		0	0	0	0	0	0	0	0	0	0	0	0
Thyroid	Number examined:	21	24	0	25	0	24	24	25	0	24	0	19
	Unremarkable:	21	24	0	25	0	24	24	25	0	23	0	19
Cyst, Follicle		0	0	0	0	0	0	0	0	0	1	0	0
C-Hematopoietic Neoplasm, see Body, Whole for type		0	0	0	0	0	0	0	0	0	0	0	0

Controls from group(s): 1 — Animal sex:	-- Animals Affected -- Males --						-- Females --					
Dosage group:	Ctls	2	3	4	5	6	Ctls	2	3	4	5	6
Tissues With Diagnoses — No. in group:	25	24	24	25	23	24	24	25	24	24	21	19
Parathyroid ....Number examined:	10	17	0	17	0	17	17	15	0	14	0	12
Unremarkable:	10	17	0	17	0	15	17	14	0	14	0	12
Cyst	0	0	0	0	0	2	0	1	0	0	0	0
Infiltrate, Lymphocytes	0	0	0	0	0	0	0	0	0	0	0	0
Heart ....Number examined:	25	24	0	25	0	24	24	25	0	24	0	19
Unremarkable:	24	24	0	25	0	24	24	25	0	24	0	17
Degeneration, Myocardium	0	0	0	0	0	0	0	0	0	0	0	0
Mineralization	1	0	0	0	0	0	0	0	0	0	0	0
C-Hematopoietic Neoplasm, see Body, Whole for type	0	0	0	0	0	0	0	0	0	0	0	0
Epicardium, inflammation	0	0	0	0	0	0	0	0	0	0	0	1
C-Vascular Neoplasm, see Body, Whole for type	0	0	0	0	0	0	0	0	0	0	0	1
Atrial dilatation	0	0	0	0	0	0	0	0	0	0	0	0
AV valve, thrombus	0	0	0	0	0	0	0	0	0	0	0	0
Aorta ....Number examined:	25	24	0	25	0	24	24	25	0	24	0	19
Unremarkable:	25	24	0	25	0	24	24	25	0	24	0	19
C-Hematopoietic Neoplasm, see Body, Whole for type	0	0	0	0	0	0	0	0	0	0	0	0
C-Vascular Neoplasm, see Body, Whole for type	0	0	0	0	0	0	0	0	0	0	0	0
Muscle, Bi Fem ....Number examined:	25	24	0	25	0	24	24	25	0	24	0	19
Unremarkable:	1	2	0	3	0	1	5	6	0	1	0	0
Degenerative myopathy	24	22	0	22	0	23	19	19	0	23	0	19
Liver ....Number examined:	25	24	0	25	0	24	24	25	0	24	0	19
Unremarkable:	13	10	0	10	0	12	0	2	0	3	0	2
Inflammation	12	11	0	15	0	12	24	22	0	21	0	17
Angiectasis	0	1	0	0	0	0	0	0	0	0	0	0
Focus, Cellular Alteration, Basophilic	0	2	0	0	0	0	0	0	0	0	0	0
Necrosis	0	3	0	2	0	0	1	2	0	1	0	0
Focus, Cellular Alteration, Mixed Cell	0	0	0	0	0	0	0	0	0	0	0	0
Mitosis, Hepatocyte, Increased	0	0	0	0	0	0	0	0	0	0	0	0
N-Carcinoma, squamous cell	0	0	0	0	0	0	0	0	0	0	0	0
Hematopoiesis, Extramedullary	0	0	0	0	0	0	0	0	0	0	0	0
C-Hematopoietic Neoplasm, see Body, Whole for type	0	0	0	0	0	0	0	1	0	0	0	0
Hepatocellular hypertrophy	0	1	0	1	0	1	0	0	0	0	0	0
Focus, Cellular Alteration, Eosinophilic	0	1	0	0	0	0	0	0	0	0	0	0
Gallbladder ....Number examined:	25	24	0	25	0	24	24	25	0	24	0	19
Unremarkable:	25	24	0	25	0	24	24	25	0	24	0	19
C-Hematopoietic Neoplasm, see Body, Whole for type	0	0	0	0	0	0	0	0	0	0	0	0

Controls from group(s): 1 — Animal sex:	-- Animals Affected -- Males --						-- Females --					
Dosage group:	Ctls	2	3	4	5	6	Ctls	2	3	4	5	6
Tissues With Diagnoses — No. in group:	25	24	24	25	23	24	24	25	24	24	21	19
Spleen ....Number examined:	25	24	0	25	1	24	24	25	1	24	1	19
Unremarkable:	17	21	0	19	0	22	14	20	0	19	0	15
Pigment	6	3	0	5	1	0	4	2	1	1	1	3
C-Vascular Neoplasm, see Body, Whole for type	3	0	0	1	0	2	2	1	0	1	0	1
Hematopoiesis, Extramedullary, Increased	0	0	0	0	0	1	4	2	0	2	0	0
Myeloproliferative change	0	0	0	0	0	0	0	0	0	1	0	0
C-Hematopoietic Neoplasm, see Body, Whole for type	0	0	0	0	0	0	0	1	0	0	0	0
Angiectasis/vascular anomaly	0	0	0	0	0	0	0	0	0	0	0	0
N-Squamous cell carcinoma	0	0	0	0	0	0	0	0	0	0	0	0
Lung ....Number examined:	25	24	0	25	0	24	24	25	0	24	0	19
Unremarkable:	15	18	0	19	0	17	19	21	0	24	0	18
B-Adenoma, Bronchiolar-Alveolar	3	2	0	2	0	1	1	2	0	0	0	1
Hemorrhage	4	4	0	4	0	5	3	0	0	0	0	0
Infiltrate, Macrophages, Alveolus	0	1	0	0	0	0	2	0	0	0	0	0
M-Carcinoma, Bronchiolar-Alveolar	1	0	0	0	0	1	0	0	0	0	0	0
Inflammation, subpleural	2	0	0	0	0	0	0	0	0	0	0	0
Hyperplasia, Epithelium, Alveolus	1	0	0	0	0	0	0	2	0	0	0	0
C-Hematopoietic Neoplasm, see Body, Whole for type	0	0	0	0	0	0	0	0	0	0	0	0
N-Squamous cell carcinoma	0	0	0	0	0	0	0	0	0	0	0	0
Inflammation	0	0	0	0	0	0	0	0	0	0	0	1
Leukocytosis	0	0	0	0	0	0	0	0	0	0	0	0
Congestion	0	0	0	0	0	0	0	0	0	0	0	0
N-Mammary adenoacanthoma	0	0	0	0	0	0	0	0	0	0	0	0
Inflammation, pleural/subpleural	0	0	0	0	0	0	0	0	0	0	0	0
N-Salivary gland carcinoma	0	0	0	0	0	0	0	0	0	0	0	0
C-Vascular Neoplasm, see Body, Whole for type	0	0	0	0	0	0	0	0	0	0	0	0
Thymus ....Number examined:	24	23	0	25	0	23	23	25	0	23	0	18
Unremarkable:	22	21	0	24	0	21	18	22	0	16	0	15
Hyperplasia, lymphoid	0	2	0	1	0	2	3	3	0	6	0	2
Hyperplasia, Epithelium	2	0	0	0	0	0	1	0	0	1	0	1
Depletion, Lymphocytes	1	0	0	0	0	0	0	0	0	0	0	0
C-Hematopoietic Neoplasm, see Body, Whole for type	0	0	0	0	0	0	0	0	0	0	0	0
C-Vascular Neoplasm, see Body, Whole for type	0	0	0	0	0	0	1	0	0	0	0	0
Necrosis, Lymphocytes	0	0	0	0	0	0	0	0	0	0	0	1
Kidney ....Number examined:	25	24	0	25	0	24	24	25	0	24	0	19
Unremarkable:	15	14	0	14	0	18	20	21	0	18	0	14
Infiltrate, Lymphocytes	1	0	0	0	0	0	0	0	0	0	0	0
Nephropathy, Chronic Progressive	9	10	0	11	0	6	4	4	0	6	0	5
Mineralization, Tubule	0	1	0	0	0	0	0	0	0	0	0	0

Controls from group(s): 1

Tissues With Diagnoses	Animal sex:	-- Animals Affected -- Males --						-- Females --					
	Dosage group:	Ctls	2	3	4	5	6	Ctls	2	3	4	5	6
	No. in group:	25	24	24	25	23	24	24	25	24	24	21	19
Kidney	Number examined:	25	24	0	25	0	24	24	25	0	24	0	19
	Unremarkable:	15	14	0	14	0	18	20	21	0	18	0	14
Angiectasis		0	0	0	1	0	0	0	0	0	0	0	0
Vacuolation, Tubule Cell		0	0	0	0	0	0	0	0	0	0	0	0
C-Hematopoietic Neoplasm, see Body, Whole for type		0	0	0	0	0	0	0	0	0	0	0	0
Cyst		0	0	0	0	0	0	0	0	0	0	0	0
Dilatation, Tubule(s)		0	0	0	0	0	0	0	0	0	0	0	0
Necrosis, Tubule		0	0	0	0	0	0	1	0	0	0	0	0
Hyperplasia, pelvis		0	0	0	0	0	0	0	0	0	0	0	0
Inflammation, pelvis		0	0	0	0	0	0	0	0	0	0	0	0
Necrosis, Papilla		0	0	0	0	0	0	0	0	0	0	0	0
Pyelonephritis		0	0	0	0	0	0	0	0	0	0	0	0
Regeneration, Tubule Cell		0	0	0	0	0	0	0	0	0	0	0	0
Urinary Bladder	Number examined:	24	24	1	25	0	23	24	24	0	24	0	19
	Unremarkable:	24	24	0	25	0	23	24	24	0	24	0	19
Dilatation		0	0	1	0	0	0	0	0	0	0	0	0
Hyperplasia, Transitional Cell		0	0	0	0	0	0	0	0	0	0	0	0
C-Hematopoietic Neoplasm, see Body, Whole for type		0	0	0	0	0	0	0	0	0	0	0	0
Stomach, Gl	Number examined:	25	24	0	25	0	24	24	25	1	24	0	19
	Unremarkable:	23	20	0	25	0	22	24	24	1	24	0	19
Inflammation		2	4	0	0	0	2	0	0	0	0	0	0
Hyperplasia, Epithelium		0	0	0	0	0	2	0	0	0	0	0	0
Mineralization		0	0	0	0	0	0	0	1	0	0	0	0
I-squamous cell carcinoma		0	0	0	0	0	0	0	0	0	0	0	0
Stomach, Nongl	Number examined:	25	24	0	25	1	24	24	25	0	24	0	19
	Unremarkable:	25	24	0	25	1	24	24	25	0	24	0	19
M-Carcinoma, Squamous Cell		0	0	0	0	0	0	0	0	0	0	0	0
B-Papilloma, Squamous Cell		0	0	0	0	0	0	0	0	0	0	0	0
Acanthosis		0	0	0	0	0	0	0	0	0	0	0	0
Hyperkeratosis		0	0	0	0	0	0	0	0	0	0	0	0
Duodenum	Number examined:	25	24	0	25	0	24	24	25	0	24	0	19
	Unremarkable:	25	24	0	25	0	24	24	25	0	24	0	19
M-Carcinoma		0	0	0	0	0	0	0	0	0	0	0	0
Ileum	Number examined:	25	23	0	25	0	24	24	25	0	24	0	19
	Unremarkable:	25	23	0	25	0	24	24	25	0	24	0	19

Controls from group(s): 1

Tissues With Diagnoses	Animal sex:	-- Animals Affected -- Males --						-- Females --					
	Dosage group:	Ctls	2	3	4	5	6	Ctls	2	3	4	5	6
	No. in group:	25	24	24	25	23	24	24	25	24	24	21	19
Colon	Number examined:	25	23	0	25	0	24	24	25	0	24	0	19
	Unremarkable:	25	23	0	25	0	24	24	25	0	24	0	19
Cecum	Number examined:	25	23	0	25	0	24	24	25	0	24	0	19
	Unremarkable:	25	23	0	25	0	24	24	25	0	24	0	19
Jejunum	Number examined:	25	24	0	25	0	23	24	25	0	24	0	19
	Unremarkable:	25	24	0	25	0	23	24	25	0	24	0	19
Intussusception		0	0	0	0	0	0	0	0	0	0	0	0
M-Carcinoma		0	0	0	0	0	0	0	0	0	0	0	0
Atypical hyperplasia		0	0	0	0	0	0	0	0	0	0	0	0
Rectum	Number examined:	24	23	0	25	0	24	23	25	0	24	0	19
	Unremarkable:	24	23	0	25	0	24	23	25	0	24	0	19
Perirectal skin ulceration/inflammation		0	0	0	0	0	0	0	0	0	0	0	0
LN, Mesenteric	Number examined:	25	24	0	25	0	22	23	25	0	24	0	19
	Unremarkable:	25	24	0	25	0	22	23	25	0	24	0	18
C-Vascular Neoplasm, see Body, Whole for type		0	0	0	0	0	0	0	0	0	0	0	1
Congestion		0	0	0	0	0	0	0	0	0	0	0	0
C-Hematopoietic Neoplasm, see Body, Whole for type		0	0	0	0	0	0	0	0	0	0	0	0
LN, Mandibular	Number examined:	22	24	0	23	0	23	22	24	0	23	0	18
	Unremarkable:	22	24	0	23	0	23	22	24	0	23	0	18
C-Hematopoietic Neoplasm, see Body, Whole for type		0	0	0	0	0	0	0	0	0	0	0	0
Gl, Mandib Saliv	Number examined:	25	24	0	25	0	24	24	25	0	24	0	19
	Unremarkable:	24	24	0	25	0	22	21	24	0	22	0	19
Inflammation		1	0	0	0	0	2	2	1	0	2	0	0
Atrophy		0	0	0	0	0	0	1	0	0	0	0	0
M-Squamous cell carcinoma		0	0	0	0	0	0	0	0	0	0	0	0
M-Carcinoma		0	0	0	0	0	0	0	0	0	0	0	0
Pancreas	Number examined:	25	24	0	25	0	24	24	25	0	24	0	19
	Unremarkable:	23	24	0	25	0	23	24	25	0	24	0	18
Inflammation		2	0	0	0	0	1	0	0	0	0	0	1
Pigment		1	0	0	0	0	0	0	0	0	0	0	0
Atrophy		0	0	0	0	0	0	0	0	0	0	0	0
Inflammation, Vessel		0	0	0	0	0	0	0	0	0	0	0	0
C-Hematopoietic Neoplasm, see Body, Whole for type		0	0	0	0	0	0	0	0	0	0	0	0

Controls from group(s): 1

Tissues With Diagnoses	Animal sex:	-- Animals Affected -- Males --						-- Females --					
	Dosage group:	Ctls	2	3	4	5	6	Ctls	2	3	4	5	6
	No. in group:	25	24	24	25	23	24	24	25	24	24	21	19
Gl, Harderian ....Number examined:		25	24	0	25	0	22	24	25	0	24	0	19
Unremarkable:		24	24	0	23	0	21	24	23	0	24	0	18
B-Adenoma		1	0	0	0	0	1	0	1	0	0	0	1
Inflammation		0	0	0	2	0	0	0	0	0	0	0	0
M-Carcinoma		0	0	0	0	0	0	0	1	0	0	0	0
C-Hematopoietic Neoplasm, see Body, Whole for type		0	0	0	0	0	0	0	0	0	0	0	0
Nerve, Optic ....Number examined:		22	22	0	24	0	21	23	24	0	20	0	16
Unremarkable:		22	22	0	24	0	21	23	24	0	20	0	16
Eye ....Number examined:		25	24	0	25	0	22	24	25	0	24	0	19
Unremarkable:		25	24	0	25	0	22	24	25	0	24	0	19
Atrophy, Retina		0	0	0	0	0	0	0	0	0	0	0	0
Rupture		0	0	0	0	0	0	0	0	0	0	0	0
Seminal Vesicle ....Number examined:		25	24	0	25	0	24						
Unremarkable:		25	24	0	25	0	24						
C-Hematopoietic Neoplasm, see Body, Whole for type		0	0	0	0	0	0						
Prostate ....Number examined:		25	24	0	25	0	24						
Unremarkable:		25	24	0	25	0	24						
Testis ....Number examined:		25	24	0	25	0	23						
Unremarkable:		24	24	0	25	0	21						
Atrophy/Degeneration, unilateral		0	0	0	0	0	2						
C-Vascular Neoplasm, see Body, Whole for type		1	0	0	0	0	0						
Epididymis ....Number examined:		25	24	0	25	0	23						
Unremarkable:		25	23	0	25	0	21						
Granuloma, Sperm		0	1	0	0	0	1						
C-Vascular Neoplasm, see Body, Whole for type		0	0	0	0	0	1						
M-Sarcoma, NOS		0	0	0	0	0	0						
Mammary, Female ....Number examined:								24	25	0	23	0	19
Unremarkable:								24	25	0	23	0	19
C-Hematopoietic Neoplasm, see Body, Whole for type								0	0	0	0	0	0
M-Carcinoma								0	0	0	0	0	0
Ovary ....Number examined:								24	25	0	24	0	19
Unremarkable:								24	25	0	24	0	19
C-Hematopoietic Neoplasm, see Body, Whole for type								0	0	0	0	0	0
C-Vascular Neoplasm, see Body, Whole for type								0	0	0	0	0	0

Controls from group(s): 1

Tissues With Diagnoses	Animal sex:	-- Animals Affected -- Males --						-- Females --					
	Dosage group:	Ctls	2	3	4	5	6	Ctls	2	3	4	5	6
	No. in group:	25	24	24	25	23	24	24	25	24	24	21	19
Uterus ....Number examined:								24	25	0	24	0	19
Unremarkable:								17	20	0	20	0	14
Hyperplasia, Cystic Endometrial								7	5	0	4	0	5
C-Hematopoietic Neoplasm, see Body, Whole for type								0	0	0	0	0	0
Cervix ....Number examined:								24	25	0	24	0	19
Unremarkable:								24	25	0	23	0	17
Hyperplasia, Cystic Endometrial								0	0	0	1	0	2
C-Hematopoietic Neoplasm, see Body, Whole for type								0	0	0	0	0	0
Vagina ....Number examined:								24	25	0	24	0	19
Unremarkable:								24	25	0	24	0	19
C-Vascular Neoplasm, see Body, Whole for type								0	0	0	0	0	0
C-Hematopoietic Neoplasm, see Body, Whole for type								0	0	0	0	0	0
M-Carcinoma, Squamous Cell								0	0	0	0	0	0
B-Papilloma, Squamous Cell								0	0	0	0	0	0
Vascular anomaly								0	0	0	0	0	0
Bone, Femur ....Number examined:		25	24	0	25	0	24	24	25	0	24	0	19
Unremarkable:		25	24	0	25	0	24	24	25	0	24	0	19
Marrow, Femur ....Number examined:		25	24	0	25	0	24	24	25	0	24	0	19
Unremarkable:		25	24	0	25	0	24	24	25	0	24	0	19
Hyperplasia, Myeloid		0	0	0	0	0	0	0	0	0	0	0	0
C-Hematopoietic Neoplasm, see Body, Whole for type		0	0	0	0	0	0	0	0	0	0	0	0
Hypocellular		0	0	0	0	0	0	0	0	0	0	0	0
Increased fat		0	0	0	0	0	0	0	0	0	0	0	0
Bone, Sternum ....Number examined:		25	24	0	25	0	24	24	25	0	24	0	19
Unremarkable:		25	24	0	25	0	24	24	25	0	24	0	19
C-Hematopoietic Neoplasm, see Body, Whole for type		0	0	0	0	0	0	0	0	0	0	0	0
Marrow, Sternum ....Number examined:		25	24	0	25	0	24	24	25	0	24	0	19
Unremarkable:		25	24	0	25	0	24	24	25	0	24	0	19
Hyperplasia, Myeloid		0	0	0	0	0	0	0	0	0	0	0	0
C-Hematopoietic Neoplasm, see Body, Whole for type		0	0	0	0	0	0	0	0	0	0	0	0
Hypocellular		0	0	0	0	0	0	0	0	0	0	0	0
Congestion/Hemorrhage		0	0	0	0	0	0	0	0	0	0	0	0

Controls from group(s): 1	Animal sex:	-- Males --						-- Females --					
		-- Animals						Affected --					
	Dosage group:	Ctls	2	3	4	5	6	Ctls	2	3	4	5	6
Tissues With Diagnoses	No. in group:	25	24	24	25	23	24	24	25	24	24	21	19
Untreat Skin/SQ	Number examined:	25	24	0	25	0	24	24	25	0	24	0	19
	Unremarkable:	20	19	0	21	0	18	16	19	0	19	0	12
Inflammation		5	5	0	4	0	6	8	6	0	5	0	7
B-Papilloma, Squamous Cell		0	0	0	0	0	0	0	0	0	0	0	0
Fibrosis		0	1	0	0	0	0	1	0	0	1	0	0
Hyperkeratosis		0	0	0	0	0	0	0	0	0	0	0	0
Pigment		0	0	0	0	0	1	1	0	0	0	0	0
M-Carcinoma, Squamous Cell		0	0	0	0	0	0	0	0	0	0	0	0
Vascular anomaly		0	0	0	0	0	0	0	0	0	0	0	0
C-Hematopoietic Neoplasm, see Body, Whole for type		0	0	0	0	0	0	0	0	0	0	0	0
B-Keratoacanthoma		0	0	0	0	0	0	0	0	0	0	0	0
Ureter	Number examined:	23	22	0	20	0	23	22	23	0	21	0	17
	Unremarkable:	23	22	0	20	0	22	22	23	0	20	0	17
Dilatation		0	0	0	0	0	1	0	0	0	1	0	0
C-Hematopoietic Neoplasm, see Body, Whole for type		0	0	0	0	0	0	0	0	0	0	0	0
Urethra	Number examined:	25	22	0	25	0	24	24	20	0	23	0	19
	Unremarkable:	25	22	0	25	0	24	24	20	0	23	0	19
Hyperplasia, Transitional Cell		0	0	0	0	0	0	0	0	0	0	0	0
B-Papilloma, Transitional Cell		0	0	0	0	0	0	0	0	0	0	0	0
Inflammation		0	0	0	0	0	0	0	0	0	0	0	0
C-Hematopoietic Neoplasm, see Body, Whole for type		0	0	0	0	0	0	0	0	0	0	0	0
Gl, Zymbal's	Number examined:	23	23	0	24	0	23	22	23	0	23	0	16
	Unremarkable:	23	23	0	24	0	23	22	23	0	23	0	16
Ectasia, Duct		0	0	0	0	0	0	0	0	0	0	0	0
Inflammation		0	0	0	0	0	0	0	0	0	0	0	0
M-Carcinoma		0	0	0	0	0	0	0	0	0	0	0	0
Subcutan Site	Number examined:	25	24	0	25	0	24	24	25	0	24	0	19
	Unremarkable:	1	1	0	1	0	0	2	2	0	1	0	1
Fibrosis		1	1	0	0	0	1	0	3	0	3	0	1
Inflammation		24	23	0	24	0	24	22	22	0	23	0	18
Granuloma, Hair Shaft		1	0	0	0	0	2	1	0	0	0	0	1
Keratin cyst		0	0	0	1	0	0	0	0	0	0	0	0
Necrosis		0	0	0	1	0	0	0	0	0	0	0	0
C-Vascular Neoplasm, see Body, Whole for type		0	0	0	0	0	0	0	1	0	0	0	0
Foreign material		0	0	0	0	0	0	1	0	0	2	0	1
Acanthosis		0	0	0	0	0	0	0	0	0	0	0	1
Skeletal muscle, focal degeneration/regeneration		0	0	0	0	0	0	0	0	0	1	0	0

Controls from group(s): 1	Animal sex:	-- Males --						-- Females --					
		-- Animals						Affected --					
	Dosage group:	Ctls	2	3	4	5	6	Ctls	2	3	4	5	6
Tissues With Diagnoses	No. in group:	25	24	24	25	23	24	24	25	24	24	21	19
Death Comment	Number examined:	25	24	0	25	0	24	24	25	0	24	0	19
	Unremarkable:	0	0	0	0	0	0	0	0	0	0	0	0
Scheduled Sacrifice		25	24	0	25	0	24	24	25	0	24	0	19
Hemangiosarcoma		0	0	0	0	0	0	0	0	0	0	0	0
Undetermined		0	0	0	0	0	0	0	0	0	0	0	0
Stomach, squamous cell carcinoma		0	0	0	0	0	0	0	0	0	0	0	0
Lymphoma		0	0	0	0	0	0	0	0	0	0	0	0
Myeloproliferative neoplasm		0	0	0	0	0	0	0	0	0	0	0	0
Perirectal skin ulceration/inflammation		0	0	0	0	0	0	0	0	0	0	0	0
Heart, AV valve thrombosis/atrial dilatation		0	0	0	0	0	0	0	0	0	0	0	0
Mammary malignant adenocacanthoma		0	0	0	0	0	0	0	0	0	0	0	0
Intestinal carcinoma		0	0	0	0	0	0	0	0	0	0	0	0
Salivary gland, squamous cell carcinoma		0	0	0	0	0	0	0	0	0	0	0	0
Salivary gland carcinoma		0	0	0	0	0	0	0	0	0	0	0	0
Body, Whole/Cav	Number examined:	4	0	0	1	0	3	2	2	0	1	0	2
	Unremarkable:	0	0	0	0	0	0	0	0	0	0	0	0
B- Hemangioma		2	0	0	0	0	0	1	1	0	0	0	1
M-Hemangiosarcoma		2	0	0	1	0	3	1	1	0	1	0	1
M-Lymphosarcoma		0	0	0	0	0	0	0	0	0	0	0	0
M-Myeloproliferative neoplasia		0	0	0	0	0	0	0	1	0	0	0	0
Tail	Number examined:	0	0	0	0	0	0	0	1	0	0	0	0
	Unremarkable:	0	0	0	0	0	0	0	1	0	0	0	0
Cavity, Abdomin	Number examined:	0	0	0	0	0	0	0	0	0	0	0	0
	Unremarkable:	0	0	0	0	0	0	0	0	0	0	0	0
Foot/Foot Pad	Number examined:	0	0	0	0	0	0	0	0	0	0	0	1
	Unremarkable:	0	0	0	0	0	0	0	0	0	0	0	0
B-Papilloma, Squamous Cell		0	0	0	0	0	0	0	0	0	0	0	1
Penis	Number examined:	0	0	0	0	0	0	0	0	0	0	0	0
	Unremarkable:	0	0	0	0	0	0	0	0	0	0	0	0
Mammary, Male	Number examined:	0	0	0	0	0	0	0	0	0	0	0	0
	Unremarkable:	0	0	0	0	0	0	0	0	0	0	0	0
Cavity, Thoracic	Number examined:	0	0	0	0	0	0	0	0	0	0	0	0
	Unremarkable:	0	0	0	0	0	0	0	0	0	0	0	0

Controls from group(s): 1	Animal sex:	-- Males --						-- Females --					
		-- Animals						Affected --					
	Dosage group:	Ctls	2	3	4	5	6	Ctls	2	3	4	5	6
Tissues With Diagnoses	No. in group:	25	24	24	25	23	24	24	25	24	24	21	19
Tongue	Number examined:	0	0	0	0	0	0	0	0	0	0	0	0
	Unremarkable:	0	0	0	0	0	0	0	0	0	0	0	0
LN, Other	Number examined:	0	0	0	0	0	0	0	0	0	0	0	0
	Unremarkable:	0	0	0	0	0	0	0	0	0	0	0	0
Skin/Subcutis	Number examined:	1	1	0	0	0	0	0	0	0	0	0	0
	Unremarkable:	0	0	0	0	0	0	0	0	0	0	0	0
B-Papilloma, Squamous Cell		0	1	0	0	0	0	0	0	0	0	0	0
M-Carcinoma, Squamous Cell		0	0	0	0	0	0	0	0	0	0	0	0
C-Hematopoietic Neoplasm, see Body, Whole for type		0	0	0	0	0	0	0	0	0	0	0	0
Hyperkeratosis		1	0	0	0	0	0	0	0	0	0	0	0

Incidence of microscopic observations – unscheduled deaths:

Note: Findings with Group 7 (positive control, MNU) was not included.

Incidence of Microscopic Observations – Unscheduled Deaths

Controls from group(s): 1	Animals Affected										
	-- Males --						-- Females --				
Tissues With Diagnoses / Animal sex:											
Dosage group:	Ctls	2	3	4	5	6	Ctls	2	3	4	5	6
No. in group:	0	1	1	0	2	1	1	0	1	1	4	6
Brain ..... Number examined:	0	1	1	0	2	1	1	0	1	1	4	6
Unremarkable:	0	1	1	0	2	1	1	0	1	1	4	6
Inflammation	0	0	0	0	0	0	0	0	0	0	0	0
C-Hematopoietic Neoplasm, see Body, Whole for type	0	0	0	0	0	0	0	0	0	0	0	0
Spinal Cord ..... Number examined:	0	1	1	0	2	1	1	0	1	1	4	6
Unremarkable:	0	1	1	0	2	1	1	0	1	1	4	6
Cyst, Keratin	0	0	0	0	0	0	0	0	0	0	0	0
Adrenal, Cortex ..... Number examined:	0	1	1	0	2	1	1	0	1	1	4	6
Unremarkable:	0	1	0	0	2	1	0	0	0	1	2	2
Hyperplasia, Subcapsular Cell	0	0	1	0	0	0	1	0	1	0	2	4
C-Hematopoietic Neoplasm, see Body, Whole for type	0	0	0	0	0	0	0	0	0	0	0	0
Adrenal, Medulla ..... Number examined:	0	1	1	0	2	1	1	0	1	1	4	6
Unremarkable:	0	1	1	0	2	1	1	0	1	1	4	6
Pituitary ..... Number examined:	0	1	1	0	2	1	1	0	1	1	4	6
Unremarkable:	0	1	1	0	2	1	1	0	1	1	4	6
Hyperplasia	0	0	0	0	0	0	0	0	0	0	0	0
C-Hematopoietic Neoplasm, see Body, Whole for type	0	0	0	0	0	0	0	0	0	0	0	0
Nerve, Sciatic ..... Number examined:	0	1	1	0	2	1	1	0	1	1	4	6
Unremarkable:	0	1	1	0	2	1	1	0	1	1	4	6
Inflammation, perineural	0	0	0	0	0	0	0	0	0	0	0	0
Inflammation	0	0	0	0	0	0	0	0	0	0	0	0
Trachea ..... Number examined:	0	1	1	0	2	1	1	0	1	1	4	6
Unremarkable:	0	1	1	0	2	1	1	0	1	1	4	6
Esophagus ..... Number examined:	0	1	1	0	2	1	1	0	1	1	4	6
Unremarkable:	0	1	1	0	2	1	1	0	1	1	4	6
B-Papilloma, Squamous Cell	0	0	0	0	0	0	0	0	0	0	0	0
Thyroid ..... Number examined:	0	1	1	0	2	1	1	0	1	1	4	6
Unremarkable:	0	1	1	0	2	1	1	0	1	1	4	6
Cyst, Follicle	0	0	0	0	0	0	0	0	0	0	0	0
C-Hematopoietic Neoplasm, see Body, Whole for type	0	0	0	0	0	0	0	0	0	0	0	0

Controls from group(s): 1	Animals Affected										
	-- Males --						-- Females --				
Tissues With Diagnoses / Animal sex:											
Dosage group:	Ctls	2	3	4	5	6	Ctls	2	3	4	5	6
No. in group:	0	1	1	0	2	1	1	0	1	1	4	6
Parathyroid ..... Number examined:	0	1	1	0	2	1	0	0	1	1	2	6
Unremarkable:	0	1	0	0	2	1	0	0	1	1	2	6
Cyst	0	0	0	0	0	0	0	0	0	0	0	0
Infiltrate, Lymphocytes	0	0	1	0	0	0	0	0	0	0	0	0
Heart ..... Number examined:	0	1	1	0	2	1	1	0	1	1	4	6
Unremarkable:	0	1	1	0	2	1	1	0	1	1	1	6
Degeneration, Myocardium	0	0	0	0	0	0	0	0	0	0	1	0
Mineralization	0	0	0	0	0	0	0	0	0	0	0	0
C-Hematopoietic Neoplasm, see Body, Whole for type	0	0	0	0	0	0	0	0	0	0	0	0
Epicardium, inflammation	0	0	0	0	0	0	0	0	0	0	1	0
C-Vascular Neoplasm, see Body, Whole for type	0	0	0	0	0	0	0	0	0	0	0	0
Atrial dilatation	0	0	0	0	0	0	0	0	0	0	1	0
AV valve, thrombus	0	0	0	0	0	0	0	0	0	0	1	0
Aorta ..... Number examined:	0	1	1	0	2	1	1	0	1	1	4	6
Unremarkable:	0	1	1	0	2	1	1	0	1	1	4	5
C-Hematopoietic Neoplasm, see Body, Whole for type	0	0	0	0	0	0	0	0	0	0	0	0
C-Vascular Neoplasm, see Body, Whole for type	0	0	0	0	0	0	0	0	0	0	0	1
Muscle, Bi Fem ..... Number examined:	0	1	1	0	2	1	1	0	1	1	4	6
Unremarkable:	0	0	1	0	1	0	0	0	0	0	0	1
Degenerative myopathy	0	1	0	0	1	1	1	0	1	1	4	5
Liver ..... Number examined:	0	1	1	0	2	1	1	0	1	1	4	6
Unremarkable:	0	0	0	0	2	1	0	0	1	0	2	2
Inflammation	0	1	0	0	0	0	0	0	0	0	1	4
Angiectasis	0	0	0	0	0	0	0	0	0	0	0	0
Focus, Cellular Alteration, Basophilic	0	0	0	0	0	0	0	0	0	0	0	0
Necrosis	0	1	0	0	0	0	0	0	0	0	0	0
Focus, Cellular Alteration, Mixed Cell	0	0	0	0	0	0	0	0	0	0	0	0
Mitosis, Hepatocyte, Increased	0	0	1	0	0	0	0	0	0	0	0	0
N-Carcinoma, squamous cell	0	0	0	0	0	0	0	0	0	0	0	0
Hematopoiesis, Extramedullary	0	0	0	0	0	0	0	0	0	1	1	0
C-Hematopoietic Neoplasm, see Body, Whole for type	0	0	0	0	0	0	1	0	0	0	0	0
Hepatocellular hypertrophy	0	0	0	0	0	0	0	0	0	0	0	0
Focus, Cellular Alteration, Eosinophilic	0	0	0	0	0	0	0	0	0	0	0	0
Gallbladder ..... Number examined:	0	1	1	0	1	1	1	0	1	1	4	6
Unremarkable:	0	1	1	0	1	1	1	0	1	1	4	6
C-Hematopoietic Neoplasm, see Body, Whole for type	0	0	0	0	0	0	0	0	0	0	0	0

Controls from group(s): 1	Animal sex:	-- Males --						-- Females --					
		-- Animals Affected --											
	Dosage group:	Ctls	2	3	4	5	6	Ctls	2	3	4	5	6
Tissues With Diagnoses	No. in group:	0	1	1	0	2	1	1	0	1	1	4	6
Spleen	Number examined:	0	1	1	0	2	1	1	0	1	1	4	6
	Unremarkable:	0	0	0	0	1	0	0	0	0	0	1	1
Pigment		0	1	0	0	1	0	0	0	0	0	1	1
C-Vascular Neoplasm, see Body, Whole for type		0	1	1	0	0	1	0	0	0	0	0	1
Hematopoiesis, Extramedullary, Increased		0	1	1	0	0	0	0	0	1	1	1	3
Myeloproliferative change		0	0	0	0	0	0	0	0	0	0	2	1
C-Hematopoietic Neoplasm, see Body, Whole for type		0	0	0	0	0	0	1	0	0	0	0	0
Angiectasis/vascular anomaly		0	0	0	0	0	0	0	0	0	0	0	0
N-Squamous cell carcinoma		0	0	0	0	0	0	0	0	0	0	0	0
Lung	Number examined:	0	1	1	0	2	1	1	0	1	1	4	6
	Unremarkable:	0	1	1	0	1	1	1	0	1	0	1	2
B-Adenoma, Bronchiolar-Alveolar		0	0	0	0	0	0	0	0	0	0	0	0
Hemorrhage		0	0	0	0	0	0	0	0	0	0	2	0
Infiltrate, Macrophages, Alveolus		0	0	0	0	0	0	0	0	0	0	1	0
M-Carcinoma, Bronchiolar-Alveolar		0	0	0	0	0	0	0	0	0	0	0	0
Inflammation, subpleural		0	0	0	0	0	0	0	0	0	0	0	0
Hyperplasia, Epithelium, Alveolus		0	0	0	0	0	0	0	0	0	0	0	0
C-Hematopoietic Neoplasm, see Body, Whole for type		0	0	0	0	0	0	0	0	0	0	0	0
N-Squamous cell carcinoma		0	0	0	0	0	0	0	0	0	1	0	0
Inflammation		0	0	0	0	0	0	0	0	0	1	0	0
Leukocytosis		0	0	0	0	0	0	0	0	0	1	0	0
Congestion		0	0	0	0	0	0	0	0	0	0	2	3
N-Mammary adenoacanthoma		0	0	0	0	0	0	0	0	0	0	0	0
Inflammation, pleural/subpleural		0	0	0	0	0	0	0	0	0	0	0	0
N-Salivary gland carcinoma		0	0	0	0	1	0	0	0	0	0	0	0
C-Vascular Neoplasm, see Body, Whole for type		0	0	0	0	0	0	0	0	0	0	0	1
Thymus	Number examined:	0	1	1	0	2	1	1	0	1	0	4	6
	Unremarkable:	0	1	1	0	0	1	0	0	1	0	4	3
Hyperplasia, lymphoid		0	0	0	0	0	0	0	0	0	0	0	1
Hyperplasia, Epithelium		0	0	0	0	0	0	0	0	0	0	0	0
Depletion, Lymphocytes		0	0	0	0	2	0	1	0	0	0	0	1
C-Hematopoietic Neoplasm, see Body, Whole for type		0	0	0	0	0	0	0	0	0	0	0	0
C-Vascular Neoplasm, see Body, Whole for type		0	0	0	0	0	0	0	0	0	0	0	1
Necrosis, Lymphocytes		0	0	0	0	0	0	0	0	0	0	0	0
Kidney	Number examined:	0	1	1	0	2	1	1	0	1	1	4	6
	Unremarkable:	0	1	0	0	1	1	1	0	0	1	4	6
Infiltrate, Lymphocytes		0	0	0	0	0	0	0	0	0	0	0	0
Nephropathy, Chronic Progressive		0	0	0	0	1	0	0	0	1	0	0	0
Mineralization, Tubule		0	0	0	0	0	0	0	0	0	0	0	0

Controls from group(s): 1	Animal sex:	-- Males --						-- Females --					
		-- Animals Affected --											
	Dosage group:	Ctls	2	3	4	5	6	Ctls	2	3	4	5	6
Tissues With Diagnoses	No. in group:	0	1	1	0	2	1	1	0	1	1	4	6
Kidney	Number examined:	0	1	1	0	2	1	1	0	1	1	4	6
	Unremarkable:	0	1	0	0	1	1	1	0	0	1	4	6
Angiectasis		0	0	0	0	0	0	0	0	0	0	0	0
Vacuolation, Tubule Cell		0	0	1	0	0	0	0	0	0	0	0	0
C-Hematopoietic Neoplasm, see Body, Whole for type		0	0	0	0	0	0	0	0	0	0	0	0
Cyst		0	0	0	0	0	0	0	0	0	0	0	0
Dilatation, Tubule(s)		0	0	0	0	0	0	0	0	0	0	0	0
Necrosis, Tubule		0	0	0	0	0	0	0	0	0	0	0	0
Hyperplasia, pelvis		0	0	0	0	0	0	0	0	0	0	0	0
Inflammation, pelvis		0	0	0	0	0	0	0	0	0	0	0	0
Necrosis, Papilla		0	0	0	0	0	0	0	0	0	0	0	0
Pyelonephritis		0	0	0	0	0	0	0	0	0	0	0	0
Regeneration, Tubule Cell		0	0	0	0	0	0	0	0	0	0	0	0
Urinary Bladder	Number examined:	0	1	1	0	2	1	1	0	1	1	4	6
	Unremarkable:	0	1	1	0	2	1	1	0	1	1	4	6
Dilatation		0	0	0	0	0	0	0	0	0	0	0	0
Hyperplasia, Transitional Cell		0	0	0	0	0	0	0	0	0	0	0	0
C-Hematopoietic Neoplasm, see Body, Whole for type		0	0	0	0	0	0	0	0	0	0	0	0
Stomach, Gl	Number examined:	0	1	1	0	2	1	1	0	1	1	4	6
	Unremarkable:	0	1	1	0	2	1	1	0	1	0	4	6
Inflammation		0	0	0	0	0	0	0	0	0	0	0	0
Hyperplasia, Epithelium		0	0	0	0	0	0	0	0	0	0	0	0
Mineralization		0	0	0	0	0	0	0	0	0	0	0	0
I-squamous cell carcinoma		0	0	0	0	0	0	0	0	0	1	0	0
Stomach, Nongl	Number examined:	0	1	1	0	2	1	1	0	1	1	4	6
	Unremarkable:	0	1	1	0	2	1	1	0	1	0	4	6
M-Carcinoma, Squamous Cell		0	0	0	0	0	0	0	0	0	1	0	0
B-Papilloma, Squamous Cell		0	0	0	0	0	0	0	0	0	0	0	0
Acanthosis		0	0	0	0	0	0	0	0	0	0	0	0
Hyperkeratosis		0	0	0	0	0	0	0	0	0	0	0	0
Duodenum	Number examined:	0	1	1	0	2	1	1	0	1	1	4	4
	Unremarkable:	0	1	1	0	2	1	1	0	1	1	4	4
M-Carcinoma		0	0	0	0	0	0	0	0	0	0	0	0
Ileum	Number examined:	0	1	1	0	2	1	0	0	1	0	4	5
	Unremarkable:	0	1	1	0	2	1	0	0	1	0	4	5

Controls from group(s): 1	Animal sex:	-- Animals Affected -- Males --						-- Females --					
	Dosage group:	Ctls	2	3	4	5	6	Ctls	2	3	4	5	6
Tissues With Diagnoses	No. in group:	0	1	1	0	2	1	1	0	1	1	4	6
Colon	Number examined:	0	1	1	0	2	1	1	0	1	1	4	6
	Unremarkable:	0	1	1	0	2	1	1	0	1	1	4	6
Cecum	Number examined:	0	1	1	0	1	1	1	0	1	1	4	4
	Unremarkable:	0	1	1	0	1	1	1	0	1	1	4	4
Jejunum	Number examined:	0	1	1	0	2	1	0	0	1	0	4	5
	Unremarkable:	0	1	1	0	2	1	0	0	1	0	4	5
Intussusception		0	0	0	0	0	0	0	0	0	0	0	0
M-Carcinoma		0	0	0	0	0	0	0	0	0	0	0	0
Atypical hyperplasia		0	0	0	0	0	0	0	0	0	0	0	0
Rectum	Number examined:	0	1	1	0	2	1	1	0	1	1	4	5
	Unremarkable:	0	1	1	0	2	1	1	0	0	1	4	5
Perirectal skin ulceration/inflammation		0	0	0	0	0	0	0	0	1	0	0	0
LN, Mesenteric	Number examined:	0	1	1	0	2	1	1	0	1	1	4	6
	Unremarkable:	0	1	1	0	2	1	1	0	1	1	4	6
C-Vascular Neoplasm, see Body, Whole for type		0	0	0	0	0	0	0	0	0	0	0	0
Congestion		0	0	0	0	0	0	0	0	0	0	0	0
C-Hematopoietic Neoplasm, see Body, Whole for type		0	0	0	0	0	0	0	0	0	0	0	0
LN, Mandibular	Number examined:	0	1	1	0	2	1	0	0	1	1	4	5
	Unremarkable:	0	1	1	0	2	1	0	0	1	1	4	5
C-Hematopoietic Neoplasm, see Body, Whole for type		0	0	0	0	0	0	0	0	0	0	0	0
Gl, Mandib Saliv	Number examined:	0	1	1	0	2	1	1	0	1	1	4	6
	Unremarkable:	0	1	1	0	1	1	1	0	1	1	3	6
Inflammation		0	0	0	0	0	0	0	0	0	0	0	0
Atrophy		0	0	0	0	0	0	0	0	0	0	1	0
M-Squamous cell carcinoma		0	0	0	0	0	0	0	0	0	0	0	0
M-Carcinoma		0	0	0	0	1	0	0	0	0	0	0	0
Pancreas	Number examined:	0	1	1	0	2	1	1	0	1	1	4	6
	Unremarkable:	0	0	1	0	2	1	1	0	1	1	4	6
Inflammation		0	1	0	0	0	0	0	0	0	0	0	0
Pigment		0	0	0	0	0	0	0	0	0	0	0	0
Atrophy		0	0	0	0	0	0	0	0	0	0	0	0
Inflammation, Vessel		0	0	0	0	0	0	0	0	0	0	0	0
C-Hematopoietic Neoplasm, see Body, Whole for type		0	0	0	0	0	0	0	0	0	0	0	0

Controls from group(s): 1	Animal sex:	-- Animals Affected -- Males --						-- Females --					
	Dosage group:	Ctls	2	3	4	5	6	Ctls	2	3	4	5	6
Tissues With Diagnoses	No. in group:	0	1	1	0	2	1	1	0	1	1	4	6
Gl, Harderian	Number examined:	0	1	1	0	2	1	1	0	1	1	4	6
	Unremarkable:	0	1	1	0	2	1	1	0	1	1	4	6
B-Adenoma		0	0	0	0	0	0	0	0	0	0	0	0
Inflammation		0	0	0	0	0	0	0	0	0	0	0	0
M-Carcinoma		0	0	0	0	0	0	0	0	0	0	0	0
C-Hematopoietic Neoplasm, see Body, Whole for type		0	0	0	0	0	0	0	0	0	0	0	0
Nerve, Optic	Number examined:	0	1	1	0	2	0	1	0	1	0	2	5
	Unremarkable:	0	1	1	0	2	0	1	0	1	0	2	5
Eye	Number examined:	0	1	1	0	2	1	1	0	1	1	4	6
	Unremarkable:	0	1	1	0	2	1	1	0	1	1	4	6
Atrophy, Retina		0	0	0	0	0	0	0	0	0	0	0	0
Rupture		0	0	0	0	0	0	0	0	0	0	0	0
Seminal Vesicle	Number examined:	0	1	1	0	2	1						
	Unremarkable:	0	1	1	0	2	1						
C-Hematopoietic Neoplasm, see Body, Whole for type		0	0	0	0	0	0						
Prostate	Number examined:	0	1	1	0	2	1						
	Unremarkable:	0	1	1	0	2	1						
Testis	Number examined:	0	1	1	0	2	1						
	Unremarkable:	0	1	1	0	2	1						
Atrophy/Degeneration, unilateral		0	0	0	0	0	0						
C-Vascular Neoplasm, see Body, Whole for type		0	0	0	0	0	0						
Epididymis	Number examined:	0	1	1	0	2	1						
	Unremarkable:	0	1	1	0	2	1						
Granuloma, Sperm		0	0	0	0	0	0						
C-Vascular Neoplasm, see Body, Whole for type		0	0	0	0	0	0						
M-Sarcoma, NOS		0	0	0	0	0	0						
Mammary, Female	Number examined:							1	0	1	1	4	6
	Unremarkable:							1	0	1	1	4	6
C-Hematopoietic Neoplasm, see Body, Whole for type								0	0	0	0	0	0
M-Carcinoma								0	0	0	0	0	0
Ovary	Number examined:							1	0	1	1	4	6
	Unremarkable:							1	0	1	1	4	6
C-Hematopoietic Neoplasm, see Body, Whole for type								0	0	0	0	0	0
C-Vascular Neoplasm, see Body, Whole for type								0	0	0	0	0	0

Controls from group(s): 1	Animal sex:	-- Males --						-- Females --					
	Dosage group:	Ctls	2	3	4	5	6	Ctls	2	3	4	5	6
Tissues With Diagnoses	No. in group:	0	1	1	0	2	1	1	0	1	1	4	6
Uterus	Number examined:							1	0	1	1	4	6
	Unremarkable:							1	0	1	1	4	6
Hyperplasia, Cystic Endometrial								0	0	0	0	0	0
C-Hematopoietic Neoplasm, see Body, Whole for type								0	0	0	0	0	0
Cervix	Number examined:							1	0	1	1	4	6
	Unremarkable:							1	0	1	1	4	6
Hyperplasia, Cystic Endometrial								0	0	0	0	0	0
C-Hematopoietic Neoplasm, see Body, Whole for type								0	0	0	0	0	0
Vagina	Number examined:							1	0	1	1	4	6
	Unremarkable:							0	0	1	1	4	6
C-Vascular Neoplasm, see Body, Whole for type								0	0	0	0	0	0
C-Hematopoietic Neoplasm, see Body, Whole for type								0	0	0	0	0	0
M-Carcinoma, Squamous Cell								0	0	0	0	0	0
B-Papilloma, Squamous Cell								0	0	0	0	0	0
Vascular anomaly								1	0	0	0	0	0
Bone, Femur	Number examined:	0	1	1	0	2	1	1	0	1	1	4	6
	Unremarkable:	0	1	1	0	2	1	1	0	1	1	4	6
Marrow, Femur	Number examined:	0	1	1	0	2	1	1	0	1	1	4	6
	Unremarkable:	0	1	1	0	1	1	1	0	0	0	4	6
Hyperplasia, Myeloid		0	0	0	0	1	0	0	0	1	1	0	0
C-Hematopoietic Neoplasm, see Body, Whole for type		0	0	0	0	0	0	0	0	0	0	0	0
Hypocellular		0	0	0	0	0	0	0	0	0	0	0	0
Increased fat		0	0	0	0	0	0	0	0	0	0	0	0
Bone, Sternum	Number examined:	0	1	1	0	2	1	1	0	1	1	4	6
	Unremarkable:	0	1	1	0	2	1	1	0	1	1	4	6
C-Hematopoietic Neoplasm, see Body, Whole for type		0	0	0	0	0	0	0	0	0	0	0	0
Marrow, Sternum	Number examined:	0	1	1	0	2	1	1	0	1	1	4	6
	Unremarkable:	0	1	1	0	1	1	1	0	0	0	4	6
Hyperplasia, Myeloid		0	0	0	0	1	0	0	0	1	1	0	0
C-Hematopoietic Neoplasm, see Body, Whole for type		0	0	0	0	0	0	0	0	0	0	0	0
Hypocellular		0	0	0	0	0	0	0	0	0	0	0	0
Congestion/Hemorrhage		0	0	0	0	0	0	0	0	0	0	0	0

Controls from group(s): 1	Animal sex:	-- Males --						-- Females --					
	Dosage group:	Ctls	2	3	4	5	6	Ctls	2	3	4	5	6
Tissues With Diagnoses	No. in group:	0	1	1	0	2	1	1	0	1	1	4	6
Untreat Skin/SQ	Number examined:	0	1	1	0	2	1	1	0	1	1	3	6
	Unremarkable:	0	1	1	0	2	0	1	0	0	1	2	6
Inflammation		0	0	0	0	0	1	0	0	1	0	1	0
B-Papilloma, Squamous Cell		0	0	0	0	0	0	0	0	0	0	0	0
Fibrosis		0	0	0	0	0	0	0	0	0	0	0	0
Hyperkeratosis		0	0	0	0	0	0	0	0	0	0	0	0
Pigment		0	0	0	0	0	0	0	0	0	0	0	0
M-Carcinoma, Squamous Cell		0	0	0	0	0	0	0	0	0	0	0	0
Vascular anomaly		0	0	0	0	0	0	0	0	0	0	0	0
C-Hematopoietic Neoplasm, see Body, Whole for type		0	0	0	0	0	0	0	0	0	0	0	0
B-Keratoacanthoma		0	0	0	0	0	0	0	0	0	0	0	0
Ureter	Number examined:	0	1	1	0	2	1	0	0	0	0	2	6
	Unremarkable:	0	1	1	0	1	1	0	0	0	0	2	6
Dilatation		0	0	0	0	1	0	0	0	0	0	0	0
C-Hematopoietic Neoplasm, see Body, Whole for type		0	0	0	0	0	0	0	0	0	0	0	0
Urethra	Number examined:	0	0	1	0	2	1	1	0	1	1	4	6
	Unremarkable:	0	0	1	0	2	1	1	0	1	1	4	6
Hyperplasia, Transitional Cell		0	0	0	0	0	0	0	0	0	0	0	0
B-Papilloma, Transitional Cell		0	0	0	0	0	0	0	0	0	0	0	0
Inflammation		0	0	0	0	0	0	0	0	0	0	0	0
C-Hematopoietic Neoplasm, see Body, Whole for type		0	0	0	0	0	0	0	0	0	0	0	0
Gl, Zymbal's	Number examined:	0	1	1	0	2	1	1	0	1	0	4	6
	Unremarkable:	0	1	1	0	1	1	1	0	1	0	4	6
Ectasia, Duct		0	0	0	0	1	0	0	0	0	0	0	0
Inflammation		0	0	0	0	0	0	0	0	0	0	0	0
M-Carcinoma		0	0	0	0	0	0	0	0	0	0	0	0
Subcutan Site	Number examined:	0	1	1	0	2	1	1	0	1	1	3	6
	Unremarkable:	0	1	0	0	2	0	1	0	0	1	1	0
Fibrosis		0	0	0	0	0	0	0	0	0	0	0	0
Inflammation		0	0	1	0	0	1	0	0	1	0	2	6
Granuloma, Hair Shaft		0	0	1	0	0	0	0	0	1	0	0	1
Keratin cyst		0	0	0	0	0	0	0	0	0	0	0	0
Necrosis		0	0	0	0	0	0	0	0	0	0	0	0
C-Vascular Neoplasm, see Body, Whole for type		0	0	0	0	0	0	0	0	0	0	0	0
Foreign material		0	0	0	0	0	0	0	0	0	0	0	0
Acanthosis		0	0	0	0	0	0	0	0	0	0	0	0
Skeletal muscle, focal degeneration/regeneration		0	0	0	0	0	0	0	0	0	0	0	0

Controls from group(s): 1	Animal sex:	-- Males --						-- Females --					
	Dosage group:	Ctls	2	3	4	5	6	Ctls	2	3	4	5	6
Tissues With Diagnoses	No. in group:	0	1	1	0	2	1	1	0	1	1	4	6
Death Comment	Number examined:	0	1	1	0	2	1	1	0	1	1	4	6
	Unremarkable:	0	0	0	0	0	0	0	0	0	0	0	0
Scheduled Sacrifice		0	0	0	0	0	0	0	0	0	0	0	0
Hemangiosarcoma		0	1	1	0	0	1	0	0	0	0	0	1
Undetermined		0	0	0	0	1	0	0	0	0	0	3	5
Stomach, squamous cell carcinoma		0	0	0	0	0	0	0	0	0	1	0	0
Lymphoma		0	0	0	0	0	0	0	0	0	0	0	0
Myeloproliferative neoplasm		0	0	0	0	0	0	1	0	0	0	0	0
Perirectal skin ulceration/inflammation		0	0	0	0	0	0	0	0	1	0	0	0
Heart, AV valve thrombosis/atrial dilatation		0	0	0	0	0	0	0	0	0	0	1	0
Mammary malignant adenoacanthoma		0	0	0	0	0	0	0	0	0	0	0	0
Intestinal carcinoma		0	0	0	0	0	0	0	0	0	0	0	0
Salivary gland, squamous cell carcinoma		0	0	0	0	0	0	0	0	0	0	0	0
Salivary gland carcinoma		0	0	0	0	1	0	0	0	0	0	0	0
Body, Whole/Cav	Number examined:	0	1	1	0	0	1	1	0	0	0	1	2
	Unremarkable:	0	0	0	0	0	0	0	0	0	0	1	0
B- Hemangioma		0	0	0	0	0	0	0	0	0	0	0	0
M-Hemangiosarcoma		0	1	1	0	0	1	0	0	0	0	0	2
M-Lymphosarcoma		0	0	0	0	0	0	0	0	0	0	0	0
M-Myeloproliferative neoplasia		0	0	0	0	0	0	1	0	0	0	0	0
Tail	Number examined:	0	0	0	0	0	0	0	0	0	0	0	0
	Unremarkable:	0	0	0	0	0	0	0	0	0	0	0	0
Cavity, Abdomin	Number examined:	0	0	0	0	0	0	0	0	0	1	0	0
	Unremarkable:	0	0	0	0	0	0	0	0	0	0	0	0
I-Squamous cell carcinoma		0	0	0	0	0	0	0	0	0	1	0	0
Inflammation		0	0	0	0	0	0	0	0	0	0	0	0
Foot/Foot Pad	Number examined:	0	0	0	0	0	0	0	0	0	0	0	0
	Unremarkable:	0	0	0	0	0	0	0	0	0	0	0	0
Penis	Number examined:	0	0	0	0	0	0	0	0	0	0	0	0
	Unremarkable:	0	0	0	0	0	0	0	0	0	0	0	0
Mammary, Male	Number examined:	0	0	0	0	0	0	0	0	0	0	0	0
	Unremarkable:	0	0	0	0	0	0	0	0	0	0	0	0

Controls from group(s): 1	Animal sex:	-- Males --						-- Females --					
	Dosage group:	Ctls	2	3	4	5	6	Ctls	2	3	4	5	6
Tissues With Diagnoses	No. in group:	0	1	1	0	2	1	1	0	1	1	4	6
Cavity, Thoracic	Number examined:	0	0	0	0	0	0	0	0	0	0	1	1
	Unremarkable:	0	0	0	0	0	0	0	0	0	0	0	0
N-squamous cell carcinoma		0	0	0	0	0	0	0	0	0	0	0	0
Inflammation		0	0	0	0	0	0	0	0	0	0	0	0
C-Hematopoietic Neoplasm, see Body, Whole for type		0	0	0	0	0	0	0	0	0	0	0	0
C-Vascular Neoplasm, see Body, Whole for type		0	0	0	0	0	0	0	0	0	0	0	1
Hyperplasia, Mesothelium		0	0	0	0	0	0	0	0	0	0	1	0
Tongue	Number examined:	0	0	0	0	0	0	0	0	0	0	0	0
	Unremarkable:	0	0	0	0	0	0	0	0	0	0	0	0
LN, Other	Number examined:	0	0	0	0	0	0	0	0	0	0	0	0
	Unremarkable:	0	0	0	0	0	0	0	0	0	0	0	0
Skin/Subcutis	Number examined:	0	0	0	0	0	0	0	0	0	0	0	0
	Unremarkable:	0	0	0	0	0	0	0	0	0	0	0	0

Toxicokinetics:

Exposure to FDKP increased with the increase in TI from 2.5 to 5.0 mg/kg/day (males) and 0.6 to 1.25 mg/kg/day (females) on Day 1 and during Week 26. The increases in C_{max} and $AUC_{0\text{-}120}$ were dose-proportional. Less than 2 fold sex differences were observed in FDKP C_{max} and $AUC_{0\text{-}120}$ values. FDKP $t_{1/2}$ values ranged from 10.8 to 34.8 min on Day 1, and from 14.9 to 46.1 min during Week 26. Moreover, no accumulation of FDKP was seen after multiple dosing of T or TI in males and T in females. Accumulation of FDKP in females was not able to estimate because of the decreased doses of TI administered on the beginning on Day 77.

Dose Proportionality Ratios of FDKP in Mouse Serum

Interval	Test Article	Males			Females		
		Dose Level Increase	C_{max} Ratio	AUC_{0-120} Ratio	Dose Level Increase	C_{max} Ratio	AUC_{0-120} Ratio
Day 1	Technosphere® Particles	1.0:3.0-fold	1.0:3.5-fold	1.0:3.1-fold	1.0:3.0-fold	1.0:2.4-fold	1.0:2.8-fold
	Technosphere®/ Insulin	1.0:2.0-fold	1.0:1.8-fold	1.0:2.0-fold	1.0:2.0-fold	1.0:1.5-fold	1.0:1.6-fold
Week 26	Technosphere® Particles	1.0:3.0-fold	1.0:3.0-fold	1.0:2.5-fold	1.0:3.0-fold	1.0:2.9-fold	1.0:2.9-fold
	Technosphere®/ Insulin	1.0:2.0-fold	1.0:2.2-fold	1.0:1.9-fold	1.0:2.1-fold	1.0:2.0-fold	1.0:1.9-fold

Exposure to insulin increased with the increase in TI from 2.5 to 5.0 mg/kg/day (males) and 0.6 to 1.25 mg/kg/day (females) on Day 1 and during Week 26. The increases in C_{max} and AUC_{0-120} were dose-proportional in males and greater than dose proportional in females. Males had higher insulin C_{max} and AUC_{0-120} values than females. Insulin $t_{1/2}$ values were ranged from 10.8 to 21.5 min on Day 1. Due to the lack of a distinct elimination phase on Week 26, insulin $t_{1/2}$ value was only estimated in Group 11 (2.5 mg/kg/day TI) males at 18.5 min. Moreover, no accumulation of insulin was seen after multiple dosing of T or TI in males. Accumulation of insulin in females could not be estimated because of the decreased doses of TI administered on the beginning of Day 77.

Dose Proportionality Ratios of Insulin in Mouse Serum

Interval	Test Article	Males			Females		
		Dose Level Increase	C_{max} Ratio	AUC_{0-120} Ratio	Dose Level Increase	C_{max} Ratio	AUC_{0-120} Ratio
Day 1	Technosphere®/ Insulin	1.0:2.0-fold	1.0:2.1-fold	1.0:2.7-fold	1.0:2.0-fold	1.0:3.6-fold	1.0:2.5-fold
Week 26	Technosphere®/ Insulin	1.0:2.0-fold	1.0:1.7-fold	1.0:1.8-fold	1.0:2.1-fold	1.0:4.6-fold	1.0:5.5-fold

All in all, in transgenic ras H2 mice via sc injection once daily for 26 weeks at 25 or 75 mg/kg/day T, or 2.5 and 5 mg/kg/day TI (male) [0.6 and 1.25 mg/kg/day TI in female], there was no conclusive evidence that either T or TI caused increased oncogenicity or remarkable organ weight, macroscopic, or non-neoplastic microscopic changes. In Spraque-Dawley rats via inhalation once daily for 104 week, T or TI was well-tolerated. There were no indications that T or TI had carcinogenic potential. Also, there were no PCNA activity changes in bronchial or alveolar lung cells across all groups.

2.6.6.6 Reproductive and developmental toxicology

7 studies were submitted by the sponsor.

- PDC-PC-2001-0006 (fertility rat)
- MKC-PC-2004-0003 (Seg II rabbit)
- PDC-PC-2002-0012 (Seg II rat)
- PDC-PC-2001-0003 (Seg II rat)
- MKC-PC-2004-0004 (Seg II rabbit)

- MKC-PC-2006-0010 (TK rat fetus)
- MKC-PC-2004-0008 (Pre-/Perinatal rat)

Fertility and early embryonic development

Pivotal Fertility Rat - PDC-PC-2001-0006: A Subcutaneous Injection Fertility Study of Fumaryl-Technosphere™ in Rats [By (b) (4) Study No. 98064, reviewed by Herman Rhee and his review was dated 01/06/03 in DARRTS]

This study examined the potential effects on FDPK upon the reproduction and fertility of male and female albino rats of the F0 generation. Parameters evaluated included clinical observations, body weights, and food consumption. Complete necropsy examination were conducted on all males approx. 2 weeks after the end of the mating period, and on all females on GD 13. A uterine examination was conducted for each female and the number of live/dead embryos, and resorptions were recorded. Reproductive assessments were performed for all males. Testicular histopathology was done for males of the control and 100 mg/kg/day treatment groups.

Group No. Identification	Dose Level (mg/kgday)	Dose Level (mg/kg/dose)	Dose Conc. (mg/mL)	Injection Volume* (mL/kg/dose)	No. of Animals Males	No. of Animals Females
1 Vehicle control	0	0	0	5	22	22
2 Fumaryl Technosphere™	10	5	1	5	22	22
3 Fumaryl Technosphere™	30	15	3	5	22	22
4 Fumaryl Technosphere™	100	50	10	5	22	22

* Animals were dosed *b.i.d.* with approximately 6 hours interval between the first and second daily dose.

Findings: No treatment-related mortality and no clinical signs attributable to FDKP exposure. No effects were seen upon body weight, body weight gains, or food consumption. Necropsy revealed no treatment-related macroscopic changes.

For the females: The number of corpora lutea, implantation sites, resorptions, live/dead embryos, and pre- and post-implantation losses for the control and treated groups were similar. In addition, for the females, ovarian and uterine weights were unaffected by treatment with FDKP. For the males, there were no significant differences in the epididymis, prostate, seminal vesicle and testis weights of the control and treated males. For the males, there were also no effects upon the sperm counts, motility, or morphology. The spermatogenic cycle was not affected by FDKP treatment.

TABLE NO. 14 GROUP MEAN PARENTAL PERFORMANCE PROJECT NO. 98064

	NO. PLACED FOR MATING MALES	FEMALES	NO. MATING	MEAN (S.D.) DAY TO MATING	NO. FEMALES PREGNANT	MATING INDEX (%)	FERTILITY INDEX (%)	CONCEPTION RATE (%)
GROUP 1 - VEHICLE CONTROL	22	22	22	2.5 (1.22)	22	100.0	100.0	100.0
GROUP 2 - FUMARYL TECHNOSPHERE™ 10 MG/KG/DAY (5 MG/KG bid)	22	22	22	4.0 (4.14)	22	100.0	100.0	100.0
GROUP 3 - FUMARYL TECHNOSPHERE™ 30 MG/KG/DAY (15 MG/KG bid)	22	22	22	2.8 (1.01)	21	100.0	95.5	95.5
GROUP 4 - FUMARYL TECHNOSPHERE™ 100 MG/KG/DAY (50 MG/KG bid)	22	22	22	2.0 (0.97)	22	100.0	100.0	100.0

TABLE NO. 15 GROUP MEAN (S.D.) UTERINE FINDINGS PROJECT NO :98064

	TOTAL NO. OF CORPORA LUTEA	NO. OF IMPLAN-TATION SITES	NO. OF LIVE EMBRYOS	NO. OF DEAD EMBRYOS
GROUP 1 - VEHICLE CONTROL	17.0 1.76	16.1 2.32	15.4 2.28	.0 .21
GROUP 2 - FUMARYL TECHNOSPHERE™ 10 MG/KG/DAY (5 MG/KG bid)	17.6 1.59	15.5 3.29	15.1 3.27	.0 .21
GROUP 3 - FUMARYL TECHNOSPHERE™ 30 MG/KG/DAY (15 MG/KG bid)	17.3 1.91	16.1 1.67	15.1 2.13	.0 .22
GROUP 4 - FUMARYL TECHNOSPHERE™ 100 MG/KG/DAY (50 MG/KG bid)	18.8 2.77	17.0 1.34	16.4 1.29	0.0 0.00

TABLE NO. 15 GROUP MEAN (S.D.) UTERINE FINDINGS PROJECT NO :98064

	NO. OF EARLY RESORP-TIONS	SUM OF EARLY RESORPTIONS & DEAD EMBRYOS	PRE-IMPLAN-TATION LOSS %	POST IMPLAN-TATION LOSS %
GROUP 1 - VEHICLE CONTROL	.7 .84	.7 .94	5.6 6.83	4.5 5.45
GROUP 2 - FUMARYL TECHNOSPHERE™ 10 MG/KG/DAY (5 MG/KG bid)	.4 .66	.4 .67	11.9 17.12	2.5 4.21
GROUP 3 - FUMARYL TECHNOSPHERE™ 30 MG/KG/DAY (15 MG/KG bid)	.9 .83	1.0 .92	6.7 8.25	6.2 6.37
GROUP 4 - FUMARYL TECHNOSPHERE™ 100 MG/KG/DAY (50 MG/KG bid)	.6 .93	.6 .93	8.4 8.37	3.2 5.17

TABLE NO. 16 GROUP MEAN (S.D.) EPIDIDYMAL SPERM EVALUATION PROJECT NO :98064

	CAUDA EPIDIDYMIS WEIGHT (G)	SPERMATOZOA PER GRAM (MILLIONS)	MOTILITY (%)
GROUP 1 - VEHICLE CONTROL	.309 .0307	689.12 167.570	63.0 10.18
GROUP 2 - FUMARYL TECHNOSPHERE™ 10 MG/KG/DAY (5 MG/KG bid)	.307 .0447	659.53 158.392	67.4 10.38
GROUP 3 - FUMARYL TECHNOSPHERE™ 30 MG/KG/DAY (15 MG/KG bid)	.291 .0457	673.62 172.754	63.0 12.74
GROUP 4 - FUMARYL TECHNOSPHERE™ 100 MG/KG/DAY (50 MG/KG bid)	.305 .0248	687.26 118.972	64.0 11.14

Overall, twice-daily SC injection of FDKP at doses up to 100 mg/kg/day was well-tolerated by male and pregnant female rats and resulted in no evidence of paternal or maternal toxicity. There was no evidence of effects upon fertility or early embryonic development at dose levels up to 100 mg/kg/day.

Herman Rhee's review on this study:

Conclusions: In order to examine the reproductive toxicity of Fumaryl Technosphere ™, the sponsor performed Segment 1 study in SD rats. A total of 100 male and 100 female rats were randomly assigned to the vehicle control, 10, 30 or 100 mg/kg/day groups. The study was performed acceptably according to reproductive study protocol, of which results indicate that Fumaryl Technosphere ™ did not produce remarkable toxicity in all 4 groups at the doses tested. However, there was an increased incidence of dark mucoid material in the vagina in HD females (17 out of 22 animals vs. 8 out of 22 in the control, which needs to be included in investigator's brochure and drug label. The mode of drug administration was subcutaneous instead of inhalation, the intended route to achieve sufficient blood levels of the drug.

FEMALE

ORGAN/FINDING	DOSE GROUP:	1	2	3	4
	ANIMAL EXAMINED:	22	22	22	22
LIVER					
- -Area pale-		-	1	-	-
THYMUS					
- -Area dark-		2	-	1	-
VAGINA					
- -Material dark-		8	8	7	17

MKC-PC-2004-0003: Subcutaneous Dosage-Range Development Toxicity Study of Technosphere® in Rabbits [By (b) (4), Study No. LCL00001]

This study was to select dosages for the later development toxicity study of FDKP in New Zealand White, presumed pregnant female rabbits. Twenty five rabbits were assigned to 5 dosage groups (0, 1, 10, 30, and 100 mg/kg/day). These rabbits were given FDKP once daily via sc injection on GDs 7 through 19. Clinical observations and body weights were recorded. All rabbits were sacrificed on GD 29 and examined for the number and distribution of corpora lutea, implantation sites and uterine contents. A gross necropsy of the thoracic, abdominal and pelvic viscera was conducted. Fetuses were weighted and examined for gross external alternations and sex.

Maternal findings: All rabbits survived to the scheduled sacrifice. One adverse clinical sign that appeared to be test article-related was scant feces in all treated groups. Other observations, including localized alopecia on the underside and limbs, soft or liquid feces, and ungroomed coat, were considered not test-article related due to the incidence was not dose-dependent. All rabbits appeared normal at necropsy (except one at 100 mg/kg/day had dark red and mottled lobes of the lungs and pink and frothy liquid in the trachea). A body weight loss was recorded on GDs 7 to 10 in the 10, 30, and 100 mg/kg/day dosage groups. Body weight gains were reduced in each of the treated groups for the entire dosing period (GDs 7-20). This is indicative of maternal toxicity as during pregnancy dams should increase weight at all doses (NOAEL< 1 mg/kg/day). Food consumption were reduced in the 30 and 100 mg/kg/day groups on GDs 7-10, 10-13, and 13-16, and for the entire dosing period.

TABLE 7 (PAGE 1): CAESAREAN-SECTIONING OBSERVATIONS - SUMMARY

DOSAGE GROUP DOSAGE (MG/KG/DAY)a		I 0 (VEHICLE)	II 1	III 10	IV 30	V 100
RABBITS TESTED	N	5	5	5	5	5
PREGNANT	N(%)	5(100.0)	5(100.0)	4(80.0)	4(80.0)	5(100.0)
RABBITS PREGNANT AND CAESAREAN-SECTIONED ON DAY 29 OF GESTATION	N	5	5	4	4	5
CORPORA LUTEA	MEAN±S.D.	10.4 ± 1.5	10.0 ± 1.4	10.0 ± 2.2	11.2 ± 2.4	10.8 ± 1.6
IMPLANTATIONS	MEAN±S.D.	9.6 ± 1.5	9.6 ± 0.9	10.0 ± 2.2	8.8 ± 2.5	9.8 ± 2.2
LITTER SIZES	MEAN±S.D.	9.2 ± 1.6	9.6 ± 0.9	9.5 ± 1.9	8.8 ± 2.5	9.4 ± 1.8
LIVE FETUSES	N	46	48	38	35	47
	MEAN±S.D.	9.2 ± 1.6	9.6 ± 0.9	9.5 ± 1.9	8.8 ± 2.5	9.4 ± 1.8
DEAD FETUSES	N	0	0	0	0	0
RESORPTIONS	MEAN±S.D.	0.4 ± 0.5	0.0 ± 0.0	0.5 ± 0.6	0.0 ± 0.0	0.4 ± 0.9
EARLY RESORPTIONS	N	2	0	0	0	0
	MEAN±S.D.	0.4 ± 0.5	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
LATE RESORPTIONS	N	0	0	2	0	2
	MEAN±S.D.	0.0 ± 0.0	0.0 ± 0.0	0.5 ± 0.6	0.0 ± 0.0	0.4 ± 0.9
DOES WITH ANY RESORPTIONS	N(%)	2(40.0)	0(0.0)	2(50.0)	0(0.0)	1(20.0)
DOES WITH ALL CONCEPTUSES RESORBED	N(%)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)
DOES WITH VIABLE FETUSES	N(%)	5(100.0)	5(100.0)	4(100.0)	4(100.0)	5(100.0)
PLACENTAE APPEARED NORMAL	N(%)	5(100.0)	5(100.0)	4(100.0)	4(100.0)	5(100.0)

a. Dosage occurred on days 7 through 19 of gestation.

Fetal findings: When caesarean-sectioned on GD29, live fetal body weights were slightly reduced (91-93% of VH values) in the 100 mg/kg/day dose group, when compared to the VH control group. All other C-section and littler parameters were not affected by FDKP. The litter averages for corpora lutea, implantations, litter size, early and late resorptions, and % resorbed conceptuses were comparable among dose groups. Moreover, no fetal gross external alternations were observed in live fetuses.

TABLE 8 (PAGE 1): LITTER OBSERVATIONS (CAESAREAN-DELIVERED FETUSES) - SUMMARY

DOSAGE GROUP DOSAGE (MG/KG/DAY)a		I 0 (VEHICLE)	II 1	III 10	IV 30	V 100
LITTERS WITH ONE OR MORE LIVE FETUSES	N	5	5	4	4	5
IMPLANTATIONS	MEAN±S.D.	9.6 ± 1.5	9.6 ± 0.9	10.0 ± 2.2	8.8 ± 2.5	9.8 ± 2.2
LIVE FETUSES	N	46	48	38	35	47
	MEAN±S.D.	9.2 ± 1.6	9.6 ± 0.9	9.5 ± 1.9	8.8 ± 2.5	9.4 ± 1.8
LIVE MALE FETUSES	N	19	26	17	12	22
% LIVE MALE FETUSES/LITTER	MEAN±S.D.	41.1 ± 15.6	53.5 ± 14.1	43.3 ± 22.8	37.5 ± 15.9	47.4 ± 20.9
LIVE FETAL BODY WEIGHTS (GRAMS)/LITTER	MEAN±S.D.	43.66 ± 1.79	44.80 ± 3.43	44.86 ± 4.00	44.44 ± 7.11	40.20 ± 4.77
MALE FETUSES	MEAN±S.D.	44.18 ± 2.42	44.77 ± 3.75	45.88 ± 4.08	46.66 ± 7.16	41.16 ± 5.29
FEMALE FETUSES	MEAN±S.D.	43.23 ± 2.19	45.00 ± 2.84	44.30 ± 4.60	43.66 ± 6.78	39.21 ± 4.63
% RESORBED CONCEPTUSES/LITTER	MEAN±S.D.	4.2 ± 5.8	0.0 ± 0.0	4.7 ± 5.6	0.0 ± 0.0	3.3 ± 7.5
		NO FETAL ALTERATIONS ON LIVE FETUSES WERE IDENTIFIED AT GROSS EXTERNAL EXAMINATION				

a. Dosage occurred on days 7 through 19 of gestation.

TK findings: Mean FDKP exposure (serum conc., C_{max}, and mean $AUC_{0\text{-last}}$) was approx proportional to the dose on both GDs 7-19. The t½ mean values were approx. 1 hr when reportable. Administration of FDKP as a daily sc injection to pregnant rabbits from GDs 7 to 19,

showed dose proportional serum exposure (C_{max} and $AUC_{0\text{-last}}$), indicating that there was no evidence of accumulation.

TABLE 7A. FDKP Serum Mean Toxicokinetic Metrics Following Daily SC Injection of Technosphere® on Gestation Days Through 19 (5 mL/kg in buffered saline) to Pregnant New Zealand White Rabbits (n=5/group)

		Group II 1.0 mg/kg/day		Group III 10 mg/kg/day		Group IV 30 mg/kg/day		Group V 100 mg/kg/day	
		Day 7	Day 19	Day 7	Day 19	Day 7	Day 19	Day 7	Day 19
C_{max} (ng/mL)	Mean	1726	1578	17365	16960	26952	21761	90808	74753
	SD	211	208	2215	5632	7632	7243	33482	20091
	CV (%)	12.2	13.2	12.8	33.2	28.3	33.3	36.9	26.9
t_{max} (h)	Mean	1.0	0.9	1.0	1.0	2.8	1.0	4.0	3.3
	SD	0	0.224	0	0	1.64	0	0	1.57
	CV (%)	0.0	24.8	0.0	0.0	58.7	0.0	0.0	47.4
$AUC_{0\text{-last}}$ (h*ng/mL)	Mean	5010	4716	51004	50494	126708	95876	459982	389508
	SD	563	794	4198	9546	20299	25296	186721	101602
	CV (%)	11.2	16.8	8.23	18.9	16.0	26.4	40.6	26.1
$AUC_{0\text{-inf}}$ (h*ng/mL)	Mean	5034	4759	51234	50796	133828[a]	97676[b]	NR[c]	NR[c]
	SD	562	788	4227	9370	NA	29915		
	CV (%)	11.2	16.6	8.25	18.4	NA	30.6		
$t_{1/2}$ (h)	Mean	1.01	1.17	0.99	1.05	1.17[a]	1.20[b]	NR[c]	NR[c]
	SD	0.139	0.113	0.143	0.210	NA	0.180		
	CV (%)	13.7	9.63	14.4	19.9	NA	15.0		

a) N=1, data insufficient in all other animals to determine terminal metrics; b) N=4, data in one animal insufficient to determine terminal metrics
c) NR - not reportable, insufficient data to determine terminal metrics in all of the animals in the group.

All in all, based on this study, dosages of 0, 2, 10, and 100 mg/kg/day of FDKP were selected for the subsequent developmental toxicity study in rabbits. The 2 mg/kg/day dosage is expected to be a NOAEL for both maternal and embryo-fetal toxicity, whereas 100 mg/kg/day dosage is expected to produce maternal toxicity and little or no developmental toxicity.

Embryofetal development

PDC-PC-2002-0012**:** A Range-Finding Subcutaneous Injection Toxicity and Embryo-Fetal developments Study of Fumaryl Technosphere™ in the Rat [By (b) (4) Study No. 96921]

Note from the reviewer: This title was provided by the sponsor, even though it was really not a dose range finding study. Comparing to PDC-PC-2001-2003, these two studies look like the pivotal Seg II rat studies, which are the combination of fertility/embryo-fetal development.

Key study findings:

- All animals survived to scheduled termination. There were no treatment-related clinical signs and no effects upon body weight or food consumption attributable to FDKP in rats given FDKP up to 100 mg/kg/day for 21 days (in males) or during GDs 6-17 (in females).
- No macroscopic changes and organ weights changed were observed in FDKP-treated rats.
- The numbers of corpora lutea, implantation sites, number of fetal resorptions, live/dead fetuses, sex ratio, and pre and post-implantation losses remained within the range of historical control data for all treatment groups.
- Once daily SC injection of FDKP at doses of 10, 30, or 100 mg/kg/day was well-tolerated by male and pregnant female rats and resulted in no evidence of maternal toxicity.

- In the teratology phase, there was similarly no evidence of toxic effects upon embryo-fetal development at dose levels of FDKP up to 100 mg/kg/day.

Study no.: 96921
Volume # and page #: eEDR Module 4.2.3.5.2, 232 pages
Conducting laboratory and location: (b) (4)
Date of study initiation: July 24th 2001
GLP compliance: No
QA reports: yes ( ), no (x)
Drug, lot #, and % purity: FDKP - PDC 035A060501 with 98.2% purity

Methods

Doses: 0, 10, 30, and 100 mg/kg/day FDKP

Group No. Identification	Dose Level (mg/kg/day)	Dose Conc. (mg/mL)	Dose Volume (mL/kg)	No. of Animals Main Study M[a]	Main Study F[b]	TK[c] F[b]
1 Vehicle control	0	0	10	6	6	-
2 Fumaryl Technosphere™	10	1	10	6	6	-
3 Fumaryl Technosphere™	30	3	10	6	6	-
4 Fumaryl Technosphere™	100	10	10	6[c]	6	6[c]

[a] Males (M) were dosed by subcutaneous injection in the mid-scapular region for 21 consecutive days.
[b] Females (F) were similarly dosed from Days 6 to 17 of gestation, inclusive.
[c] Toxicokinetic (TK) blood sampling was confined to Group 4. Samples were collected from an additional subset of 6 females and from the main study males in this group.

Species/strain: Spraque Dawley CD (Crl:CD®(SD)BR) rats
Number/sex/group: 6/sex/group
Route, formulation, volume, and infusion rate: sc injection with a dose vol. of 10 ml/kg
Satellite groups used for toxicokinetics: TK n=6 females in 100 mg/kg/day group
Study design: VH or FDKP were given to males daily by SC injection in the mid scapular region for 21 days. Females were similarly dosed on GDs 6-17.
Parameters and endpoints evaluated: clinical observations, body weights and food consumption was performed and the fetuses examined externally and weighed. Males were euthanized on Day 22 following collection of TK blood samples to confirm exposure. All animals were subjected to macroscopic examination and organ weight assessment at necropsy. A satellite group of high dose females was bled for TKs on GDs 6-17 and thereafter euthanized and their pregnancy status confirmed. No uterine or fetal examination was performed for these females.

Results

Mortality (dams): All animals survived to scheduled termination.

Clinical signs (dams): Clinical signs, relating to skin and/or fur condition, broken toe nails and/or swollen forepaws, were noted in a few individuals. However, no clinical signs attributable to FDKP were observed.

TABLE NO. 2 INCIDENCE OF CLINICAL OBSERVATIONS PROJECT NO. 96921

GROUP 1 - VEHICLE CONTROL
GROUP 2 - FUMARYL TECHNOSPHERE™ 10 MG/KG/DAY
GROUP 3 - FUMARYL TECHNOSPHERE™ 30 MG/KG/DAY
GROUP 4 - FUMARYL TECHNOSPHERE™ 100 MG/KG/DAY

	NO. OF ANIMALS EXHIBITING CLINICAL OBSERVATIONS								
	MALES				SEX	FEMALES			
OBSERVATIONS	1	2	3	4	GROUP	1	2	3	4
N =	6	6	6	6		6	6	6	6
Skin, red, tail	1	0	0	0		0	0	0	0
Skin, scab/red, forepaw(s)/forelimb(s)	1	1	2	0		0	0	0	1
Skin, lesion, forelimb(s)	0	0	1	0		0	0	0	0
Skin, lesion with discharge, forelimb(s)	0	0	1	0		0	0	0	0
Fur, staining, red, urogenital region	0	1	0	0		0	0	0	0
Fur, staining, black, periorbital region	0	0	0	0		0	1	0	0
Fur, thin cover, forepaw(s)/forelimb(s)	0	2	2	1		0	1	0	0
Broken toenail, forepaw(s)	1	1	0	0		0	0	0	0
Swollen, forepaw(s)	0	1	0	0		0	0	0	0

Body weight (dams): No clear effects of FDKP upon body weights or body weight gains were observed. A slight reduction in body weight gain was evident during the first 3 days of treatment (especially in females from GDs 6-9). In addition, there seemed to have a clear decrease in body weight in group 3 and 4 males compared to the other groups throughout the study.

FIGURE NO. 1 PROJECT NO. 96921

GROUP MEAN BODY WEIGHTS (G)
MALES

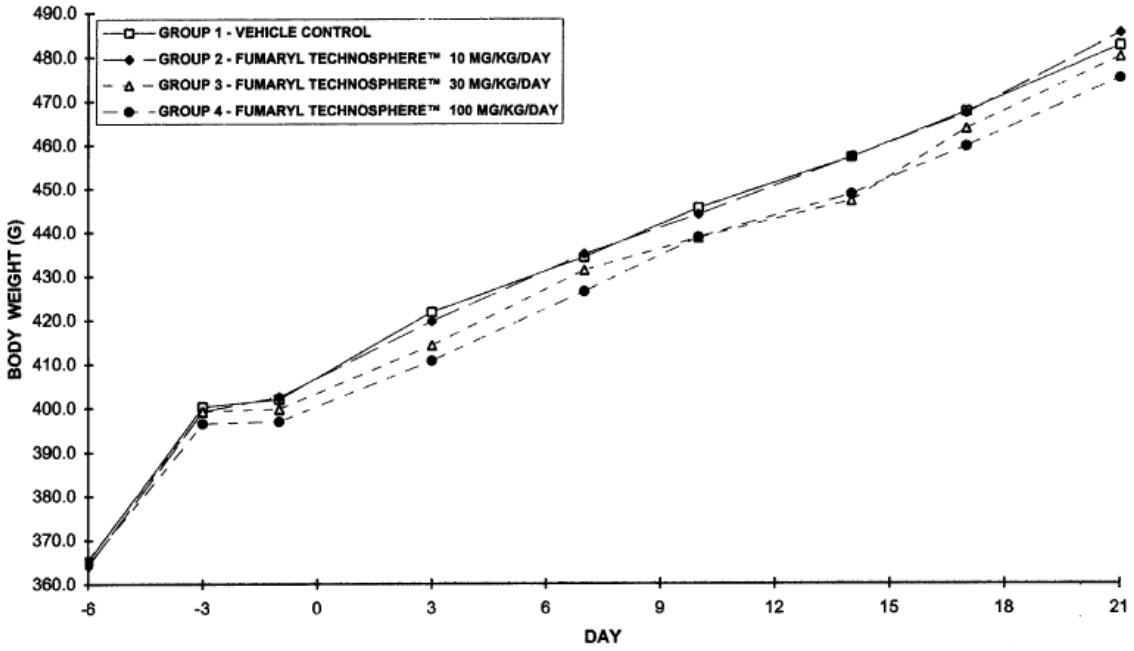
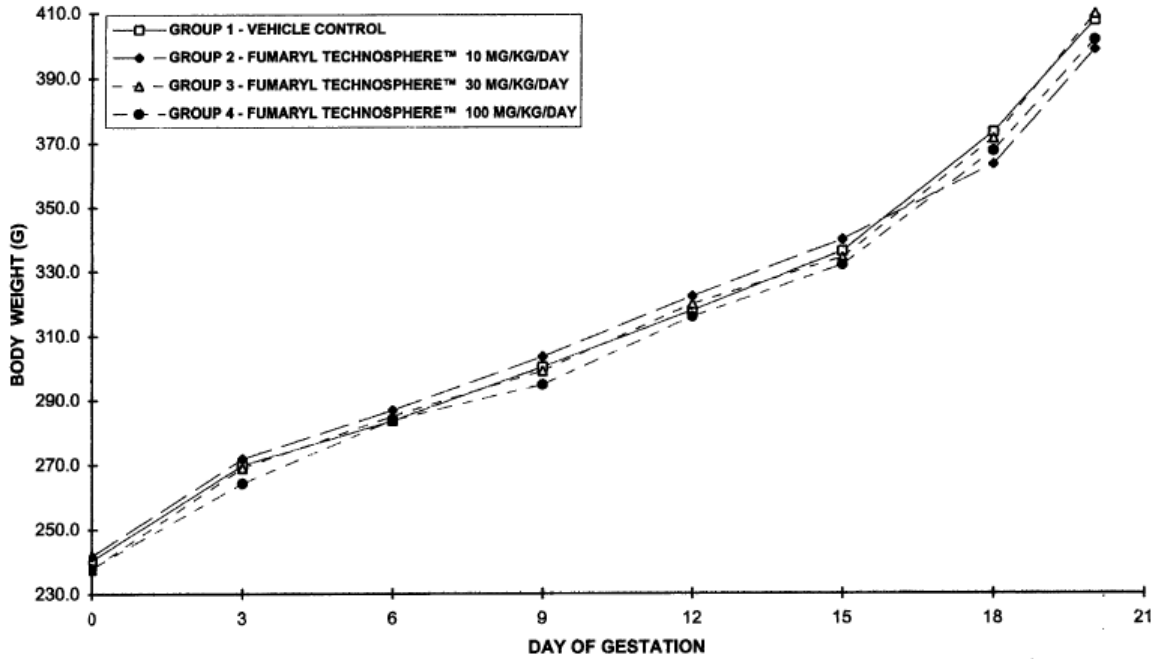


FIGURE NO. 2 PROJECT NO. 96921

GROUP MEAN BODY WEIGHTS (G) OF PREGNANT FEMALES



Food consumption (dams): FDKP had no effect on food consumption.

FOOD CONSUMPTION (GRAMS/ANIMAL), SUMMARY OF MEANS

GROUP 1 VEHICLE CONTROL
GROUP 2 FUMARYL TECHNOSPHERE™ 10 MG/KG/DAY
GROUP 3 FUMARYL TECHNOSPHERE™ 30 MG/KG/DAY
GROUP 4 FUMARYL TECHNOSPHERE™ 100 MG/KG/DAY

SEX	MALES			
GROUP	1	2	3	4
DAY -6- -3	72.0	74.3	73.7	70.2
DAY -3- -1	59.8	59.0	59.5	56.8
DAY -1- 3	91.0	87.8	90.6	86.2
DAY 3- 7	117.3	114.3	117.3	111.5
DAY 7- 10	87.2	84.2	84.0	82.7
DAY 10- 14	126.0	117.8	120.5	113.3
DAY 14- 17	94.3	81.5	97.5	88.0
DAY 17- 21	122.0	125.3	126.5	120.2

FOOD CONSUMPTION (GRAMS/ANIMAL), SUMMARY OF MEANS OF PREGNANT FEMALES

GROUP 1 VEHICLE CONTROL
GROUP 2 FUMARYL TECHNOSPHERE 10 MG/KG/DAY
GROUP 3 FUMARYL TECHNOSPHERE 30 MG/KG/DAY
GROUP 4 FUMARYL TECHNOSPHERE 100 MG/KG/DAY

SEX	FEMALES			
GROUP	1	2	3	4
DAY 0- 3	62.0	65.8	65.2	63.0
DAY 3- 6	73.7	79.2	77.0	82.7
DAY 6- 9	76.3	84.6	68.0	79.3
DAY 9- 12	76.8	85.8	82.6	85.3
DAY 12- 15	80.5	84.8	84.4	92.7
DAY 15- 18	91.2	100.6	107.2	96.5
DAY 18- 20	60.3	57.0	58.0	64.0

Terminal and necroscopic evaluations: C-section data (implantation sites, pre- and post-implantation loss, etc.):

Gross pathology: No macroscopic changes were observed in FDKP treated rats. Minor areas of dark discolorations at the injection sites of a small number of treated and control animals were the result of the injection procedure and not associated with the FDKP treatment. Dark areas in the ventral subcutis of a number of high dose male animals were the consequence of blood sampling and were not associated with FDKP treatment. Remaining gross changes seen at necropsy were considered to be spontaneous.

INCIDENCE OF NECROPSY FINDINGS BY ORGAN/GROUP/SEX

MALE

ORGAN/FINDING	DOSE GROUP:	1	2	3	4
	ANIMAL EXAMINED:	6	6	6	6
INJECTION SITE	:				
- -Area dark-	:	1	3	2	2
KIDNEY	:				
- -Dilatation pelvis-	:	-	-	1	-
LIVER	:				
- -Area pale-	:	-	-	1	-
SKIN MISCELLANEOUS	:				
- -Ulceration-	:	-	-	1	-
SUBCUTANEOUS TISSUE	:				
- -Area dark-	:	-	-	-	6
THYMUS	:				
- -Area dark-	:	1	1	1	-
- -Mottled-	:	-	-	-	1

FEMALE

ORGAN/FINDING	DOSE GROUP:	1	2	3	4
	ANIMAL EXAMINED:	6	6	6	6
ADRENAL	:				
- -Area dark-	:	1	-	-	-
INJECTION SITE	:				
- -Area dark-	:	1	-	1	1
THYMUS	:				
- -Area dark-	:	-	1	1	1
- -Foci dark-	:	-	-	-	1

Organ weights: Epidydymis, prostate, testis and ovary weights were similar for control and treated animals with no treatment related effect of FDKP evident within the absolute and relative to body organ weights.

SUMMARY OF ABSOLUTE ORGAN WEIGHTS (g)

Group Sex		FINAL BODY WEIGHT	EPIDIDYMIS	PROSTATE	SEMINAL VESICLE
1 Male	Mean	480.155	1.2005	1.3385	0.8467
	S.D.	37.2098	0.05657	0.23040	0.22132
	N	6	6	6	6
2 Male	Mean	474.058	1.1802	1.2658	1.0152
	S.D.	18.7590	0.10405	0.18816	0.26385
	N	6	6	6	6
3 Male	Mean	475.935	1.1932	1.2040	0.8075
	S.D.	10.3824	0.08875	0.18023	0.11122
	N	6	6	6	6
4 Male	Mean	461.565	1.2357	1.3752	1.2397
	S.D.	26.8405	0.03264	0.40668	0.64316
	N	6	6	6	6

Group Sex		FINAL BODY WEIGHT	OVARY
1 Female	Mean	407.833	0.12043
	S.D.	29.7887	0.017643
	N	6	6
2 Female	Mean	384.333	0.11665
	S.D.	41.2634	0.013257
	N	6	6
3 Female	Mean	396.500	0.12362
	S.D.	38.2400	0.018042
	N	6	6
4 Female	Mean	401.833	0.12677
	S.D.	10.6286	0.016823
	N	6	6

Uterine findings: the pregnancy rate ranged from 83.3 (10 and 30 mg/kg/day) to 100 % (control and 100 mg/kg/day) for the main study females. All the TK females assigned to Group 4 were also confirmed to be pregnant. The number of corpora lutea, implantation sites, number of resorptions, live/dead fetuses, sex ratio, and pre and post implantation losses remained within the range of historical control data for all groups, according to the sponsor.

TABLE NO. 1 GROUP MATERNAL PERFORMANCE* PROJECT NO. 96921

	GROUP 1 VEHICLE CONTROL	GROUP 2 FUMARYL TECHNOSPHERE™ 10 MG/KG/DAY	GROUP 3 FUMARYL TECHNOSPHERE™ 30 MG/KG/DAY	GROUP 4 FUMARYL TECHNOSPHERE™ 100 MG/KG/DAY
NO. OF RATS MATED	6	6	6	6
NO. OF PREGNANT RATS	6	5	5	6
NO. OF RATS IDENTIFIED AS PREGNANT BY AMMONIUM SULFIDE	0	0	0	0
NO. OF PREGNANCY STATUS NOT DETERMINABLE	0	0	0	0
NO. OF RATS DYING/EUTHANIZED ON STUDY	0	0	0	0
NO. OF RATS WITH TOTAL RESORPTIONS	0	0	0	0
NO. OF RATS LITTERING PRIOR TO SCHEDULED CESAREAN	0	0	0	0
PREGNANCY RATE (%)	100.0	83.3	83.3	100.0

* Excluding toxicokinetic animals

TABLE NO. 13 GROUP MEAN (S.D.) UTERINE FINDINGS PROJECT NO :96921

	TOTAL NO. OF CORPORA LUTEA	TOTAL IMPLAN-TATION SITES	MALE FETUSES	FEMALE FETUSES	SEX RATIO (% MALES)
GROUP 1 - VEHICLE CONTROL	16.8 1.33	15.2 2.56	7.2 1.47	7.8 2.86	48.9 12.76
GROUP 2 - FUMARYL TECHNOSPHERE ™ 10 MG/KG/DAY	15.0 2.45	13.2 1.30	5.2 2.28	6.8 2.05	43.1 15.67
GROUP 3 - FUMARYL TECHNOSPHERE ™ 30 MG/KG/DAY	16.4 1.52	14.8 .84	7.0 1.87	6.4 1.82	52.3 12.19
GROUP 4 - FUMARYL TECHNOSPHERE ™ 100 MG/KG/DAY	17.2 3.82	14.7 1.21	7.5 3.94	6.2 2.86	53.4 23.39

TABLE NO. 13 GROUP MEAN (S.D.) UTERINE FINDINGS PROJECT NO :96921

	LIVE FETUSES	DEAD FETUSES	EARLY RESORP-TIONS	MIDDLE RESORP-TIONS	LATE RESORP-TIONS
GROUP 1 - VEHICLE CONTROL	15.0 2.37	0.0 0.00	.2 .41	0.0 0.00	0.0 0.00
GROUP 2 - FUMARYL TECHNOSPHERE ™ 10 MG/KG/DAY	12.0 1.58	0.0 0.00	1.2 1.30	0.0 0.00	0.0 0.00
GROUP 3 - FUMARYL TECHNOSPHERE ™ 30 MG/KG/DAY	13.4 1.67	0.0 0.00	1.4 1.14	0.0 0.00	0.0 0.00
GROUP 4 - FUMARYL TECHNOSPHERE ™ 100 MG/KG/DAY	13.7 1.51	0.0 0.00	1.0 .63	0.0 0.00	0.0 0.00

Note from the reviewer: A slight decrease in live fetuses was seen in all T-treated pregnant dams compared to control.

TABLE NO. 13 GROUP MEAN (S.D.) UTERINE FINDINGS PE

	SUM OF RESORP-TIONS	PRE-IMPLAN-TATION LOSS (%)	POST IMPLAN-TATION LOSS (%)	GRAVID UTERUS WEIGHT (G)
GROUP 1 - VEHICLE CONTROL	.2 .41	9.8 13.87	.9 2.29	88.0 13.28
GROUP 2 - FUMARYL TECHNOSPHERE TM 10 MG/KG/DAY	1.2 1.30	11.1 8.26	8.9 9.74	72.6 8.50
GROUP 3 - FUMARYL TECHNOSPHERE TM 30 MG/KG/DAY	1.4 1.14	9.4 6.59	9.6 8.10	82.4 9.04
GROUP 4 - FUMARYL TECHNOSPHERE TM 100 MG/KG/DAY	1.0 .63	12.3 13.19	6.9 4.23	82.5 10.25

Note from the reviewer: Group 4 shows an increase in preimplantation and post-implantation loss compared to controls, suggesting some issue at 100 mg/kg/day. Normally, these findings would not be considered drug-related in a Seg II study. However, due to the timing of the dosing, this is a combined Seg I/II study. Therefore, this could be related to drug.

Offspring (malformations, variations, etc.): Fetal weights were similar for control and treated groups and considered unaffected by treatment. There were also no external findings for the fetuses in any group.

TABLE NO. 14 GROUP LITTER MEAN (S.D.) FETAL WEIGHTS (G) PROJECT NO :96

	MALES	FEMALES	TOTAL
GROUP 1 - VEHICLE CONTROL	3.80 .169	3.61 .212	3.71 .178
GROUP 2 - FUMARYL TECHNOSPHERE TM 10 MG/KG/DAY	3.91 .255	3.68 .298	3.76 .294
GROUP 3 - FUMARYL TECHNOSPHERE TM 30 MG/KG/DAY	3.98 .076	3.73 .168	3.87 .103
GROUP 4 - FUMARYL TECHNOSPHERE TM 100 MG/KG/DAY	3.92 .148	3.74 .199	3.83 .152

TABLE NO. 15 GROUP INCIDENCE OF FETAL EXTERNAL FINDINGS MAJOR MALFORMATIONS AND MINOR ANOMALIES PROJECT NO. 96921

	GROUP 1 VEHICLE CONTROL		GROUP 2 FUMARYL TECHNOSPHERE™ 10 MG/KG/DAY		GROUP 3 FUMARYL TECHNOSPHERE™ 30 MG/KG/DAY		GROUP 4 FUMARYL TECHNOSPHERE™ 100 MG/KG/DAY	
	L/E	F/E	L/E	F/E	L/E	F/E	L/E	F/E
EXTERNAL	6	90	5	60	5	67	6	82
	L/A	F/A	L/A	F/A	L/A	F/A	L/A	F/A
MAJOR MALFORMATIONS (TOTAL)	0	0	0	0	0	0	0	0
MINOR ANOMALIES (TOTAL)	0	0	0	0	0	0	0	0

L/E = Litters examined
F/E = Fetuses examined
L/A = Litters affected
F/A = Fetuses affected

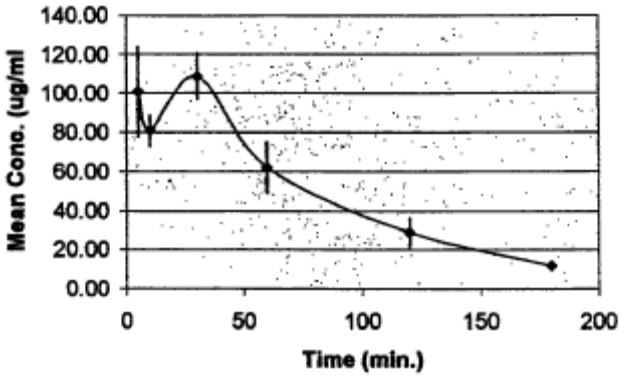
Note from the reviewer: Embryo-fetal studies usually look at external, visceral and skeletal malformations/variations in all dose groups. These parameters were not examined in this study, hence this study was considered as a dose-range finding study by the sponsor.

Toxicokinetics: The high dose animals achieved a peak concentration in the plasma of ~ 125 µg/ml FDKP in a single dose of 100 mg/kg/day. Previous studies on humans following a pulmonary dose of 20 mg Technosphere (1 of 3 daily doses) by SC administration showed an average peak plasma concentration of 0.46 µg/ml. The peak concentration achieved in the rat was ~ 270x of a single 20 mg dose in man or ~ 100x the total daily dose. However, a dose of 20 mg is 2-4x what is typically required therapeutically in humans. Therefore, it's more likely that 5-10 mg would be given 3x a day, allowing an even greater margin over typical daily dosing.

Day 7

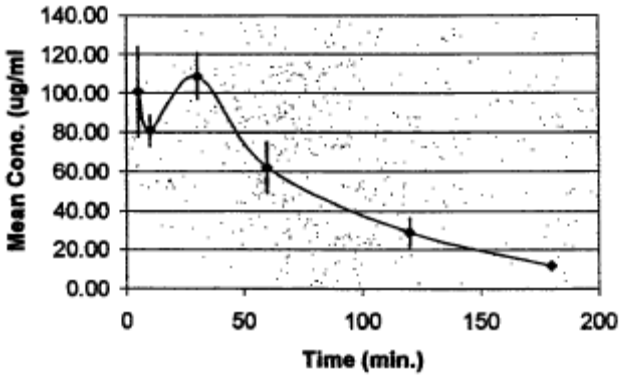
Time (min.)	D7 S#4507	D7 S#4508	D7 S#4509	D7 S#4510	D7 S#4511	D7 S#4512	Mean Conc. (ug/ml)	Std. Dev. (ug/ml)
5	88.69	93.62	65.63				82.65	14.94
10				63.81	74.57	75.09	71.16	6.36
30	109.87	181.80	86.40				126.03	49.71
60				76.82	62.24	59.14	66.07	9.44
120	33.33	59.57	34.08				42.32	14.94
180				11.09	5.70	5.97	7.58	3.04

Mean Technosphere concentration in Female Rat Plasma
Day 17, Assay 8/21/01



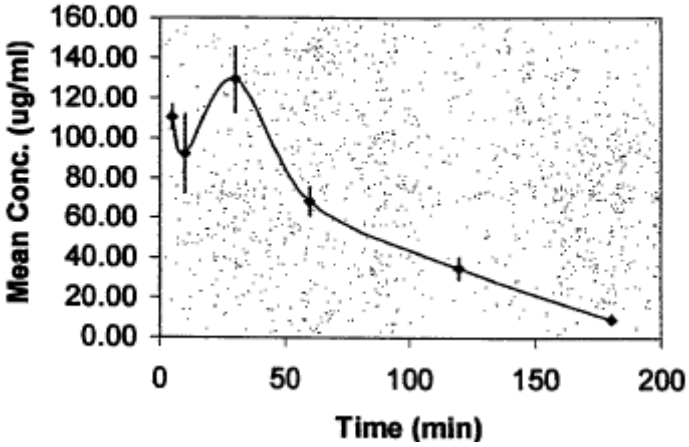
Time (min.)	D17 S#4507	D17 S#4508	D17 S#4509	D17 S#4510	D17 S#4511	D17 S#4512	Mean Conc. (ug/ml)	Std. Dev. (ug/ml)
5	84.67	117.13	Blank				100.90	22.96
10				74.38	89.72	78.73	80.94	7.90
30	95.41	112.28	118.29				108.66	11.86
60				76.77	57.04	52.21	62.01	13.01
120	26.37	37.15	23.16				28.89	7.33
180					12.09	Blank	12.09	0

Mean Technosphere concentration in Female Rat Plasma
Day 17, Assay 8/21/01



5	105.33	108.38	117.02				110.24	6.06
10				70.96	110.07	94.81	91.95	19.71
30	147.15	115.47	124.68				129.10	16.29
60				71.47	73.03	60.20	68.23	7.00
120	32.49	31.07	41.32				34.96	5.55
180				11.93	6.86	8.38	9.06	2.60

Mean Technosphere concentration male Rat Plasma
Day 21, Assay 8/30/01



Pivotal Seg II rat study – Study Title: A Subcutaneous Injection Teratology Study of Fumaryl-Technosphere™ in Rats [PDC-PC-2001-0003]

Key study findings:

- Twice daily SC injection of FDPK at doses of 10, 30, or 100 mg/kg/day was well-tolerated by pregnant females rats, resulting in no evidence of maternal toxicity. There was no evidence of effects upon embryo-fetal development at doses up to 100 mg/kg/day.
- Two animals died due TK bleeds. No effects on maternal food consumption, body weight, or body weight gains. No treatment related macroscopic change was noted.
- For the mothers, there were also no effects on the numbers of corpora lutea, implantation sites, resorptions, pre- and post-implantation losses, the numbers of live/dead fetuses, and the sex ratio.
- For the fetuses, fetal weights were not affected by FDPK and there were no major malformations. The group incidences of litters and fetuses with both overall and individual minor anomalies (primarily dilated ureters) were low with no differences between the control and treated groups. In addition, overall minor skeletal anomalies were observed at similar or lower incidences for litters and fetuses of treated groups when compared to the control group. The group percentages of fetuses with common skeletal variants were similar for all groups.
- The NOAEL of FDKP for F0 male/female was 100 mg/kg/day.
- The NOAEL of FDKP for F1 litter was 100 mg/kg/day.

Study no.: 98061
Volume # and page #: eEDR module 4.2.3.5.1., 393 pages
Conducting laboratory and location: (b) (4)
Date of study initiation: November 19, 2001
GLP compliance: Yes
QA reports: yes (x) no ( )
Drug, lot #, and % purity: See below.

Identity	Fumaryl Technosphere™
Batch/Lot No.	PDC 035A060501
Purity	98.2%. Treated as 100% for dose calculation purposes.
Expiry Date	June 7, 2004
Description	White lyophilized powder
Storage Conditions	Controlled room temperature (*ca.* 18 to 24°C), protected from light
Handling Precautions	Routine laboratory precautions
Supplier	Pharmaceutical Discovery Corporation

Methods

Doses: 0, 10, 30, and 100 mg/kg/day, twice daily on GDs 6 to 17
Species/strain: Spraque Dawley CD (Crl: CD(SD) IGS BR) rats
Number/sex/group: 136 female rats, 22/group (Group I through IV)
Route, formulation, volume, and infusion rate: SC injection in the volume of 5 mL/kg
Satellite groups used for toxicokinetics: 9 rats for TK, 3/group (Group I through IV)
Study design: Dosages were selection on the basis of a dosage-range study ((b) (4) No. 96921).

Group No. Identification	Dose Level (mg/kg/day)	(mg/kg/dose)	Conc. (mg/mL)	Volume* (mL/kg/dose)	No. of Females Main Study	TK
1 Vehicle control	0	0	0	5	22	9
2 Fumaryl Technosphere™	10	5	1	5	22	9
3 Fumaryl Technosphere™	30	15	3	5	22	9
4 Fumaryl Technosphere™	100	50	10	5	22	9

* Animals were dosed *b.i.d.* with approximately 6 hours interval between the first and second daily dose.

Parameters and endpoints evaluated: Viabilities, clinical observations, body weights, and feed consumption values were recorded. Gross pathology was performed for main study females on GD 20. Ovaries were removed and the corpora lutea were counted. The gravid uterus was weighted and the uterine contents, including the placentas, were examined. The numbers and positions of live fetuses, dead fetuses, and early/middle/late resorptions were recorded. In addition, necropsy consisted of an external examination, including identification of all clinically recorded lesions, as well as detailed internal examination.

Moreover, each fetus was weighed. Detailed external examination was performed. A detailed internal examination was performed on approx. ½ of the fetuses in each litter. The heads of these fetuses were removed and examined by the technique of Wilson. The remaining ½ of fetuses in each litter was stained with alizarin red S and skeletal examination.

Results

Mortality: Two control females found dead on GDs 6 and 17, following a TK bleed. Otherwise, there were no compound-related deaths.

Clinical signs: There were no FDKP-related adverse clinical observations. Some clinical signs, including skin and/or fur condition were noted for a few rats from all groups. These findings could be due to experimental procedures.

TABLE NO. 2 INCIDENCE OF CLINICAL OBSERVATIONS FEMALES PROJECT NO. 98061

GROUP 1 - VEHICLE CONTROL
GROUP 2 - FUMARYL TECHNOSPHERE™ 10 MG/KG/DAY (5 MG/KG bid)
GROUP 3 - FUMARYL TECHNOSPHERE™ 30 MG/KG/DAY (15 MG/KG bid)
GROUP 4 - FUMARYL TECHNOSPHERE™ 100 MG/KG/DAY (50 MG/KG bid)

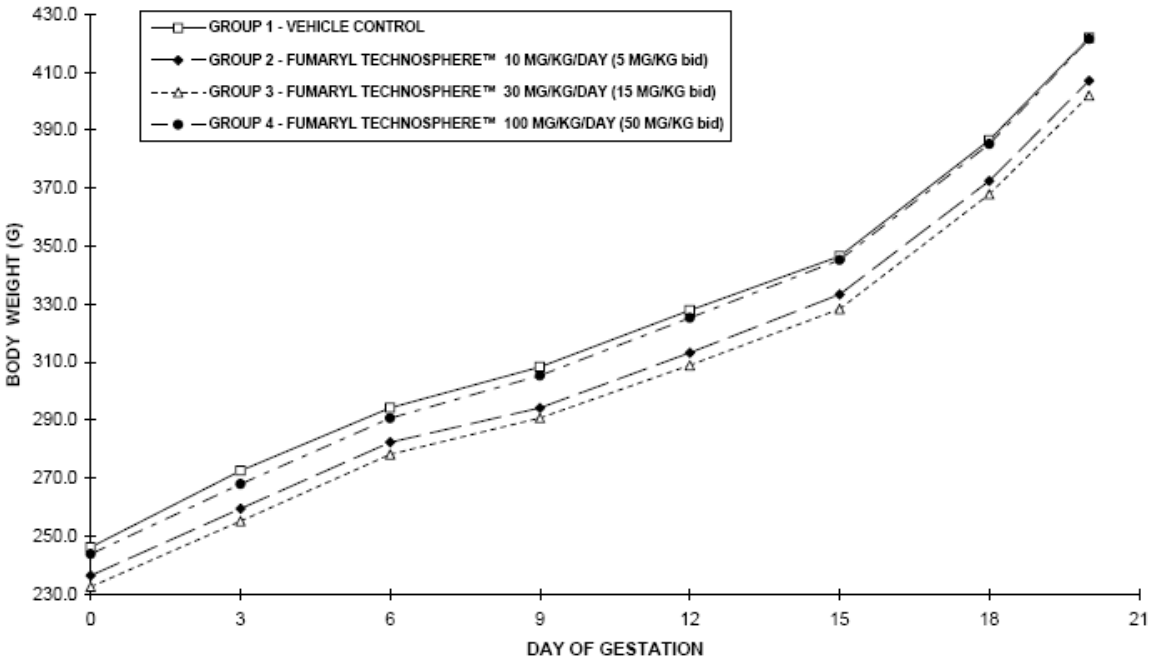
	NO. OF ANIMALS EXHIBITING CLINICAL OBSERVATIONS			
	GROUP			
OBSERVATIONS	1	2	3	4
N =	22	22	22	22
Fur, staining, red, pinna(e)	1	0	0	0
Fur, thin cover, forepaw(s)/forelimb(s)	3	2	1	4
Fur, thin cover, abdominal region	0	0	0	1
Fur, thin cover, ventral thoracic region	0	0	0	1
Skin, lesion with discharge, liquid, moderate, red	0	0	0	1
Skin, red, forepaw(s)	0	0	0	1
Skin, scab, pinna(e)	0	1	0	0

Body weight: There was a statistical difference between the 30 mg/kg/day dose group and control group on GD 6 (278.2±15.5 vs. 294.3±14.3 respectively). Since the magnitude of the body weight difference remained similar throughout the treatment period and did not seem to be affected by dose, the subsequent statistical differences in body weights and corrected mean body

weights might be due to biological variation. In addition, the transient statistical differences observed between 10 mg/kg/day group and control group mean body weights were probably also due to the inter-individual variability in body weights and body weight gains.

FIGURE NO. 1 PROJECT NO. 98061

GROUP MEAN BODY WEIGHTS (G) OF PREGNANT FEMALES



Food consumption: There were no dose-related effects on food consumption.

Toxicokinetics: Not found in the study report. Note from the reviewer: However, the result was later mentioned in MKC-PC-2006-0010 (see table below).

In a developmental toxicity study of subcutaneously injected Technosphere® Particles in rats ([redacted] (b) (4) Project No. 98061, A Subcutaneous Injection Teratology Study of Fumaryl Technosphere™ in Rats), analysis of plasma samples collected on DGs 6 and 17 yielded the C_{max} values identified below in Text Table 2.

Text Table 2	Mean Exposure Levels (mcg/mL) at C_{max}			
	Dosage Level (mg/kg/day)			
Day of Gestation	0 (Vehicle)	10	30	100
DG 6	BLQ	6.28	17.5	70.8
DG 17	BLQ	6.73	17.6	61.6

BLQ – Below the limit of quantitation

Necropsy: No treatment related macroscopic findings were observed. There was a low incidence of injection site reactions in the 100 mg/kg/day groups (2/22), with occasional individuals with dark material in the vagina in treated-groups. There were some gross changes in liver, kidney, and thymus, observed in both control and treated animals.

INCIDENCE OF NECROPSY FINDINGS BY ORGAN/GROUP

FEMALE

ORGAN/FINDING	DOSE GROUP:	1	2	3	4
	ANIMAL EXAMINED:	22	22	22	22
INJECTION SITE	:				
- -Area dark-	:	-	-	-	1
I.S. SCAPULAR, LEFT	:				
- -Area dark-	:	-	-	-	1
JEJUNUM	:				
- -Diverticulum-	:	1	-	-	-
KIDNEY	:				
- -Dilatation pelvis-	:	-	1	-	1
LIVER	:				
- -Area pale-	:	-	3	1	-
LYMPH NODE	:				
- -Discoloration dark-	:	-	1	-	1
THYMUS	:				
- -Area dark-	:	4	2	2	2
- -Foci dark-	:	3	3	2	-
VAGINA	:				
- -Material dark-	:	-	1	1	1

Uterine Findings: The pregnancy rate ranged from 90.9 to 100% for main study females. All TK females, except one female (in 10 mg/kg/day group) were also confirmed to be pregnant. The number of corpora lutea, implantation sites, number of resorptions, live and dead fetuses, sex ratio, and pre- and post-implantation losses for the control and treated groups were similar

TABLE NO. 1 GROUP MATERNAL PERFORMANCE * PROJECT NO. 98061

	GROUP 1 VEHICLE CONTROL	GROUP 2 FUMARYL TECHNOSPHERE™ 10 MG/KG/DAY (5 MG/KG bid)	GROUP 3 FUMARYL TECHNOSPHERE™ 30 MG/KG/DAY (15 MG/KG bid)	GROUP 4 FUMARYL TECHNOSPHERE™ 100 MG/KG/DAY (50 MG/KG bid)
NO. OF RATS MATED	22	22	22	22
NO. OF PREGNANT RATS	21	22	21	20
NO. OF RATS IDENTIFIED AS PREGNANT BY AMMONIUM SULFIDE	0	0	0	0
NO. OF PREGNANCY STATUS NOT DETERMINABLE	0	0	0	0
NO. OF RATS DYING/EUTHANIZED ON STUDY	0	0	0	0
NO. OF RATS WITH TOTAL RESORPTIONS	0	0	0	0
NO. OF RATS LITTERING PRIOR TO SCHEDULED CESAREAN	0	0	0	0
PREGNANCY RATE (%)	95.5	100.0	95.5	90.9

* Excluding toxicokinetic animals

TABLE NO. 8 GROUP MEAN (S.D.) UTERINE FINDINGS PROJECT NO :98061

	TOTAL NO. OF CORPORA LUTEA	TOTAL IMPLAN-TATION SITES	MALE FETUSES	FEMALE FETUSES	SEX RATIO (% MALES)
GROUP 1 - VEHICLE CONTROL	16.5	14.8	6.3	7.4	46.3
	2.46	2.50	1.59	2.13	10.93
GROUP 2 - FUMARYL TECHNOSPHERE™ 10 MG/KG/DAY (5 MG/KG bid)	15.7	13.8	6.8	6.3	52.7
	3.18	3.31	1.90	2.37	12.22
GROUP 3 - FUMARYL TECHNOSPHERE™ 30 MG/KG/DAY (15 MG/KG bid)	15.3	13.8	6.2	7.2	49.0
	3.16	3.33	2.16	2.80	18.06
GROUP 4 - FUMARYL TECHNOSPHERE™ 100 MG/KG/DAY (50 MG/KG bid)	16.5	14.9	6.6	7.4	47.1
	2.84	2.38	1.93	1.73	11.20

Fetal Findings:

- Fetal weights: There were similar for control and treated groups, with mean fetal total weights of 3.72, 3.79, 3.74, and 3.71 g for Groups 1 to 4 respectively).

TABLE NO. 9 GROUP LITTER MEAN (S.D.) FETAL WEIGHTS (G) PROJECT NO :98061

	MALES	FEMALES	TOTAL
GROUP 1 - VEHICLE CONTROL	3.81	3.63	3.72
	.355	.342	.335
GROUP 2 - FUMARYL TECHNOSPHERE™ 10 MG/KG/DAY (5 MG/KG bid)	3.87	3.72	3.79
	.312	.291	.294
GROUP 3 - FUMARYL TECHNOSPHERE™ 30 MG/KG/DAY (15 MG/KG bid)	3.83	3.61	3.74
	.330	.256	.336
GROUP 4 - FUMARYL TECHNOSPHERE™ 100 MG/KG/DAY (50 MG/KG bid)	3.82	3.62	3.71
	.252	.249	.226

- Major/minor malformations: No major malformations were noted. The group incidences of litters and fetuses with both overall and individual minor anomalies (mainly dilated ureters) were low with no differences between the incidences of control or treated groups.

TABLE NO. 10 GROUP INCIDENCE OF FETAL EXTERNAL, VISCERAL AND SKELETAL FINDINGS MAJOR MALFORMATIONS, MINOR ANOMALIES AND COMMON VARIANTS PROJECT NO. 98061

	GROUP 1 VEHICLE CONTROL		GROUP 2 FUMARYL TECHNOSPHERE™ 10 MG/KG/DAY (5 MG/KG bid)		GROUP 3 FUMARYL TECHNOSPHERE™ 30 MG/KG/DAY (15 MG/KG bid)		GROUP 4 FUMARYL TECHNOSPHERE™ 100 MG/KG/DAY (50 MG/KG bid)	
	L/E	F/E	L/E	F/E	L/E	F/E	L/E	F/E
EXTERNAL (EXT)	21	288	22	287	21	281	20	279
VISCERAL (VIS)	21	145	22	142	21	140	20	140
SKELETAL (SKE)	21	143	22	145	21	141	20	139
TECHNIQUE OF WILSON (WT)	21	145	22	142	21	140	20	140
	L/A	F/A	L/A	F/A	L/A	F/A	L/A	F/A
MAJOR MALFORMATIONS (TOTAL)	0	0	0	0	0	0	0	0
MINOR EXTERNAL AND VISCERAL ANOMALIES (TOTAL)	4	5	2	4	5	6	3	3
EYE(S) Lens(es) oval (WT)	0	0	0	0	0	0	1	1
KIDNEY(S) Reduction of the renal papilla(e) (VIS)	0	0	1	1	0	0	0	0
URETER(S) Dilated (VIS)	4	5	2	4	5	6	2	2

L/E = Litters examined L/A = Litters affected
F/E = Fetuses examined F/A = Fetuses affected

- Minor skeletal anomalies: Overall minor skeletal anomalies were seen at similar or lower incidences for litters and fetuses of treated groups when compared to the control group. The lower incidences of litters and fetuses affected in the 10 mg/kg/day attained statistical differences. This was the result of lower incidences of individual findings, including reduced ossification of the hyoid bones and irregular ossification of the interparietal bones. There differences might be due to biological variation.

TABLE NO. 10 GROUP INCIDENCE OF FETAL EXTERNAL, VISCERAL AND SKELETAL FINDINGS MAJOR MALFORMATIONS, MINOR ANOMALIES AND COMMON VARIANTS PROJECT NO. 98061

	GROUP 1 VEHICLE CONTROL		GROUP 2 FUMARYL TECHNOSPHERE™ 10 MG/KG/DAY (5 MG/KG bid)		GROUP 3 FUMARYL TECHNOSPHERE™ 30 MG/KG/DAY (15 MG/KG bid)		GROUP 4 FUMARYL TECHNOSPHERE™ 100 MG/KG/DAY (50 MG/KG bid)	
	L/E	F/E	L/E	F/E	L/E	F/E	L/E	F/E
EXTERNAL (EXT)	21	288	22	287	21	281	20	279
VISCERAL (VIS)	21	145	22	142	21	140	20	140
SKELETAL (SKE)	21	143	22	145	21	141	20	139
TECHNIQUE OF WILSON (WT)	21	145	22	142	21	140	20	140
	L/A	F/A	L/A	F/A	L/A	F/A	L/A	F/A
MINOR SKELETAL ANOMALIES (TOTAL)	18	60	12 A	31 C	19	51	17	53
SKULL								
Hyoid bone: Reduced ossification	10	24	5	12 A	15	31	12	35
Parietal bone(s): Reduced ossification	2	2	4	6	3	5	3	3
Parietal bone(s): Irregular ossification	1	1	1	1	0	0	0	0
Interparietal bone: Reduced ossification	3	5	4	5	4	7	3	4
Interparietal bone: Irregular ossification	13	30	7	12 B	11	17	11	19
Supraoccipital bone: Reduced ossification	2	3	3	4	1	1	1	1
Supraoccipital bone: Irregular ossification	7	10	3	5	8	10	6	7
VERTEBRAL COLUMN								
Extra pre-sacral vertebrae	1	1	0	0	0	0	0	0
Caudal vertebra(e): Reduced no.	1	1	0	0	0	0	0	0
Lumbar centrum: Semi-bipartite	0	0	0	0	2	2	0	0
Ossification center(s) on 1st lumbar vertebra or 14th thoracic vertebra	11	12	7	8	5	5	6	8
Sacral vertebral arch(es): Reduced ossification	1	1	1	2	1	1	1	1

SIGNIFICANTLY DIFFERENT FROM CONTROL (GROUP 1) VALUE: A P<=0.05 B P<=0.01 C P<=0.001 (FISHER'S)

L/E = Litters examined L/A = Litters affected

F/E = Fetuses examined F/A = Fetuses affected

TABLE NO. 10 GROUP INCIDENCE OF FETAL EXTERNAL, VISCERAL AND SKELETAL FINDINGS MAJOR MALFORMATIONS, MINOR ANOMALIES AND COMMON VARIANTS PROJECT NO. 98061

	GROUP 1 VEHICLE CONTROL		GROUP 2 FUMARYL TECHNOSPHERE™ 10 MG/KG/DAY (5 MG/KG bid)		GROUP 3 FUMARYL TECHNOSPHERE™ 30 MG/KG/DAY (15 MG/KG bid)		GROUP 4 FUMARYL TECHNOSPHERE™ 100 MG/KG/DAY (50 MG/KG bid)	
	L/E	F/E	L/E	F/E	L/E	F/E	L/E	F/E
EXTERNAL (EXT)	21	288	22	287	21	281	20	279
VISCERAL (VIS)	21	145	22	142	21	140	20	140
SKELETAL (SKE)	21	143	22	145	21	141	20	139
TECHNIQUE OF WILSON (WT)	21	145	22	142	21	140	20	140
MINOR SKELETAL ANOMALIES (CONT'D)	L/A	F/A	L/A	F/A	L/A	F/A	L/A	F/A
RIBS								
Reduced	1	1	0	0	0	0	1	1
Notched	0	0	1	1	0	0	0	0
Rudimentary 14th rib(s)	2	3	0	0	1	1	0	0
Rudimentary 14th rib with contralateral ossification center	1	1	1	1	1	1	0	0
Ossification center(s) on 7th cervical vertebra	1	1	0	0	0	0	0	0
Extra 14th rib(s)	0	0	0	0	1	2	0	0
Extra 14th rib with contralateral rudimentary rib	1	1	0	0	0	0	0	0
Extra 14th rib with contralateral ossification center	0	0	1	1	0	0	0	0
PELVIC GIRDLE								
Pubic bone(s): Reduced ossification	4	4	3	5	3	5	4	4
Ischial bone(s): Reduced ossification	0	0	0	0	0	0	1	1

L/E = Litters examined L/A = Litters affected

F/E = Fetuses examined F/A = Fetuses affected

- Common skeletal variants: The group percentages of fetuses with common skeletal variants were similar for all groups.

TABLE NO. 10 GROUP INCIDENCE OF FETAL EXTERNAL, VISCERAL AND SKELETAL FINDINGS MAJOR MALFORMATIONS, MINOR ANOMALIES AND COMMON VARIANTS PROJECT NO. 98061

	GROUP 1 VEHICLE CONTROL	GROUP 2 FUMARYL TECHNOSPHERE™ 10 MG/KG/DAY (5 MG/KG bid)	GROUP 3 FUMARYL TECHNOSPHERE™ 30 MG/KG/DAY (15 MG/KG bid)	GROUP 4 FUMARYL TECHNOSPHERE™ 100 MG/KG/DAY (50 MG/KG bid)
	AFFECTED FETUSES/LITTERS MEAN % (S.D.)	AFFECTED FETUSES/LITTERS MEAN % (S.D.)	AFFECTED FETUSES/LITTERS MEAN % (S.D.)	AFFECTED FETUSES/LITTERS MEAN % (S.D.)
COMMON SKELETAL VARIANTS				
Thoracic centrum variants (absent/reduced/ irregular/semi-bipartite/bipartite)	37.4 (26.87)	37.8 (23.36)	35.2 (32.49)	36.0 (23.75)
Sternebrae 1 to 4 (absent/reduced/irregular/ semi-bipartite/bipartite)	15.6 (15.79)	7.1 (11.37)	14.4 (17.84)	16.1 (15.52)
Sternebra 5 and Xiphisternum (absent/reduced/ irregular/semi-bipartite/bipartite)	83.3 (23.75)	82.6 (19.90)	90.2 (17.49)	90.3 (15.36)

Pivotal Seg II Rabbit Study - Study title: Subcutaneous Developmental Toxicity Study of Technosphere® in Rabbits [MKC-PC-2004-2004]

Key study findings:

- The 100 mg/kg/day FDKP caused mortality, increased numbers with adverse clinical signs of scant and soft or liquid feces, reduced body weight gain, and decreased absolute and relative feed consumption in pregnant rabbits.
- The small reductions in feed consumption were observed in the 10 mg/kg/day group. These were not considered related to the test article because they were transient and did not affect body weight. However, decreased body weight was significant at 2 mg/kg/day, suggesting maternal toxicity.
- The maternal NOAEL of FDKP was < 2 mg/kg/day.
- No C-sectioning or litter parameters were affected and no fetal gross external, soft tissue or skeletal alternations (malformations and variations) were caused by FDKP, as high as 100 mg/kg/day.
- The developmental NOAEL of FDKP was 100 mg/kg/day.
- FDKP exposure, as measured by mean serum C_{max} and AUC_{last}, were approx. proportional to the dose on both GDs 7 and 19. There was no evidence of accumulation of FDKP in serum following repeated dosing. The t ½ for FDKP was estimated to be < 2 hrs.

Study no.: LCL0002
Volume # and page #: eEDR module 4.2.3.5.1., 317 pages
Conducting laboratory and location: (b) (4)
Date of study initiation: August 13th 2004
GLP compliance: Yes
QA reports: yes (x) no ( )
Drug, lot #, and % purity: See below.

PRODUCT NAME/ DESCRIPTION: (b) (4) Fumaryl Technosphere®
3,6-Bis [N-Fumaryl N-(n-Butyl) amino]-2,5-Diketopiperazine

PACKAGING DESCRIPTION: Bulk Powder in Bottle(s)
LOT NUMBER: D035U.03.012

PRODUCT STAGE: Finished Product
EXPIRY DATE: 11/2004
SPECIFICATION #: S035-002
STORAGE CONDITIONS: Keep Tightly sealed, Out of Light, Store at Room Temperature (15-30)°C

ITEM NUMBER	ATTRIBUTE	TEST METHOD/ SOURCE	SPECIFICATION	RESULTS
1	Technosphere Assay (HPLC)	TM# 5319	Minimum 98.0% Area %	99.5 %
2	Impurities Assay (HPLC)	TM# 5319	Record All Impurities Area% and RT with an Area% greater than (b) (4)%	Impurity RRT Area % (b) (4)% % %
3	Identification (MS)	TM# 5304	MS Electrospray: M+1 = (b) (4) m/z Or M-1 = m/z	M+1= (b) (4) m/z Confirmed
4	Particle Size by Cascade Impactor	TM# 5318	MMAD (b) (4) Percent Respirable Fraction	(b) (4)
5	Microbial Limits	Outside Contractor	Limit ≤ (b) (4) CFU/g for aerobic plate count, mold and yeast. No Salmonella, Staphylococcus aureus, Escherichia coli, or Pseudomonas aeruginosa detected	Pass
6	(b) (4)			
7	Appearance	TM# 5303	White Powder	White Powder
8	Particle Size by Light Scatter	TM# 5311	(b) (4)	(b) (4)

Methods

Doses: 0, 2, 10, and 100 mg/kg/day, once daily on GDs 7 to 19
Species/strain: Rabbit/Hra:(NZW)SPF
Number/sex/group: 91 rabbits, Time-mated females, 20/group (Group I through IV)
Route, formulation, volume, and infusion rate: SC injection in the volume of 5 mL/kg
Satellite groups used for toxicokinetics: 9 rabbits for TK, 3/group (Group II through IV)
Study design: Dosages were selection on the basis of a dosage-range study (LCL0001).

Dosage Group	Dosage[a] (mg/kg/day)	Concentration (mg/mL)	Dosage Volume (mL/kg)	Number of Rabbits	Assigned Rabbit Numbers	
					Main Study	Toxicokinetic Sample Collections
I	0 (Vehicle)	0	5	20	8201 - 8220	NA
II	2	0.4	5	20 + 3[b]	8221 - 8240	8281 - 8283
III	10	2	5	20 + 3[b]	8241 - 8260	8284 - 8286
IV	100	20	5	20 + 3[b]	8261 - 8280	8287 - 8289

a. The test article was considered 100% active/pure for the purpose of dosage calculations.
b. Three rabbits assigned for toxicokinetic sample collections.
NA - Not applicable

Parameters and endpoints evaluated: Viabilities, clinical observations, body weights, and feed consumption values were recorded. Blood samples were collected from the additional rabbits assigned to Groups II to IV on GDs 7 and 19. All surviving rabbits were sacrificed on GD 29, c-sectioned, and examined for the number and distribution of corpora lutea, implantation sites and uterine contents. A gross necropsy of the thoracic, abdominal and pelvic viscera was performed. Fetuses were weighted and examined for gross external, visceral, and skeletal alternations, and sex.

Results

Mortality: One rabbit in the 100 mg/kg/day group was found dead on GD 26. Adverse clinical signs, including scant feces on GDs 11-25, loss of body weight from GDs 7-17 and GDs 18-25, a reduction in food consumption throughout the dosing period, were observed in this rabbit. There were 9 fetuses in utero, and all fetuses appeared normal for their developmental age. This death was considered related to the test article. Note from the reviewer: Since it occurred in the highest dosage group, this suggests that 100 mg/kg/day given during organogenesis might exceed the maternal MTD.

Clinical signs: Scant feces were observed in a significantly increased number of rabbits in the 100 mg/kg/day group. Soft or liquid feces were observed in 5 and 4 rabbits in each 10 and 100 mg/kg/day groups, respectively. Other clinical signs (e.g. localized alopecia on the underside, ungroomed coat, swollen vulva, scab on the mouth or right hindpaw, no feces in the cage pan, and ulceration on the right hindpaw) were not considered to be test article-related because a similar number of rabbits in the control groups were affected, and only 1-3 rabbits in each dose group were affected with the clinical sign in non-dose dependent manner.

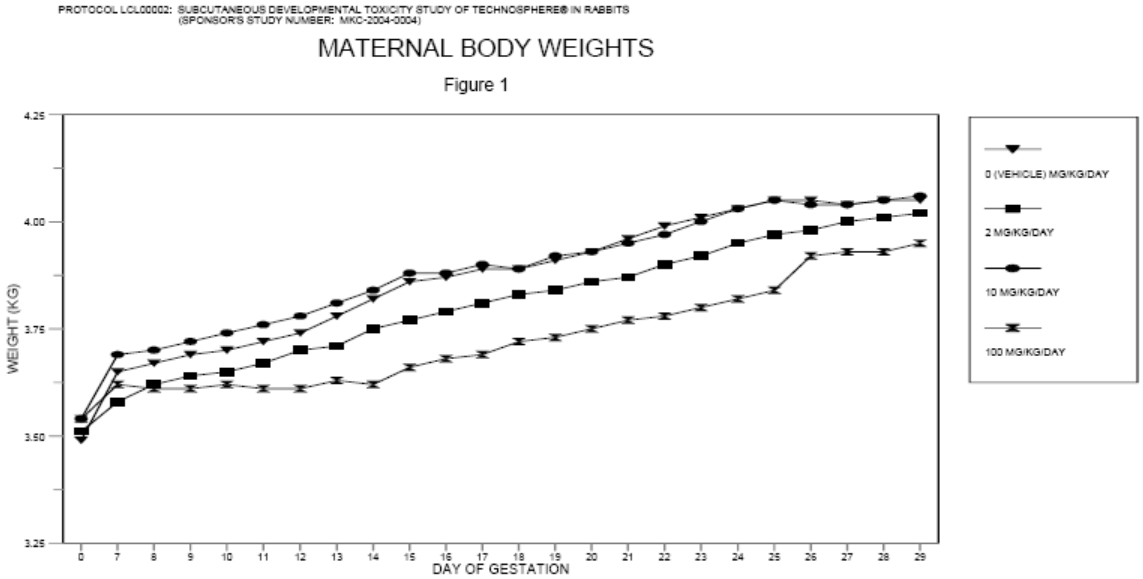
TABLE 1 (PAGE 1): CLINICAL OBSERVATIONS - SUMMARY

DOSAGE GROUP DOSAGE (MG/KG/DAY)a	I 0 (VEHICLE)	II 2	III 10	IV 100
MAXIMUM POSSIBLE INCIDENCE	460/ 20	460/ 20	460/ 20	457/ 20
FOUND DEAD	0	0	0	1b
SCANT FECES	7/ 3	3/ 2	6/ 3	64/ 11**b
SOFT OR LIQUID FECES	3/ 2	3/ 2	9/ 5	8/ 4
LOCALIZED ALOPECIA: UNDERSIDE	14/ 1	0/ 0	0/ 0	31/ 3
UNGROOMED COAT	5/ 1	1/ 1	2/ 1	19/ 2
VULVA: SWOLLEN	0/ 0	0/ 0	10/ 1	20/ 1
MOUTH OR RIGHT HINDPAW: SCAB	0/ 0	0/ 0	13/ 1	8/ 1
NO FECES IN CAGE PAN	0/ 0	0/ 0	0/ 0	8/ 1
RIGHT HINDPAW: ULCERATION	0/ 0	0/ 0	0/ 0	3/ 1

PERSISTENT ADVERSE CLINICAL OBSERVATIONS WERE CONFIRMED AT NECROPSY, NO ADDITIONAL GROSS LESIONS WERE IDENTIFIED

STATISTICAL ANALYSES OF CLINICAL OBSERVATION DATA WERE RESTRICTED TO THE NUMBER OF RABBITS WITH OBSERVATIONS.
MAXIMUM POSSIBLE INCIDENCE = (DAYS x RABBITS)/NUMBER OF RABBITS EXAMINED PER GROUP ON DAYS 7 THROUGH 29 OF PRESUMED GESTATION.
N/N = TOTAL NUMBER OF OBSERVATIONS/NUMBER OF RABBITS WITH OBSERVATION.
a. Dosage occurred on days 7 through 19 of presumed gestation.
b. Doe 8262 was found dead on day 26 of gestation.
** Significantly different from the vehicle control group value ($p \leq 0.01$).

Body weight: There were significant reductions in body weight gain observed in the 2 and 100 mg/kg/day groups throughout gestation, which suggests maternal toxicity at these doses. However, it is unusual in that the 10 mg/kg/day group did not have reduced body weight gain. Body weight gains were reduced in the 100 mg/kg/day group on GDs 7 through 20 (during dosing period, 46% of the control group value), as well as GDs 7-29 (during the gestation period following initiation of dosing) and GDs 0-29 (the entire gestation period), when compared with the respective control group values. Body weight gains were comparable among the groups during the post-dosing period.



Food consumption: A significant reduction in absolute feed consumption and a reduction in relative feed consumption occurred in the 100 mg/kg/day group on GDs 7-10, following initiation of dosing. Feed consumption remained reduced in the 100 mg/kg/day group from GD 10, as well as in the 10 mg/kg/day group on GDs 24-29.

TABLE 5 (PAGE 1): MATERNAL ABSOLUTE FEED CONSUMPTION VALUES (G/DAY) - SUMMARY

DOSAGE GROUP		I	II	III	IV
DOSAGE (MG/KG/DAY)a		0 (VEHICLE)	2	10	100
RABBITS TESTED	N	20	20	20	20
PREGNANT	N	18	19	20	20
MATERNAL FEED CONSUMPTION (G/DAY)					
DAYS 7 - 10	MEAN±S.D.	178.4 ± 8.1	172.9 ± 16.1 [18]b	168.9 ± 19.8	138.3 ± 50.0*
DAYS 10 - 13	MEAN±S.D.	169.2 ± 17.8	168.2 ± 16.1	162.4 ± 22.7	114.5 ± 74.0
DAYS 13 - 16	MEAN±S.D.	171.2 ± 13.1	161.2 ± 26.1	152.6 ± 35.2	129.6 ± 68.3 [19]b
DAYS 16 - 20	MEAN±S.D.	171.2 ± 16.7 [17]b	166.6 ± 28.8 [18]b	162.4 ± 23.8 [19]b	140.4 ± 68.6
DAYS 7 - 20	MEAN±S.D.	172.1 ± 12.7 [17]b	167.7 ± 19.6 [18]b	161.5 ± 22.9 [19]b	131.0 ± 61.3
DAYS 20 - 24	MEAN±S.D.	161.6 ± 23.4 [17]b	158.5 ± 27.9 [18]b	152.4 ± 35.2 [18]b	139.1 ± 60.3 [19]b
DAYS 24 - 29	MEAN±S.D.	123.1 ± 63.0 [15]b	111.2 ± 30.9 [14]b	98.8 ± 44.8 [13]b	110.9 ± 52.6 [14]b,c
DAYS 20 - 29	MEAN±S.D.	140.6 ± 42.1 [15]b	133.0 ± 22.7 [14]b	124.8 ± 38.0 [13]b	123.9 ± 48.8 [14]b,c
DAYS 7 - 29	MEAN±S.D.	160.1 ± 20.0 [15]b	152.8 ± 16.9 [14]b	146.9 ± 24.7 [13]b	124.0 ± 50.7 [14]b,c

DAYS = DAYS OF GESTATION
[ ] = NUMBER OF VALUES AVERAGED
a. Dosage occurred on days 7 through 19 of gestation.
b. Excludes values that were incorrectly recorded or associated with spillage.
c. Excludes values for doe 8262, which was found dead on day 26 of gestation.
* Significantly different from the vehicle control group value (p≤0.05).

Toxicokinetics: The mean exposure (peak serum concentration, C_{max}, and AUC_{last}) for FDKP was approximately proportional to dose on both GDs 7 and 19. The t ½ mean value was less than 2 hrs. There was no evidence of accumulation. In all but one rabbit, AUC_{last} obtained on GD 7 was higher compared to that obtained on GD 19. However, due to the small numbers of rabbits in each group and high intragroup variability in AUC, the decrease in mean AUC_{last} was only significant for rabbits given FDKP at 2 mg/kg/day.

Based on the projected human AUC of approx. 46,000 min•ng/mL, rabbits were dosed in the range of 10 to 1000 fold the projected human daily exposure (105 mg TI).

Text Table 2. Mean Toxicokinetic Metrics – DG 7 and DG 19

Metric	2 mg/kg		10 mg/kg		100 mg/kg	
	DG 7	DG 19	DG 7	DG 19	DG 7	DG 19
C_{max} (ng/mL)	3460	2247	16166	13154	203011	91156
T_{max} (min)	50.0	60.0	60.0	120	240	260
AUC_{last} (min*ng/mL)	631161	482240	3730360	2689334	57417845	28731895
AUC_{inf} (min*ng/mL)	640236	506098	4124864 [N=2]	2540954 [N=1]	NR	30617160
$T_{1/2}$ (min)	72.5	108	71.4 [N=2]	56.8 [N=1]	NR	74.7

DG - Day of Gestation
NR - not reportable

Necropsy: No gross lesions were seen.

Fertility parameters (mating/fertility index, corpora lutea, preimplantation loss, etc.): No C-sectioning or litter parameters were affected and no gross external, soft tissue or skeletal fetal alternations were caused by T dosages, up to 100 mg/kg/day.

- *C-sectioning and litter observations*: Pregnancy occurred in 18-20 of the 20 in each group. C-sectioning observations on GD 29 were based on 18, 19, 20, and 19 surviving pregnant rabbits with one or more live fetuses in the 4 respective dosages groups (0, 2, 10, and 100 mg/kg/day). There was 1 dead fetus in the 2 mg/kg/day group. Note from the reviewer: this dead fetus might account for the increase seen in table 8 where 6.7% vs. 5.4% dead or resorbed/litter in 2 mg/kg/day and control groups respectively. No historical control was provided by the sponsor.

TABLE 7 (PAGE 1): CAESAREAN-SECTIONING OBSERVATIONS - SUMMARY

DOSAGE GROUP		I	II	III	IV
DOSAGE (MG/KG/DAY)a		0 (VEHICLE)	2	10	100
RABBITS TESTED	N	20	20	20	20
PREGNANT	N(%)	18(90.0)	19(95.0)	20(100.0)	20(100.0)
FOUND DEAD	N(%)	0(0.0)	0(0.0)	0(0.0)	1(5.0)
RABBITS PREGNANT AND CAESAREAN-SECTIONED ON DAY 29 OF GESTATION	N	18	19	20	19
CORPORA LUTEA	MEAN±S.D.	9.7 ± 1.4	10.5 ± 2.3	10.2 ± 2.2	9.9 ± 2.0
IMPLANTATIONS	MEAN±S.D.	8.8 ± 1.4	9.4 ± 2.4	9.6 ± 2.4	8.6 ± 2.6
LITTER SIZES	MEAN±S.D.	8.3 ± 1.1	8.7 ± 2.4	9.3 ± 2.2	8.2 ± 2.4
LIVE FETUSES	N	149	165	186	155
	MEAN±S.D.	8.3 ± 1.1	8.7 ± 2.4	9.3 ± 2.2	8.2 ± 2.4
DEAD FETUSES	N	0	1	0	0
	MEAN±S.D.	0.0 ± 0.0	0.0 ± 0.2	0.0 ± 0.0	0.0 ± 0.0
RESORPTIONS	MEAN±S.D.	0.6 ± 1.0	0.6 ± 1.2	0.4 ± 0.7	0.4 ± 0.8
EARLY RESORPTIONS	N	5	3	3	2
	MEAN±S.D.	0.3 ± 0.6	0.2 ± 0.4	0.2 ± 0.4	0.1 ± 0.3
LATE RESORPTIONS	N	5	9	4	6
	MEAN±S.D.	0.3 ± 0.8	0.5 ± 1.2	0.2 ± 0.6	0.3 ± 0.8
DOES WITH ANY RESORPTIONS	N(%)	6(33.3)	6(31.6)	5(25.0)	5(26.3)
DOES WITH ALL CONCEPTUSES DEAD OR RESORBED	N(%)	0(0.0)	0(0.0)	0(0.0)	0(0.0)
DOES WITH VIABLE FETUSES	N(%)	18(100.0)	19(100.0)	20(100.0)	19(100.0)
PLACENTAE APPEARED NORMAL	N(%)	18(100.0)	19(100.0)	20(100.0)	19(100.0)

a. Dosage occurred on days 7 through 19 of gestation.

No c-sectioning or litter parameters were affected by FDKP, as high as 100 mg/kg/day. The litter averages for corpora lutea, implantation, littler sizes, live fetuses, resorptions, % live male fetuses, fetal body weights, and % resorbed conseptuses were comparable among all groups. No dam had a littler consisting of only resorbed conceptuses. All placentae appeared normal.

TABLE 8 (PAGE 1): LITTER OBSERVATIONS (CAESAREAN-DELIVERED FETUSES) - SUMMARY

DOSAGE GROUP		I	II	III	IV
DOSAGE (MG/KG/DAY)a		0 (VEHICLE)	2	10	100
LITTERS WITH ONE OR MORE LIVE FETUSES	N	18	19	20	19
IMPLANTATIONS	MEAN±S.D.	8.8 ± 1.4	9.4 ± 2.4	9.6 ± 2.4	8.6 ± 2.6
LIVE FETUSES	N	149	165	186	155
	MEAN±S.D.	8.3 ± 1.1	8.7 ± 2.4	9.3 ± 2.2	8.2 ± 2.4
LIVE MALE FETUSES	N	73	90	85	77
% LIVE MALE FETUSES/LITTER	MEAN±S.D.	48.8 ± 17.5	54.6 ± 17.3	45.8 ± 21.0	49.7 ± 16.4
LIVE FETAL BODY WEIGHTS (GRAMS)/LITTER	MEAN±S.D.	43.08 ± 5.24	41.53 ± 5.61	42.30 ± 4.49	42.20 ± 8.28
MALE FETUSES	MEAN±S.D.	43.40 ± 5.17	41.53 ± 6.19	42.44 ± 4.59 [19]b	42.64 ± 9.10
FEMALE FETUSES	MEAN±S.D.	42.76 ± 5.44	41.72 ± 5.72	41.45 ± 5.09	41.39 ± 7.46
% DEAD OR RESORBED CONCEPTUSES/LITTER	MEAN±S.D.	5.4 ± 9.3	6.7 ± 11.2	3.2 ± 6.0	4.1 ± 8.6

[ ] = NUMBER OF VALUES AVERAGED
a. Dosage occurred on days 7 through 19 of gestation.
b. Litter 8243 had no male fetuses.

- *Fetal alternations:* Fetal alterations were defined as malformation or variation. Litter averages were calculated for specific fetal ossification sites as part of the evaluation of the degree of fetal ossification. Fetal evaluations were based on 149, 165, 186, and 155 live, GD 29 C-sectioned delivered fetuses in 18, 19, 20, and 19 litters in the 0, 2, 10, and 100 mg/kg/day groups, respectively. Each of these fetuses were examined for gross external, soft tissue, and skeletal alterations, fetal ossification site averages.

Table 9 – summary of fetal alterations: In Group I to IV, 61.1%, 68.4%, 75%, and 15.8% of litters with fetuses were seen with alterations respectively. There were 14.1%, 15.8%, 14.5%, and 1.9% fetuses with alterations in these same respective dosage groups. All of the values reported for these parameters in the 100 mg/kg/day groups were significantly decreased when compared to the respective control group values. In addition, none of the alterations occurred in more than 1 litter in any group and/or in a dosage dependent manner. Skeletal ossification averages per fetus per litter did not significantly differ among groups. Note from the reviewer: There was an increase (fetuses with alternations) at 2 mg/kg/day group when compared with controls. This dose was the lowest dose tested, which was also associated with maternal toxicity. This finding is most likely attributed to maternal toxicity.

TABLE 9 (PAGE 1): FETAL ALTERATIONS - SUMMARY

DOSAGE GROUP		I	II	III	IV
DOSAGE (MG/KG/DAY)a		0 (VEHICLE)	2	10	100
LITTERS EVALUATED	N	18	19	20	19
FETUSES EVALUATED	N	149	166	186	155
LIVE	N	149	165	186	155
DEAD	N	0	1b	0	0
LITTERS WITH FETUSES WITH ANY ALTERATION OBSERVED	N(%)	11(61.1)	13(68.4)	15(75.0)	3(15.8)**
FETUSES WITH ANY ALTERATION OBSERVED	N(%)	21(14.1)	26(15.8)	27(14.5)	3(1.9)**
% FETUSES WITH ANY ALTERATION/LITTER	MEAN±S.D.	14.0 ± 15.8	15.1 ± 14.7	15.0 ± 14.2	1.5 ± 3.5**

a. Dosage occurred on days 7 through 19 of gestation.
b. Dead fetus was excluded from group averages and statistical analyses. Observations for this conceptus are cited on Table 21.
** Significantly different from the vehicle control group value (p≤0.01).

Table 10 – fetal gross external alterations: Total of 149, 165, 186, and 155 live fetuses were examined for gross external alterations. One fetus in the 10 mg/kg/day group had the right forepaw flexed downward (see table below). One dead fetus in the 2 mg/kg/day group had both

forepaws flexed downward, both hindpaws rotated medially, cleft palate, and non-patent nares. Note from the reviewer: These were major malformation associated with mortality. In addition, skeletal examination of this dead fetus showed that nasals contained an internasal, the interparietals were not ossified, the palate was incompletely ossified, and the scapular bodies were irregularly shaped.

TABLE 10 (PAGE 1): FETAL GROSS EXTERNAL ALTERATIONS - SUMMARY

DOSAGE GROUP		I	II	III	IV
DOSAGE (MG/KG/DAY)a		0 (VEHICLE)	2	10	100
LITTERS EVALUATED	N	18	19	20	19
FETUSES EVALUATED	N	149	166	186	155
LIVE	N	149	165	186	155
DEAD	N	0	1b	0	0
FOREPAWS: FLEXED DOWNWARD					
LITTER INCIDENCE	N(%)	0(0.0)	0(0.0)	1(5.0)	0(0.0)
FETAL INCIDENCE	N(%)	0(0.0)	0(0.0)	1(0.5)	0(0.0)

a. Dosage occurred on days 7 through 19 of gestation.
b. Dead fetus was excluded from group averages and statistical analyses. Observations for this conceptus are cited on Table 21.

Table 11 – fetal soft tissue alterations:

Malformations: 1) diaphragm – 1 fetus in the 0 mg/kg/day had a diaphragmatic hernia, 2) heart – 1 fetus in the 0 mg/kg/day group had a large heart, a septal defect, a distended aorta, and a constricted pulmonary artery, as well as an absent intermediate lobe of the lung, and 3) vessels – 1 fetus in the 0 mg/kg/day group had a distended aorta and a constricted pulmonary artery.

More Malformations/variations: 1) lungs – absence of the intermediate lobe of the lung (common in this strain) was seen in 1 fetus in each of 0, 2, and 10 mg/kg/day groups, and 2) gallbladder – absent from 3 littermates in 2 mg/kg/day dose group. Note from the reviewer: Historical control was not provided by the sponsor to support that absent intermediate lung lobes and gall bladder were common findings for this rabbit strain. These findings at 2 mg/kg/day suggest that the dose of 2 mg/kg/day is associated with malformations. This dose was also associated with maternal toxicity (weight loss).

TABLE 11 (PAGE 1): FETAL SOFT TISSUE ALTERATIONS - SUMMARY

DOSAGE GROUP		I	II	III	IV
DOSAGE (MG/KG/DAY)a		0 (VEHICLE)	2	10	100
LITTERS EVALUATED	N	18	19	20	19
FETUSES EVALUATED	N	149	166	186	155
LIVE	N	149	165	186	155
DEAD	N	0	1b	0	0
DIAPHRAGM: HERNIA					
LITTER INCIDENCE	N(%)	1(5.6)	0(0.0)	0(0.0)	0(0.0)
FETAL INCIDENCE	N(%)	1(0.7)	0(0.0)	0(0.0)	0(0.0)
HEART: LARGE					
LITTER INCIDENCE	N(%)	1(5.6)	0(0.0)	0(0.0)	0(0.0)
FETAL INCIDENCE	N(%)	1(0.7)c	0(0.0)	0(0.0)	0(0.0)
HEART: INTRAVENTRICULAR SEPTAL DEFECT					
LITTER INCIDENCE	N(%)	1(5.6)	0(0.0)	0(0.0)	0(0.0)
FETAL INCIDENCE	N(%)	1(0.7)c	0(0.0)	0(0.0)	0(0.0)
VESSELS: DISTENDED AORTA					
LITTER INCIDENCE	N(%)	1(5.6)	0(0.0)	0(0.0)	0(0.0)
FETAL INCIDENCE	N(%)	1(0.7)c	0(0.0)	0(0.0)	0(0.0)
VESSELS: PULMONARY ARTERY CONSTRICTED					
LITTER INCIDENCE	N(%)	1(5.6)	0(0.0)	0(0.0)	0(0.0)
FETAL INCIDENCE	N(%)	1(0.7)c	0(0.0)	0(0.0)	0(0.0)
LUNGS: INTERMEDIATE LOBE ABSENT					
LITTER INCIDENCE	N(%)	1(5.6)	1(5.3)	1(5.0)	0(0.0)
FETAL INCIDENCE	N(%)	1(0.7)c	1(0.6)d	1(0.5)	0(0.0)
GALLBLADDER: ABSENT					
LITTER INCIDENCE	N(%)	0(0.0)	1(5.3)	0(0.0)	0(0.0)
FETAL INCIDENCE	N(%)	0(0.0)	3(1.8)**d	0(0.0)	0(0.0)

a. Dosage occurred on days 7 through 19 of gestation.
b. Dead fetus was excluded from group averages and statistical analyses. Observations for this conceptus are cited on Table 21.
c. Fetus 8210-6 had other soft tissue alterations.
d. Fetus 8229-6 had other soft tissue alterations.
** Significantly different from the vehicle control group value (p≤0.01).

- Tables 12 and 13 – fetal skeletal alterations:

Malformations: 1) vertebrae/ribs – 1 fetus in each of 0 and 2 mg/kg/day groups had interrelated vertebral/rib malformations (i.e. small and fused arches and fused centra in the cervical vertebrae, hemivertebrea as well as split ribs, misaligned caudal vertebrate, and irregularly shaped ribs).

Variations: 1) skull – small irregularities in ossification of the skull were observed in 9, 14, 20, and 2 fetuses in 6, 9, 13, and 2 litters in the 0, 2, 10, and 100 mg/kg/day groups, respectively. The litter and fetal incidences of these irregularities were significantly increased in the 10 mg/kg/day group, whereas the fetal incidence of these irregularities was significantly decreased in the 100 mg/kg/day group, 2) hyoid – one or both hyoid were angulated in 3, 5, 3, and 1 fetuses from 2, 4, 2, and 1 litters in the 0, 2, 10, and 100 mg/kg/day dose groups, respectively, 3) vertebrae – bifid centra in cervical vertebrae occurred in 1 fetus in the 2 mg/kg/day group, 4), sternum – delayed sternal ossification occurred in 3 fetuses from 2 litters in the 2 mg/kg/day group and 1 fetus in the 100 mg/kg/day. Fused sternal centra also occurred in 3, 1, and 2 fetuses from 2, 1, and 2 litters in the 0, 2, and 10 mg/kg/day groups respectively. Asymmetric sternal centra occurred in 1 fetus in the 0 mg/kg/day group, 5) scapulae – irregularly shaped ala of the scapular bodies occurred in 3, 1, and 1 fetuses from 1, 1, and 1 litter in the 0, 2, and 10 mg/kg/day groups, respectively, and 6) fetal ossification site averages – No significant differences among the 4 dose groups in the average numbers of ossification sites per fetus for the hyoid, vertebrae, ribs, sternum, forelimbs, or hindlimbs but unossified skull.

TABLE 12 (PAGE 1): FETAL SKELETAL ALTERATIONS - SUMMARY
(See footnotes on the last page of this table.)

DOSAGE GROUP		I	II	III	IV
DOSAGE (MG/KG/DAY)a		0 (VEHICLE)	2	10	100
LITTERS EVALUATED	N	18	19	20	19
FETUSES EVALUATED	N	149	166	186	155
LIVE	N	149	165	186	155
DEAD	N	0	1b	0	0
SKULL - IRREGULAR OSSIFICATION:c					
(SUMMARIZATION OF ALL IRREGULAR OSSIFICATION OF THE SKULL d; MIDLINE SUTURE DISPLACED, CONTAINED AN INTERNASAL, SUTURE IRREGULAR CONTAINED AN INTRANASAL AND FUSED)					
LITTER INCIDENCE	N(%)	6(33.3)	9(47.4)	13(65.0)*	2(10.5)
FETAL INCIDENCE	N(%)	9(6.0)	14(8.5)	20(10.8)*	2(1.3)*
SKULL: NASALS, MIDLINE SUTURE DISPLACED					
LITTER INCIDENCE	N(%)	6(33.3)	5(26.3)	11(55.0)*	2(10.5)*
FETAL INCIDENCE	N(%)	7(4.7)	8(4.8)	15(8.1)*	2(1.3)*h
SKULL: NASALS, CONTAINED AN INTERNASAL					
LITTER INCIDENCE	N(%)	2(11.1)	3(15.8)	3(15.0)	0(0.0)
FETAL INCIDENCE	N(%)	2(1.3)	3(1.8)	3(1.6)	0(0.0)
SKULL: NASAL - FRONTAL, SUTURE IRREGULAR					
LITTER INCIDENCE	N(%)	1(5.6)	1(5.3)	1(5.0)	0(0.0)
FETAL INCIDENCE	N(%)	1(0.7)	1(0.6)	1(0.5)	0(0.0)
SKULL: NASAL, CONTAINED AN INTRANASAL					
LITTER INCIDENCE	N(%)	0(0.0)	2(10.5)	1(5.0)	0(0.0)
FETAL INCIDENCE	N(%)	0(0.0)	2(1.2)	1(0.5)	0(0.0)
SKULL: NASALS, FUSED					
LITTER INCIDENCE	N(%)	0(0.0)	0(0.0)	1(5.0)	0(0.0)
FETAL INCIDENCE	N(%)	0(0.0)	0(0.0)	1(0.5)	0(0.0)
SKULL: OTHER ALTERATIONS:					
HYOID: ALA, ANGULATED					
LITTER INCIDENCE	N(%)	2(11.1)	4(21.0)	2(10.0)	1(5.3)
FETAL INCIDENCE	N(%)	3(2.0)f	5(3.0)	3(1.6)	1(0.6)

TABLE 12 (PAGE 2): FETAL SKELETAL ALTERATIONS - SUMMARY
(See footnotes on the last page of this table.)

DOSAGE GROUP		I	II	III	IV
DOSAGE (MG/KG/DAY)a		0 (VEHICLE)	2	10	100
LITTERS EVALUATED	N	18	19	20	19
FETUSES EVALUATED	N	149	166	186	155
LIVE	N	149	165	186	155
DEAD	N	0	1b	0	0
CERVICAL VERTEBRAE: ARCH, SMALL					
LITTER INCIDENCE	N(%)	0(0.0)	1(5.3)	0(0.0)	0(0.0)
FETAL INCIDENCE	N(%)	0(0.0)	1(0.6)g	0(0.0)	0(0.0)
CERVICAL VERTEBRAE: ARCHES, FUSED					
LITTER INCIDENCE	N(%)	0(0.0)	1(5.3)	0(0.0)	0(0.0)
FETAL INCIDENCE	N(%)	0(0.0)	1(0.6)g	0(0.0)	0(0.0)
CERVICAL VERTEBRAE: CENTRUM, BIFID					
LITTER INCIDENCE	N(%)	0(0.0)	1(5.3)	0(0.0)	0(0.0)
FETAL INCIDENCE	N(%)	0(0.0)	1(0.6)g	0(0.0)	0(0.0)
CERVICAL VERTEBRAE: CENTRA, FUSED					
LITTER INCIDENCE	N(%)	0(0.0)	1(5.3)	0(0.0)	0(0.0)
FETAL INCIDENCE	N(%)	0(0.0)	1(0.6)g	0(0.0)	0(0.0)
THORACIC VERTEBRAE: HEMIVERTEBRA					
LITTER INCIDENCE	N(%)	1(5.6)	0(0.0)	0(0.0)	0(0.0)
FETAL INCIDENCE	N(%)	1(0.7)e	0(0.0)	0(0.0)	0(0.0)
CAUDAL VERTEBRAE: MISALIGNED					
LITTER INCIDENCE	N(%)	2(11.1)	1(5.3)	0(0.0)	0(0.0)
FETAL INCIDENCE	N(%)	2(1.3)	1(0.6)	0(0.0)	0(0.0)
RIBS: IRREGULARLY SHAPED					
LITTER INCIDENCE	N(%)	0(0.0)	0(0.0)	0(0.0)	1(5.3)
FETAL INCIDENCE	N(%)	0(0.0)	0(0.0)	0(0.0)	1(0.6)h
RIBS: SPLIT					
LITTER INCIDENCE	N(%)	1(5.6)	0(0.0)	0(0.0)	0(0.0)
FETAL INCIDENCE	N(%)	1(0.7)e	0(0.0)	0(0.0)	0(0.0)
RIBS: 11 PRESENT					
LITTER INCIDENCE	N(%)	1(5.6)	0(0.0)	0(0.0)	0(0.0)
FETAL INCIDENCE	N(%)	1(0.7)e	0(0.0)	0(0.0)	0(0.0)

TABLE 12 (PAGE 3): FETAL SKELETAL ALTERATIONS - SUMMARY

DOSAGE GROUP		I	II	III	IV
DOSAGE (MG/KG/DAY)a		0 (VEHICLE)	2	10	100
LITTERS EVALUATED	N	18	19	20	19
FETUSES EVALUATED	N	149	166	186	155
LIVE	N	149	165	186	155
DEAD	N	0	1b	0	0
STERNAL CENTRA: INCOMPLETELY OSSIFIED					
LITTER INCIDENCE	N(%)	0(0.0)	2(10.5)	0(0.0)	1(5.3)
FETAL INCIDENCE	N(%)	0(0.0)	3(1.8)g	0(0.0)	1(0.6)h
STERNAL CENTRA: FUSED					
LITTER INCIDENCE	N(%)	2(11.1)	1(5.3)	2(10.0)	0(0.0)
FETAL INCIDENCE	N(%)	3(2.0)e	1(0.6)	2(1.1)	0(0.0)
STERNAL CENTRA: ASYMMETRIC					
LITTER INCIDENCE	N(%)	1(5.6)	0(0.0)	0(0.0)	0(0.0)
FETAL INCIDENCE	N(%)	1(0.7)e	0(0.0)	0(0.0)	0(0.0)
SCAPULAE: ALA, IRREGULARLY SHAPED					
LITTER INCIDENCE	N(%)	1(5.6)	1(5.3)	1(5.0)	0(0.0)
FETAL INCIDENCE	N(%)	3(2.0)f	1(0.6)	1(0.5)	0(0.0)

a. Dosage occurred on days 7 through 19 of gestation.
b. Dead fetus was excluded from group averages and statistical analyses. Observations for this conceptus are cited on Table 21.
c. Fetuses with alterations of the skull and/or hyoid are not separately identified in this summarization, except when alterations of other ossification sites were also present.
d. Includes all alterations noted for the skull except hyoid, ala, angulated. This category is excluded because this alteration does not result from irregular ossification.
e. Fetus 8214-1 had other skeletal alterations.
f. Fetus 8217-8 had other skeletal alterations.
g. Fetus 8229-6 had other skeletal alterations.
h. Fetus 8265-11 had other skeletal alterations.
* Significantly different from the vehicle control group value ($p \leq 0.05$).

TABLE 13 (PAGE 1): FETAL OSSIFICATION SITES - CAESAREAN-DELIVERED LIVE FETUSES (DAY 29 OF GESTATION) - SUMMARY

DOSAGE GROUP		I	II	III	IV
DOSAGE (MG/KG/DAY)a		0 (VEHICLE)	2	10	100
LITTERS EXAMINED	N	18	19	20	19
FETUSES EXAMINED	N	149	165	186	155
OSSIFICATION SITES PER FETUS PER LITTER					
HYOID	MEAN±S.D.	1.00 ± 0.00	0.99 ± 0.02	1.00 ± 0.00	1.00 ± 0.00
VERTEBRAE					
CERVICAL	MEAN±S.D.	7.00 ± 0.00	7.00 ± 0.00	7.00 ± 0.00	7.00 ± 0.00
THORACIC	MEAN±S.D.	12.62 ± 0.29	12.64 ± 0.28	12.70 ± 0.30	12.73 ± 0.29
LUMBAR	MEAN±S.D.	6.37 ± 0.30	6.36 ± 0.27	6.30 ± 0.30	6.27 ± 0.29
SACRAL	MEAN±S.D.	3.00 ± 0.00	3.00 ± 0.00	3.00 ± 0.00	3.00 ± 0.00
CAUDAL	MEAN±S.D.	16.99 ± 0.42	16.87 ± 0.28	17.02 ± 0.30	16.94 ± 0.44
RIBS (PAIRS)	MEAN±S.D.	12.56 ± 0.30	12.55 ± 0.28	12.63 ± 0.29	12.64 ± 0.29
STERNUM					
MANUBRIUM	MEAN±S.D.	1.00 ± 0.00	1.00 ± 0.00	1.00 ± 0.00	1.00 ± 0.00
STERNAL CENTERS	MEAN±S.D.	3.98 ± 0.14	3.95 ± 0.17	3.94 ± 0.17	3.88 ± 0.25
XIPHOID	MEAN±S.D.	0.97 ± 0.06	0.94 ± 0.13	0.96 ± 0.07	0.91 ± 0.19
FORELIMB b					
CARPALS	MEAN±S.D.	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
METACARPALS	MEAN±S.D.	4.99 ± 0.03	4.98 ± 0.05	5.00 ± 0.02	4.96 ± 0.14
DIGITS	MEAN±S.D.	5.00 ± 0.00	5.00 ± 0.00	5.00 ± 0.00	5.00 ± 0.00
PHALANGES	MEAN±S.D.	13.93 ± 0.17	13.77 ± 0.27	13.96 ± 0.09	13.87 ± 0.24
HINDLIMB b					
TARSALS	MEAN±S.D.	2.00 ± 0.00	2.00 ± 0.00	2.00 ± 0.00	1.99 ± 0.04
METATARSALS	MEAN±S.D.	4.00 ± 0.00	4.00 ± 0.00	4.00 ± 0.00	4.00 ± 0.00
DIGITS	MEAN±S.D.	4.00 ± 0.00	4.00 ± 0.00	4.00 ± 0.00	4.00 ± 0.00
PHALANGES	MEAN±S.D.	12.00 ± 0.00	12.00 ± 0.00	12.00 ± 0.00	11.99 ± 0.04

a. Dosage occurred on days 7 through 19 of gestation.
b. Calculated as average per limb.

MKC-PC-2006-0010: Fetal Toxicokinetic Study of Subcutaneous Technosphere® Particles in Rats [By (b) (4), Study No. LCL00009]

This study was to determine exposure levels in fetuses following twice daily FDKP treatment of pregnant female rats. 40 pregnant Crl:CD(SD) female rats (10 rats per group) were given FDKP twice daily via sc injection on GDs 6-17 at dosages of 0, 10, 30, and 100 mg/kg/day. Viabilities, clinical observations, body weights, and feed consumption values were measured. The female rats were euthanized on GD 18. Fetuses were removed from the uterus, and fetal blood samples were collected.

Maternal and fetal findings: No mortality and no clinical signs were observed during the course of the study. Mean maternal body weights and body weight gains and absolute and relative feed consumption values were not affected by the FDKP treatment.

TK findings: Fetal TK samples were collected from 8, 9, 10, and 10 pregnant dams in 0, 10, 30, and 100 mg/kg/day groups, respectively. Fetal plasma samples were collected on GD 18, approx. 24 hrs after the final SC injection of the treated dam on GD 17, yielded the exposure values of 0.031356, 0.06833, and 0.2637 mg/kg/day in 10, 30, and 100 mg/kg/day groups, respectively (see Table 1 below).

Text Table 1	Mean Fetal Exposure Levels (mcg/mL)			
	Maternal Dosage Level (mg/kg/day)			
Day of Gestation	0 (Vehicle)	10	30	100
DG 18	a	0.031356	0.06833	0.2637

a. Four of eight control group samples had detectible levels of test article. These levels ranged from 0.000327 to 0.00207 mcg/mL. The levels were well below those obtained in the lowest dosage group and were believed to have been introduced during collection and/or processing of the samples. These detectable levels were not considered to have affected the outcome of the study.

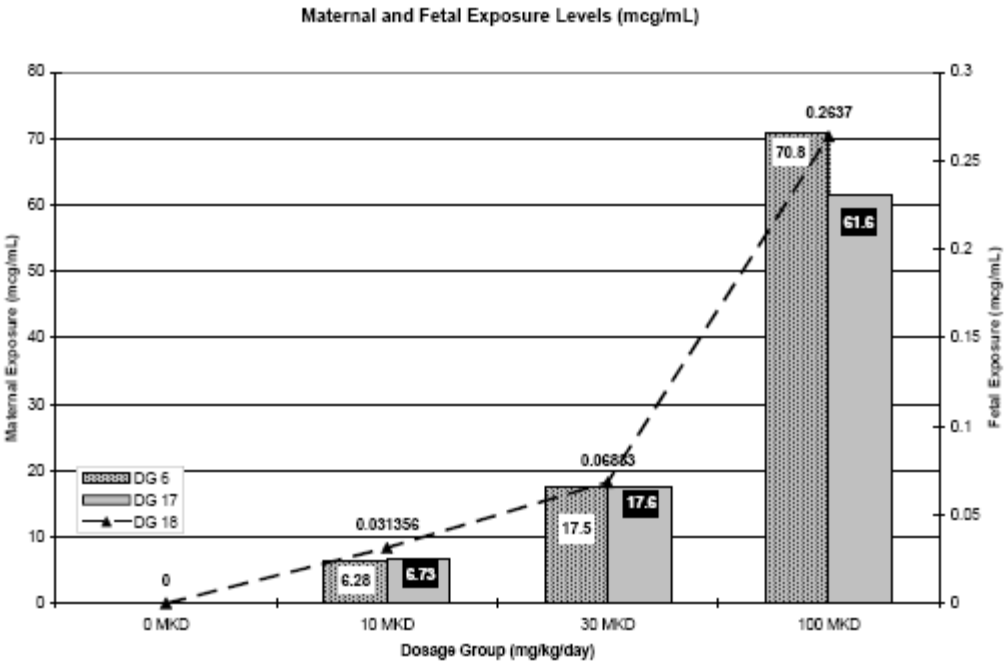
Fetal plasma level in the 10 mg/kg/day group was 45.9% of the level in the 30 mg/kg/day group, which was 25.9% of the level in 100 mg/kg/day group. This suggested the levels were roughly dose proportional. It also indicated that FDKP levels remained present in fetal plasma at least 24 hrs after treatment of the dam.

When the fetal exposure levels were compared to maternal C_{max} values obtained (in a separate development toxicity study in rats, see table below), fetal plasma levels represented 0.47% (0.031356 / 6.73), 0.39% (0.06833 / 17.6), and 0.43% (0.2637 / 61.6) of maternal plasma levels of 10, 30, and 100 mg/kg/day respectively.

In a developmental toxicity study of subcutaneously injected Technosphere® Particles in rats (b) (4) Project No. 98061, A Subcutaneous Injection Teratology Study of Fumaryl Technosphere™ in Rats), analysis of plasma samples collected on DGs 6 and 17 yielded the C_{max} values identified below in Text Table 2.

Text Table 2	Mean Exposure Levels (mcg/mL) at C_{max}			
	Dosage Level (mg/kg/day)			
Day of Gestation	0 (Vehicle)	10	30	100
DG 6	BLQ	6.28	17.5	70.8
DG 17	BLQ	6.73	17.6	61.6

BLQ – Below the limit of quantitation



Overall, this study indicated that fetal exposure occurred as a result of treatment of the F0 generation dams twice daily via SC injection on GDs 6 through 17.

Prenatal and postnatal development

Study title: Developmental and Perinatal/Postnatal Reproduction Toxicity Study of Subcutaneous Technosphere® Particles in Rats, Including a Postnatal Behavioral/Functional Evaluation [MKC-PC-2004-0008]

Key study findings:

- Pregnant rats (F0 generation) were given Technosphere® at 0, 10, 30, or 100 mg/kg/day twice daily via SC injection from GD (gestation day) 7 to LD (lactation day) 20. The study showed that there were no clinical or necropsy observation in the F0 generation female rats that were considered treatment related. Maternal body weights and body weight gains, absolute/relative food consumption values during gestation and lactation, and natural delivery and litter parameters were not affected by T particles as high as 100 mg/kg/day. No clinical or necropsy observations in the F1 generation pups were attributed to T particles as high as 100 mg/kg/day.
- No deaths were observed in F1 generation related to treatment of the F0 generation dams with the test article. All clinical and necropsy observations in the F1 generation male and female rats were considered unrelated to treatment of the F0 generation dams with T particles.
- Doses up to 100 mg/kg/day also did not affect the absolute weights of testes or epididymides or the ratios of these weights to the terminal body weight and resulted in no microscopic changes in F1 generation male rats. Although there were reductions in the absolute weights of the testes and epididymides in the maternal 100 mg/kg/day group, as well as the ratios of these organ weights to terminal body weights, there reductions were not considered to be related to the T articles because there were no corresponding effects in mating and fertility and no microscopic changes revealed by histopathological evaluation.
- Body weights and body weight gains, absolute/relative food consumption values, sexual maturation, and performance in the passive avoidance and water maze tests for the F1

generation rats were not affected by maternal dosage of T particles up to 100 mg/kg/day. However, there were 3 pups that failed to learn from the 10 and 30 mg/kg/day groups. Mating and fertility parameters and C-sectioning and litter parameters for the F1 generation were not affected and no fetal gross external alterations were caused by maternal dosages of T particles up to 100 mg/kg/day.

- TK analyses showed that administration of T particles in saline as a SC injection increased the exposure with increasing dose. Following the first of the 2 daily injections on GD7, the increase was proportional to the dose. However, following the first of the 2 daily injections on LD 20, the increase was proportional to the dose in 10 and 30 mg/kg/day groups. There was no difference between GD 7 and LD 20. For the 100 mg/kg/day group, the total AUC_{inf}, due to the twice daily dosing, on GD 7 than on LD 20 were 8,340 and 5,870 µg•min/mL respectively, indicating the NOAEL (maternal) of 8.34 mg•min/mL.
- In addition, the t_{max} occurred at 30 min post injection for the 10 mg/kg/day group and 60 min for the 30 and 100 mg/kg/day groups. The t ½ ranged from 27.4 to 36.6 min for all groups, except the 100 mg/kg/day group on LD 20, where the value was 64.9 min.
- Moreover, the mean concentrations found in the mother's milk at 4 hrs postdose on LD 12 were dose proportional at 308, 886, and 3515 ng/mL for the 10, 30, and 100 mg/kg/day groups, respectively. This concentration was approx. 10% of the serum concentration at 1 hr (approx. the t_{max}), suggesting that secretion of T into milk.
- The maternal NOEL for Technosphere® was 100 mg/kg/day, the highest dose given. The reproductive NOEL in the dams was also 100 mg/kg/day. However, the F0 females were dosed from GD 7 (during organogenesis). Therefore, the T particles were not on board to evaluate parameters such as implantation, resorptions, and etc.
- The NOEL for viability and growth in the offspring to weaning and continuing through maturation was 100 mg/kg/day. The reproductive and developmental NOEL in the F1 generation dams was also 100 mg/kg/day. Note from the reviewer: Decreased male reproductive organ weights and impaired learning were observed in F1 pups at 30 and/or 100 mg/kg/day, suggesting that the developmental NOAEL is probably 10 mg/kg/day.

Study no.: LCL00003
Volume # and page #: eEDR Module 4.2.3.5.3., 508 pages
Conducting laboratory and location: (b) (4)
Date of study initiation: Feb 25th, 2005
GLP compliance: Yes
QA reports: yes (x) no ( )
Drug, lot #, and % purity: D-035U-04-006 (see table below) – Technosphere® particles

PRODUCT NAME/ DESCRIPTION:	(b) (4) Fumaryl Technosphere® 3,6-Bis [N-Fumaryl N-(n-Butyl) amino]-2,5-Diketopiperazine
PACKAGING DESCRIPTION:	Bulk Powder in Bottle(s)
LOT NUMBER:	D035U04006
PRODUCT STAGE:	Finished Product
EXPIRY DATE:	07/2006
SPECIFICATION #:	S035-002
STORAGE CONDITIONS:	Keep Tightly Sealed, Out of Light, Store at Room Temperature (15-30)°C

ITEM NUMBER	ATTRIBUTE	TEST METHOD/ SOURCE	SPECIFICATION	RESULTS
1	Technosphere Assay (HPLC)	TM# 5319	Minimum 98.0% Area %	99.3 %
2	Impurities Assay (HPLC)	TM# 5319	Record All Impurities Area% and RT with an Area% greater than (b) (4) %	Impurity RRT Area % (b) (4) % % % %
3	Identification (MS)	TM# 5304	MS Electrospray: M+1 = (b) (4) m/z Or M-1 = m/z	M+1= (b) (4) m/z Confirmed
4	Particle Size by Cascade Impactor	TM# 5318	MMAD (b) (4) Percent Respirable Fraction	(b) (4)
5	Microbial Limits	Outside Contractor	Limit ≤ (b) (4) CFU/g for aerobic plate count, mold and yeast. No Salmonella, Staphylococcus aureus, Escherichia coli, or Pseudomonas aeruginosa detected	Pass
6	(b) (4)			
7	Appearance	TM# 5303	White Powder	White Powder
8	Particle Size by Light Scatter	TM# 5311	(b) (4)	(b) (4)

Identification[a]	Description	Lot Numbers	Dates Received	Suppliers[b]	Storage Conditions	Expiration Dates
Phosphate Buffered Saline (pH 7.4 or 7.2[c]) without magnesium chlorite or calcium chlorite	Clear, colorless liquid	1195947	26 FEB	(b) (4)	Room temperature	DEC 05
		1191210	10 DEC		Room temperature	OCT 05
		1182381	07 OCT		Room temperature	AUG 05
		1252966	22 MAR		Room temperature	JAN 07

a. The vehicle consisted of potassium phosphate monobasic (CAS Number 7778-77-0), sodium chloride (CAS Number 7647-14-5) and sodium phosphate dibasic (CAS Number 7558-79-4).
b. (b) (4)
c. See APPENDIX E, item 2.

Methods

Doses: 0, 10, 30, and 100 mg/kg/day twice daily via SC injection from GD 7 (gestation day) through LD (lactation day) 20 or GD 24 (rats that did not deliver a litter).

2.7.1.1. F0 Generation Rats

Dosage Group	Dosage[a,b] (mg/kg/day)	Dosage[a,b] (mg/kg/dosage)	Concentration (mg/mL)	Dosage Volume (mL/kg)	Number of Rats	Assigned F0 Generation Female Rat Numbers	
						Main Study	Toxicokinetic Sample Collection
I	0 (Vehicle)	0 (Vehicle)	0	5	25	5501 - 5525	NA
II	10	5	1	5	25 + 9[c]	5526 - 5550	2974 - 2982
III	30	15	3	5	25 + 9[c]	5551 - 5575	2983 - 2991
IV	100	50	10	5	25 + 9[c]	5576 - 5600	2992 - 3000

a. The test article was considered 100% active for the purpose of dosage calculations.
b. The two daily subcutaneous injections were separated by approximately 6 hours ± 30 minutes (see APPENDIX E, items 4 and 5).
c. Twenty-seven additional mated female rats assigned nine per group in Groups II through IV for toxicokinetic sample collections.
NA - Not applicable

2.7.1.2. F1 Generation Rats

Dosage Group	Maternal Dosage		Number of Rats Per Sex	Assigned F1 Generation Rat Numbers	
	Dosage (mg/kg/day)	Dosage (mg/kg/dosage)		Male	Female
I	0 (Vehicle)	0 (Vehicle)	25	8001 - 8025	8301 - 8325
II	10	5	25	8026 - 8050	8326 - 8350
III	30	15	25	8051 - 8075	8351 - 8375
IV	100	50	25	8076 - 8100	8376 - 8400

Species/strain: Crl:(CD)SD VAF/Plus® rats
Number/sex/group: Females; 25 per group (for both F0 and F1)
Route, formulation, volume, and infusion rate: sc injection with volume of 5 mL/kg
Satellite groups used for toxicokinetics: TK 9 per group (II to IV for F0)

Study design: 100 pregnant female rats were randomly assigned to 4 groups (25 rats/group). An additional 27 pregnant females rats were randomly assigned to Group 2 through 4 (9 rats/group) for TK sample collections. Test article and or VH were administrated via sc injection twice daily from GD 7 through LD 20 for rats that delivered a litter, or GD 24 for rats that did not deliver a litter.

Parameters and endpoints evaluated: F0 generation rats: viabilities, clinical observations, and observations of maternal behavior were recorded. Blood samples were collected from the female rats assigned for TK sample collections from GD 7 and either GD 25 (if necessary) or PN 20. Blood and milk samples were collected from these females rats on either LD 12 or LD 13. F1 generation rats: viabilities, clinical observations, and body weights were recorded. On LD 21, all F0 generation dams and all F1 generation pups not selected for continued evaluation were sacrificed by CO2 and examined for gross lesions.

Approx. 200 F1 generations rats (25/sex/group) were chosen for continued evaluation. Viabilities, clinical observations, body weights, and food consumption were recorded. These rats were also evaluated for sexual maturation and learning and memory by passive avoidance and water maze tests.

At approx. 90 days of age, the F1 generation rats were assigned to cohabitation. Male rats were sacrificed after completion of the cohabitation period and a gross necropsy was performed. Tests and epididymides weights were recorded. Female rats were sacrificed on GD 21, C-sectioned and a gross necropsy was performed. Each fetus was weighted and examined for sec and gross external alterations.

Results

F0 in-life findings:

Mortality: All rats survived to scheduled sacrifice.

Clinical signs: All signs were considered unrelated to the test article because 1) a similar number of rats were affected in the control group and 2) signs were observed in only 1 rat in any dose group. The observations included soft or liquid feces, localized alopecia, chromorhinorrhea, dehydration, urine-stained abdominal fur, portion of tail missing, swollen digit, incisors misaligned, red perivaginal substance, sparse hair coat, and ptosis. In addition, localized alopecia on the forelimbs occurred in a significant number of F0 generation female rats in the 30 mg/kg/day group during the gestation period.

TABLE B1 (PAGE 1): CLINICAL OBSERVATIONS - SUMMARY - F0 GENERATION FEMALE RATS

DOSAGE GROUP	I	II	III	IV
DOSAGE (MG/KG/DAY)a	0 (VEHICLE)	10	30	100
MORTALITY	0	0	0	0
PRESUMED GESTATION:				
MAXIMUM POSSIBLE INCIDENCE	410/ 25	409/ 25	412/ 24b	410/ 25
SOFT OR LIQUID FECES	2/ 2	2/ 1	2/ 2	4/ 3
LOCALIZED ALOPECIA: TOTAL	10/ 1	0/ 0	34/ 4	2/ 1
LIMB(S)	0/ 0	0/ 0	34/ 4**	2/ 1
UNDERSIDE	10/ 1	0/ 0	0/ 0	0/ 0
CHROMORHINORRHEA	0/ 0	0/ 0	0/ 0	2/ 1
DEHYDRATION	0/ 0	0/ 0	0/ 0	2/ 1
URINE-STAINED ABDOMINAL FUR	0/ 0	1/ 1	1/ 1	0/ 0
PORTION OF TAIL MISSING	0/ 0	0/ 0	15/ 1	0/ 0
LEFT FOREPAW: 4TH DIGIT, SWOLLEN	0/ 0	0/ 0	8/ 1	0/ 0
INCISOR(S): MISALIGNED	0/ 0	10/ 1	0/ 0	0/ 0

STATISTICAL ANALYSES OF CLINICAL OBSERVATION DATA WERE RESTRICTED TO THE NUMBER OF RATS WITH OBSERVATIONS.
MAXIMUM POSSIBLE INCIDENCE = (DAYS x RATS)/NUMBER OF RATS EXAMINED PER GROUP.
N/N = TOTAL NUMBER OF OBSERVATIONS/NUMBER OF RATS WITH OBSERVATION.
a. Dosage occurred on day 7 of presumed gestation through day 20 of lactation.
b. Excludes rat 5561, which had 10 or less sperm observed in the vaginal smear and was inadvertently assigned to the study.
** Significantly different from the vehicle control group value ($p \leq 0.01$).

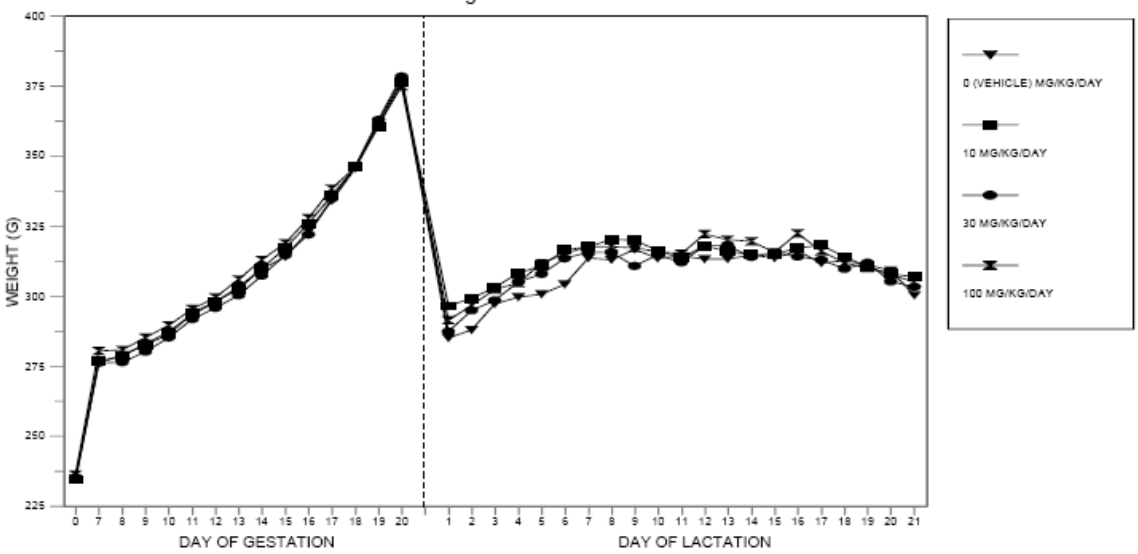
TABLE B1 (PAGE 2): CLINICAL OBSERVATIONS - SUMMARY - F0 GENERATION FEMALE RATS

DOSAGE GROUP	I	II	III	IV
DOSAGE (MG/KG/DAY)a	0 (VEHICLE)	10	30	100
MORTALITY	0	0	0	0
LACTATION:				
MAXIMUM POSSIBLE INCIDENCE	504/ 24	504/ 24	483/ 23	504/ 24
DEHYDRATION	1/ 1	0/ 0	2/ 2	7/ 4
URINE-STAINED ABDOMINAL FUR	7/ 2	0/ 0	1/ 1	6/ 3
SOFT OR LIQUID FECES	9/ 5	2/ 2	2/ 2	2/ 2
LOCALIZED ALOPECIA: TOTAL	23/ 2	53/ 4	55/ 3	21/ 1
LIMB(S)	23/ 2	53/ 4	55/ 3	21/ 1
UNDERSIDE	21/ 1	0/ 0	0/ 0	0/ 0
RED PERIVAGINAL SUBSTANCE	1/ 1	0/ 0	0/ 0	1/ 1
CHROMORHINORRHEA	0/ 0	0/ 0	0/ 0	3/ 1
SPARSE HAIR COAT	0/ 0	0/ 0	0/ 0	1/ 1
LEFT FOREPAW: 4TH DIGIT, SWOLLEN	0/ 0	0/ 0	21/ 1	0/ 0
PORTION OF TAIL MISSING	0/ 0	0/ 0	21/ 1	0/ 0
INCISOR(S): MISALIGNED	0/ 0	21/ 1	0/ 0	0/ 0
PTOSIS	0/ 0	1/ 1	0/ 0	0/ 0

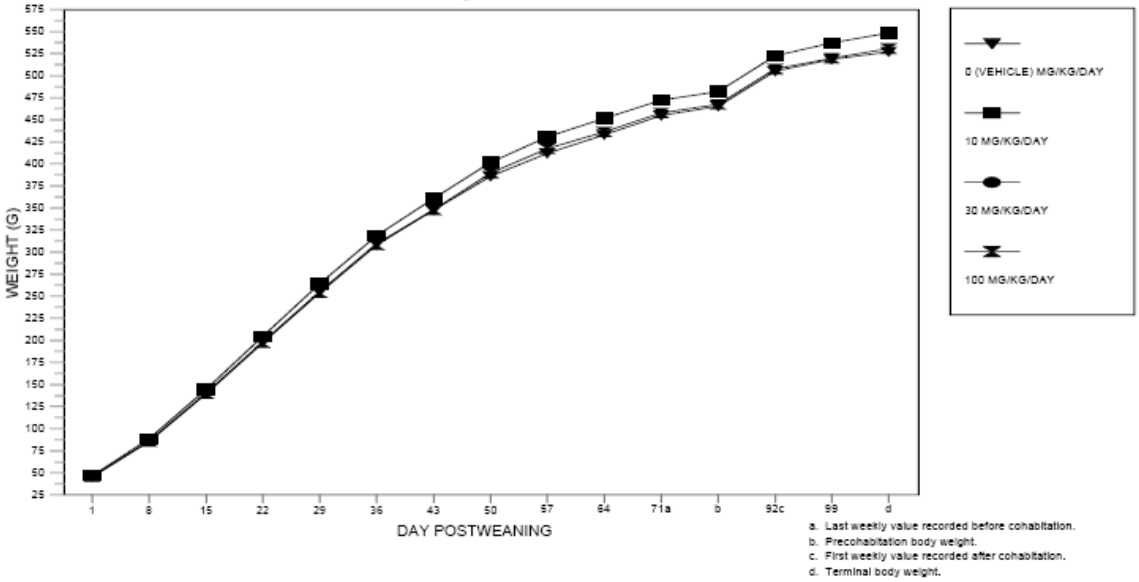
STATISTICAL ANALYSES OF CLINICAL OBSERVATION DATA WERE RESTRICTED TO THE NUMBER OF RATS WITH OBSERVATIONS.
MAXIMUM POSSIBLE INCIDENCE = (DAYS x RATS)/NUMBER OF RATS EXAMINED PER GROUP.
N/N = TOTAL NUMBER OF OBSERVATIONS/NUMBER OF RATS WITH OBSERVATION.
a. Dosage occurred on day 7 of gestation through day 20 of lactation.

Maternal body weights: Maternal body weights and body weight gains during gestation and lactation were not affected by T particles as high as 100 mg/kg/day. All values were comparable among the 4 dose groups. In addition, there were maternal body weight losses occurred on LDs 7 through 10 in the 10, 30, and 100 mg/kg/day group, on LDs 10 through 14 in the 10 and 30 mg/kg/day groups, and on LDs 14 through 21 in all groups, including the control. The losses in the treated groups either coincided with comparable reductions in body weight gain or comparable body weight losses in the control group during each of the respective intervals.

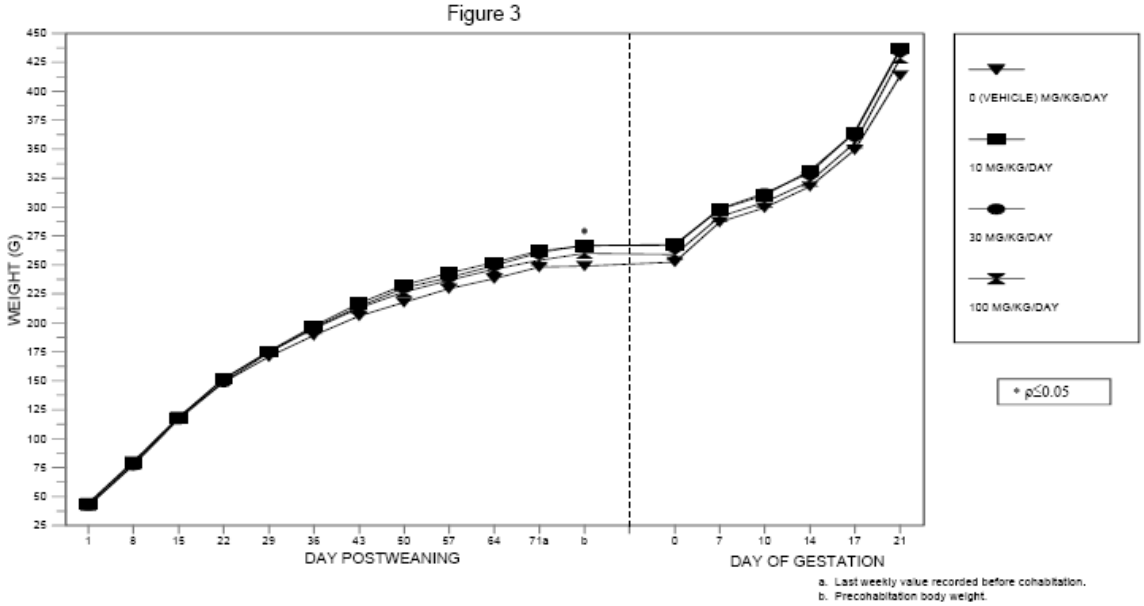
MATERNAL BODY WEIGHTS
F0 GENERATION FEMALE RATS
Figure 1



BODY WEIGHTS
F1 GENERATION MALE RATS
Figure 2



BODY WEIGHTS
F1 GENERATION FEMALE RATS
Figure 3



Food consumption: Relative food consumption values were significantly increased in the 10 and 100 mg/kg/day groups and increased in the 30 mg/kg/day group on GDs 12 though 15. These transient increases were not considered related to the test article because the increases were not dose-dependent.

F0 necropsy: There were no necropsy observations related to the test article in the F0 generation female rats. One rat in the 100 mg/kg/day group had a constriction 1.0 cm from the distal portion of the vagina and slight bilateral hydrometra. This rat was not

pregnant. No other gross lesions were observed. Also, absolute (g/day) and relative (g/kg/day) food consumption values during gestation and lactation were unaffected by doses of T particles as high as 100 mg/kg/day.

TABLE B2 (PAGE 1): NECROPSY OBSERVATIONS - SUMMARY - F0 GENERATION FEMALE RATS

DOSAGE GROUP		I	II	III	IV
DOSAGE (MG/KG/DAY)a		0 (VEHICLE)	10	30	100
RATS EXAMINED b	N	25	25	24c	25
MORTALITY	N	0	0	0	0
APPEARED NORMAL	N	25	25	24	24
VAGINA:					
CONSTRICTED	N	0	0	0	1
UTERUS:					
BILATERAL, HYDROMETRA, SLIGHT	N	0	0	0	1

a. Dosage occurred on day 7 of presumed gestation through day 20 of lactation.
b. Refer to the individual clinical observations table (Table B15) for external observations confirmed at necropsy.
c. Excludes rat 5561, which had 10 or less sperm observed in the vaginal smear and was inadvertently assigned to the study.

Natural delivery and litter observation: Pregnancy occurred in 24, 24, 23, and 24 of the 25 mated female rats in the 0, 10, 30, and 100 mg/kg/day groups respectively. All pregnant dams delivered litters. Natural delivery observations were not affected by T particles as high as 100 mg/kg/day. Numbers of dams delivering litters, the duration of gestation, average for implantation sites per delivered litter, the gestation index, the numbers of dams with still born pups and of dams with all pups dying were comparable among the 4 groups.

TABLE B11 (PAGE 1): NATURAL DELIVERY OBSERVATIONS - SUMMARY - F0 GENERATION FEMALE RATS

DOSAGE GROUP		I	II	III	IV
DOSAGE (MG/KG/DAY)a		0 (VEHICLE)	10	30	100
RATS ASSIGNED TO NATURAL DELIVERY	N	25	25	24b	25
PREGNANT	N(%)	24(96.0)	24(96.0)	23(95.8)	24(96.0)
DELIVERED LITTERS	N(%)	24(100.0)	24(100.0)	23(100.0)	24(100.0)
DURATION OF GESTATION c	MEAN±S.D.	22.7 ± 0.5	22.8 ± 0.4	22.7 ± 0.4	22.8 ± 0.4
IMPLANTATION SITES	N	373	349	340	373
PER DELIVERED LITTER	MEAN±S.D.	15.5 ± 1.7	14.5 ± 1.8	14.8 ± 1.8	15.5 ± 2.9
DAMS WITH STILLBORN PUPS	N(%)	1(4.2)	2(8.3)	2(8.7)	1(4.2)
DAMS WITH NO LIVEBORN PUPS	N(%)	0(0.0)	0(0.0)	0(0.0)	0(0.0)
GESTATION INDEX d	%	100.0	100.0	100.0	100.0
	N/N	24/ 24	24/ 24	23/ 23	24/ 24
DAMS WITH ALL PUPS DYING DAYS 1-4 POSTPARTUM	N(%)	0(0.0)	0(0.0)	0(0.0)	0(0.0)
DAMS WITH ALL PUPS DYING DAYS 5-21 POSTPARTUM	N(%)	0(0.0)	0(0.0)	0(0.0)	0(0.0)

a. Dosage occurred on day 7 of gestation through day 20 of lactation.
b. Excludes rat 5561, which had 10 or less sperm observed in the vaginal smear and was inadvertently assigned to the study.
c. Calculated as the time (in days) elapsed between confirmed mating (arbitrarily defined as day 0) and the time (in days) the first pup was delivered.
d. Number of rats with live offspring/number of pregnant rats.

TABLE B12 (PAGE 1): LITTER OBSERVATIONS (NATURALLY DELIVERED PUPS) - SUMMARY - F1 GENERATION LITTERS

MATERNAL DOSAGE GROUP		I	II	III	IV
MATERNAL DOSAGE (MG/KG/DAY)a		0 (VEHICLE)	10	30	100
DELIVERED LITTERS WITH ONE OR MORE LIVEBORN PUPS	N	24	24	23	24
PUPS DELIVERED (TOTAL)	N	344	318	326	358
	MEAN±S.D.	14.3 ± 3.1	13.2 ± 2.4	14.2 ± 1.7	14.9 ± 3.0
LIVEBORN	MEAN±S.D.	14.2 ± 3.1	13.1 ± 2.5	14.1 ± 1.7	14.9 ± 2.9
	N(%)	342(99.4)	314(98.7)	324(99.4)	357(99.7)
STILLBORN	MEAN±S.D.	0.1 ± 0.4	0.2 ± 0.6	0.1 ± 0.3	0.0 ± 0.2
	N(%)	2(0.6)	4(1.2)	2(0.6)	1(0.3)
MORIBUND SACRIFICED	N	0	1	0	0
PUPS FOUND DEAD, MORIBUND SACRIFICED OR PRESUMED CANNIBALIZED					
DAY 1	N/N(%)	1/342(0.3)	1/314(0.3)	1/324(0.3)	2/357(0.6)
DAYS 2- 4	N/N(%)	0/341(0.0)	1/313(0.3)	0/323(0.0)	1/355(0.3)
DAYS 5- 7	N/N(%)	2/341(0.6)	1/312(0.3)	0/323(0.0)	1/354(0.3)
DAYS 8-10	N/N(%)	3/339(0.9)	0/311(0.0)	0/323(0.0)	1/353(0.3)
DAYS 11-14	N/N(%)	0/336(0.0)	0/311(0.0)	0/323(0.0)	0/352(0.0)
DAYS 15-17	N/N(%)	1/336(0.3)	0/310(0.0)	0/323(0.0)	0/352(0.0)
DAYS 18-21	N/N(%)	0/335(0.0)	0/310(0.0)	0/323(0.0)	0/352(0.0)
VIABILITY INDEX b	%	99.7	99.4	99.7	99.2
	N/N	341/342	312/314	323/324	354/357
LACTATION INDEX c	%	98.2	99.4	100.0	99.4
	N/N	335/341	310/312	323/323	352/354

DAY(S) = DAY(S) POSTPARTUM
a. Dosage occurred on day 7 of gestation through day 20 of lactation.
b. Number of live pups on day 4 postpartum/number of liveborn pups on day 1 postpartum.
c. Number of live pups on day 21 (weaning) postpartum/number of live pups on day 4 postpartum.

TABLE B12 (PAGE 2): LITTER OBSERVATIONS (NATURALLY DELIVERED PUPS) - SUMMARY - F1 GENERATION LITTERS

MATERNAL DOSAGE GROUP		I	II	III	IV
MATERNAL DOSAGE (MG/KG/DAY)a		0 (VEHICLE)	10	30	100
DELIVERED LITTERS WITH ONE OR MORE LIVEBORN PUPS	N	24	24	23	24
SURVIVING PUPS/LITTER b					
DAY 1c	MEAN±S.D.	14.2 ± 3.1	13.1 ± 2.5	14.1 ± 1.7	14.9 ± 2.9
DAY 4	MEAN±S.D.	14.2 ± 3.1	13.0 ± 2.5	14.0 ± 1.7	14.8 ± 2.9
DAY 7	MEAN±S.D.	14.1 ± 3.0	13.0 ± 2.5	14.0 ± 1.7	14.7 ± 2.9
DAY 10	MEAN±S.D.	14.0 ± 3.0	13.0 ± 2.5	14.0 ± 1.7	14.7 ± 2.9
DAY 14	MEAN±S.D.	14.0 ± 3.0	13.0 ± 2.5	14.0 ± 1.7	14.7 ± 2.9
DAY 17	MEAN±S.D.	14.0 ± 3.0	12.9 ± 2.5	14.0 ± 1.7	14.7 ± 2.9
DAY 21	MEAN±S.D.	14.0 ± 3.0	12.9 ± 2.5	14.0 ± 1.7	14.7 ± 2.9
PERCENT MALE PUPS PER NUMBER OF PUPS SEXED					
DAY 1c	MEAN±S.D.	49.1 ± 14.9	51.7 ± 16.3	50.3 ± 15.7	47.3 ± 17.8
DAY 4	MEAN±S.D.	48.9 ± 15.0	52.1 ± 16.7	50.5 ± 15.6	47.4 ± 17.9
DAY 7	MEAN±S.D.	48.9 ± 15.1	52.2 ± 16.6	50.5 ± 15.6	47.5 ± 18.0
DAY 10	MEAN±S.D.	49.0 ± 15.0	52.2 ± 16.6	50.5 ± 15.6	47.4 ± 18.0
DAY 14	MEAN±S.D.	49.0 ± 15.0	52.2 ± 16.6	50.5 ± 15.6	47.4 ± 18.0
DAY 17	MEAN±S.D.	48.8 ± 15.1	52.1 ± 16.5	50.5 ± 15.6	47.4 ± 18.0
DAY 21	MEAN±S.D.	48.8 ± 15.1	52.1 ± 16.5	50.5 ± 15.6	47.4 ± 18.0

DAY = DAY POSTPARTUM
a. Dosage occurred on day 7 of gestation through day 20 of lactation.
b. Average number of live pups per litter, including litters with no surviving pups.
c. Includes pups born alive, found dead day 1 postpartum.

TABLE B12 (PAGE 3): LITTER OBSERVATIONS (NATURALLY DELIVERED PUPS) - SUMMARY - F1 GENERATION LITTERS

MATERNAL DOSAGE GROUP		I	II	III	IV
MATERNAL DOSAGE (MG/KG/DAY)a		0 (VEHICLE)	10	30	100
DELIVERED LITTERS WITH ONE OR MORE LIVEBORN PUPS	N	24	24	23	24
LIVE LITTER SIZE AT WEIGHING					
DAY 1	MEAN±S.D.	14.2 ± 3.1	13.0 ± 2.5	14.0 ± 1.7	14.8 ± 2.9
DAY 4	MEAN±S.D.	14.2 ± 3.1	13.0 ± 2.5	14.0 ± 1.7	14.8 ± 2.9
DAY 7	MEAN±S.D.	14.1 ± 3.0	13.0 ± 2.5	14.0 ± 1.7	14.7 ± 2.9
DAY 10	MEAN±S.D.	14.0 ± 3.0	13.0 ± 2.5	14.0 ± 1.7	14.7 ± 2.9
DAY 14	MEAN±S.D.	14.0 ± 3.0	13.0 ± 2.5	14.0 ± 1.7	14.7 ± 2.9
DAY 17	MEAN±S.D.	14.0 ± 3.0	12.9 ± 2.5	14.0 ± 1.7	14.7 ± 2.9
DAY 21	MEAN±S.D.	14.0 ± 3.0	12.9 ± 2.5	14.0 ± 1.7	14.7 ± 2.9
PUP WEIGHT/LITTER (GRAMS)					
DAY 1	MEAN±S.D.	6.7 ± 0.9	6.8 ± 0.6	6.7 ± 0.5	6.6 ± 0.9
DAY 4	MEAN±S.D.	9.4 ± 1.6	9.6 ± 1.2	9.4 ± 1.0	9.2 ± 1.5
DAY 7	MEAN±S.D.	13.2 ± 2.8	13.8 ± 2.0	13.2 ± 1.5	13.0 ± 2.5
DAY 10	MEAN±S.D.	17.9 ± 3.8	18.6 ± 3.1	17.7 ± 2.0	17.6 ± 3.5
DAY 14	MEAN±S.D.	24.6 ± 5.6	25.4 ± 4.2	24.2 ± 2.8	24.1 ± 4.7
DAY 17	MEAN±S.D.	29.3 ± 7.2	30.1 ± 5.2	28.4 ± 3.5	28.1 ± 5.7
DAY 21	MEAN±S.D.	39.4 ± 9.8	40.4 ± 6.9	38.2 ± 4.8	37.2 ± 7.4

DAY = DAY POSTPARTUM
a. Dosage occurred on day 7 of gestation through day 20 of lactation.

Clinical and Necropsy Observation – F1 pups: No clinical or necropsy observations were attributed to T particle up to 100 mg/kg/day. All clinical observations were considered unrelated to the test article by the sponsor because 1) no more than 4 litters in any dose groups were affected, 2) no dose-dependent relationship, and 3) observations occurred only in litters in the control groups. The observations included scab on the lower midline or tip of tail, cold to touch, not nursing, no milk band present, dehydration, dark purple area on the ear, nose, or back, alopecia on the lower midline or head, ungroomed coat, lacrimation, dried cornea, and etc.

In addition, no milk was found in the stomachs of 1,2,1, and 3 F1 generation pups that were found dead in the 0, 10, 30, and 100 mg/kg/day groups respectively. The right forelimb and internal organs of 1 F1 generation pup in the control group that was found dead were missing and presumed to have been cannibalized by the dam. No additional gross lesions were revealed by necropsy of the F1 generation pups that were found dead. Moreover, 1 F1 generation pup in each of 30 and 100 mg/kg/day groups had moderate dilation of the pelvis of the right or left kidney, and 1 F1 generation pup in the 10 mg/kg/day group had marked dilation of the lateral ventricles of the brain. No other gross lesions were revealed by necropsy of the F1 generation pups not selected for continued evaluation.

TABLE B13 (PAGE 1): CLINICAL OBSERVATIONS FROM BIRTH TO DAY 21 POSTPARTUM - SUMMARY - F1 GENERATION PUPS

MATERNAL DOSAGE GROUP		I	II	III	IV
MATERNAL DOSAGE (MG/KG/DAY)a		0 (VEHICLE)	10	30	100
LITTERS EXAMINED (N)	N	24	24	23	24
TRANSIENT CLINICAL OBSERVATIONS:		TOTAL FREQUENCY (DAYS X PUPS)/LITTERS WITH OBSERVATIONS			
LOWER MIDLINE OR TIP OF TAIL:					
SCAB	N/N	6/ 1	0/ 0	0/ 0	8/ 1
COLD TO TOUCH	N/N	8/ 1	0/ 0	3/ 1	4/ 3
NOT NURSING	N/N	1/ 1	0/ 0	0/ 0	2/ 2
NO MILK BAND PRESENT	N/N	1/ 1	0/ 0	0/ 0	2/ 2
DEHYDRATION	N/N	40/ 4	1/ 1	0/ 0	2/ 2
DARK PURPLE OR PURPLE AREA b	N/N	2/ 1	6/ 2	4/ 1	0/ 0
LOWER MIDLINE OR HEAD:					
ALOPECIA	N/N	4/ 1	6/ 1	0/ 0	0/ 0
UNGROOMED COAT	N/N	7/ 1	0/ 0	0/ 0	0/ 0
EXOPHTHALMOS	N/N	0/ 0	1/ 1	0/ 0	0/ 0
LACRIMATION	N/N	0/ 0	1/ 1	0/ 0	0/ 0
DRIED CORNEA	N/N	0/ 0	1/ 1	0/ 0	0/ 0
CORNEAL ULCERATION	N/N	0/ 0	1/ 1	0/ 0	0/ 0
LOWER MIDLINE: LESION	N/N	1/ 1	0/ 0	0/ 0	0/ 0
PERSISTENT CLINICAL OBSERVATIONS:		TOTAL FREQUENCY (DAYS X PUPS)/LITTERS WITH OBSERVATIONS			
TIP OF TAIL MISSING	N/N	21/ 1	0/ 0	0/ 0	0/ 0

a. Dosage occurred on day 7 of gestation through day 20 of lactation.
b. Located on the right ear, nose or back.

TABLE B14 (PAGE 1): NECROPSY OBSERVATIONS - SUMMARY - F1 GENERATION PUPS

MATERNAL DOSAGE GROUP		I	II	III	IV
MATERNAL DOSAGE (MG/KG/DAY)a		0 (VEHICLE)	10	30	100
LITTERS EXAMINED	N	24	24	23	24
TOTAL PUPS EXAMINED	N	7	7	3	4
FOUND DEAD b	N	5	2	1	3
STILLBORN	N	2	4	2	1
MORIBUND SACRIFICED	N	0	1	0	0
NO MILK IN STOMACH c	N(%)	1(20.0)	2(100.0)	1(100.0)	3(100.0)
RIGHT FORELIMB AND INTERNAL ORGANS: PRESUMED CANNIBALIZED	N(%)	1(16.7)	0(0.0)	0(0.0)	0(0.0)
PUPS SACRIFICED AND NECROPSIED ON DAY 21 POSTPARTUM b					
LITTERS EVALUATED	N	23	24	23	24
PUPS EVALUATED	N	285	260	273	302
APPEARED NORMAL					
LITTER INCIDENCE	N(%)	23(100.0)	23(95.8)	22(95.6)	23(95.8)
PUP INCIDENCE	N(%)	285(100.0)	259(99.6)	272(99.6)	301(99.7)
KIDNEYS: RIGHT OR LEFT, PELVIS, MODERATE DILATION					
LITTER INCIDENCE	N(%)	0(0.0)	0(0.0)	1(4.3)	1(4.2)
PUP INCIDENCE	N(%)	0(0.0)	0(0.0)	1(0.4)	1(0.3)
BRAIN: BILATERAL, LATERAL VENTRICLES, MARKED DILATION					
LITTER INCIDENCE	N(%)	0(0.0)	1(4.2)	0(0.0)	0(0.0)
PUP INCIDENCE	N(%)	0(0.0)	1(0.4)	0(0.0)	0(0.0)

a. Dosage occurred on day 7 of gestation through day 20 of lactation.
b. Restricted to pups in which complete necropsies were performed. Complete necropsies were not performed on pups in which autolysis or cannibalization precluded evaluation.
c. Analysis restricted to pups found dead and necropsied.

TK analysis: Administration of T particles in saline as sc injection increased exposure with increasing dose. Following the first of the 2 daily injections on GD 7, the increase was proportional to the dose, whereas following the first of the two injections on LD 20, dose proportionality was seen for the 10 and 30 mg/kg/day groups. No accumulation for the two lower dose groups. For the 100 mg/kg/day group, the AUC_{inf} was lower on LD

20 than on GD 7 (2,930,000 vs 4,170,000 ng•min/mL). The total daily AUC_{inf} would be double these values due to the twice daily dosing. The T_{max} occurred at 30 min post injection for 10 mg/kg/day group and 60 min for 30 and 100 mg/kg/day groups. The t ½ ranged from 24.7 to 36.6 min for all groups, except the 100 mg/kg/day group on LD 20 was 64.9 min.

TABLE 1. FDKP Mean-Serum Toxicokinetic Metrics Following the First of Twice Daily Subcutaneous Injection of Technosphere® Particles (T) from GD 7 Through LD 20

	10 mg/kg/day		30 mg/kg/day		100 mg/kg/day	
	GD 7	LD 20	GD 7	LD 20	GD 7	LD 20
C_{max} (ng/mL)	4928	4115	12750	9405	37881	20625
t_{max} (min)	30.0	30.0	60.0	60.0	60.0	60.0
AUC_{last} (ng*min/mL)	391617	374947	1229315	1098107	4166216	2840264
AUC_{inf} (ng*min/mL)	392413	375197	1230083	1099723	4167927	2933841
$t_{1/2}$ (min)	31.7	32.8	31.0	36.6	27.4	64.9

TABLE 2. FDKP Extrapolated Exposure for a Twice Daily Subcutaneous Injection of Technosphere® Particles (T) from GD 7 Through LD 20

	10 mg/kg/day		30 mg/kg/day		100 mg/kg/day	
	GD 7	LD 20	GD 7	LD 20	GD 7	LD 20
AUC_{last} (ng*min/mL)	783234	749894	2458629	2196214	8332431	5680527
AUC_{inf} (ng*min/mL)	784827	750395	2460167	2199446	8335854	5867682

TABLE 3. FDKP Mean Serum Concentrations Following the First of Twice Daily Subcutaneous Injection of Technosphere® Particles (T) from GD 7 Through LD 20 to Pregnant Sprague-Dawley Rats (N=3/time point for GD 7 and N=2 for LD 20)

Time (min)		10 mg/kg/day		30 mg/kg/day		100 mg/kg/day	
		GD 7	LD 20	GD 7	LD 20	GD 7	LD 20
Pre-dose	mean		1.36		1.74[a]		13.9[a]
	SD		NA				
15	mean	2490		7087		11201	
	SD	2228		2194		3892	
30	mean	4928	4115	9931	4729	17503	12044[b]
	SD	578	NA	2050	NA	2686	NA
60	mean	3461	3112	12750	9405	37881	20625
	SD	79.6	NA	1189	NA	5275	NA
120	mean	873	1080	3394	4823[a]	18778	12997[a]
	SD	611	NA	904		3630	
240	mean	66.9	116	161	809	1726	2674[b]
	SD	38.7	NA	40.3	NA	1187	NA
360	mean	17.4	5.28	17.2	30.6	43.3	1000[b]
	SD	21.6	NA	3.62	NA	17.1	NA

Blank spaces indicate that no blood sample was taken at that time point.

a) N=1

b) N=2, but highly divergent numbers.

c) NA, not applicable to N= 2.

In addition, the mean concentrations found in the mother's milk at 4 hrs postdose on LD 12 were dose proportional at 308, 886, and 3515 ng/mL for the 10, 30, and 100 mg/kg/day groups, respectively. This concentration was approx. 10% of the serum concentration at 1 hr (approx. the T_{max}).

TABLE 4. FDKP Serum and Corresponding Milk Concentrations Following the First of Twice Daily Subcutaneous Injection of Technosphere® Particles (T) on LD 12 to Pregnant Sprague-Dawley Rats. Blood Was Obtained 1 Hour After Dosing; Milk Was Obtained 4 Hours After Dosing.

Dose Group	Animal Number	Cs (ng/mL)	Cm (ng/mL)
10 mg/kg/day	2980	3015	207
	2975	3694	357
	2982	2491	235
	2976	3206	429
	2977	3841	243
	2979	3130	379
	Mean	**3230**	**308**
	SD	488	91.5
30 mg/kg/day	2986	9038	875
	2987	10746	686
	2989	12658	1270
	2991	8108	562
	2983	12955	1039
	Mean	**10701**	**886**
	SD	2145	281
100 mg/kg/day	2993	25646	2921
	2995	35946	3238
	2997	20073	2374
	2998	27417	5275
	3000	31125	3768
	Mean	**28041**	**3515**
	SD	5950	1106

F1 in life & physical development findings:

Mortality: All F1 generation rats selected for further evaluation, survived to the day of scheduled sacrifice. One F1 generation dam in the maternal 10 mg/kg/day group began to deliver and was sacrificed on GD 21. This occurrence was not attributed to treatment of the F0 generation dams with the test article because it was a single occurrence in the low dose group.

Clinical observations: All clinical observations in the F1 generation male and female rats were considered unrelated to treatment of the F0 generation dams with T particle because the incidences were not dose dependent, occurred in a relatively small number of rat. The observations were also common in this strain provided by the sponsor, with historical

data generated from (b) (4) These observations included misaligned and/or missing/broken incisors, chromodacryorrhea, sparse hair coat, soft or liquid feces, scab, swollen or bent snout, dehydration, localized alopecia, ulceration, and etc.

TABLE C1 (PAGE 1): CLINICAL OBSERVATIONS - SUMMARY - F1 GENERATION MALE RATS

MATERNAL DOSAGE GROUP	I	II	III	IV
MATERNAL DOSAGE (MG/KG/DAY)	0 (VEHICLE)	10	30	100
MAXIMUM POSSIBLE INCIDENCE	375/ 25	375/ 25	375/ 25	375/ 25
MORTALITY	0	0	0	0
INCISOR(S): TOTAL	26/ 5	53/ 8	28/ 7	30/ 4
MISALIGNED	23/ 5	45/ 8	20/ 5	27/ 4
MISSING/BROKEN	4/ 1	8/ 3	10/ 4	3/ 1
CHROMODACRYORRHEA	1/ 1	23/ 5	6/ 2	10/ 3
SPARSE HAIR COAT	0/ 0	3/ 1	0/ 0	6/ 2
SOFT OR LIQUID FECES	0/ 0	2/ 2	2/ 2	1/ 1
SCAB a	0/ 0	3/ 1	6/ 2	4/ 1
SNOUT: SWOLLEN	0/ 0	8/ 1	1/ 1	1/ 1
CHROMORHINORRHEA	2/ 2	1/ 1	0/ 0	1/ 1
BENT SNOUT	0/ 0	0/ 0	0/ 0	1/ 1
DEHYDRATION	1/ 1	1/ 1	1/ 1	0/ 0
LOCALIZED ALOPECIA: TOTAL	8/ 1	0/ 0	8/ 2	0/ 0
LIMB(S)	8/ 1	0/ 0	7/ 1	0/ 0
NECK	0/ 0	0/ 0	1/ 1	0/ 0
RED SUBSTANCE IN CAGE	0/ 0	0/ 0	1/ 1	0/ 0
RED PERIORAL SUBSTANCE	0/ 0	0/ 0	1/ 1	0/ 0
ULCERATION b	0/ 0	9/ 2	0/ 0	0/ 0
EXCESS SALIVATION - SLIGHT	0/ 0	2/ 2	0/ 0	0/ 0

STATISTICAL ANALYSES OF CLINICAL OBSERVATION DATA WERE RESTRICTED TO THE NUMBER OF RATS WITH OBSERVATIONS.
MAXIMUM POSSIBLE INCIDENCE = (WEEKS x RATS)/NUMBER OF RATS EXAMINED PER GROUP.
N/N = TOTAL NUMBER OF OBSERVATIONS/NUMBER OF RATS WITH OBSERVATION.
a. Located on the neck, right ear or left side of back.
b. Located on the mouth (palate), right ear or left side of back.

TABLE C1 (PAGE 2): CLINICAL OBSERVATIONS - SUMMARY - F1 GENERATION MALE RATS

MATERNAL DOSAGE GROUP	I	II	III	IV
MATERNAL DOSAGE (MG/KG/DAY)	0 (VEHICLE)	10	30	100
MAXIMUM POSSIBLE INCIDENCE	375/ 25	375/ 25	375/ 25	375/ 25
MORTALITY	0	0	0	0
LEFT SIDE OF BACK: MODERATE ERYTHEMA	0/ 0	3/ 1	0/ 0	0/ 0
RIGHT EAR: SWOLLEN	0/ 0	3/ 1	0/ 0	0/ 0
GASPING	1/ 1	0/ 0	0/ 0	0/ 0

STATISTICAL ANALYSES OF CLINICAL OBSERVATION DATA WERE RESTRICTED TO THE NUMBER OF RATS WITH OBSERVATIONS.
MAXIMUM POSSIBLE INCIDENCE = (WEEKS x RATS)/NUMBER OF RATS EXAMINED PER GROUP.
N/N = TOTAL NUMBER OF OBSERVATIONS/NUMBER OF RATS WITH OBSERVATION.

TABLE C4 (PAGE 1): CLINICAL OBSERVATIONS - SUMMARY - F1 GENERATION FEMALE RATS

MATERNAL DOSAGE GROUP	I	II	III	IV
MATERNAL DOSAGE (MG/KG/DAY)	0 (VEHICLE)	10	30	100
MORTALITY	0	0	0	0
DELIVERED AND SACRIFICED	0	1a	0	0
PRECOHABITATION (DAY 1 OF STUDY TO THE DAY OF COHABITATION):				
MAXIMUM POSSIBLE INCIDENCE	275/ 25	275/ 25	275/ 25	275/ 25
INCISOR(S): TOTAL	0/ 0	2/ 2	0/ 0	8/ 4
MISALIGNED	0/ 0	1/ 1	0/ 0	6/ 3
MISSING/BROKEN	0/ 0	1/ 1	0/ 0	3/ 2
CHROMODACRYORRHEA	0/ 0	0/ 0	3/ 1	4/ 2
EXCESS SALIVATION - SLIGHT	0/ 0	0/ 0	1/ 1	2/ 2
LOCALIZED ALOPECIA: TOTAL	0/ 0	0/ 0	0/ 0	4/ 2
NECK	0/ 0	0/ 0	0/ 0	3/ 2
HEAD	0/ 0	0/ 0	0/ 0	1/ 1
RED SUBSTANCE IN CAGE PAN	0/ 0	0/ 0	0/ 0	2/ 2
SPARSE HAIR COAT	7/ 2	7/ 3	2/ 1	3/ 1
PORTION OF TAIL MISSING	0/ 0	0/ 0	0/ 0	11/ 1
SNOUT: SWOLLEN	0/ 0	0/ 0	0/ 0	3/ 1
ERYTHEMA: GRADE 1	0/ 0	0/ 0	2/ 1	0/ 0
RED PERIORAL SUBSTANCE	0/ 0	0/ 0	1/ 1	0/ 0
DEHYDRATION	0/ 0	0/ 0	1/ 1	0/ 0

STATISTICAL ANALYSES OF CLINICAL OBSERVATION DATA WERE RESTRICTED TO THE NUMBER OF RATS WITH OBSERVATIONS.
MAXIMUM POSSIBLE INCIDENCE = (WEEKS x RATS)/NUMBER OF RATS EXAMINED PER GROUP.
N/N = TOTAL NUMBER OF OBSERVATIONS/NUMBER OF RATS WITH OBSERVATION.
a. Dam 8344 partially delivered a litter before scheduled sacrifice on day 21 of gestation.

TABLE C4 (PAGE 2): CLINICAL OBSERVATIONS - SUMMARY - F1 GENERATION FEMALE RATS

MATERNAL DOSAGE GROUP	I	II	III	IV
MATERNAL DOSAGE (MG/KG/DAY)	0 (VEHICLE)	10	30	100
MORTALITY	0	0	0	0
DELIVERED AND SACRIFICED	0	1a	0	0
PRECOHABITATION (DAY 1 OF STUDY TO THE DAY OF COHABITATION): (CONT.)				
MAXIMUM POSSIBLE INCIDENCE	275/ 25	275/ 25	275/ 25	275/ 25
NECK: SCAB	2/ 1	0/ 0	0/ 0	0/ 0
SOFT OR LIQUID FECES	1/ 1	0/ 0	0/ 0	0/ 0
CHROMORHINORRHEA	1/ 1	0/ 0	0/ 0	0/ 0

STATISTICAL ANALYSES OF CLINICAL OBSERVATION DATA WERE RESTRICTED TO THE NUMBER OF RATS WITH OBSERVATIONS.
MAXIMUM POSSIBLE INCIDENCE = (WEEKS x RATS)/NUMBER OF RATS EXAMINED PER GROUP.
N/N = TOTAL NUMBER OF OBSERVATIONS/NUMBER OF RATS WITH OBSERVATION.
a. Dam 8344 partially delivered a litter before scheduled sacrifice on day 21 of gestation.

Necropsy observations: All necropsy observations in the F1 generation rats were considered unrelated to treatment of F0 generation dams with T particles because the incidences were not dose-dependent and only occurred in 1 rat. In addition, slight dilation of the left and/or right kidney occurred in the one F1 generation male in each of the maternal 10, 30, and 100 mg/kg/day groups. Note from the reviewer: this kidney dilation finding that appeared as single incidence in all treated groups because T could be excreted unchanged from the kidney and could across into the rat fetus. One F1 generation male rat in the maternal 100 mg/kg/day group had small testes and small epididymides. An additional male in the same maternal group had a mass in the abdominal adipose tissue above the right testis. Moreover, moderate dilation and marked dilation of the pelvis of the right kidney was observed in F1 generation dams in the maternal 0 and 30 mg/kg/day groups. The placentas for fetuses 2 and 3 were fused in a

dam in the maternal 10 mg/kg/day group. No other gross lesions were seen by necropsy examination of the F1 generation rats.

TABLE C2 (PAGE 1): NECROPSY OBSERVATIONS - SUMMARY - F1 GENERATION MALE RATS

MATERNAL DOSAGE GROUP		I	II	III	IV
MATERNAL DOSAGE (MG/KG/DAY)		0 (VEHICLE)	10	30	100
RATS EXAMINED a	N	25	25	25	25
MORTALITY	N	0	0	0	0
APPEARED NORMAL	N	25	24	24	22
KIDNEYS: LEFT AND/OR RIGHT, SLIGHT DILATION	N	0	1	1	1
ABDOMINAL ADIPOSE: ABOVE RIGHT TESTIS, MASS	N	0	0	0	1
TESTES: BILATERAL, SMALL	N	0	0	0	1
EPIDIDYMIDES: BILATERAL, SMALL	N	0	0	0	1

a. Refer to the individual clinical observations table (Table C26) for external observations confirmed at necropsy.

Terminal body weights, epididymides and testes weights and ratios (%) of epididymides and testes weight to terminal body weight: These parameters were examined on pups on Day 90 after birth. Terminal body weights in the F1 generation male rats were comparable among the 4 groups. Absolute weights for paired epididymides and paired testes, as well as the ratio of these organ weights to terminal body weight were significantly reduced in the maternal 100 mg/kg/day group. Absolute weights for paired testes, as well as the ratios of these organs to terminal body weights, were also significantly reduced in the maternal 30 mg/kg/day group. However, these observations were not considered toxicologically important because there were no microscopic changes on testes or epididymides and there were no effects on the mating or fertility of the F1 generation male rats. Moreover, absolute weights for paired epididymides were significantly increased in the maternal 10 mg/kg/day group. This observation was not toxicologically important because the increase was not dose dependent. There was no corresponding increase in the ratio of these organ weights to terminal body weights.

TABLE C3 (PAGE 1): TERMINAL BODY WEIGHTS, EPIDIDYMIDES AND TESTES WEIGHTS AND RATIOS (%) OF EPIDIDYMIDES AND TESTES WEIGHT TO TERMINAL BODY WEIGHT - SUMMARY - F1 GENERATION MALE RATS

MATERNAL DOSAGE GROUP		I	II	III	IV
MATERNAL DOSAGE (MG/KG/DAY)		0 (VEHICLE)	10	30	100
RATS TESTED	N	25	25	25	25
TERMINAL BODY WEIGHT	MEAN±S.D.	526.8 ± 47.2	548.4 ± 45.8	530.4 ± 67.4	529.0 ± 58.8
EPIDIDYMIDES PAIRED	MEAN±S.D.	1.61 ± 0.15	1.76 ± 0.17**	1.52 ± 0.17	1.46 ± 0.14** [24]a
EPIDIDYMIDES PAIRED (%)	MEAN±S.D.	0.306 ± 0.035	0.322 ± 0.037	0.289 ± 0.031	0.277 ± 0.025** [24]a
TESTES PAIRED	MEAN±S.D.	3.55 ± 0.27	3.64 ± 0.26	3.33 ± 0.26**	3.33 ± 0.31** [24]a
TESTES PAIRED (%)	MEAN±S.D.	0.677 ± 0.068	0.666 ± 0.064	0.632 ± 0.060*	0.631 ± 0.047* [24]a

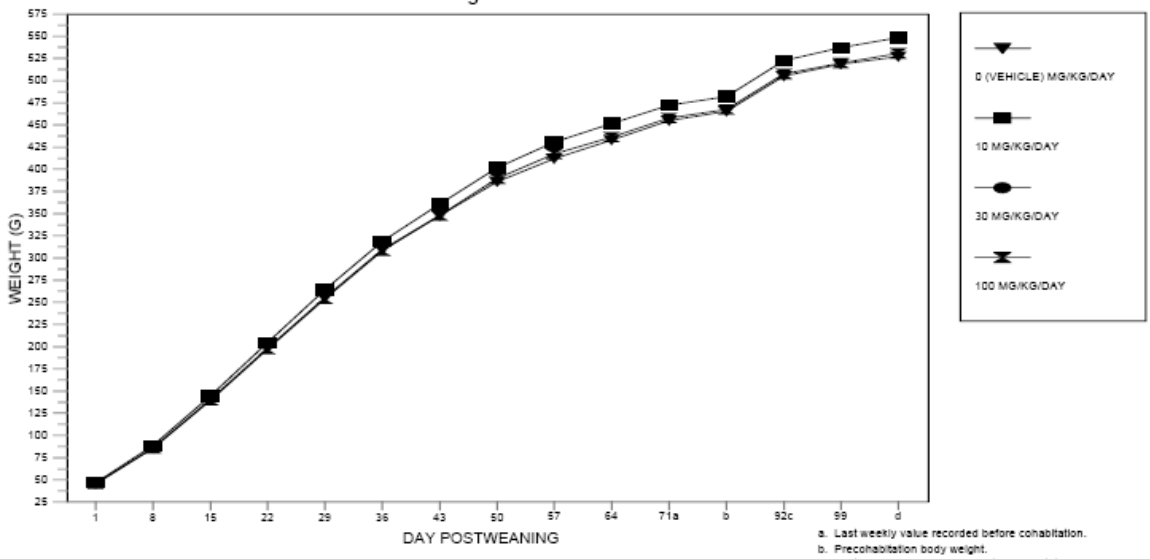
ALL WEIGHTS WERE RECORDED IN GRAMS (G). RATIOS (%) = (ORGAN WEIGHT/TERMINAL BODY WEIGHT) X 100.
[ ] = NUMBER OF VALUES AVERAGED
a. Excludes values for rat 8078, which had abnormal organs.
* Significantly different from the vehicle control group value ($p \leq 0.05$).
** Significantly different from the vehicle control group value ($p \leq 0.01$).

Histopathological Evaluations: Treatment of the F0 generation dams with T particles up to 100 mg/kg/day did not produce microscopic changes in F1 generation male rats, with regards to only testes/epididymis weight, thoracic/abdominal/pelvic viscera examinations. A standard battery of tissues was not evaluated in this study.

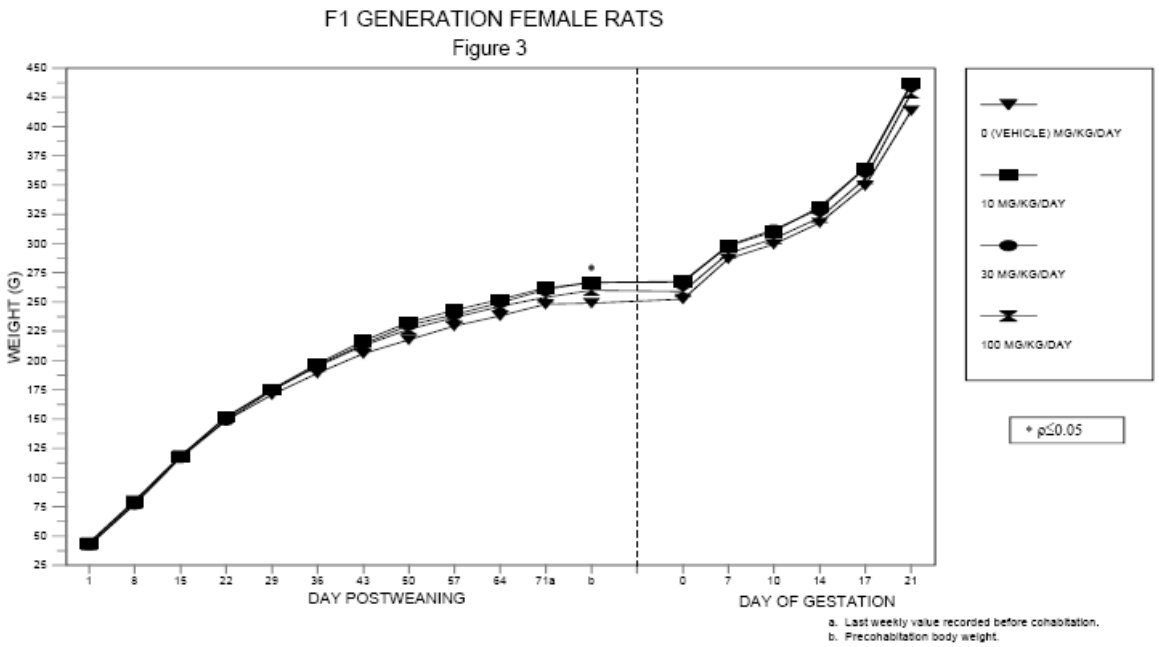
Body weights and body weight changes: Not affected by maternal dosages of T particles up to 100 mg/kg/day. There were no significant differences observed among the 4 dosage groups for the F1 generation male rats during the post-weaning period. In addition, body weight recorded for the F1 generation female rats on the day that cohabitation began, as well as body weight gains during the entire pre-cohabitation period, were significantly increased in the maternal 10 and 30 mg/kg/day groups. These increases were not attributed to maternal treatment with the test article because these increases did not occur in the dose-dependent manner. Moreover, body weight gains for F1 generation dams were significantly increased in the maternal 10, 30, and 100 mg/kg/day groups on GDs 17 to 20. These were not attributed to maternal treatment because body weight gain during the entire gestation period (GDs 0 to 21) was comparable among the 4 groups.

F1 GENERATION MALE RATS

Figure 2



a. Last weekly value recorded before cohabitation.
b. Precohabitation body weight.
c. First weekly value recorded after cohabitation.
d. Terminal body weight.



Absolute and relative food consumption: Not affected by maternal doses of T particles up to 100 mg/kg/day.

Sexual maturation: Not affected by maternal doses of T particles up to 100 mg/kg/day. The average day on which preputial separation occurred was comparable among the 4 groups. The average number of days required for vaginal patency was significantly increased in the 30 mg/kg/day group compared to the average time for the control group. However, it was not toxicologically significant because the increase did not occur in a dose dependent manner.

TABLE C18 (PAGE 1): SEXUAL MATURATION - SUMMARY - F1 GENERATION RATS

MATERNAL DOSAGE GROUP		I	II	III	IV
MATERNAL DOSAGE (MG/KG/DAY)		0 (VEHICLE)	10	30	100
MALE RATS	N	25	25	25	25
PREPUTIAL SEPARATION a	MEAN±S.D.	44.9 ± 1.8	44.9 ± 2.9	45.4 ± 2.0	45.6 ± 2.4
FEMALE RATS	N	25	25	25	25
VAGINAL PATENCY b	MEAN±S.D.	32.0 ± 1.6	32.7 ± 2.0 [24]c	33.5 ± 2.5* [24]c	32.2 ± 1.4 [24]c

[ ] = NUMBER OF VALUES AVERAGED
a. Average day postpartum that the prepuce was observed to be separated.
b. Average day postpartum that the vagina was observed to be patent.
c. Excludes rats in which exact day of maturity can not be determined.
* Significantly different from the vehicle control group value (p≤0.05).

F1 in life & behavioral evaluation findings:

Passive Avoidance: Treatment of T particles had no effect on learning, short-term retention, long-term retention, or response inhibition in the F1 generation male and female rats. One F1 generation male rat in the maternal 30 mg/kg/day group and 1 F1 generation female rat in the maternal 100 mg/kg/day failed to learn. These occurrences were not attributed to the F0 generation dams with the test article because the incidences were not dose-dependent by the sponsor.

TABLE C19 (PAGE 1): PASSIVE AVOIDANCE PERFORMANCE - SUMMARY - F1 GENERATION RATS

MATERNAL DOSAGE GROUP		I	II	III	IV
MATERNAL DOSAGE (MG/KG/DAY)		0 (VEHICLE)	10	30	100
MALE RATS					
SESSION 1a	N	24	24	23	24
TRIALS TO CRITERION	MEAN±S.D.	4.7 ± 1.3	5.1 ± 1.8	4.8 ± 1.2	4.4 ± 1.6
LATENCY TRIAL 1b	MEAN±S.D.	4.9 ± 2.4	7.9 ± 11.9	5.5 ± 3.4	5.5 ± 4.1
LATENCY TRIAL 2b	MEAN±S.D.	29.8 ± 22.2	31.2 ± 23.4	24.5 ± 19.2	34.0 ± 23.1
FAILED TO LEARN c	N(%)	0(0.0)	0(0.0)	1(4.3)	0(0.0)
SESSION 2a	N	24	24	22	24
TRIALS TO CRITERION	MEAN±S.D.	3.5 ± 2.3	3.1 ± 1.5	3.1 ± 1.0	3.5 ± 1.4
LATENCY TRIAL 1b	MEAN±S.D.	31.8 ± 24.3	37.4 ± 24.4	37.3 ± 23.7	28.6 ± 22.6
FEMALE RATS					
SESSION 1a	N	24	23	23	24
TRIALS TO CRITERION	MEAN±S.D.	4.5 ± 1.2	4.8 ± 1.6	4.4 ± 1.1	5.4 ± 2.8
LATENCY TRIAL 1b	MEAN±S.D.	5.9 ± 6.8	5.1 ± 2.5	7.0 ± 3.6	6.5 ± 3.5
LATENCY TRIAL 2b	MEAN±S.D.	31.1 ± 21.9	25.5 ± 21.8	27.1 ± 20.9	28.3 ± 22.0
FAILED TO LEARN c	N(%)	0(0.0)	0(0.0)	0(0.0)	1(4.2)
SESSION 2a	N	24	23	23	23
TRIALS TO CRITERION	MEAN±S.D.	4.0 ± 3.6	3.8 ± 3.0	3.2 ± 0.8	3.1 ± 1.1
LATENCY TRIAL 1b	MEAN±S.D.	35.4 ± 23.7	34.0 ± 24.6	29.3 ± 23.2	26.8 ± 24.0

a. Sessions 1 (Learning Phase) and 2 (Retention Phase) of testing were separated by a one-week interval.
b. The latency was recorded in seconds.
c. Number of rats that did not meet the criterion in Session 1 (Learning Phase); Session 2 (Retention Phase) values for these rats were excluded from summarization and statistical analyses.

Water maze performance: Treatment of T particles had no effect on water maze performance of the F1 generation rats regarding learning, short-term retention, long-term retention, or response inhibition. Two F1 generation male rats in the maternal 30 mg/kg/day group and one F1 generation female rat in each of the maternal 10 and 30 mg/kg/day groups failed to learn. These observations were not attributed to treatment of the F0 generation dams with T particles because the incidences occurred in no more than 2 rats/sex/group and there incidences were not dose-dependent.

TABLE C20 (PAGE 1): WATERMAZE PERFORMANCE - SUMMARY - F1 GENERATION RATS

MATERNAL DOSAGE GROUP		I	II	III	IV
MATERNAL DOSAGE (MG/KG/DAY)		0 (VEHICLE)	10	30	100
MALE RATS					
SESSION 1a	N	24	24	23	24
TRIALS TO CRITERION	MEAN±S.D.	9.3 ± 3.2	8.9 ± 2.2	8.6 ± 2.7	9.0 ± 2.7
ERRORS PER TRIAL	MEAN±S.D.	0.35 ± 0.15	0.52 ± 0.36	0.39 ± 0.28	0.44 ± 0.27
LATENCY TRIAL 2b	MEAN±S.D.	12.6 ± 9.1	17.2 ± 12.9	14.8 ± 11.6	15.2 ± 12.9
FAILED TO LEARN c	N(%)	1(4.2)	0(0.0)	2(8.7)	0(0.0)
SESSION 2a	N	23	24	21	24
TRIALS TO CRITERION	MEAN±S.D.	6.6 ± 2.0	5.9 ± 1.5	6.1 ± 1.8	6.4 ± 2.3
ERRORS PER TRIAL	MEAN±S.D.	0.17 ± 0.19	0.14 ± 0.24	0.12 ± 0.17	0.13 ± 0.17
LATENCY TRIAL 1b	MEAN±S.D.	8.5 ± 4.2	8.6 ± 6.7	8.4 ± 4.2	9.3 ± 5.7
FEMALE RATS					
SESSION 1a	N	24	23	23	24
TRIALS TO CRITERION	MEAN±S.D.	9.8 ± 2.8	9.3 ± 2.6	9.3 ± 2.6	9.3 ± 3.0
ERRORS PER TRIAL	MEAN±S.D.	0.40 ± 0.17	0.51 ± 0.27	0.43 ± 0.23	0.42 ± 0.27
LATENCY TRIAL 2b	MEAN±S.D.	15.5 ± 9.8	15.2 ± 9.5	17.5 ± 13.5	13.6 ± 12.6
FAILED TO LEARN c	N(%)	0(0.0)	1(4.3)	1(4.3)	0(0.0)
SESSION 2a	N	24	22	22	24
TRIALS TO CRITERION	MEAN±S.D.	7.7 ± 3.0	8.0 ± 3.5	6.5 ± 2.5	7.1 ± 3.5
ERRORS PER TRIAL	MEAN±S.D.	0.26 ± 0.28	0.20 ± 0.18	0.11 ± 0.16	0.12 ± 0.22
LATENCY TRIAL 1b	MEAN±S.D.	10.1 ± 8.4	11.4 ± 7.5	9.7 ± 5.0	9.1 ± 6.0

a. Sessions 1 (Learning Phase) and 2 (Retention Phase) of testing were separated by a one-week interval.
b. The latency was recorded in seconds.
c. Number of rats that did not meet the criterion in Session 1 (Learning Phase); Session 2 (Retention Phase) values for these rats were excluded from summarization and statistical analyses.

F1 in life & reproduction findings:

There were no effects on the mating and fertility parameters evaluated in the F1 generation rats. Values for the number of days in cohabitation, the number of rats that mated, the fertility index, the number of rats with confirmed mating dates during the 1st, 2nd, and/or 3rd weeks of cohabitation, and the number of pregnancies per number of rats in cohabitation were comparable among the 4 groups and did not significantly differ.

TABLE C21 (PAGE 1): MATING AND FERTILITY - SUMMARY - F1 GENERATION MALE RATS

MATERNAL DOSAGE GROUP		I	II	III	IV
MATERNAL DOSAGE (MG/KG/DAY)		0 (VEHICLE)	10	30	100
RATS IN COHABITATION	N	25	25	25	25
DAYS IN COHABITATION a	MEAN±S.D.	3.6 ± 3.3	3.2 ± 3.0	4.1 ± 3.6	3.3 ± 3.1
RATS THAT MATED b	N(%)	24(96.0)	24(96.0)	23(92.0)	25(100.0)
FERTILITY INDEX c,d	N/N	23/ 24	22/ 24	21/ 23	24/ 25
	(%)	(95.8)	(91.7)	(91.3)	(96.0)
RATS WITH CONFIRMED MATING DATES	N	24	24	23	25
MATED WITH FEMALE e					
DAYS 1-7	N(%)	23(95.8)	23(95.8)	22(95.6)	22(88.0)
DAYS 8-14	N(%)	1(4.2)	1(4.2)	1(4.3)	3(12.0)
RATS PREGNANT/RATS IN COHABITATION d	N/N	23/ 25	22/ 25	21/ 25	24/ 25
	(%)	(92.0)	(88.0)	(84.0)	(96.0)

a. Restricted to rats with a confirmed mating date and rats that did not mate.
b. Includes only one mating for each male rat.
c. Number of pregnancies/number of rats that mated.
d. Includes only one pregnancy for each rat that impregnated more than one female rat.
e. Restricted to rats with a confirmed mating date.

TABLE C22 (PAGE 1): MATING AND FERTILITY - SUMMARY - F1 GENERATION FEMALE RATS

MATERNAL DOSAGE GROUP		I	II	III	IV
MATERNAL DOSAGE (MG/KG/DAY)		0 (VEHICLE)	10	30	100
RATS IN COHABITATION	N	25	25	25	25
DAYS IN COHABITATION a	MEAN±S.D.	3.7 ± 3.7	3.2 ± 3.1	4.3 ± 4.1	3.3 ± 3.1
RATS THAT MATED	N(%)	25(100.0)	25(100.0)	25(100.0)	25(100.0)
FERTILITY INDEX b	N/N	24/ 25	23/ 25	23/ 25	24/ 25
	(%)	(96.0)	(92.0)	(92.0)	(96.0)
RATS WITH CONFIRMED MATING DATES	N	25	25	25	25
MATED BY FIRST MALE c					
DAYS 1-7	N(%)	23(92.0)	23(92.0)	22(88.0)	22(88.0)
DAYS 8-14	N(%)	1(4.0)	1(4.0)	1(4.0)	3(12.0)
MATED BY SECOND MALE c					
DAYS 15-21	N(%)	1(4.0)	1(4.0)	2(8.0)	0(0.0)
RATS PREGNANT/RATS IN COHABITATION	N/N	24/ 25	23/ 25	23/ 25	24/ 25
	(%)	(96.0)	(92.0)	(92.0)	(96.0)

a. Restricted to rats with a confirmed mating date and rats that did not mate.
b. Number of pregnancies/number of rats that mated.
c. Restricted to rats with a confirmed mating date.

C-sectioning and litter observations: No c-sectioning or litter parameters were affected by maternal dosages of T particles up to 100 mg/kg/day. C-sectioning observations were based on 24, 22, 23, and 24 pregnant rats with 1 or more live fetuses in Groups I through IV, respectively. Also, the mean number of early resorptions, the mean % of dams with any resorptions and the mean % of resorbed conceptuses per litter were significantly reduced in the maternal 10 mg/kg/day group. The mean % of dams with any resorptions was significantly reduced in the maternal 30 mg/kg/day corresponding increase in each of these parameters in the control group. Therefore, the sponsor did not considered these

observations to be toxicologically relevant. Moreover, the litter averages for corpora lutea, implantations, litter sizes, live fetuses, late resorptions, fetal body weights, and % live male fetuses were comparable among the 4 groups. No dam had a litter consisting only resorbed conceptuses, and there were no dead fetuses. All placentae appeared normal.

TABLE C23 (PAGE 1): CAESAREAN-SECTIONING OBSERVATIONS - SUMMARY - F1 GENERATION FEMALE RATS

MATERNAL DOSAGE GROUP		I	II	III	IV
MATERNAL DOSAGE (MG/KG/DAY)		0 (VEHICLE)	10	30	100
RATS TESTED	N	25	25	25	25
PREGNANT	N(%)	24(96.0)	23(92.0)	23(92.0)	24(96.0)
DELIVERED AND SACRIFICED	N(%)	0(0.0)	1(4.3)	0(0.0)	0(0.0)
RATS PREGNANT AND CAESAREAN-SECTIONED ON DAY 21 OF GESTATION	N	24	22	23	24
CORPORA LUTEA	MEAN±S.D.	15.7 ± 1.6	16.0 ± 2.8	16.4 ± 3.0	16.0 ± 2.2
IMPLANTATIONS	MEAN±S.D.	15.1 ± 1.5	14.9 ± 2.4	15.6 ± 2.5	15.0 ± 2.0
LITTER SIZES	MEAN±S.D.	13.5 ± 2.2	14.6 ± 2.5	14.9 ± 2.1	14.2 ± 2.1
LIVE FETUSES	N	325	321	342	342
	MEAN±S.D.	13.5 ± 2.2	14.6 ± 2.5	14.9 ± 2.1	14.2 ± 2.1
DEAD FETUSES	N	0	0	0	0
RESORPTIONS	MEAN±S.D.	1.6 ± 2.2	0.3 ± 0.7**	0.7 ± 0.9	0.8 ± 0.9
EARLY RESORPTIONS	N	36	7	17	19
	MEAN±S.D.	1.5 ± 2.1	0.3 ± 0.7**	0.7 ± 0.9	0.8 ± 0.9
LATE RESORPTIONS	N	2	0	0	0
	MEAN±S.D.	0.1 ± 0.3	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
DAMS WITH ANY RESORPTIONS	N(%)	17(70.8)	5(22.7)**	11(47.8)*	14(58.3)
DAMS WITH ALL CONCEPTUSES RESORBED	N(%)	0(0.0)	0(0.0)	0(0.0)	0(0.0)
DAMS WITH VIABLE FETUSES	N(%)	24(100.0)	22(100.0)	23(100.0)	24(100.0)
PLACENTAE APPEARED NORMAL	N(%)	24(100.0)	21(95.4)	23(100.0)	24(100.0)

* Significantly different from the vehicle control group value ($p \leq 0.05$).
** Significantly different from the vehicle control group value ($p \leq 0.01$).

TABLE C24 (PAGE 1): LITTER OBSERVATIONS (CAESAREAN-DELIVERED FETUSES) - SUMMARY - F1 GENERATION FEMALE RATS

MATERNAL DOSAGE GROUP		I	II	III	IV
MATERNAL DOSAGE (MG/KG/DAY)		0 (VEHICLE)	10	30	100
LITTERS WITH ONE OR MORE LIVE FETUSES	N	24	22	23	24
IMPLANTATIONS	MEAN±S.D.	15.1 ± 1.5	14.9 ± 2.4	15.6 ± 2.5	15.0 ± 2.0
LIVE FETUSES	N	325	321	342	342
	MEAN±S.D.	13.5 ± 2.2	14.6 ± 2.5	14.9 ± 2.1	14.2 ± 2.1
LIVE MALE FETUSES	N	170	160	169	185
% LIVE MALE FETUSES/LITTER	MEAN±S.D.	52.1 ± 13.4	50.4 ± 14.9	50.1 ± 13.1	53.9 ± 15.0
LIVE FETAL BODY WEIGHTS (GRAMS)/LITTER	MEAN±S.D.	5.32 ± 0.36	5.37 ± 0.30	5.31 ± 0.26	5.36 ± 0.28
MALE FETUSES	MEAN±S.D.	5.45 ± 0.41	5.49 ± 0.33	5.43 ± 0.27	5.48 ± 0.30
FEMALE FETUSES	MEAN±S.D.	5.17 ± 0.35	5.25 ± 0.28	5.18 ± 0.28	5.20 ± 0.32
% RESORBED CONCEPTUSES/LITTER	MEAN±S.D.	10.0 ± 12.9	2.1 ± 4.4**	4.3 ± 5.2	5.3 ± 5.8

** Significantly different from the vehicle control group value ($p \leq 0.01$).

F2 in life findings:

None of the gross external alternations in the F2 generation fetuses were attributed to treatment of the F0 generation dams with T particles as high as 100 mg/kg/day. Fetus (8379-14) in the

maternal 100 mg/kg/day group had a depressed left eye bulge and whole body edema, whereas fetus (8320-5) in the maternal 10 mg/kg/day group had thread-like tails, which did not exceed concurrent controls. All other fetuses appeared normal at gross external examination. These alterations however were not attributed to treatment of the F0 generation dams with the T particles because the alternations occurred in single fetus.

TABLE C25 (PAGE 1): FETAL GROSS EXTERNAL ALTERATIONS - SUMMARY - F2 GENERATION LITTERS/FETUSES

MATERNAL DOSAGE GROUP		I	II	III	IV
MATERNAL DOSAGE (MG/KG/DAY)		0 (VEHICLE)	10	30	100
LITTERS EVALUATED	N	24	22	23	24
FETUSES EVALUATED	N	325	321	342	342
LIVE	N	325	321	342	342
EYES: LEFT, BULGE DEPRESSED					
LITTER INCIDENCE	N(%)	0(0.0)	0(0.0)	0(0.0)	1(4.2)
FETAL INCIDENCE	N(%)	0(0.0)	0(0.0)	0(0.0)	1(0.3)a
BODY: WHOLE BODY EDEMA					
LITTER INCIDENCE	N(%)	0(0.0)	0(0.0)	0(0.0)	1(4.2)
FETAL INCIDENCE	N(%)	0(0.0)	0(0.0)	0(0.0)	1(0.3)a
TAIL: THREAD-LIKE					
LITTER INCIDENCE	N(%)	1(4.2)	1(4.5)	0(0.0)	0(0.0)
FETAL INCIDENCE	N(%)	1(0.3)	1(0.3)	0(0.0)	0(0.0)

a. Fetus 8379-14 had other gross external alterations.

2.6.6.7 Local tolerance

MKC-PC-2007-0019: A Single Dose Primary Skin Irritation Study in Rabbits with Technosphere® Particles and Technosphere®/Insulin [By (b) (4) Study No. LCL00021]

The potential irritant and/or corrosive effects of T and TI were evaluated in the skin of New Zealand White rabbits. Initially, 1 animal per test article received a single dermal application to one intact and one abraded test sites. Since no severe irritation was observed, 5 additional animals per test article were dosed for a total of 6 rabbits in each group (see table below). These doses were applied to the skin for 24 hrs. Test sites were subsequently examined and scored for dermal irritation for up to 14 days following test article application.

Group	Test Site	Treatment	Test Material	Dose Volume (g)	No. of Animals Male
1	1	Intact	Technosphere®	0.50[a]	6
	2	Abraded	Technosphere®	0.50[a]	
2	1	Intact	Technosphere®/Insulin	0.50[b]	6
	2	Abraded	Technosphere®/Insulin	0.50[b]	

[a]moistened with 21 drops (~ 0.62 mL) of deionized water to form a paste.
[b]moistened with 22 drops (~ 0.67 mL) of deionized water to form a paste.

For T on intact test sites, it produced well-defined to moderate erythema and very slight to slight edema at 6/6 sites by 1 hr scoring interval. The dermal irritation resolved at 1/6 sites by the 72 hr scoring interval, at 3/6 sites by Study Day 7, at 1/6 sites by Study Day 10, and at 1/6 sites by Study Day 14. Desquamation was also noted at 2/6 sites during the study. For T on abraded test sites, it produced well-defined to moderate to severe erythema and very slight to slight edema at 6/6 sites by 1 hr scoring interval. The dermal irritation resolved at 3/6 sites by Study Day 7, at 2/6 sites by Study Day 10, and at 1/6 test sites by Study Day 14. Desquamation was also noted at 3/6 test sites during the study.

For TI on intact test sites, it produced well-defined to moderate to severe erythema at 6/6 sites and very slight edema at 5/6 sites by 1 hr scoring interval. The dermal irritation resolved at 3/6 sites by the 72 hr scoring interval, at 2/6 sites by Study Day 7, at 1/6 sites by Study Day 10, and at 1/6 sites by Study Day 14. Desquamation was also noted at 2/6 sites during the study. For TI on abraded test sites, it produced well-defined to moderate to severe erythema and very slight to slight edema at 6/6 sites by 1 hr scoring interval. The dermal irritation resolved at 3/6 sites by Study Day 7, at 2/6 sites by Study Day 10, and at 1/6 test sites by Study Day 14. Desquamation was also noted at 3/6 test sites during the study.

All in all, both T and TI were considered to be moderate irritants to the skin of the rabbit.

Based on Primary Irritation

Indices calculated for EPA-FIFRA criteria in the table that follows, the test articles were biologically similar.

Test Material	Primary Irritation Index (P.I.I.)
Technosphere® - intact	2.38
Technosphere® - abraded	2.17
Technosphere®/Insulin –intact	2.00
Technosphere®/Insulin – abraded	2.33

Based on the EEC Evaluation Criteria, Technosphere® is classified for erythema and edema as indicated below:

Mean Irritation Scores				
Group	Erythema-Intact	Erythema-Abraded	Edema-Intact	Edema-Abraded
1	1.44	1.39	0.67	0.44
Irritation Rating	Nonirritant	Nonirritant	Nonirritant	Nonirritant

Based on the EEC Evaluation Criteria, Technosphere®/Insulin is classified for erythema and edema as indicated below:

Mean Irritation Scores				
Group	Erythema-Intact	Erythema-Abraded	Edema-Intact	Edema-Abraded
2	1.28	2.28	0.39	0.44
Irritation Rating	Nonirritant	Irritant	Nonirritant	Nonirritant

Based on Primary Irritation Indices calculated for FDA criteria in the table that follows, the test articles were biologically similar and are considered to be nonirritants to the skin of the rabbit.

Test Material	Primary Irritation Index (P.I.I.)
Technosphere® - intact	2.67
Technosphere® - abraded	2.50
Technosphere®/Insulin –intact	2.42
Technosphere®/Insulin – abraded	2.58

2.6.6.8 Special toxicology studies

13 studies were submitted by the sponsor:

- PDC-PC-2001-0001 (antigen)
- MKC-PC-2007-0004 (other: phototox)
- MKC-PC-2008-0001 (other: ecotox), Daphnia magna)
- MKC-PC-2008-0002 (other: ecotox, green alga)
- MKC-PC-2008-0005 (other: PS80 tox/TK profile)
- MKC-PC-2008-2006 (impurities)
- MKC-PC-2008-0016 (other: skin irritation w/ 3 FDKP intermediates)
- MKC-PC-2008-0018 (other: delayed contact hypersensitivity w/ 3 FDKP intermediates)
- MKC-PC-2008-0019 (other: acute oral tox w/ 3 FDKP intermediates)
- MKC-PC-2008-0020 (immunotox)
- MKC-PC-2008-0021 (other: partition coefficient of FDKP)
- MKC-PC-2008-0023 (other: eye irritation w/ 3 FDKP intermediates)
- MKC-PC-2008-0024 (other: biodegradability of FDKP)

PDC-PC-2001-0001: Dermal Sensitization w/ Technosphere in Guinea Pigs – Maximization Test [By (b) (4) Study No. 7260-100]

This study was to assess the contact sensitization potential of FDKP via the intradermal injection and topical application in guinea pigs. Total of 38 healthy young adult male albino guinea pigs were participated in this two-phased study. First phase was an irritation screening study using 8 animals to determine the irritation threshold of the test article, FDKP. This study was to find 1) the highest concentration that does not produce excessive irritation to use for intradermal injections, 2) a moderately irritating concentration to use for the topical induction application, and 3) the highest nonirritating topical concentration to use for the challenge application.

Identification	Number of Animals
Irritation screening study	
Topical application group	4
Intradermal injection group	4
Definitive study	
Test group	20
Initial irritation control group	10

For irritation screening study, 4 animals received FDKP (1% w/v in sterile water) via intradermal injection showed mild to moderate dermal reactions. Moderate dermal reactions were exhibited to the 5% w/v concentration, and moderate to intense dermal reactions were observed to the 10, 15 and 25% w/v concentrations of FDKP in sterile water. Blanching of the injection sites were also seen at concentrations > 5%. In addition, 4 other animals received FDKP via topical application. No dermal irritation was seen with either of the concentration applied (10 or 25% w/w in petrolatum).

Table 1: Individual Body Weights and Dermal Reactions - Irritation Screening Study - Intradermal Injections

		Dermal Reactions									
		Test Material Concentration (% w/v in sterile water)									
	Body	1%		5%		10%		15%		25%	
Animal	Weight	Hour		Hour		Hour		Hour		Hour	
Number	(g)	24	48	24	48	24	48	24	48	24	48
E25880	469	1	1	2[b]	2[b]	2[b]	2[b]	2[b]	2[b]	2[b]	2
E25882	474	1	1	2[b]	2	2[b]	3[b]	2[b]	3[b]	2[b]	3[b]
E25883	502	1	1	2[b]	2[b]	2	2	2[b]	2[b]	2	2
E25884	463	2	1	2	1	2[b]	2[b]	2[b]	2[b]	2	2

b Blanching.

Table 2: Individual Body Weights and Dermal Reactions - Irritation Screening Study - Topical Application

		Dermal Reactions			
		Test Material Concentration (% w/w in petrolatum)			
		10%		25%	
Animal	Body	Hour		Hour	
Number	Weight (g)	24	48	24	48
E25876	540	0	0	0	0
E25877	499	0	0	0	0
E25878	506	0	0	0	0
E25879	469	0	0	0	0

For definitive study, all animals in all groups appeared normal throughout the study with the exception of 1 animal in the initial irritation control group which exhibited a thin appearance on Days 11 through 20. All animals showed body weight gain during the study with the exception of one additional control animal that maintained the same weight from Day 36 to Day 39. With regards to dermal reaction at induction sites, the irritation was consisted of mild to moderate erythema and edema reactions with scab formation at injection sites. The irritation at the induction sites of the animals in the test group was slightly greater than the ones in the initial irritation control group. With regards to dermal reaction to FDKP at initial challenge, 10 out of 20 animals (in the test group) showed mild dermal reactions to the initial challenge application of the 25% w/w mixture of FDKP at 24 hrs. At 48 hrs, 2 out of these 10 animals no longer had dermal reactions but 3 animals that didn't have reaction at 24 hrs exhibited dermal reactions at 48 hrs. In addition, 1 out of 20 animals (in the test group) showed a mild dermal reaction to the application of petrolatum at 48 hrs only. Moreover, 2 out of 10 animals (in the initial irritation control group) showed mild dermal reactions to the 25% w/w mixture of the FDKP at the initial challenge phase at the 24 hr observation, but 1 out of these two animals no longer had a dermal reaction at 48 hrs. With regards to dermal reaction to FDKP at second challenge, 4 out of 20 animals (in the test group) showed mild to moderate dermal reactions to 25% w/w mixture of the FDKP at 24 hr observation, and in 3 of the same animals at 48 hrs. Of the 3 of 20 animals with reactions (in the test group), 2 had a scattered mild redness reaction and 1 had a moderate and diffuse redness reaction. These reactions were considered to be probable sensitization reactions. In addition, none of the 5 additional native control animals showed a dermal reaction to 25% w/w of FDKP at the second challenge.

Overall, the EC and US EPA Guidelines require a response of > 30% (i.e. 6 of 20 animals) to classify a compound as a skin sensitizer by an adjuvant-type test method. Based on the number of animals in the test group that was considered to have exhibited sensitization reactions at the second challenge (i.e. 3 of 20 animals), a risk phase was not required for dermal sensitization hazard. Note from the reviewer: It is unclear how this mild to moderate skin sensitization would relate to the clinical use by inhalation.

Table 6: Individual Dermal Reactions to Challenge Application

Animal Number	Initial Challenge				Second Challenge	
	Test Material†		Petrolatum		Test Material†	
	Hour		Hour		Hour	
	24	48	24	48	24	48
Test Group						
E26068	0	0	0	0	0	0
E26069	0	0	0	0	0	0
E26070	1	0	0	0	0	0
E26071	1	1	0	0	2	2
E26072	1	0	0	0	0	0
E26073	1	1	0	0	1	1
E26074	0	0	0	0	0	0
E26075	0	0	0	0	0	0
E26076	0	0	0	0	0	0
E26077	1	1	0	0	0	0
E26078	0	1	0	0	0	0
E26079	1	1	0	0	0	0
E26080	0	1	0	0	0	0
E26081	1	1	0	0	0	0
E26082	0	1	0	1	0	0
E26083	0	0	0	0	0	0
E26084	0	0	0	0	0	0
E26085	1	1	0	0	0	0
E26086	1	1	0	0	1	1
E26087	1	1	0	0	1	0

† Fumaryl Technosphere™ administered as a 25% w/w mixture in petrolatum.

Table 6 (continued): Individual Dermal Reactions to Challenge Application

Animal Number	Initial Challenge				Second Challenge	
	Test Material†		Petrolatum		Test Material†	
	Hour		Hour		Hour	
	24	48	24	48	24	48
Initial Irritation Control Group						
E26088	0	0	0	0	*	*
E26089	0	0	0	0	*	*
E26090	0	0	0	0	*	*
E26091	1	1	0	0	*	*
E26092	0	0	0	0	*	*
E26093	0	0	0	0	*	*
E26094	0	0	0	0	*	*
E26095	0	0	0	0	*	*
E26096	1	0	0	0	*	*
E26097	0	0	0	0	*	*
Additional Irritation Control Group						
E26333	*	*	*	*	0	0
E26334	*	*	*	*	0	0
E26335	*	*	*	*	0	0
E26336	*	*	*	*	0	0
E26337	*	*	*	*	0	0

† Fumaryl Technosphere™ administered as a 25% w/w mixture in petrolatum.
* Animal not treated during this phase of study.

MKC-PC-2007-0004: Neutral Red Uptake Phototoxicity Assay of Technosphere and Technosphere/Insulin in Balb/c 3T3 Fibroblasts [By (b) (4) Study No. LCL00016]

This study was to evaluate the phototoxicity potential of T and TI measured by the relative reduction in viability of Balb/c 3T3 mouse fibroblasts administered the test articles and exposed to UVA, as compared with those without UVA. One range-finding assay and two definitive assays were conducted.

In the range-finding assay, up to 1000 mg/l of T and up to 1000 mg/l TI were tested. However, the IC50 with or without UVA could not be achieved for T and TI. Since no IC50s were determined, the PIF (photoirritancy factor) values could not be calculated. Therefore, the PIF was designated as 1 to characterize the results. The MPE (mean photo effect) values for T and TI were 0.007 and -0.001, respectively. The CPZ (chlorpromazine) was used as positive control. Its IC50 was 1.396 mg/l with UVA and 38.96 mg/l without UVA. The PIF and MPE values for CPZ were 27.913 and 0.490, respectively.

MPE and PIF Cut-Off Values for 3T3 NRU PT Assay

Mean Photo Effect (MPE)	Photoirritancy Factor (PIF)	Phototoxic Potential
<0.1	<2	Non-phototoxic
>0.1 and <0.15	>2 and <5	Probably phototoxic
>0.15	>5	Phototoxic

Range-Finding Assay Summary

FORMULATION	MEAN PHOTO EFFECT (MPE)	PHOTOIRRITANCY FACTOR (PIF)	IC_{50} (mg/L)	
			+UVA	-UVA
Technosphere® Particles	0.007	*1 [a]	NA	NA
Technosphere®/Insulin	-0.001	*1 [a]	NA	NA
Chlorpromazine	0.490	27.913	1.396	38.96

a. Since the test article did not permit determination of either the IC_{50}(-UVR) or IC_{50}(+UVR), a PIF value could not be calculated and a photoirritancy factor designated as PIF = *1 was assigned to characterize the results.

IC_{50}: Inhibition Concentration-50, is the effective concentration of a test or control article to reduce the maximum activity/viability of the cells by 50%.

+UVA: with UVA (315-400 nm) exposure 5 J/cm^2

- UVA: without UVA exposure

NA: Not achieved

In the definitive assays, up to 1000 mg/l for T and up to 1000 mg/l for TI were tested with and without UVA exposure. The assay results showed that both T and TI did not elicit cytoxicity in the absence and presence of UVA irradiation. Again, since no IC50s were determined for T or TI, the PIF values were designated as 1 to characterize the results. The MPE values for T were -0.012 and -0.007 in definitive assays 1 and 2, respectively. The MPE values for TI were -0.013 and -0.009 in definitive assays 1 and 2, respectively. The CPZ was again used as positive control. Its IC50 was 1.419 mg/l with UVA and 37.22 mg/l without UVA. The PIF and MPE values for CPZ were 26.233 and 0.458, respectively.

Inhibition Concentration (IC_{50})
The Assay Summary

Formulation	IC_{50} (mg/L)					
	Range-Finding Assay		Definitive Assay 1		Definitive Assay 2	
	+UVA	-UVA	+UVA	-UVA	+UVA	-UVA
Technosphere® Particles	NA	NA	NA	NA	NA	NA
Technosphere®/Insulin	NA	NA	NA	NA	NA	NA
Chlorpromazine	1.396	38.96	1.419	37.22	1.419	37.22

IC_{50} = Inhibition Concentration-50, is the effective concentration of a test or control article to reduce the maximum activity/viability of the cells by 50%.

+UVA = with UVA (315-400 nm) exposure 5 J/cm^2

-UVA = without UVA exposure

NA = Not achieved

Mean Photo Effect (MPE) and Photoirritancy Factor (PIF)
The Assay Summary

Formulation	Range-Finding Assay		Definitive Assay 1		Definitive Assay 2	
	MPE	PIF	MPE	PIF	MPE	PIF
Technosphere® Particles	0.007	*1 [a]	-0.012	*1 [a]	-0.007	*1 [a]
Technosphere®/Insulin	-0.001	*1 [a]	-0.013	*1 [a]	-0.009	*1 [a]
Chlorpromazine	0.490	27.913	0.458	26.233	0.458	26.233

a. Since the test article did not permit determination of either the IC_{50}(-UVR) or IC_{50}(+UVR), a PIF value could not be calculated and a photoirritancy factor designated as PIF = *1 was assigned to characterize the results.

Overall, based on the definitive assay results and the OECD (Organization for Economic Cooperation and Development) guidelines for the conduct of the 3T3 NRU test, the phototoxic potential for both T and TI were non-phototoxic.

MKC-PC-2008-0001: Technosphere® Powder Acute Toxicity to *Daphnia Magna* [By (b) (4) Study No. MKC0002]

This study was to study the acute toxicity of T to *Daphnia magna* under static exposure conditions. Twenty Daphnia (less than 24 hrs old) was exposed to T for 48 hrs at 100 mg/l (an overall mean measured level of 97.2 mg/l. Findings: One Daphnia (5%) was immobile after 48 hrs exposure to T at 97.2 mg/l. According to the OECD guideline, an incidence rate of < 10% was not considered to be significant. This study revealed that the 48 hr IC_{50} for T was > 97.2 mg/l and the NOEL for T was 97.2 mg/l.

MKC-PC-2008-0002: Technosphere® Powder Algal Growth Inhibition Assay [By (b) (4) Study No. MKC0003]

This study was to assess the growth of the unicellular green alga *Pseudokirchneriella subcapitata* under non-axenic conditions. Six replicate algal cultures, with an initial cell density of 1 x 10^4 per ml were exposed to T at 100 mg/l (an overall mean measured level of 101 mg/l). Cell numbers were counted daily to monitor growth. Findings: When compared to control cultures, neither the AUC nor the average specific growth rate was significantly reduced at a mean measured level of 101 mg/l. Both E_bC_{50} (72 hr, AUC) and E_rC_{50} (0-72hr, average specific growth rate) were > 101 mg/l. NOEC (no observed effect concentration) was 101 mg/l.

Cell densities

Technosphere® Powder exposure concentration (mg/L) Nominal	Measured	Replicate number	Cell densities (cells/mL) 24 hours	48 hours	72 hours
Control	nd	R_1	50717	285000	1390017
		R_2	51350	259900	1382383
		R_3	46017	269733	1351683
		R_4	50150	264833	1393250
		R_5	50417	274567	1298017
		R_6	46617	271833	1376417
		Mean	*49211*	*270978*	*1365294*
100	101	R_1	54983	313633	1419917
		R_2	51917	299633	1420383
		R_3	53583	293700	1400017
		R_4	55050	296333	1384583
		R_5	57117	308767	1302883
		R_6	52883	299000	1338350
		Mean	*54256*	*301844*	*1377689*

nd : none detected (less than the limit of detection of 1 mg/L)
R_1.R_6 : replicate number

Note : the initial cell density was estimated to be 1.29 x 10^4/mL.

Inhibition of growth

Parameter	Concentration (mg/L) Nominal	Measured	Sample size	Mean	% Inhibition	*p*
Area under curve to 72 hours	0	0	6	23.5	0.0	-
	100	101	6	24.5	-4.3	0.007**
Growth rate to 72 hours	0	0	6	0.068	0.0	-
	100	101	6	0.068	-0.2	0.634
Growth Rate 0-24 hours	0	0	6	0.066	0.0	-
	100	101	6	0.070	-6.2	0.002**
Growth Rate 24-48 hours	0	0	6	0.071	0.0	-
	100	101	6	0.071	-0.6	0.713
Growth Rate 48 – 72 hours	0	0	6	0.067	0.0	-
	100	101	6	0.063	6.1	0.003**

p values are for the comparison with Control using the *t*-test
** $p < 0.01$

MKC-PC-2008-0005: A 28-Day Powder Inhalation Toxicity Study Followed by a 14-Day Recovery Period of PS80-Enriched Technosphere® Powder in Spraque-Dawley Rats [By (b) (4) Study No. 75065]

This study was to determine the toxicity and TK profile in rats given PS80-enriched T via 60 min inhalation exposure for 28 days with 14 day recovery period. Mortality, clinical signs, body weights, food consumption, ophthalmoscopy and clinical pathology were assessed. Serial blood samples were collected to determine circulating serum FDKP levels. All animals were euthanized upon completion of their respective treatment or recovery periods. Selected tissues were retained, weighted, and examined microscopically.

Group Number	Group Designation	Target Dose Level[A] (mg/kg/day)	Theoretical Target Dose Level of PS80[B] (mg/kg/day)	Toxicology Groups				Toxicokinetics Groups	
				Main		Recovery			
				Male	Female	Male	Female	Male	Female
1	Air Control	0	0	10	10	-	-	3	3
2	Low Dose	17 / 23[C]	0.51 / 0.46[C]	10	10	-	-	6	6
3	High Dose	50	1.00	10	10	10	10	6	6

[A] Dose levels are expressed in terms of Technosphere® Powder.
[B] Target values were based on a nominal purity of the PS80 in the Technosphere® Powder (3.0% May 14 to 22, 2008 and 2.0% for the remainder of the study). Achieved values were calculated based on actual PS80 content as determined in Section 5.4.
[C] Target dose levels were adjusted on Day 8 of exposure at the request of the Sponsor to ensure compatibility with the proposed clinical regimen, based on PS80 determinations conducted during Week 1 of exposure.

Tox findings: Inhalation administration of PS80-enriched FDKP was well-tolerated. There were no adverse clinical signs or ocular changes related to PS80-enriched T treatment. Following necropsy, there were also no organ weight changes or macroscopic or microscopic findings.

TK findings: The PS80-enriched T was readily absorbed with T_{max} between 0.05 to 0.25 hr across all dose groups. Exposure, based on C_{max} and AUC_{0-t} increased in a dose-dependent manner on Days 1 and 28 in males and females. Mean PS80-enriched T increased by 1.7 to 2.3 fold in males and 1.7 to 2.2 fold in females on Days 1 and 28 respectively. Mean PS80-enriched T at the high dose group were similar in males compared to females on Days 1 and 28, indicating no gender differences in exposure. In addition, the accumulation ratio (Rc) for PS80-enriched T ranged from 1.18 to 1.81 across males and females, indicating no accumulation following 28 days of daily dosing.

Overall, inhalation administration of PS80-enriched T once daily for 28 days was well-tolerated by rats. There were no indications of local and systemic effects related to the test article. The NOEL was at the high dose of 45 mg/kg/day for Technosphere® powder and 1.11 mg/kg/day for PS80.

Macroscopic Findings:

GROUP INCIDENCE: NECROPSY - ALL DATA

Kill type: TERMINAL

Tissue / Observation	Group:	1	2	3	1	2	3
	Sex:	M	M	M	F	F	F
	Number:	10	10	10	10	10	10
ADRENAL							
Pale discoloration		0	0	0	0	0	1
KIDNEY							
Depressed area		0	0	1	0	0	0
Dilatation		0	0	0	0	1	0
LYMPH NODE, MANDIBULAR							
Dark discoloration		1	0	0	0	0	0
Dark focus		0	0	0	0	1	0
LUNG							
Dark area		0	1	0	1	0	0
STOMACH							
Dark focus		0	0	1	0	0	0
Foreign body		0	0	1	0	0	0
THYMUS							
Dark area		1	1	1	0	1	0
Dark discoloration		0	0	1	1	1	1
Dark focus		1	0	0	0	0	0

GROUP INCIDENCE: NECROPSY - ALL DATA

Kill type: RECOVERY

Tissue / Observation	Group: / Sex: / Number:	3 / M / 10	3 / F / 10
KIDNEY			
Dilatation		1	1
LYMPH NODE, BRONCHIAL			
Enlargement		1	0
LIVER			
Firm		1	0
Pale area		1	0
LYMPH NODE, MANDIBULAR			
Dark focus		2	2
LUNG			
Dark area		2	0
Dark focus		2	0
SKIN & SUBCUTIS			
Gelatinous material		1	0
THYMUS			
Dark area		2	1
Dark discoloration		1	0
Mottled		1	0

Microscopic Findings:

Kill type: TERMINAL

Tissue / Observation	Group:	1	2	3	1	2	3
	Sex:	M	M	M	F	F	F
	Number:	10	10	10	10	10	10
EYE							
Number examined		10	0	10	10	0	10
Cell debris, corneal		3	0	2	3	0	2
minimal		3	0	2	3	0	2
HEART							
Number examined		10	0	10	10	0	10
Cell infiltrate, myocardial		2	0	3	1	0	0
minimal		2	0	3	1	0	0
KIDNEY							
Number examined		10	0	10	10	1	10
Dilatation, pelvic		0	0	0	0	1	0
mild		0	0	0	0	1	0
Dilatation, tubular, cystic		0	0	0	0	0	1
minimal		0	0	0	0	0	1
Regeneration, tubular		1	0	3	2	0	2
minimal		1	0	3	2	0	2
Cell infiltrate, interstitial		1	0	2	1	0	0
minimal		1	0	2	1	0	0
Inflammation, pelvic		0	0	0	0	0	1
mild		0	0	0	0	0	1
LIVER							
Number examined		10	0	10	10	0	10
Cell infiltrate, mononuclear		0	0	0	1	0	0
minimal		0	0	0	1	0	0
Microgranuloma		2	0	1	1	0	1
minimal		2	0	1	1	0	1
LYMPH NODE, MANDIBULAR							
Number examined		10	0	10	10	1	10
Congestion/hemorrhage, agonal		1	0	0	0	1	0
present		1	0	0	0	1	0
LUNG							
Number examined		10	1	10	10	0	10
Histiocytosis, alveolar		3	0	2	0	0	1
minimal		3	0	2	0	0	1
Hemorrhage, alveolar, focal		1	0	2	1	0	0
minimal		1	0	2	1	0	0
Mineralization, alveolar wall		1	0	0	0	0	0
minimal		1	0	0	0	0	0
PANCREAS							
Number examined		10	0	10	10	0	10
Atrophy, acinar, focal		0	0	0	1	0	1

Kill type: TERMINAL

Tissue / Observation	Group:	1	2	3	1	2	3
	Sex:	M	M	M	F	F	F
	Number:	10	10	10	10	10	10
PANCREAS (continued)							
Atrophy, acinar, focal (continued)							
minimal		0	0	0	1	0	1
PROSTATE							
Number examined		10	0	10	-	-	-
Cell infiltrate, interstitial		4	0	5	-	-	-
minimal		4	0	5	-	-	-
Inflammation, acinar		4	0	4	-	-	-
minimal		4	0	4	-	-	-
THYMUS							
Number examined		10	1	10	10	2	10
Congestion/hemorrhage, agonal		2	1	2	1	2	1
present		2	1	2	1	2	1
URINARY BLADDER							
Number examined		9	0	10	10	0	9
Inflammation		0	0	1	0	0	1
minimal		0	0	1	0	0	0
mild		0	0	0	0	0	1

Kill type: RECOVERY

Tissue / Observation	Group: 3 Sex: M Number: 10	Group: 3 Sex: F Number: 10
EYE		
Number examined	10	10
Cell debris, corneal	2	2
minimal	2	2
HEART		
Number examined	10	10
Cell infiltrate, myocardial	2	1
minimal	2	1
KIDNEY		
Number examined	10	10
Dilatation, pelvic	2	1
minimal	1	0
mild	1	1
Regeneration, tubular	2	2
minimal	2	2
Cell infiltrate, interstitial	4	3
minimal	4	3
Mineralization, pelvis	0	1
minimal	0	1
LYMPH NODE, BRONCHIAL		
Number examined	10	10
Erythrocytosis, sinus	1	0
minimal	1	0
LIVER		
Number examined	10	10
Necrosis, focal	0	1
minimal	0	1
Necrosis, massive	1	0
mild	1	0
Cell infiltrate, mononuclear	0	1
minimal	0	1
LYMPH NODE, MANDIBULAR		
Number examined	10	10
Congestion/hemorrhage, agonal	2	2
present	2	2
LUNG		
Number examined	10	10
Hemorrhage, alveolar, focal	2	0
minimal	2	0
Partial underexpansion	1	0
present	1	0

Kill type: RECOVERY

Tissue / Observation	Group: 3 Sex: M Number: 10	Group: 3 Sex: F Number: 10
PANCREAS		
Number examined	10	10
Cell infiltrate, interstitial	1	0
minimal	1	0
PROSTATE		
Number examined	10	-
Cell infiltrate, interstitial	3	-
minimal	3	-
Inflammation, acinar	1	-
minimal	1	-
THYMUS		
Number examined	10	10
Congestion/hemorrhage, agonal	4	1
present	4	1

MKC-PC-2008-0006: A 28-Day Subcutaneous Injection Toxicity Study Followed by a 14-Day Recovery Period of FDKP with Three Known Impurities in Spraque-Dawley Rats [By (b) (4) Study No. 70569]

This study was to determine the toxicity and TK profile of FDKP with three known impurities ((b) (4), following daily SC injections to rats for 28 days. Mortality, clinical signs, body weights, food consumption, ophthalmoscopy and clinical pathology were assessed. Serial blood samples were collected to determine circulating serum FDKP levels. All animals were euthanized upon completion of their respective treatment or recovery periods. Selected tissues were retained, weighted, and examined microscopically.

Group Number	Group Designation	Dose (mg/kg/day)	Dose Concentration (mg/mL)	Toxicology Groups				Toxicokinetic Groups	
				Main		Recovery			
				Male	Female	Male	Female	Male	Female
1	Vehicle Control	0	0	10	10	5	5	3	3
2	Low Dose	10	2.0	10	10	-	-	6	6
3	Mid Dose	35	7.0	10	10	-	-	6	6
4	High Dose	75	15.0	10	10	5	5	6	6

Test Article*:	Identity:	Fumaryl Diketopiperazine (FDKP) with impurities identified by molecular weight (b) (4)
	Description:	White Powder
	Batch/Lot No.:	FD08064
	Expiry Date:	April 2009
	Purity:	Area purity 93.1%, Weight purity 86.0%
	Storage Conditions:	15 to 30ºC, protected from light
	Handling Precautions:	Standard laboratory procedures during routine handling and the wearing of a cartridge respirator during weighing
	Supplier:	MannKind Corporation

PRODUCT NAME/ DESCRIPTION: Impurity Enriched Fumaryl diketopiperazine (E)-3,6-bis [4-(*N*-carboxy- 2-propenyl)amidobutyl]-2,5-diketopiperazine
PACKAGING DESCRIPTION: Bulk Powder in Jar(s)
LOT NUMBER: FD08064

PRODUCT STAGE: Final Product
EXPIRY DATE: April 2009
SPECIFICATION #: N/A
STORAGE CONDITIONS: 15 to 30ºC, keep tightly sealed, protect from light

NUMBER	ATTRIBUTE	TEST METHOD/ SOURCE	SPECIFICATION	RESULTS
1	FDKP Assay (HPLC)	TM5478	Report Result	93.1 % (Area %) 86.0 % (Weight %)
2	FDKP Impurities Assay (HPLC)	TM5478	Impurity Molecular Weight Area % (b) (4)	Impurity Molecular Weight Area % (b) (4)

Tox findings: Subcutaneous administration of FDKP + 3 known impurities for 28 days was well-tolerated by rats. There were no systemic effects related to the test article. Test articled changes were gross lesions at necropsy at the sites of SC administration in mid and high dose animals.

Mid-dose animals had higher incidences of subcutaneous dark areas at 1 injection site (R2) when compared to VH control animals. However, there was no correlation to any histological changes and no adverse changes were observed in any tissues from this group. In addition, high-dose animals had higher incidences of subcutaneous dark areas at 3 injection sites (R1, R2, and L2). There changes did correlate with microscopic subcutaneous focal mineralization in several animals. The mineralization was minimal to mild (low severity) and was not present following the 14-day recovery period.

Table. Incidence of dark area at the injection sites.

Injection site	Males				Females			
Dose level (mg/kg/day)	0	10	35	75	0	10	35	75
L1	0/10	1/10	0/10	0/10	0/10	0/10	0/10	0/10
L2	3/10	2/10	3/10	7/10	2/10	0/10	3/10	3/10
L3	0/10	0/10	1/10	0/10	0/10	0/10	0/10	0/10
R1	0/10	0/10	0/10	3/10	2/10	1/10	0/10	1/10
R2	7/10	6/10	9/10	10/10	1/10	2/10	6/10	6/10
R3	1/10	1/10	0/10	1/10	0/10	1/10	0/10	0/10

TK findings: FDKP was readily absorbed with T_{max} between 0.25 to 0.5 hr across all dose groups. Exposure, based on C_{max} and AUC_{0-t} increased in a dose-dependent manner from 10 to 75 mg/kg/day on Days 1 and 28 in males and females. There were no gender differences in exposure and no accumulation following 28 days of daily dosing.

Overall, subcutaneous administration of FDKP with 3 known impurities once daily for 28 days was well-tolerated by rats. There were no indications of systemic effects related to the test article. Subcutaneous dark areas were noted grossly at necropsy in mid and high dose animals (correlating to microscopic subcutaneous mineralization at high dose). Such finding was local with low severity, and it was reversible. Exposure to FDKP was dose proportional in both males and females. Lastly, the NOEL was at the low dose of 10 mg/kg/day and NOAEL was at the high dose of 74 mg/kg/day for FDKP with 3 known impurities ((b) (4)

Note from the reviewer: The 3 impurities were tested at (b) (4) of total dose (spiked) in all FDKP dose groups 10, 35, 75 mg/kg/day, which means the 75 mg/kg/day FDKP group had a higher amount of 3 impurities tested than the 10 and 30 mg/kg/day FDKP groups.

Macroscopic findings:

Kill type: TERMINAL

Tissue / Observation	Group:	1	2	3	4	1	2	3	4
	Sex:	M	M	M	M	F	F	F	F
	mg/kg/day:	0	10	35	75	0	10	35	75
	Number:	10	10	10	10	10	10	10	10
KIDNEY									
Dilatation		0	0	1	0	0	0	0	0
LYMPH NODE, MANDIBULAR									
Dark focus		0	0	1	0	1	0	0	0
LUNG									
Dark area		0	0	0	0	1	0	0	0
Dark focus		0	0	0	0	2	0	0	0
STOMACH									
Depressed area		0	0	0	0	0	0	1	0
SEMINAL VESICLE									
Small		1	0	1	0	-	-	-	-
THYMUS									
Dark area		1	2	1	1	1	1	3	5
Mottled		1	2	1	1	1	0	1	2
TESTIS									
Soft		1	0	1	2	-	-	-	-
INJECTION SITE L1									
Dark area		0	1	0	0	0	0	0	0
INJECTION SITE L2									
Dark area		3	2	3	7	2	0	3	3
Thickening		0	0	0	1	0	0	0	0
INJECTION SITE L3									
Dark area		0	0	1	0	0	0	0	0
INJECTION SITE R1									
Dark area		0	0	0	3	2	1	0	1
INJECTION SITE R2									
Dark area		7	6	9	10	1	2	6	6
Pale area		0	0	0	0	0	0	0	1
Thickening		0	1	1	3	0	0	0	1
INJECTION SITE R3									
Dark area		1	1	0	1	0	1	0	0

Kill type: RECOVERY

Tissue / Observation	Group:	1	4	1	4
	Sex:	M	M	F	F
	mg/kg/day:	0	75	0	75
	Number:	5	5	5	5
LUNG					
Dark area		0	1	0	0
STOMACH					
Dark focus		1	0	0	0

Microscopic findings:

Kill type: TERMINAL

Tissue / Observation	Group:	1	2	3	4	1	2	3	4
	Sex:	M	M	M	M	F	F	F	F
	mg/kg/day:	0	10	35	75	0	10	35	75
	Number:	10	10	10	10	10	10	10	10
EYE									
Number examined		10	0	0	10	10	0	0	10
Scab		3	0	0	2	0	0	0	2
minimal		3	0	0	2	0	0	0	2
Fold/rossette,retinal		0	0	0	1	1	0	0	1
minimal		0	0	0	1	0	0	0	1
mild		0	0	0	0	1	0	0	0
HEART									
Number examined		10	0	0	10	10	0	0	10
Cell infiltrate,mononuclear		1	0	0	1	2	0	0	0
minimal		1	0	0	0	2	0	0	0
mild		0	0	0	1	0	0	0	0
KIDNEY									
Number examined		10	0	1	10	10	0	0	10
Dilatation,pelvic		0	0	1	0	0	0	0	0
mild		0	0	1	0	0	0	0	0
Dilatation,tubular		2	0	0	2	1	0	0	0
minimal		1	0	0	2	0	0	0	0
mild		1	0	0	0	1	0	0	0
Basophilia,tubular		5	0	1	6	2	0	0	3
minimal		4	0	1	6	1	0	0	3
mild		1	0	0	0	1	0	0	0
Cell infiltrate,interstitial		2	0	0	1	1	0	0	0
minimal		2	0	0	1	1	0	0	0
Pyelitis		0	0	0	1	0	0	0	1
minimal		0	0	0	1	0	0	0	1
LIVER									
Number examined		10	0	0	10	10	0	0	10
Vacuolation,diffuse		0	0	0	0	3	0	0	3
minimal		0	0	0	0	3	0	0	2
mild		0	0	0	0	0	0	0	1
Necrosis,focal		1	0	0	0	0	0	0	0
minimal		1	0	0	0	0	0	0	0
Microgranuloma		0	0	0	0	2	0	0	3
minimal		0	0	0	0	2	0	0	3
LYMPH NODE, MANDIBULAR									
Number examined		10	0	1	10	10	0	0	10
Congestion/hemorrhage		0	0	1	0	1	0	0	0
minimal		0	0	1	0	0	0	0	0
mild		0	0	0	0	1	0	0	0

Kill type: TERMINAL

Tissue / Observation	Group:	1	2	3	4	1	2	3	4
	Sex:	M	M	M	M	F	F	F	F
	mg/kg/day:	0	10	35	75	0	10	35	75
	Number:	10	10	10	10	10	10	10	10
LUNG									
Number examined		10	0	0	10	10	0	0	10
Cell infiltrate,mixed		1	0	0	0	1	0	0	1
minimal		1	0	0	0	1	0	0	1
Cell infiltrate,perivascular		0	0	0	0	1	0	0	2
minimal		0	0	0	0	0	0	0	2
mild		0	0	0	0	1	0	0	0
PANCREAS									
Number examined		10	0	0	10	10	0	0	10
Atrophy,acinar,focal		0	0	0	0	0	0	0	1
minimal		0	0	0	0	0	0	0	1
RECTUM									
Number examined		10	0	0	10	10	0	0	10
Cell infiltrate,submucosal		1	0	0	0	0	0	0	0
minimal		1	0	0	0	0	0	0	0
SMALL INTESTINE, JEJUNUM									
Number examined		10	0	0	10	10	0	0	10
Mineralization		0	0	0	0	1	0	0	0
minimal		0	0	0	0	1	0	0	0
SPLEEN									
Number examined		10	0	0	10	10	0	0	10
Hematopoiesis,extramedullary		5	0	0	5	4	0	0	4
minimal		3	0	0	3	4	0	0	4
mild		2	0	0	2	0	0	0	0
STERNUM & MARROW									
Number examined		10	0	0	10	10	0	0	10
Hypocellularity,bone marrow		0	0	0	1	0	0	0	0
minimal		0	0	0	1	0	0	0	0
THYMUS									
Number examined		10	4	2	10	10	1	4	10
Congestion/hemorrhage		4	4	2	3	4	0	3	5
minimal		2	2	0	3	3	0	3	0
mild		2	2	2	0	1	0	0	5
TONGUE									
Number examined		10	0	0	10	10	0	0	10
Cell infiltrate,mixed		1	0	0	0	0	0	0	0
minimal		1	0	0	0	0	0	0	0

Kill type: TERMINAL

Tissue / Observation	Group:	1	2	3	4	1	2	3	4
	Sex:	M	M	M	M	F	F	F	F
	mg/kg/day:	0	10	35	75	0	10	35	75
	Number:	10	10	10	10	10	10	10	10
TESTIS									
Number examined		10	0	1	10	-	-	-	-
Atrophy, seminiferous tubular		1	0	0	0	-	-	-	-
minimal		1	0	0	0	-	-	-	-
URINARY BLADDER									
Number examined		10	0	0	10	10	0	0	9
Cell infiltrate, submucosal		1	0	0	0	0	0	0	0
minimal		1	0	0	0	0	0	0	0
Inflammation, chronic		0	0	0	0	0	0	0	1
mild		0	0	0	0	0	0	0	1
INJECTION SITE L1									
Number examined		0	1	0	0	0	0	0	0
Hemorrhage		0	1	0	0	0	0	0	0
mild		0	1	0	0	0	0	0	0
Inflammation, subcutaneous		0	1	0	0	0	0	0	0
minimal		0	1	0	0	0	0	0	0
INJECTION SITE L2									
Number examined		3	2	3	7	2	0	3	3
Mineralization		0	0	0	1	0	0	0	0
minimal		0	0	0	1	0	0	0	0
Hemorrhage		3	2	3	7	2	0	3	3
minimal		1	1	2	5	2	0	1	0
mild		2	1	1	1	0	0	1	1
moderate		0	0	0	1	0	0	1	2
Inflammation, subcutaneous		3	2	2	6	1	0	2	1
minimal		2	1	1	2	0	0	2	1
mild		1	1	1	3	1	0	0	0
moderate		0	0	0	1	0	0	0	0
INJECTION SITE L3									
Number examined		0	0	1	0	0	0	0	0
Hemorrhage		0	0	1	0	0	0	0	0
moderate		0	0	1	0	0	0	0	0
Inflammation, subcutaneous		0	0	1	0	0	0	0	0
minimal		0	0	1	0	0	0	0	0
INJECTION SITE R1									
Number examined		0	0	0	3	2	1	0	1
Regeneration, muscular		0	0	0	0	1	0	0	0
minimal		0	0	0	0	1	0	0	0
Mineralization		0	0	0	0	0	0	0	1
minimal		0	0	0	0	0	0	0	1
Hemorrhage		0	0	0	3	1	1	0	1

Kill type: TERMINAL

Tissue / Observation	Group:	1	2	3	4	1	2	3	4
	Sex:	M	M	M	M	F	F	F	F
	mg/kg/day:	0	10	35	75	0	10	35	75
	Number:	10	10	10	10	10	10	10	10
INJECTION SITE R1 (continued)									
Hemorrhage (continued)									
minimal		0	0	0	1	1	0	0	0
mild		0	0	0	1	0	1	0	1
moderate		0	0	0	1	0	0	0	0
Inflammation,subcutaneous		0	0	0	3	1	1	0	1
minimal		0	0	0	1	1	0	0	1
mild		0	0	0	2	0	1	0	0
INJECTION SITE R2									
Number examined		10	6	9	10	10	2	6	10
Regeneration,muscular		0	0	0	1	0	0	0	0
minimal		0	0	0	1	0	0	0	0
Mineralization		0	0	0	7	0	0	0	2
minimal		0	0	0	2	0	0	0	0
mild		0	0	0	5	0	0	0	2
Hemorrhage		8	6	9	10	2	2	6	6
minimal		4	2	6	0	1	0	1	3
mild		4	4	3	8	0	1	4	3
moderate		0	0	0	2	1	1	1	0
Cell infiltrate,mononuclear		1	0	0	0	0	0	0	0
mild		1	0	0	0	0	0	0	0
Microgranuloma		0	0	0	0	0	0	0	1
minimal		0	0	0	0	0	0	0	1
Inflammation,subcutaneous		10	6	9	9	10	2	6	7
minimal		0	0	1	2	4	0	2	3
mild		5	4	4	2	4	2	3	4
moderate		5	2	4	5	2	0	1	0
INJECTION SITE R3									
Number examined		1	1	0	1	0	1	0	0
Regeneration,muscular		0	0	0	0	0	1	0	0
mild		0	0	0	0	0	1	0	0
Hemorrhage		1	1	0	1	0	1	0	0
mild		1	1	0	1	0	1	0	0
Hemorrhage,focal		1	0	0	0	0	0	0	0
minimal		1	0	0	0	0	0	0	0
Inflammation,subcutaneous		1	1	0	1	0	0	0	0
minimal		1	1	0	1	0	0	0	0

Kill type: RECOVERY

Tissue / Observation	Group:	1	4	1	4
	Sex:	M	M	F	F
	mg/kg/day:	0	75	0	75
	Number:	5	5	5	5
ADRENAL					
Number examined		5	5	5	5
Mineralization, corticomedullary		1	0	0	0
mild		1	0	0	0
EPIDIDYMIS					
Number examined		5	5	-	-
Cell infiltrate, interstitial		2	0	-	-
minimal		2	0	-	-
EYE					
Number examined		5	5	5	5
Scab		1	1	0	0
minimal		1	1	0	0
Atrophy, retinal		0	1	0	0
mild		0	1	0	0
Fold/rossette, retinal		0	0	1	0
mild		0	0	1	0
HEART					
Number examined		5	5	5	5
Cell infiltrate, mononuclear		2	1	1	0
minimal		2	1	1	0
KIDNEY					
Number examined		5	5	5	5
Dilatation, pelvic		0	1	0	0
mild		0	1	0	0
Basophilia, tubular		2	2	1	1
minimal		2	2	1	1
Mineralization, papillary		0	0	0	1
mild		0	0	0	1
Cell infiltrate, interstitial		1	1	0	0
minimal		1	1	0	0
LIVER					
Number examined		5	5	5	5
Vacuolation, diffuse		0	0	2	2
minimal		0	0	2	2
Hematopoiesis, extramedullary		0	1	0	0
minimal		0	1	0	0
Microgranuloma		2	2	1	0
minimal		2	2	1	0

Kill type: RECOVERY

Tissue / Observation	Group:	1	4	1	4
	Sex:	M	M	F	F
	mg/kg/day:	0	75	0	75
	Number:	5	5	5	5
LYMPH NODE, MANDIBULAR					
Number examined		5	5	5	5
Congestion/hemorrhage		0	0	0	1
minimal		0	0	0	1
LUNG					
Number examined		5	5	5	5
Hemorrhage, focal		0	1	0	0
minimal		0	1	0	0
Cell infiltrate, mononuclear		0	1	0	0
minimal		0	1	0	0
Cell infiltrate, perivascular		0	0	0	1
minimal		0	0	0	1
PANCREAS					
Number examined		5	5	5	5
Cell infiltrate, interstitial		1	0	0	1
minimal		1	0	0	1
PROSTATE					
Number examined		5	5	-	-
Cell infiltrate, interstitial		2	1	-	-
minimal		2	1	-	-
Inflammation		0	1	-	-
minimal		0	1	-	-
RECTUM					
Number examined		5	5	5	5
Cell infiltrate, submucosal		0	0	0	1
minimal		0	0	0	1
SPLEEN					
Number examined		5	5	5	5
Hematopoiesis, extramedullary		5	5	1	1
minimal		5	5	1	1
INJECTION SITE R2					
Number examined		5	5	5	5
Regeneration, muscular		0	0	0	1
minimal		0	0	0	1

MKC-PC-2008-0016: Primary Dermal Irritation/Corrosion Study with Three FDKP Intermediates in Rabbits [By (b) (4) Study No. 7260-112]

This study was to assess the relative level of primary skin irritation/corrosion of 3 FDKP intermediates on rabbits under semioccluded conditions. Moistened test articles were applied to the intact skin area of rabbits which were covered with a semiocclusive dressing. Patches were removed approx. 4 hrs. The untreated skin of each animal served as its own control. The degree of erythema and edema was evaluated approx. 60 min, 24 hrs, 48 hrs, and 72 hrs after patch removal. Mortality, clinical observation, and body weight were assessed. Among these 3 FDKP intermediates, test article (b) (4) was starting materials, whereas test articles (b) (4) were intermediates.

Test Article	Lot No.	Storage	Purity	Re-Test Date	Reserve (Archive) Sample
(b) (4)	EM019	Room temperature	98.91%	10/2008	Not Collected
	D.011.A.06.002	Room temperature	100%	05/2009	Not Collected
	D.004.D.06.001	Room temperature	98%	05/2009	Not collected

NS Not supplied by the sponsor yet.

Findings: There was no irritation present at the 72 hr exam. No signs of erythema or edema at any observation interval. No clinical signs were observed during the study.

Overall, all 3 compound were classified as dermally non-irritating.

Individual Dermal Irritation Data

Skin Condition	Interval	Dermal Irritation Score: Animal No. F67734	Animal No. F67735	Animal No. F67736	Average Primary Dermal Irritation Score[a]	
	Erythema and Eschar Formation					
Intact	60 min[d]	0	0	0	60 min	0
	24 hr	0	0	0	24 hr	0
	48 hr	0	0	0	48 hr	0
	72 hr	0	0	0	72 hr	0
	Edema					
Intact	60 min[d]	0	0	0		
	24 hr	0	0	0		
	48 hr	0	0	0		
	72 hr	0	0	0		
Individual Index Score[b]		0	0	0		
					Primary Dermal Irritation Index (PDII)[c] = 0.0	

Skin Condition	Interval	Dermal Irritation Score: Animal No. F67737	Animal No. F67738	Animal No. F67739	Average Primary Dermal Irritation Score[a]	
	Erythema and Eschar Formation					
Intact	60 min[d]	0	0	0	60 min	0
	24 hr	0	0	0	24 hr	0
	48 hr	0	0	0	48 hr	0
	72 hr	0	0	0	72 hr	0
	Edema					
Intact	60 min[d]	0	0	0		
	24 hr	0	0	0		
	48 hr	0	0	0		
	72 hr	0	0	0		
Individual Index Score[b]		0	0	0		
					Primary Dermal Irritation Index (PDII)[c] = 0.0	

Skin Condition	Interval	Dermal Irritation Score: Animal No. F67740	Animal No. F67741	Animal No. F67742	Average Primary Dermal Irritation Score[a]	
	Erythema and Eschar Formation					
Intact	60 min[d]	0	0	0	60 min	0
	24 hr	0	0	0	24 hr	0
	48 hr	0	0	0	48 hr	0
	72 hr	0	0	0	72 hr	0
	Edema					
Intact	60 min[d]	0	0	0		
	24 hr	0	0	0		
	48 hr	0	0	0		
	72 hr	0	0	0		
Individual Index Score[b]		0	0	0		
					Primary Dermal Irritation Index (PDII)[c] = 0.0	

Appears this way on original

a The average primary dermal irritation score per interval is the total dermal irritation score for all the animals (erythema and edema) divided by the number of animals (3) at each observation period.
b The individual index scores are the total of the erythema and edema scores (8) at 60 minutes and 24, 48, and 72 hours divided by the number of observation periods (4).
c The primary dermal irritation index (PDII) is the sum of the individual index scores, divided by the number of animals (3).
d The 60 min scoring was performed 0.5 to 1 hour after patch removal.

The average score per interval, individual index scores, and primary irritation index values are all rounded to the nearest tenth.

MKC-PC-2008-0018: Dermal Sensitization Study in Guinea Pigs with Three FDKP Intermediates-Closed Patch Technique [By (b) (4) Study No. 7260-115]

This two-phase study (irritation phase and definitive study) was to assess the delayed contract hypersensitivity potential of 3 FDKP intermediates (test articles (b) (4)) in guinea pigs. Male Crl(HA)BR guinea pigs were assigned to 11 groups. The results from the Groups 1 -3 Irritation Phase animals were used to pick the induction and challenge doses for the Definitive Study animals. These animals received 4 doses each of different concentrations. Each site was treated with 37.5, 50, 75 or 95% mixed in petrolatum. The animals were then scored for dermal irritation at 24 and 48 hrs post-applications. During the induction phase, the Groups 4-6 and Group 10 animals remained untreated. The Groups 7-9 and Group 11 received 3 induction doses (95% test article #1, 86% test articles #2 and #3). All Definitive Study groups received challenge doses. At approx. 24 and 48 hrs after the induction and challenge applications, each animal was examined for a dermal irritation reaction. Toxicity was assessed based on mortality, clinical observations, and body weights. Sensitization potential was based on the induction and challenge irritation scoring.

Group	Identification	Number of Animals
	Irritation Phase	
1	Test Article #1 - (b) (4)	4
2	Test Article #2 - (b) (4)	4
3	Test Article #3 - (b) (4) (b) (4)	4
	Main (Definitive) Study	
4	Naïve Control Group for Test Article #1 - (b) (4)	5
5	Naïve Control Group for Test Article #2 - (b) (4) (b) (4)	5
6	Naïve Control Group for Test Article #3 - (b) (4) (b) (4)	5
7	Test Article #1 - (b) (4)	10
8	Test Article #2 - (b) (4)	10
9	Test Article #3 - (b) (4) (b) (4)	10
10	Naïve Positive Control Group	5
11	Positive Control Group	10

Test Article	Lot No.	Storage	Purity	Re-Test Date	Reserve (Archive) Sample
(b) (4)	EM019	Room temperature	98.91%	10/2008	Collected
	D.011.A.06.002	Room temperature	100%	05/2009	Collected
	D.004.D.06.001	Room temperature	98%	05/2009	Collected

Findings: Based on the irritation phase, 95% was selected as the induction dose for Test Article #1, and 86% was selected as the induction dose for Test Articles #2 and #3. Also, the doses selected for the challenge dose were 95%, 86%, and 86% for Test Articles #1, #2, and #3, respectively. All animals survived to their respective termination. Clinical observations and body weights were comparable between the control and treated groups.

During the induction phase, Groups 7-9 animals were normal and did not have any dermal irritation. On the other hand, Group 11 positive control animals were normal after the 1st induction dose. Very faint erythema were noted in 6/10 animals after the 2nd induction dose and in 8/10 animals after the 3rd induction dose. During the challenge phase, Groups 4-9 animals appeared to be normal with any reaction during the 24 and 48 hr challenge dermal irritation readings. On the other hand, some of Group 10 and 11 positive control animals had very faint erythema after the challenge dose.

Overall, test article #1 (b) (4) at a dose level of 95% for the induction dose and 95% for the challenge dose was not considered to be a sensitizer. Test article #2 (b) (4) at a dose level of 86% for the induction dose and 86% for the challenge dose was not considered to be a sensitizer. Test article #3 (b) (4) at a dose level of 86% for the induction dose and 86% for the challenge dose was not considered to be a sensitizer. In short, all 3 compounds were classified as non-sensitizers.

MKC-PC-2008-0019: Acute Oral Toxicity Study with Three FDKP Intermediates in Rats (Acute Toxic Class Method) [By (b) (4) Study No. 7260-114]

This study was to assess the acute oral toxicity induced by three FDKP intermediates by oral gavage to rats. Three females were dosed at 2000 mg/kg for each test article and three males were also dosed at 2000 mg/kg for each test article two days later. Each test article was mixed with corn oil and administered as a single dose. Due to unscheduled deaths in Group 1 males, Group 5 males were dosed at 300 mg/kg. Following dosing, animals were observed for 14 days prior to necropsy. Toxicity was assessed based on mortality, clinical signs, body weights, and macroscopic observations.

Group	Test Article	Treatment Dose Level	Number of Animals	
			Males	Females
1	Test Article #1 (b) (4)	2000 mg/kg	3	3
2	Test Article #2 (b) (4)	2000 mg/kg	3	3
3	Test Article #3 (b) (4)	2000 mg/kg	3	3
4[a]	Test Article #1 (b) (4)	300 mg/kg	3	0

a Three females were dosed at 2000 mg/kg for each test article, two days later 3 males per test article were dosed at 2000 mg/kg. Due to unscheduled deaths in Group 1 males, Group 4 males were dosed at 300 mg/kg.

Findings: For Test Article #1 (b) (4), 2 female rats survived to scheduled necropsy at 2000 mg/kg. Clinical observations, including hunched appearance, hypoactivity, nonformed feces, irregular and labored respiration, rough haircoat, were observed in these 2 animals. One female was found dead on SD 1 and clinical observations, including ataxia, hypoactivity, recumbency, irregular respiration, and rough haircoat, were observed prior to her death. There findings were treatment-related. In addition, 3 male rats given 2000 mg/kg were found death on SDs 1, 5, and 6. Clinical observations, including hunched appearance, ataxia, hypoactivity, red discharge from nose and/or eye, yellow oral discharge, squinted eyes, irregular and labored respiration, rough haircoat, and/or body tremors, were observed. These findings were considered treatment-related. Moreover, 3 male rats given 300 mg/kg survived to scheduled necropsy. Clinical observations, including hypoactivity, squinted eyes, irregular respiration, rough haircoat, were seen in 1 male rat on Days 1, 2, and 4, which were considered to be treatment-related. Two other male rats given 300 mg/kg were normal during clinical observations and at the time of necropsy. All 3 animals gained weight during the study. For Test Article #2 (b) (4), all 3 female and male rats at 2000 mg/kg survived to necropsy. All animals were normal during clinical observations and gained weight throughout the study. One female had white particulate matter in the stomach at the time of necropsy while all remaining animals were normal during necropsy. For Test Article #3 (b) (4), all 3 female and male rats at 2000 mg/kg survived to necropsy. All animals were normal during clinical observations and gained weight throughout the study. All animals were normal at the time of necropsy.

All in all, Test Article #1 (b) (4)) orally at 2000 mg/kg for female and male rats and 300 mg/kg for male rats was considered to be a Category 4. Test Article #2 (b) (4) orally at 2000 mg/kg for female and male rats was considered to be a Category 5. Test Article #3 (b) (4) orally at 2000 mg/kg for female and male rats was considered to be a category 5. In short, the starting material (b) (4) showed greater acute oral toxicity in rats, and was more irritating to the eye than the other 2 intermediates (b) (4) Note from the reviewer: It is unclear how the toxic dose of test article #1 related to an impurity level in T, given in a clinical setting by inhalation route.

Table 1.

GHS acute toxicity hazard categories and acute toxicity estimate values defining the respective categories.

GHS category	Dose range of LD_{50} values	Human health effect
Category 1	LD_{50} < 5 mg/kg	Fatal if swallowed
Category 2	LD_{50} > 5 mg/kg < 50 mg/kg	Fatal if swallowed
Category 3	LD_{50} > 50 mg/kg < 300 mg/kg	Toxic if swallowed
Category 4	LD_{50} > 300 mg/kg < 2000 mg/kg	Harmful if swallowed
Category 5[a]	LD_{50} > 2000 mg/kg < 5000 mg/kg and LD_{50} > 5000 mg/kg	May be harmful if swallowed not cla

[a] Criteria for category 5 are intended to enable the identification of substances which are of relatively low acute toxicity hazard but which under certain circumstances may present a danger to vulnerable populations. Recognizing the need to protect animal welfare, testing in animals in category 5 ranges is discouraged and should only be considered when there is a strong likelihood that results of such a test have a direct relevance for protecting human health (United Nations, 2007).

MKC-PC-2008-0020: A 28 Day Inhalation Immunotoxicity Study with a 28 Day Recovery Period of Technosphere® Powder and Technosphere®/Insulin in Spraque-Dawley Rats [By (b) (4) Study no. 75101]

The study was to determine the potential immunotoxicity of T and TI in male and female rats, following daily inhalation exposure for 28 days following a 28 day recovery period. There was a separate group of animals received SC administration of FDKP. Mortality, clinical signs, body weights and food consumption were performed throughout the course of the study. Immunotoxicity was assessed by immunophenotyping peripheral blood lymphocytes and by evaluating the primary IgM and IgG antibody responses to a T-cell dependent antigen (KLH).

The study was designed as follows:

Gr.	Group Designation	Phase 1: Immunophenotyping				Phase 2: TDAR measurement			
		Main 1		Recovery 1		Main 2		Recovery 2	
		Male	Female	Male	Female	Male	Female	Male	Female
1	Air Control	10	10	5	5	10	10	5	5
2	Technosphere® Powder Control Low Dose	10	10	-	-	10	10	-	-
3	Technosphere® Powder Control High Dose	10	10	5	5	10	10	5	5
4	Technosphere®/Insulin Low Dose	10	10	-	-	10	10	-	-
5	Technosphere®/Insulin High Dose	10	10	5	5	10	10	5	5
6	Fumaryl Diketopiperazine (FDKP)* Subcutaneous	10	10	5	5	10	10	5	5

* FDKP in Phosphate Buffer TDAR = T-cell dependent antibody response

The achieved aerosols and estimated dose levels were as follows:

Gr.	Group Designation	Achieved Dose Level (mg/kg/day)						Achieved Aerosol Concentration (mg/L)	
		Technosphere® Powder		Insulin (IU)		Exposure duration (min)		Technosphere®	Insulin
		Male	Female	Male	Female	Male	Female		
1	Air Control	-	-	-	-	60	33	-	-
2	Technosphere® Powder Control Low Dose	1.3775	0.8175	0	0	60	33	0.0374	0
3	Technosphere® Powder Control High Dose	44	47	0	0	60	60	1.1851	0
4	Technosphere® /Insulin Low Dose	0.7663	0.4995	0.1022	0.0666	60	36	0.0210	0.0028
5	Technosphere® /Insulin High Dose	1.3274	0.7871	0.1839	0.1090	60	33	0.0361	0.0050

Gr.	Group Designation	Technosphere® Powder Dose Level (mg/kg/day)		Dose Volume (mL/kg)	Technosphere® Powder Dose Formulation Concentration (mg/mL)
		Male	Female		
6	Fumaryl Diketopiperazine (FDKP)* Subcutaneous	75	75	5	15

* FDKP in Phosphate Buffer

Findings: All animals survived to study termination. Dosing was well-tolerated via either route of administration. No adverse clinical signs related to treatment. Body weights and food consumption were unaffected by inhaled T or TI, as well as after repeated subcutaneously injected FDKP.

- For immunotoxicity assessment: blood immunophenotyping, no changes were seen in total lymphocyte counts from all groups at the end of the main and recovery periods. In Groups 3-5, there were no clear changes in absolute numbers of lymphocytes subsets (Helper T, cytotoxic T lymphocytes, and B lymphocytes) at the end of the treatment period. There were some changes in the relative % of these lymphocytes subsets. However, without clear changes in absolute numbers, these relative changes were considered, by the sponsor, insignificant. In Group 6 (FDKP via SC), the absolute numbers and relative % of NK lymphocytes were decreased at the end of the treatment period compared to the air control group. This change was more pronounced in females than males. Partial recovery of this change was also seen in these animals. However, due to the absence of a similarly-treated VH-only SC control group, it was unclear the significance of this particular finding.

Summary Table of Significant Differences in Blood Total Lymphocyte Count

Parameter	Group	% Difference vs Control Group 1	Statistically Significant[a]	Biologically Significant[b]	Treatment Related[c]	Comments
Total Lymphocyte Counts						
Recovery Study (Day 57)						
Males	3	-36%	No	No	No	
	5	-30%	No	No	No	

a. Significantly different from control Group 1 value:
A - P ≤ 0.05 B – P ≤ 0.01 C – P ≤ 0.001 (Dunnett)
D – P ≤ 0.05 E – P ≤ 0.01 F – P ≤ 0.001 (Dunn)
b. Based on the range of results (mean and standard deviation) obtained in the control Group 1.
c. Based on interpretation of all available data taking into account individual data variability and/or absence of clear trends.

Summary Table of Significant Differences in Absolute Numbers of Each Lymphocyte Subset in Blood

Parameter	Group	% Difference vs Control Group 1	Statistically Significant[a]	Biologically Significant[b]	Treatment Related[c]	Comments
Absolute Total T Lymphocyte Numbers ($CD3^+$)						
Recovery Study (Day 57)						
Males	5	-31%	No	No	No	d,f
Absolute Cytotoxic T Lymphocyte Numbers ($CD3^+/CD8a^+$)						
Main Study (Day 29)						
Males	3	32%	No	No	No	d,e
Females	5	35%	No	No	No	d
Recovery Study (Day 57)						
Males	3	-31%	No	No	No	d,f
	5	-40%	No	No	No	d,f
Absolute B Lymphocyte Numbers ($CD3^-/CD45RA^+$)						
Recovery Study (Day 57)						
Males	3	-51%	Yes (A)	No	No	d,f
	5	-37%	No	No	No	d,f
	6	-34%	No	No	No	d
Females	3	-34%	No	No	No	d,e
	6	-49%	No	No	No	d
Absolute NK Lymphocyte Numbers ($CD3^-/CD161a^{hi}$)						
Main Study (Day 29)						
Males	3	81%	No	No	No	d,e
	6	-63%	No	No	Yes	e
Females	5	37%	No	No	No	d
	6	-52%	Yes (A)	No	Yes	e
Recovery Study (Day 57)						
Males	5	40%	No	No	No	d,f
	6	-43%	No	No	Yes	e,g
Females	6	-39%	No	No	Yes	g

a. Significantly different from control Group 1 value:
A - P ≤ 0.05 B – P ≤ 0.01 C – P ≤ 0.001 (Dunnett)
D – P ≤ 0.05 E – P ≤ 0.01 F – P ≤ 0.001 (Dunn)
b. Based on the range of results (mean and standard deviation) obtained in the control Group 1.
c. Based on interpretation of all available data taking into account individual data variability and/or absence of clear trends.
d. Although these changes in mean values are ≥ ±30%, they are not considered treatment related nor biologically relevant when taking into account individual animal variability and/or range of values obtained in the control Group 1.
e. A change in the relative percentage (percentage difference ≥ ±30%) and/or a statistically significant difference of this lymphocyte population on the same time point and in the same sex was observed.
f. Due to variability in total lymphocyte count (percentage difference ≥ ±30%) observed on the same time point and in the same sex.
g. Based on the observation of treatment-related effect during the main study.

Summary Table of Significant Differences in Relative Percentages of Each Lymphocyte Subset in Blood

Parameter	Group	% Difference vs Control Group 1	Statistically Significant[a]	Biologically Significant[b]	Treatment Related[c]	Comments
Relative Percentage of Total T Lymphocytes ($CD3^+$)						
Main Study (Day 29)						
Males	4	21%	Yes (C)	Yes	Yes	
Recovery Study (Day 57)						
Males	3	19%	Yes (A)	No	No	d
	6	17%	Yes (A)	No	No	d
Relative Percentage of Helper T Lymphocytes ($CD3^+/CD4^+$)						
Main Study (Day 29)						
Males	4	25%	Yes (C)	Yes	Yes	e
	6	16%	Yes (A)	No	Yes	
Recovery Study (Day 57)						
Males	3	24%	Yes (A)	No	No	d
	6	29%	Yes (A)	No	No	d
Relative Percentage of Cytotoxic T Lymphocytes ($CD3^+/CD8^+$)						
Main Study (Day 29)						
Males	3	16%	Yes (A)	No	Yes	e
	4	20%	Yes (B)	No	Yes	e
	5	19%	Yes (B)	No	Yes	
Relative Percentage of B Lymphocytes ($CD3^-/CD45RA^+$)						
Main Study (Day 29)						
Males	3	-21%	Yes (B)	No	Yes	
	4	-28%	Yes (C)	Yes	Yes	
	5	-22%	Yes (B)	No	Yes	
Recovery Study (Day 57)						
Females	3	-30%	No	No	No	d,e

a. Significantly different from control Group 1 value:
A - $P \leq 0.05$ B – $P \leq 0.01$ C – $P \leq 0.001$ (Dunnett)
D – $P \leq 0.05$ E – $P \leq 0.01$ F – $P \leq 0.001$ (Dunn)

b. Based on the range of results (mean and standard deviation) obtained in the control Group 1.

c. Based on interpretation of all available data taking into account individual data variability and/or absence of clear trends.

d. Although this change in mean value is $\geq \pm 30\%$ and/or statistically significant, they are not considered treatment related nor biologically relevant when taking into account individual animal variability and/or range of values obtained in the control Group 1.

e. A change in the absolute number (percentage difference $\geq \pm 30\%$ and/or statistically significant difference) of this lymphocyte population on the same time point and in the same sex was observed as described above.

Summary Table of Significant Differences in Relative Percentages of Each Lymphocyte Subset in Blood (Cont'd)

Parameter	Group	% Difference vs Control Group 1	Statistically Significant[a]	Biologically Significant[b]	Treatment Related[c]	Comments
Relative Percentage of NK Lymphocytes ($CD3^-/CD161a^{hi}$)						
Main Study (Day 29)						
Males	3	72%	No	No	No	e
	5	54%	No	No	No	d
	6	-37%	No	No	Yes	e
Females	6	-46%	Yes (A)	No	Yes	e
Recovery Study (Day 57)						
Males	3	-32%	No	No	No	d
	6	-59%	No	No	No	d,e

a. Significantly different from control Group 1 value:
A - P ≤ 0.05 B – P ≤ 0.01 C – P ≤ 0.001 (Dunnett)
D – P ≤ 0.05 E – P ≤ 0.01 F – P ≤ 0.001 (Dunn)

b. Based on the range of results (mean and standard deviation) obtained in the control Group 1.

c. Based on interpretation of all available data taking into account individual data variability and/or absence of clear trends.

d. Although this change in mean value is ≥ ±30% and/or statistically significant, they are not considered treatment related nor biologically relevant when taking into account individual animal variability and/or range of values obtained in the control Group 1.

e. A change in the absolute number (percentage difference ≥ ±30% and/or statistically significant difference) of this lymphocyte population on the same time point and in the same sex was observed as described above.

- For immunotoxicity assessment: T-cell dependent antibody response (TDAR), there were no changes in the primary IgM and IgG T-cell dependent antibody response in any treated group compared to the air inhalation control group. Moreover, there were no organ weight changes or macroscopic/microscopic findings for the animals dosed via inhalation. However, most of animals dosed via SC administration at 75 mg/kg/day showed a minimal to moderate local irritation and dark or red area/focus at necropsy and minimal to moderate subcutaneous hemorrhage and/or inflammation, occasionally associated with regeneration of subcutaneous muscle and/or necrosis of the subcutaneous connective tissues, during histological examination. These findings were not observed at the end of the recovery period.

Group Mean T-Cell Dependent Antibody Response (Anti-KLH IgM)

Main Study
Males

Group 1 - Air Control
Group 2 - Technosphere® Powder Control Low Dose
Group 3 - Technosphere® Powder Control High Dose

Group	Summary Information	Mean (µg/mL) Day 11	Day 17	Day 18	Day 19
1	Geometric Mean	0.195250	29.084	22.895	21.840
	Mean	0.195250	48.730	34.823	30.713
	SD	0.000000	42.900	31.840	29.188
	N	10	10	10	10
2	Geometric Mean	0.195250	37.930	27.548	27.324
	Mean	0.195250	58.425	38.247	37.931
	SD	0.000000	54.885	33.110	29.117
	N	10	10	10	10
3	Geometric Mean	0.195250	9.424	8.248	8.888
	Mean	0.195250	34.090	22.441	21.040
	SD	0.000000	53.107	30.627	27.676
	N	10	10	10	10

Significantly different from control group (group 1) value: D - P ≤ 0.05 E - P ≤ 0.01 F - P ≤ 0.001 (Dunn)

Group 4 - Technosphere®/Insulin Low Dose
Group 5 - Technosphere®/Insulin High Dose
Group 6 - Fumaryl Diketopiperazine (FDKP)* Subcutaneous

Group	Summary Information	Mean (µg/mL) Day 11	Day 17	Day 18	Day 19
4	Geometric Mean	0.195250	40.874	33.081	25.632
	Mean	0.195250	77.193	55.101	35.492
	SD	0.000000	105.319	66.533	32.797
	N	10	10	10	10
5	Geometric Mean	0.195250	18.042	17.671	14.394
	Mean	0.195250	21.940	20.784	15.843
	SD	0.000000	12.358	9.480	6.506
	N	10	10	10	10
6	Geometric Mean	0.195250	39.353	36.149	23.424
	Mean	0.195250	68.554	77.426	38.210
	SD	0.000000	83.696	118.364	39.294
	N	10	10	10	10

Significantly different from control group (group 1) value: D - P ≤ 0.05 E - P ≤ 0.01 F - P ≤ 0.001 (Dunn)

Group Mean T-Cell Dependent Antibody Response (Anti-KLH IgM)

Main Study
Females

Group 1 - Air Control
Group 2 - Technosphere® Powder Control Low Dose
Group 3 - Technosphere® Powder Control High Dose

Group	Summary Information	Mean (µg/mL) Day 11	Day 17	Day 18	Day 19
1	Geometric Mean	0.195250	30.241	23.146	19.236
	Mean	0.195250	128.939	106.520	70.506
	SD	0.000000	217.229	219.302	124.823
	N	10	10	10	10
2	Geometric Mean	0.195250	19.232	18.543	14.773
	Mean	0.195250	46.656	62.451	30.614
	SD	0.000000	83.419	113.161	48.734
	N	10	10	10	10
3	Geometric Mean	0.195250	33.200	19.009	26.628
	Mean	0.195250	133.037	61.518	86.996
	SD	0.000000	161.860	79.794	113.446
	N	10	10	10	10

Significantly different from control group (group 1) value: D - P ≤ 0.05 E - P ≤ 0.01 F - P ≤ 0.001 (Dunn)

Group 4 - Technosphere®/Insulin Low Dose
Group 5 - Technosphere®/Insulin High Dose
Group 6 - Fumaryl Diketopiperazine (FDKP)* Subcutaneous

Group	Summary Information	Mean (µg/mL) Day 11	Day 17	Day 18	Day 19
4	Geometric Mean	0.195250	20.340	21.169	18.928
	Mean	0.195250	61.187	51.836	44.010
	SD	0.000000	92.896	84.108	73.095
	N	10	10	10	10
5	Geometric Mean	0.195250	26.958	29.450	23.908
	Mean	0.195250	113.799	97.108	62.622
	SD	0.000000	197.178	169.058	106.112
	N	10	10	10	10
6	Geometric Mean	0.195250	18.554	18.024	21.181
	Mean	0.195250	42.920	45.550	47.007
	SD	0.000000	54.989	66.588	52.728
	N	10	10	10	10

Significantly different from control group (group 1) value: D - P ≤ 0.05 E - P ≤ 0.01 F - P ≤ 0.001 (Dunn)

Group Mean T-Cell Dependent Antibody Response (Anti-KLH IgM)

Recovery
Males

Group 1 - Air Control
Group 3 - Technosphere® Powder Control High Dose
Group 5 - Technosphere®/Insulin High Dose
Group 6 - Fumaryl Diketopiperazine (FDKP)* Subcutaneous

Group	Summary Information	Mean (µg/mL) Day 39	Day 45	Day 46	Day 47
1	Geometric Mean	0.195250	28.312	29.214	24.533
	Mean	0.195250	35.880	34.774	38.558
	SD	0.000000	32.865	22.597	38.596
	N	5	5	5	5
3	Geometric Mean	0.195250	8.547	9.167	8.344
	Mean	0.195250	11.634	10.978	10.376
	SD	0.000000	12.278	8.407	8.690
	N	5	5	5	5
5	Geometric Mean	0.195250	28.504	29.702	21.262
	Mean	0.195250	43.030	44.138	27.714
	SD	0.000000	36.593	38.755	19.337
	N	5	5	5	5
6	Geometric Mean	0.195250	12.671	9.966	8.897
	Mean	0.195250	44.580	41.587	26.584
	SD	0.000000	61.848	54.937	39.417
	N	5	5	5	5

Significantly different from control group (group 1) value: D - P ≤ 0.05 E - P ≤ 0.01 F - P ≤ 0.001 (Dunn)

Group Mean T-Cell Dependent Antibody Response (Anti-KLH IgM)

Recovery
Females

Group 1 - Air Control
Group 3 - Technosphere® Powder Control High Dose
Group 5 - Technosphere®/Insulin High Dose
Group 6 - Fumaryl Diketopiperazine (FDKP)* Subcutaneous

Group	Summary Information	Mean (µg/mL) Day 39	Day 45	Day 46	Day 47
1	Geometric Mean	0.195250	10.687	8.303	6.280
	Mean	0.195250	14.592	11.048	8.418
	SD	0.000000	8.766	5.911	4.680
	N	5	5	5	5
3	Geometric Mean	0.233734	31.012	30.951	23.166
	Mean	0.252200	45.710	61.296	44.556
	SD	0.127344	38.730	68.327	53.803
	N	5	5	5	5
5	Geometric Mean	0.271765	9.302	8.057	7.529
	Mean	0.360200	10.678	9.136	7.868
	SD	0.368839	7.292	5.517	2.613
	N	5	5	5	5
6	Geometric Mean	0.195250	45.775	45.117	32.038
	Mean	0.195250	179.426	184.188	74.478
	SD	0.000000	249.742	274.844	77.310
	N	5	5	5	5

Significantly different from control group (group 1) value: D - P ≤ 0.05 E - P ≤ 0.01 F - P ≤ 0.001 (Dunn)

Group Mean T-Cell Dependent Antibody Response (Anti-KLH IgG)

Main Study
Males

Group 1 - Air Control
Group 2 - Technosphere® Powder Control Low Dose
Group 3 - Technosphere® Powder Control High Dose

Group	Summary Information	Mean (µg/mL) Day 11	Day 26	Day 29
1	Geometric Mean	0.19550	2.618	4.301
	Mean	0.19550	4.749	7.711
	SD	0.00000	3.978	6.074
	N	10	10	10
2	Geometric Mean	0.19550	4.149	7.520
	Mean	0.19550	6.758	12.343
	SD	0.00000	5.910	9.845
	N	10	10	10
3	Geometric Mean	0.19550	2.577	4.170
	Mean	0.19550	6.463	12.159
	SD	0.00000	7.650	15.742
	N	10	10	10

Significantly different from control group (group 1) value: D - P ≤ 0.05 E - P ≤ 0.01 F - P ≤ 0.001 (Dunn)

Group 4 - Technosphere®/Insulin Low Dose
Group 5 - Technosphere®/Insulin High Dose
Group 6 - Fumaryl Diketopiperazine (FDKP)* Subcutaneous

Group	Summary Information	Mean (µg/mL) Day 11	Day 26	Day 29
4	Geometric Mean	0.19550	6.944	11.915
	Mean	0.19550	10.625	17.861
	SD	0.00000	9.615	15.251
	N	10	10	10
5	Geometric Mean	0.19550	7.424	15.548
	Mean	0.19550	8.428	18.328
	SD	0.00000	3.860	11.581
	N	10	10	10
6	Geometric Mean	0.19550	9.414	17.683
	Mean	0.19550	17.731	31.600 D
	SD	0.00000	19.333	30.232
	N	10	10	10

Significantly different from control group (group 1) value: D - P ≤ 0.05 E - P ≤ 0.01 F - P ≤ 0.001 (Dunn)

Group Mean T-Cell Dependent Antibody Response (Anti-KLH IgG)

Main Study
Females

Group 1 - Air Control
Group 2 - Technosphere® Powder Control Low Dose
Group 3 - Technosphere® Powder Control High Dose

Group	Summary Information	Mean (µg/mL) Day 11	Day 26	Day 29
1	Geometric Mean	0.19550	3.981	8.349
	Mean	0.19550	12.641	27.150
	SD	0.00000	15.309	30.164
	N	10	10	10
2	Geometric Mean	0.19550	4.288	7.122
	Mean	0.19550	8.203	15.107
	SD	0.00000	6.776	14.437
	N	10	10	10
3	Geometric Mean	0.19550	11.047	18.984
	Mean	0.19550	17.626	34.755
	SD	0.00000	17.539	42.698
	N	10	10	10

Significantly different from control group (group 1) value: D - P ≤ 0.05 E - P ≤ 0.01 F - P ≤ 0.001 (Dunn)

Group 4 - Technosphere®/Insulin Low Dose
Group 5 - Technosphere®/Insulin High Dose
Group 6 - Fumaryl Diketopiperazine (FDKP)* Subcutaneous

Group	Summary Information	Mean (µg/mL) Day 11	Day 26	Day 29
4	Geometric Mean	0.19550	14.103	23.455
	Mean	0.19550	23.340	37.942
	SD	0.00000	27.147	43.667
	N	10	10	10
5	Geometric Mean	0.19550	4.163	9.884
	Mean	0.19550	11.876	24.993
	SD	0.00000	16.575	31.625
	N	10	10	10
6	Geometric Mean	0.19550	7.282	13.279
	Mean	0.19550	11.922	19.680
	SD	0.00000	10.242	16.818
	N	10	10	10

Significantly different from control group (group 1) value: D - P ≤ 0.05 E - P ≤ 0.01 F - P ≤ 0.001 (Dunn)

Group Mean T-Cell Dependent Antibody Response (Anti-KLH IgG)

Recovery
Males

Group 1 - Air Control
Group 3 - Technosphere® Powder Control High Dose
Group 5 - Technosphere®/Insulin High Dose
Group 6 - Fumaryl Diketopiperazine (FDKP)* Subcutaneous

Group	Summary Information	Mean (µg/mL) Day 39	Day 54	Day 57
1	Geometric Mean	0.19550	1.997	2.533
	Mean	0.19550	3.186	3.764
	SD	0.00000	3.195	3.413
	N	5	5	5
3	Geometric Mean	0.19550	0.397	0.728
	Mean	0.19550	0.593	1.103
	SD	0.00000	0.590	1.108
	N	5	5	5
5	Geometric Mean	0.19550	14.915	15.261
	Mean	0.19550	52.970	31.398
	SD	0.00000	69.428	43.186
	N	5	5	5
6	Geometric Mean	0.19550	1.276	2.624
	Mean	0.19550	1.865	5.041
	SD	0.00000	1.332	4.698
	N	5	5	5

Significantly different from control group (group 1) value: D - P ≤ 0.05 E - P ≤ 0.01 F - P ≤ 0.001 (Dunn)

Group Mean T-Cell Dependent Antibody Response (Anti-KLH IgG)

Recovery
Females

Group 1 - Air Control
Group 3 - Technosphere® Powder Control High Dose
Group 5 - Technosphere®/Insulin High Dose
Group 6 - Fumaryl Diketopiperazine (FDKP)* Subcutaneous

Group	Summary Information	Mean (µg/mL) Day 39	Day 54	Day 57
1	Geometric Mean	0.19550	1.806	2.782
	Mean	0.19550	2.098	3.286
	SD	0.00000	1.066	1.819
	N	5	5	5
3	Geometric Mean	0.19550	2.295	6.084
	Mean	0.19550	6.637	11.940
	SD	0.00000	7.695	9.785
	N	5	5	5
5	Geometric Mean	0.19550	1.442	2.779
	Mean	0.19550	2.333	5.019
	SD	0.00000	2.060	3.922
	N	5	5	5
6	Geometric Mean	0.19550	4.454	8.401
	Mean	0.19550	13.026	26.126
	SD	0.00000	16.160	33.868
	N	5	5	5

Significantly different from control group (group 1) value: D - P ≤ 0.05 E - P ≤ 0.01 F - P ≤ 0.001 (Dunn)

Overall, daily inhalation of either T or TI powder in male and female rats after 28 days of exposure via inhalation or SC administration resulted in treatment-related changes in the relative percentage of some lymphocyte subsets but had no effect on their absolute numbers, in a decrease in NK lymphocytes, and no changes in the IgM and IgG T-cell dependent antibody response. Note from the reviewer: Anti-drug antibody responses were not looked at in chronic toxicology studies with neither T nor TI.

MKC-PC-2008-0021: FDKP (as Technosphere® Particles) Partition Coefficient [By (b) (4) , Study No. MKC0004]

This study was to determine the partition coefficient FDKP. As the test substance is an ionisable compound, separate tests were conduced at each of pH 4, 7, and 9. Findings: FDKP was determined to have $\log_{10}P_{ow}$ values of -2.4, <-3.4, and <-3.4 at pH 4, 7, and 9 respectively. Note from the reviewer: This study should be included in the PK section as this has to do with lung absorption but it is unclear how based on the study findings.

MKC-PC-2008-0023: Primary Eye Irritation/Corrosion Study with Three FDKP Intermediates in Rabbits [By (b) (4) Study no. 7260-113]

This study assessed the relative level of irritation/corrosion produced following a single exposure of 3 FDKP intermediates to one eye of albino rabbits. Male Hra:(NZW)SPF rabbits received a single ocular instillation of 1 of 3 test articles: #1 (b) (4), #2 (b) (4) or #3 (b) (4) for 24 hrs before rinsed with saline. The treated eyes of all animals were examined for ocular irritation at approx. 1, 24, 48, and 48 hrs after treatment. Mortality, clinical observation, and body weights were assessed.

Findings: There were no treatment-related changes in body weights. For Test Article #1 (b) (4), ocular instillation caused mild irritation to the iris and severe irritation to the conjunctiva,

including swelling, redness, and discharge that progressed in severity from Total Irritation Score of 21 at 1 hr after treatment to 25 at 24 hrs after treatment. The severe irritation and other observations such as hyperemia, blepharspasm, and photophobia, led to the animals being euthanized on Day 2. The irritancy category of severely irritating for test article #1 was also based on the appearance of the animals and the observations, such as severe peri-orbital swelling.

Individual and Mean Eye Irritation Data

Group 1 – Test Article #1

Animal Number	Cornea		Iris	Conjunctivae			Total Score*
	A	B**	A	A	B	C	
			1 Hr				
F67743 [a]	0	0	1	2	3	3	21
F67444 [a]	0	0	1	2	3	3	21
F67745 [a]	0	0	1	2	3	3	21
						Mean	**21.0**
			24 Hr				
F67743 [a, d, b, g, h, e, f]	0	0	1	3	4	3	25
F67744 [a, d, b, e, f, g, h]	0	0	1	3	4	3	25
F67745 [a, d, b, e, f, g, h]	0	0	1	3	4	3	25
						Mean	**25.0**

a Test material present on eye
b Right eye was washed with saline
d Sodium fluorescein test performed (-).
e Folds Above normal
f Congestion
g Swelling
h Circumcorneal injection

Cornea	Iris	Conjunctivae
A – Degree of opacity B – Area of involvement	A – Degree of iridal irritation	A – Redness B – Chemosis C – Discharge

* Total Score: Corneal Score (A x B x 5) + Iridal Score (A x 5) + Conjunctival Score (A + B + C) x 2
** Data entered as '0' as a representation of '—'

For Test Article #2 (b) (4), it caused mild signs of irritation of the conjunctivae at 1 hr after treatment with Total Irritation Score of 6. The treated eyes were normal after 24 hrs and thereafter with the Total Irritation Score of 0.

Group 2 – Test Article #2

Animal Number	Cornea A	Cornea B**	Iris A	Conjunctivae A	Conjunctivae B	Conjunctivae C	Total Score*
			1 Hr				
F67746 [a]	0	0	0	1	0	1	4
F67447 [a]	0	0	0	1	1	2	8
F67748 [a]	0	0	0	1	1	1	6
						Mean	**6.0**
			24 Hr				
F67746 [a, d, b]	0	0	0	0	0	0	0
F67447 [a, d, b]	0	0	0	0	0	0	0
F67748 [a, d, b]	0	0	0	0	0	0	0
						Mean	**0.0**
			48 Hr				
F67746	0	0	0	0	0	0	0
F67447	0	0	0	0	0	0	0
F67748	0	0	0	0	0	0	0
						Mean	**0.0**
			72 Hr				
F67746	0	0	0	0	0	0	0
F67447	0	0	0	0	0	0	0
F67748	0	0	0	0	0	0	0
						Mean	**0.0**

a Test material present on eye.
b Right eye was washed with saline.
d Sodium fluorescein test performed (-).

Cornea	Iris	Conjunctivae
A – Degree of opacity B – Area of involvement	A – Degree of iridal irritation	A – Redness B – Chemosis C – Discharge

* Total Score: Corneal Score (A x B x 5) + Iridal Score (A x 5) + Conjunctival Score (A + B + C) x 2
** Data entered as '0' as a representation of '—'

For Test Article #3 (b) (4), it caused mild signs of irritation to the conjunctivae at 1 hr after treatment with Total Irritation Score of 3.3. The treated eyes were normal after 24 hrs and thereafter with the Total Irritation Score of 0. The irritancy of mildly irritating was based on all scores were not 0 at the 48 hr reading.

Group 3 – Test Article #3

Animal Number	Cornea A	Cornea B**	Iris A	Conjunctivae A	Conjunctivae B	Conjunctivae C	Total Score*
			1 Hr				
F67749 [a]	0	0	0	1	0	0	2
F67450 [a]	0	0	0	1	0	1	4
F67751 [a]	0	0	0	1	0	1	4
						Mean	**3.3**
			24 Hr				
F67749 [d, b]	0	0	0	0	0	0	0
F67450 [d, b]	0	0	0	0	0	0	0
F67751 [a, d, b]	0	0	0	0	0	0	0
						Mean	**0.0**
			48 Hr				
F67749	0	0	0	0	0	1	2
F67450	0	0	0	0	0	0	0
F67751	0	0	0	0	0	0	0
						Mean	**0.7**
			72 Hr				
F67749	0	0	0	0	0	0	0
F67450	0	0	0	0	0	0	0
F67751	0	0	0	0	0	0	0
						Mean	**0.0**

a Test material present on eye.
b Right eye was washed with saline.
d Sodium fluorescein test performed (-).

Cornea	Iris	Conjunctivae
A – Degree of opacity B – Area of involvement	A – Degree of iridal irritation	A – Redness B – Chemosis C – Discharge

* Total Score: Corneal Score (A x B x 5) + Iridal Score (A x 5) + Conjunctival Score (A + B + C) x 2
** Data entered as '0' as a representation of '—'

Overall, Test article #1 was severely irritating with maximum mean primary ocular irritation index of 25 at 24 hr postinstillation. Test article #2 was minimally irritating and non-corrosive with the maximum mean primary ocular irritation index of 6 at 1 hr postinstillation, and with the additional observations of hyperemia, blepharspasm, and photophobia, led to the animals being euthanized on Day 2 of the study. Test article #3 was mildly irritating and noncorrosive with a maximum mean primary ocular irritation index of 3.3 at 1 hr postinstillation. In short, the starting material (b) (4) showed a higher ocular irritancy score in rabbits than either of the other 2 intermediates (b) (4) Note from the reviewer: it is unclear how these three FDKP intermediates relative to the MRHD in a clinical setting.

MKC-PC-2008-0024: Technosphere® Powder Assessment of Ready Biodegradability: Modified Strum Test [By (b) (4) Study No. MKC0006]

This study was to assess the ready biodegradability of Technosphere® powder in the CO2 Evolution test. The test duration was 43 days. Findings: substances were considered to be readily biodegradable in such test if CO2 production is equal to or greater than 60% of the theoretical value within 10 days of the level, therefore, achieving 10% Technosphere® powder, cannot be considered readily biodegradable. However, the test substance may be considered as ultimately biodegradable.

2.6.7 TOXICOLOGY TABULATED SUMMARY

2.6.7.1 Toxicology Overview Technosphere Insulin (TI)

Type of Study	Species and Strain	Method of Admin	Duration of Dosing	Doses (mg/kg)	GLP	Testing Facility	Study No.	Location Vol.	Page
Single-Dose Toxicity									
	SD Rat	Oral gavage	Single + 14-day observation period	T : 5,000	Yes	(b) (4)	PDC-PC-1993-0001 (b) (4) No.: 93-8117-21		
	SD Rat	Oral gavage	Single + 14-day observation period	T : 50 (± calcitonin; 1% loaded)	Yes		PDC-PC-1993-0002 (b) (4) No.: 93-8118-21		
	Beagle Dog	Oral gelatin capsules	Single + 14-day observation period	T : 5,000	Yes		PDC-PC-1994-0002 (b) (4) No 94452		
Repeat-Dose Toxicity									
	Hybrid Mouse	sc	28 days	T : 10, 35, 75 TI : 2.5, 5, 10, 15, & 35	Yes		MKC-PC-2006-0011 AB29DV/-AB29DW.253R (b) (4)		
	CD1 Mouse	sc	13 weeks	T : 10, 75 TI: 10, 35	Yes		MKC-PC-2005-0011 (b) (4) No. 7679		
	Chbb Rat	Inhalation	4 weeks	T: 0, 0.5, 14.4, & 61.7	Yes		PDC-PC-1996-0002 (b) (4) No. I96032		
	SD Rat	Inhalation	13/26 weeks	T: 0, 5.3 (M), & 51.3 (F)	Yes		PDC-PC-1999-0002 (b) (4) No. 91740		
	SD Rat	Inhalation	26 weeks	T: 11.7 (M, F) TI: 1.1 or 2.9 U/kg (M) TI: 0.4 or 1.1 U/kg (F) TI: 1.9 or 5.2 U/kg (M) TI: 1.3 or 3.5 U/kg (F)	Yes		MKC-PC-2003-0002 (b) (4) No. 78235		

Type of Study	Species and Strain	Method of Admin	Duration of Dosing	Doses (mg/kg)	GLP	Testing Facility	Study No.	Location Vol.	Page
Repeat-Dose Toxicity									
	Beagle Dog	Inhalation	5 days	T: 15.18 (M); 16.74 (F)	No	(b) (4)	PDC-PC-1996-0001 (b) (4) No. I96009		
	Beagle Dog	Inhalation	5 days	T: 1.966 TI: 0.265, 0.894, 1.966	Yes		MKC-PC-2003-0022 (b) (4) 5031		
	Beagle Dog	Inhalation, sc	4 weeks	T: 0, 0.3, 4.0, & 40.3 Technosphere	Yes		PDC-PC-1997-0001 (b) (4) No. I97003		
	Beagle Dog	Oral	30 days	Technosphere 0, 50	Yes		PDC-PC-1994-0001 (b) (4) No. 94443		
		Inhalation	13, 26, & 39 weeks	T: 0, 5, & 38 TI: 1.9	Yes		PDC-PC-2000-0003 (b) (4) No. 91739		
		Inhalation	26 & 39 weeks	T: 2.4 T: 10.9 TI: 0.39 or 0.04 U/kg TI: 1.92 or 0.22 U/kg	Yes		MCK-PC-2003-0004 (b) (4) No. 2973		

Type of Study	Species and Strain	Method of Admin	Duration of Dosing	Doses (mg/kg)	GLP	Testing Facility	Study No.	Location Vol.	Page
Genotoxicity									
	Salmonella typhimurium *Escherichia coli*	In Vitro	-	T: 0, 50, 150, 500, 1500, & 5000 µg/plate TI: 0, 50, 150, 500, 1500, & 5000 µg/plate	Yes	(b) (4)	MKC-PC-2004-0002 AA89DG-DH503 (b) (4)		
	Human Lymphocyte	In Vitro	4 hours incubation 4 hours incubation 20 hours	T: -S9: 0, 281, 562.5, & 1125 µg/mL T: +S9: 0, 562.5, 1125, & 2250 µg/mL T: -S9: 0, 35, 70, & 140 µg/mL	Yes		PDC-PC-2000-0006 AA37RS.341 (b) (4)		
	Human Lymphocyte	In Vitro	4 hours incubation 4 hours incubation 20 hours	TI: -S9: 0, 1250, 2500, & 5000 µg/mL TI: +S9: 0, 1250, 2500, & 5000 µg/mL TI: -S9: 0, 1250, 2500, & 5000 µg/mL	Yes		PDC-PC-2002-0003 AA60RE.341 (b) ( )		
	ICR Mice	ip	Single dose	T: 24 hours (M): 400, 800, & 1600 mg/kg T: 24 hours (F): 450, 900, & 1800 mg/kg T: 48 hours both sexes: 1600 (M), & 1800 (F) mg/kg	Yes		PDC-PC-2002-0011 AA68EH.123 (b) ( )		

Type of Study	Species and Strain	Method of Admin	Duration of Dosing	Doses (mg/kg)	GLP	Testing Facility	Study No.	Location Vol.	Page
Carcinogenicity									
	Hybrid (rasH2) Mouse	sc	26 weeks	T: 0, 25, & 75 TI: Low: 2.5 (M), 2.5→0.6 (F) High:5.0 (M), 5.0→1.25 (F)	Yes	(b) (4)	MKC-PC-2007-0003 (b) (4) No. 7260-107		
	SD Rat	Inhalation	104 weeks	T: 0, Low: 1.104 (M), 0.678 (F) High: 41 (M), 46 (F) TI: Low: 0.6572 or 0.0787 U/kg (M) 0.4413 or 0.0529 U/kg (F) High: 1.2313 or 0.1457 U/kg (M) 0.7608 or 0.0900 U/kg (F)	Yes		MKC-PC-2004-0001 (b) (4) Study No. 575 1		

Type of Study	Species and Strain	Method of Admin	Duration of Dosing	Doses (mg/kg)	GLP	Testing Facility	Study No.	Location Vol.	Page
Reprotoxicology									
	SD Rat	sc	(M): 4 weeks prior to mating; & with mating F: 2 weeks prior to mating; & with mating to G7	T: 0, 10, 30, & 100	Yes	(b) (4)	PDC-PC-2001-0006 (b) (4) No. 98064		
	SD Rat	sc	G6-G17	T: 0, 10, 30, & 100	No		PDC-PC-2002-0012 (b) (4) No. 96921		
	SD Rat	sc	G6-G17	T: 0, 10, 30, & 100	Yes		PDC-PC-2001-0003 (b) (4) No. 98061		
	NZW Rabbit	sc	G7-G19	T: 0, 1, 10, 30, & 100	Yes		MKC-PC-2004-0003 (b) (4) 100001		
	NZW Rabbit	sc	G7-G19	T: 0, 2, 10, & 100	Yes		MKC-PC-2004-0004 (b) (4) 100002		
	SD Rat	sc	G7 – L20 postpartum; G24- rats not delivering	T: 0, 10, 30, & 100	Yes		MKC-PC-2004-0008 (b) (4) 100003		
	SD Rat	sc	G7 – L20 postpartum; G24- rats not delivering	T: 0, 10, 30, & 100	Yes		MKC-PC-2004-0008 (b) (4) 100003		

Type of Study	Species and Strain	Method of Admin	Duration of Dosing	Doses (mg/kg)	GLP	Testing Facility	Study No.	Location Vol.	Page
Local Tolerance									
	NZW Rabbit	Dermal Abraded & intact	Single	T: 0.5 g TI: 0.5 g	Yes	(b) (4)	MKC-PC-2007-0019 (b) (4) No. LCL00021		
Other									
Antigen	Guinea Pig	id	Single & 2 bi-monthly challenges	T, TI: 5% w/v dermal inj T, TI: 25% w/w dermal application	Yes		PDC-PC-2001-0001 (b) (4) 7260-100		
Immunotox	Rat	Inhalation / sc	28 days	Low T: 1.38 (M); 0.82 (F) High T: 44.0 (M); 47.0 (F) Low TI: 0.77 (M) Low TI: 0.50 (F) High TI: 1.33 (M) High TI: 0.79 (F) T: sc: 75 (M) & (F)	Yes		MKC-PC-2008-0020 (b) (4) dy No. 75101		
Impurities	Rat	sc	28 days	FDKP Impurities: 0, 10, 35, & 75	Yes		MKC-PC-2008-0006 (b) Study No. 70569		
PS80	Rat	Inhalation	28 days	T: 12→16 & 45 PS80:0.29→0.40 & 1.11	Yes		MKC-PC-2008-0005 (b) Study No. 75065		
Phototox	3T3 Fibro-Blasts	In Vitro	20-60 minutes	T & TI: 10, 15, 50, 60, 100, 150, 300, 500, 600, & 1000 mg/L ± UVA	Yes		MKC-PC-2007-0004 (b) (4) No. LCL00016		

Type of Study	Species and Strain	Method of Admin	Duration of Dosing	Doses (mg/kg)	GLP	Testing Facility	Study No.	Location Vol.	Page
Other									
Ecotox	*Daphnia magna*	Exposure in medium	24 & 48 hours	T: Nominal = 100 mg/L T: Actual = 97.2 mg/L	Yes	(b) (4)	MKC-PC-2008-0001 (b) (4) No. MKC0002		
	Pseudokirchneriella subcapitata	Exposure in medium	24, 48, & 72 hours	T: Nominal = 100 mg/L T: Actual = 101 mg/L	Yes		MKC-PC-2008-0002 (b) (4) No. MKC0003		
Intermediate	NZW Rabbit	Topical; dermal	Single dose: 1, 24, 48, & 72-hour evaluations	Intermed 1, 2, 3 : 0.5 g, moistened with distilled water	Yes		MKC-PC-2008-0016 (b) (4) No. 7260-112		
	Guinea Pig	Topical: dermal	3 weekly induction doses; challenge dose 2 weeks later	Intermed 1: 95% Intermed 2: 86% Intermed 3: 86%	Yes		MKC-PC-2008-0018 (b) (4) No. 7260-115		
	SD Rat	Oral Gavage	Single	Intermed 1: 300, 2000 Intermed 2: 2000 Intermed 3: 2000	Yes		MKC-PC-2008-0019 (b) (4) No. 7260-114		
	NZW Rabbit	Topical: Ocular	Single	Intermed 1: 0.0671 g Intermed 2: 0.0553 g Intermed 3: 0.0388 g	Yes		MKC-PC-2008-0023 (b) (4) No. 7260-113		

Type of Study	Species and Strain	Method of Admin	Duration of Dosing	Doses (mg/kg)	GLP	Testing Facility	Study No.	Location Vol.	Page
Other									
Log P	In Vitro -	In Vitro -	-	T	Yes	(b) (4)	MKC-PC-2008-0021 (b) (4) No. MKC0004		
Biodegrada-tion	In Vitro -	In Vitro	-	T	Yes		MKC-PC-2008-0024 (b) (4) No. MKC0006		

G = Gestation Day
L = Lactation Day
(b) (4)
T = Technosphere particles
TI = Technosphere Insulin
sc = subcutaneous route

2.6.7.2 Toxicokinetics: Overview of Toxicokinetics Studies

Test Article: Technosphere or Technosphere Insulin Inhalation Powder

Type of Study	Test System	Method of Admin.	Doses (mg/kg)	GLP	Study Number	Location Vol.	Page
28-day range-finding study	Hybrid Mice	sc	T: 10, 35, & 75 TI: 2.5, 5, 10, 15, & 35	Yes	MKC-PC-2006-0011 AB29DV/AB29DW.2S3R (b) (4)		
13-week toxicity study	Mice	sc	T: 10, 75 TI: 10, 35	Yes	MKC-PC-2005-0011 (b) (4) No. 7679		
28-day toxicity study	Rats	Inhalation	T: 0, 0.5, 14.4, & 61.7	Yes	PDC-PC-1996-0002 (b) (4) No. I96032		
26-week toxicity study	Rats	Inhalation	T: 11.7 (M,F) TI: 1.1 or 3 U/kg (M); 0.4 or 1 U/kg f TI: 1.9 or 5 U/kg (M); 1.3 or 3 U/kg (F)	Yes	MKC-PC-2003-0002 (b) (4) No. 78235		
5-day tolerability study	Dogs	Inhalation	T: 15.18 (M); 16.74 (F)	No	PDC-PC-1996-0001 (b) (4) No. I96009		
4-week toxicity study	Dogs	Inhalation; sc	T: 0, 0.3, 4.0, & 40.3 T/C: 7.4 Calcitonin: 0.1→0.05 sc	Yes	PDC-PC-1997-0001 (b) (4) No. I97003		
13/26/39-week toxicity study	Dogs	Inhalation	T: 2.4 & 10.9 TI: 0.39 or 0.04 U/kg; 1.92 or 0.22 U/kg	Yes	MKC-PC-2003-0004 (b) (4) No. 2973		

Type of Study	Test System	Method of Admin.	Doses (mg/kg)	GLP	Study Number	Location Vol.	Page
Carcinogenicity study	Hybrid Mice	sc	T: 0, 25, & 75 TI: Low: 2.5 (M), 2.5→0.6 (F) TI High: 5.0 (M), 5.0→1.25 (F)	Yes	MKC-PC-2007-0003 (b) (4) No. 7260-107		
Carcinogenicity study	Rats	Inhalation	T: Low: 1.104 (M), 0.6784 (F) High: 41 (M), 46 (F) TI: Low: 0.6572 / 0.0787 mg/kg (insulin) (M); 0.4413 / 0.0529 mg/kg (insulin) (F) High: 1.2313 / 0.1457 mg/kg (insulin) (M) 0.7608 or 0.0900 mg/kg (insulin) (F)	Yes	MKC-PC-2004-0001 (b) (4) Study No. 5751		
Pilot teratology study	Rabbit	sc	T: 1, 10, 30, & 100	Yes	MKC-PC-2004-0003 (b) (4) 100001		
Teratology study	Rabbit	sc	T: 2, 10 & 100	Yes	MKC-PC-2004-0004 (b) (4) 100002		
Peri/postnatal development	Rat	sc	T: 10, 30 & 100 (BID = 5, 15,50)	Yes	MKC-PC-2004-0008 (b) (4) 100003		
Fetal Toxicokinetic study	Rat	sc	T: 10, 30 & 100 (BID = 5, 15, 50)	Yes	MKC-PC-2005-0010 (b) (4) 00009		

2.6.7.3 Toxicokinetics Overview of Toxicokinetics Data

Test Article: Technosphere or Technosphere Insulin Inhalation Powder

AUC (ng·min/mL)									
Daily Dose (mg/kg)	Route	Mice		Rats		Dogs		Female Rabbits	Humans
		M	F	M	F	M	F		
0.39 TI	Inhalation					16233[f]	21373[f]		
0.4 TI	Inhalation			-	97895[e]				
0.44 TI	Inhalation			-	(133)[h]				
0.657 TI	Inhalation			(285)[h]	-				
0.678 T	Inhalation			-	(262)[h]				
0.761 TI	Inhalation			-	(160)[h]				
1.0 T	sc							282960[f]	
1.1 TI	Inhalation			140457[e] (465)[h]	- -				
1.2 TI	Inhalation			(545)[h]	-				224245
1.3 TI	Inhalation				176373[e]				
1.9 TI	Inhalation			191492[e]	-	72512[f]	76284[f]		
2.0 T	sc							484240[f]	
2.4 T	Inhalation					102604[f]	112946[f]		
2.5 TI	sc	99695[g]	22771[g]						
4.0 T	Inhalation					191400[e] 154800[e]	212400[e] 172800[e]		
5.0 TI	sc	189459[g]	44147[g]						
5.0 T	sc				374947[k]				

AUC (ng·min/mL)									
Daily Dose (mg/kg)	Route	Mice		Rats		Dogs		Female Rabbits	Humans
		M	F	M	F	M	F		
7.4 T/C	Inhalation					288000[e] 90000[e]	261000[e] 212400[e]		
10 TI	sc	340833[a]	364224[a]						
10 T	sc	495810[a]	460799[a]					3029640[i] 2689334[j]	
10.9 T	Inhalation					395824[f]	527344[f]		
11.7 T	Inhalation			514362[c]	703445[c]				
15.0 TI	sc								
15.0 T	sc.				1098107[k]				
15.2 T	Inhalation					(4.56)[d]	-		
16.7 T	Inhalation					-	(12.14)[d]		
25 T	sc	1173006[g]	962262[g]						
30.0 T	sc							5752560[i]	
35 TI	sc	1394130[a]	1576578[a]						
35 T	sc								
40.3 T	Inhalation					1416000[e] 1158000[e]	1776000[e] 1512000[e]		
41.0 T	Inhalation			(4488)[c]	-				
46.0 T	Inhalation			-	(6260)[c]				
50 T	sc				2840264[k]				
61.7 T	Inhalation			381600[b]	401400[b]				

AUC (ng·min/mL)									
Daily Dose (mg/kg)	Route	Mice		Rats		Dogs		Female Rabbits	Humans
		M	F	M	F	M	F		
75 T	sc	3551454[a]	4610297[a]						
75 T	sc	2968476[g]	2753474[g]						
100 T	sc							23370480[i] 28731895[j]	

Notes: 1) Values in parenthesis are C_{max} in µg/mL
2) Human exposure based on maximum human daily dose of 105 mg TI per day (93 mg T) with 11.4% Insulin = 1.3 mg/kg T

[a] at Day 91
[b] at Day 24
[c] at Day 182
[d] at Day 5
[e] at Day 29; values for FDKP-1 and FDKP-2
[f] at Week 39
[g] at Week 26; female doses: 2.5→0.6 mg/kg, 5.0→1.25 mg/kg (mouse sc carcinogenicity study)
[h] at Week 104; C_{max} available only (rat inhalation carcinogenicity study)
[i] at Gestation Day 19 (range-finding)
[j] at Gestation Day 19 (GLP)
[k] at Lactation Day 20, after first of two daily doses

2.6.7.4 Toxicokinetic Data (Ranking)

Overview of Toxicokinetics Data
FDKP Toxicokinetics

Test Article: Technosphere or Technosphere Insulin Inhalation Powder

FDKP AUC (ng-min/mL)	Species	Sex	Daily Dose (mg/kg)	Route	Type of Study	Study Interval	NOAEL[a] (T only)	Study No.
16,233	Dog	M	0.39 TI	Inhalation	39-week Tox	Week 39	Below	MKC-PC-2003-0004
21,373	Dog	F	0.39 TI	Inhalation	39-week Tox	Week 39	Below	MKC-PC-2003-0004
22,771	Mouse	F	2.5→0.625 TI	sc	26-week Carcino	Week 26	Below	MKC-PC-2007-0003
44,147	Mouse	F	5.0→1.25 TI	sc	26-week Carcino	Week 26	Below	MKC-PC-2007-0003
72,512	Dog	M	1.91 TI	Inhalation	39-week Tox	Week 39	Below	MKC-PC-2003-0004
76,284	Dog	F	1.91 TI	Inhalation	39-week Tox	Week 39	Below	MKC-PC-2003-0004
97,895	Rat	F	0.4 TI	Inhalation	6-month Tox	Day 182	Below	MKC-PC-2003-0002
99,695	Mouse	M	2.5 TI	sc	26-week Carcino	Week 26	Below	MKC-PC-2007-0003
102,604	Dog	M	2.4 T	Inhalation	39-week Tox	Week 39	Below	MKC-PC-2003-0004
112,946	Dog	F	2.4 T	Inhalation	39-week Tox	Week 39	Below	MKC-PC-2003-0004
140,457	Rat	M	1.1 TI	Inhalation	6-month Tox	Day 182	Below	MKC-PC-2003-0002
176,373	Rat	F	1.3 TI	Inhalation	6-month Tox	Day 182	Below	MKC-PC-2003-0002
189,459	Mouse	M	5.0 TI	sc	26-week Carcino	Week 26	Below	MKC-PC-2007-0003
191,492	Rat	M	1.9 TI	Inhalation	6-month Tox	Day 182	Below	MKC-PC-2003-0002
224,245	Human		1.3 TI	Inhalation	Max Daily Dose		-	Clinical Study 110
282,960	Rabbit	F	1.0 T	sc	RF Teratology	GD19	Below	MKC-PC-2004-0003
340,833	Mouse	M	10 TI	sc	90-day Tox	Day 91	Below	MKC-PC-2005-0011
346,200	Dog	M	4.0 T	Inhalation	1-month Tox	Day 29	Below	PDC-PC-1997-0001
364,224	Mouse	F	10 TI	sc	90-day Tox	Day 91	Below	MKC-PC-2005-0011
374,947	Rat	F	5.0 T	sc	Peri/postnatal Repro	DL 20	Below	MKC-PC-2004-0008
378,000	Dog	M	7.4 T/C	Inhalation	1-month Tox	Day 29	Below	PDC-PC-1997-0001
381,600	Rat	F	61.7 T	Inhalation	1-month Tox	Day 24	NOAEL	PDC-PC-1996-0002

FDKP AUC (ng-min/mL)	Species	Sex	Daily Dose (mg/kg)	Route	Type of Study	Study Interval	NOAEL[a] (T only)	Study No.
385,200	Dog	F	4.0 T	Inhalation	1-month Tox	Day 29	Below	PDC-PC-1997-0001
395,824	Dog	M	10.9 T	Inhalation	39-week Tox	Week 39	NOAEL	MKC-PC-2003-0004
401,400	Rat	M	61.7 T	Inhalation	1-month Tox	Day 24	NOAEL	PDC-PC-1996-0002
460,799	Mouse	F	10 T	sc	90-day Tox	Day 91	Below	MKC-PC-2005-0011
473,400	Dog	F	7.4 T/C	Inhalation	1-month Tox	Day 29	Below	PDC-PC-1997-0001
482,240	Rabbit	F	2.0 T	sc	Teratology	GD19	Below	MKC-PC-2004-0004
495,810	Mouse	M	10 T	sc	90-day Tox	Day 91	Below	MKC-PC-2005-0011
514,362	Rat	M	11.7	Inhalation	6-month Tox	Day 182	NOAEL	MKC-PC-2003-0002
527,344	Dog	F	10.9 T	Inhalation	39-week Tox	Week 39	NOAEL	MKC-PC-2003-0004
703,445	Rat	F	11.7 T	Inhalation	6-month Tox	Day 182	NOAEL	MKC-PC-2003-0002
962,262	Mouse	F	25.0 T	sc	26-week Carcino	Week 26	Below	MKC-PC-2007-0003
1,098,107	Rat	F	15.0 T	sc	Peri/postnatal Repro	DL20	Below	MKC-PC-2004-0008
1,173,006	Mouse	M	25.0 T	sc	26-week Carcino	Week 26	Below	MKC-PC-2007-0003
1,394,130	Mouse	M	35.0 TI	sc	90-day Tox	Day 91	Below	MKC-PC-2005-0011
1,576,578	Mouse	F	35.0 TI	sc	90-day Tox	Day 91	Below	MKC-PC-2005-0011
2,574,000	Dog	M	40.3 T	Inhalation	1-month Tox	Day 29	NOAEL	PDC-PC-1997-0001
2,689,334	Rabbit	F	10.0 T	sc	Teratology	GD19	NOAEL	MKC-PC-2004-0004
2,753,474	Mouse	F	75.0 T	sc	26-week Carcino	Week 26	NOAEL	MKC-PC-2007-0003
2,840,264	Rat	F	50.0 T	sc	Peri/postnatal Repro	DL20	NOAEL	MKC-PC-2004-0008
2,968,476	Mouse	M	75.0 T	sc	26-week Carcino	Week 26	NOAEL	MKC-PC-2007-0003
3,029,640	Rabbit	F	10.0 T	sc	RF Teratology	GD19	NOAEL	MKC-PC-2004-0003
3,288,000	Dog	F	40.3 T	Inhalation	1-month Tox	Day 29	NOAEL	PDC-PC-1997-0001
3,551,454	Mouse	M	75.0 T	sc	90-day Tox	Day 91	NOAEL	MKC-PC-2005-0011
4,610,297	Mouse	F	75.0 T	sc	90-day Tox	Day 91	NOAEL	MKC-PC-2005-0011

FDKP AUC (ng·min/mL)	Species	Sex	Daily Dose (mg/kg)	Route	Type of Study	Study Interval	NOAEL[a] (T only)	Study No.
5,752,560	Rabbit	F	30.0 T	sc	RF Teratology	GD19	Above	MKC-PC-2004-0003
23,370,480	Rabbit	F	100.0 T	sc	RF Teratology	GD19	Above	MKC-PC-2004-0003
28,731,895	Rabbit	F	100.0 T	sc	Teratology	GD19	Above	MKC-PC-2004-0004

[a] Exposure either below, at, or above NOAEL exposure for each study

Notes: 1) Study No. MKC-PC-2004-0008: at Lactation Day 20, after first of two daily doses
2) Human exposure based on maximum human daily dose of 105 mg TI per day (b) (4)
3) Study No. PDC-PC-1997-0001: AUCs are total of values for FDKP1 and FDKP2

2.6.7.4A Toxicology: Drug Substance Test Article Batch Analysis Test Article: Technosphere or Technosphere Insulin Inhalation Powder

Test Article	FDKP Assay[a] (Area %)	Specified Impurity (Area %)[a]	FDKP Lot No.	FDKP[a] Purity (Area %)	Study No.	Type of Study
PDC 098-012	(b) (4)	(b) (4)	001E.96.02	(b) (4)	PDC-PC-1996-0002	Technosphere 4-week Inhalation Tox in Rats
			001E.96.03			
			001E.96.04			
PDC 102-002			001E.96.06		PDC-PC-1997-001	Technosphere/ Calcitonin: 4-week Inhalation Tox in Dogs (with SC Calcitonin arm)
			001E.96.07			
			001E.96.08			
			001E.96.09			
			001R.96.02			
			001R.96.03			
PDC 35A.060501			001.RW.0160		PDC-PC-2001-0003	Technosphere Teratology Rats
PDC 35A.060501			001.RW.0160		PDC-PC-2001-0006	Technosphere: Fertility in Rats
043R.02.024			FPPV08.02.02[b]			

Test Article	FDKP Assay[a] (Area %)	Specified Impurity (Area %)[a]	FDKP Lot No.	FDKP[a] Purity (Area %)	Study No.	Type of Study
D035U.02.006		(b) (4)	FPPV.08.02.02[b]	(b) (4)	PDC-PC-2002-0005	Technosphere Insulin: CV Safety in Dogs
PDC 35B0022			001.E00.52			
			001.E00.51			
			001.R00.02			
			001.R00.01			
D035U.02.002			FPPV072002-01[b]			
			FPPV072002-02[b]			
			FPPV072002-03[b]			
			FPPV072002-04[b]			
PDC 224.022 Tween-free Tech			FPPV.09.2002[b]			
			FPPV.09.004[b]			

NT = Not Tested NA = Not Applicable ND = Not Detected
NR = None above reporting threshold of (b) (4)% Tox = Toxicology w = week(s) NQ = Not Quantified

[a] Results generated via TM5319.

[b] Blended lot, not tested

Test Article	FDKP Assay [a] (Area %)	Specified Impurity (Area %)[a] (b) (4)	FDKP Lot No.	FDKP[a] Purity (Area %)	Study No.	Type of Study
D035U.02.006		(b) (4)	FPPV.08.02.02[b]	(b) (4)	PDC-PC-2002-0007	Technosphere Insulin: CNS Safety in Rats
D035U.02.002			FPPV072002-01[b]			
			FPPV072002-02[b]			
			FPPV072002-03[b]			
			FPPV072002-04[b]			
043R.02.024			FPPV08.02.02[b]			
D035U.02.002			FPPV072002-01[b]		PDC-PC-2002-0011	Technosphere: Gene Tox -Rat Micronucleus
			FPPV072002-02[b]			
			FPPV072002-03[b]			
			FPPV072002-04[b]			
PDC 35A.060501			001.RW.0160		PDC-PC-2002-0012	Technosphere: Pilot RF Teratology in Rats
D035U.03.010			D0001.EG.03.001		MKC-PC-2003-0004	Technosphere Insulin: 39-week Inhalation Tox in Dogs
D035U.03.011			D0001.EG.03.002			
D035U.03.012			D0001.EG.03.002			
			D0001.EG.03.001			
043RD.03.001			D0001.EG.03.002			

NT = Not Tested NA = Not Applicable ND = Not Detected
NR = None above reporting threshold of (b) (4) % Tox = Toxicology w = week(s) NQ = Not Quantified
[a] Results generated via TM5319.
[b] Blended lot, not tested

Test Article	FDKP Assay [a] (Area %)	Specified Impurity (Area %)[a] (b) (4)	FDKP Lot No.	FDKP [a] Purity (Area %)	Study No.	Type of Study
D035U.02.009		(b) (4)	FPPV.09.02.01[b]	(b) (4)	MKC-PC-2004-0001	Technosphere Insulin: 104-week Inhalation Carcinogenicity in Rats
D035U.03.008			FPML.073003[b]			
D035U.03.011			D0001.EG.03.002			
D035U.03.012			D0001.EG.03.002			
			D0001.EG.03.001			
D035U.04.001			D0001.EG.03.003			
D035U.04.002			D0001.EG.03.004			
D035U.04.003			D0001.EG.03.004			
			D0001.EG.03.005			
D035U.04.004			D0001.EG.04.001			
D035U.04.005			D0001.EG.04.001			
D035U.04.006			D0001.EG.04.001			
D035U.04.007			D0001.EG.04.001			
			D0001.EG.03.005			
D035U.04.008			D0001.EG.03.005			
D035U.04.009			D0001.EG.03.005			
			D0001.EG.04.002			
D035U.05.001			N1500			
D035U.05.004			N1502			
D035UA.04.002			D0001.EG.03.003			

Test Article	FDKP Assay [a] (Area %)	Specified Impurity (Area %)[a]	FDKP Lot No.	FDKP [a] Purity (Area %)	Study No.	Type of Study
		(b) (4)				
PM5069A		(b) (4)	D0001.EG.04.002	(b) (4)		
			D0001.EG.04.003			
PM5283A			N1501			
D232.06.001			N1871			
D232.06.002			N1871			
D043RD.03.001			D0001.EG.03.002			
D043RD.04.001			D0001.EG.03.004			
D043RD.04.003			D0001.EG.03.004			
D043RD.04.005			D0001.EG.03.004			
D043RD.04.006			D0001.EG.03.003			
D043RD.04.007			D0001.EG.03.003			
D043RD.04.008			D0001.EG.04.001			
D043RD.05.002			D0001.EG.04.003			

NT = Not Tested NA = Not Applicable ND = Not Detected
NR = None above reporting threshold of (b) (4) % Tox = Toxicology w = week(s)
[a] Results generated via TM5319.
[b] Blended lot, not tested

Test Article	FDKP Assay [a] (Area %)	Specified Impurity (Area %)[a]	FDKP Lot No.	FDKP[a] Purity (Area %)	Study No.	Type of Study
		(b) (4)				
D035U.03.008		(b) (4)	FPML073003[b]	(b) (4)	MKC-PC-2004-0002	Technosphere/ Insulin: Genetox - Bacterial Reverse Mutation Assay
D043RD.03.001			D0001.EG.03.002			
D035U.03.012			D0001.EG.03.002 D0001.EG.03.001		MKC-PC-2004-0003	Technosphere Pilot RF Teratology in Rabbits
D035U.03.012			D0001.EG.03.002 D0001.EG.03.001		MKC-PC-2004-0004	Technosphere Teratology in Rabbits
D035U.04.006			D0001.EG.04.001		MKC-PC-2004-0008	Technosphere Peri/postnatal in Rats
D035U.04.008 D043RD.03.003			D0001.EG.03.005 D0001.EG.03.003		MKC-PC-2005-0017	Technosphere Insulin: hERG assay
D232.06.001			N1871		MKC-PC-2006-0010	Technosphere Rat Fetal TK Study
D232.06.002 D043RD.05.002			N1871 D0001.EG.04.003		MKC-PC-2006-0038	Technosphere Insulin: Renal Function in Rats

Test Article	FDKP Assay [a] (Area %)	Specified Impurity (Area %)[a]	FDKP Lot No.	FDKP[a] Purity (Area %)	Study No.	Type of Study
		(b) (4)				
D232.06.004		(b) (4)	N1871	(b) (4)	MKC-PC-2007-0003	Technosphere Insulin: 26-weeks sc Carcinogenicity in Mice
D231.07.001			N2259			
D232.06.003			N1871 N1872		MKC-PC-2007-0021	Technosphere: Respiratory Safety
D232.06.003			N1871 N1872		MKC-PC-2007-0023	Technosphere Pulmonary Function Rats

NT = Not Tested NA = Not Applicable ND = Not Detected
NR = None above reporting threshold of (b) (4) % Tox = Toxicology w = week(s)
[a] Results generated via TM5319.
[b] Blended lot, not tested

Test Article	FDKP Assay Purity (Area %)	Specified Impurity (Area %)	FDKP Lot No.	FDKP (Area %)	Study No.	Type of Study
		(b) (4)				
FD 080.36A	(b) (4)	(b) (4)	N2259	(b) (4)	MKC-PC-2008-0005	Technosphere +PS80 28-day Inhalation Rat
			N2260			
FD 080.36B			N2259			
			N2260			
FD 080.64			NA		MKC-PC-2008-0006	Technosphere + Spiked Impurities 28-day Inhalation Rat
D232.06.002			N1871		MKC-PC-2008-0020	Technosphere Insulin: 28-day Inhalation Immunotox in Rats
D232.06.003			N1871			
			N1872			
D232.06.004			N1871			
D231.07.001			N2259			

NT = Not Tested NA = Not Applicable ND = Not Detected
NR = None above reporting threshold of (b) (4)% Tox = Toxicology w = week(s)

Additional Characterization Data – TM5478 Technosphere and Technosphere Insulin Inhalation Powder

Lot No.	Assay Purity (Wt %)	Specified Impurity (Area %) (RRT)
		(b) (4)
PDC 098-012		(b) (4)
D035U.02.002		
D035U.02.009		
D035U.03.008		
D035U.03.011		
D035U.03.012		
D035U.04.001		
D035U.04.002		
D035U.04.003		
D035U.04.004		
D035U.04.005		
D035U.04.006		
D035U.04.007		
D035U.04.008		
D035U.04.009		
D035U.05.004		

Additional Characterization Data – TM5478 Technosphere and Technosphere Insulin Inhalation Powder

Lot No.	Assay Purity (Wt %)	Specified Impurity (Area %) (RRT)
		(b) (4)
PM5069A		(b) (4)
D232.06.001		
D232.06.002		
D035UA.04.002		

NT = Not Tested NA = Not Applicable ND = Not Detected NR = None above reporting threshold of (b) (4)%
Tox = Toxicology w = week(s)
* Result reported in area%

Lot No.	Assay Purity (Wt %)	Specified Impurity (Area %) (RRT)
		(b) (4)
D043RD.03.001		(b) (4)
D043RD.04.001		
D043RD.04.003		
D043RD.04.005		
D043RD.04.006		
D043RD.04.007		
D043RD.04.008		
D231.07.001		

NT = Not Tested NA = Not Applicable ND = Not Detected NR = None above reporting threshold of (b) (4)%
Tox = Toxicology w = week(s)
[a] Result reported in area%

2.6.7.4B Toxicology **Novel Excipient (FDKP) Analysis** **Test Article: Technosphere or Technosphere Insulin Inhalation Powder**

Novel Excipient (FDKP) TM5478 Data				
Lot No.	Assay Purity[a] (Wt %)	Specified Impurity (Area %) (RRT)[a]	Study No.[a]	Type of Study
		(b) (4)		
N1500		(b) (4)	2004-0001	Rat Inhalation Carc
N1501			2004-0001	Rat Inhalation Carc
N1502			2004-0001	Rat Inhalation Carc
N1871			2004-0001	Rat Inhalation Carc
			2006-0010	Rat Fetal TK
			2006-0038	Rat Renal Fct
			2007-0021	Respiratory Safety
			2007-0023	Rat Pulmonary Fct
			2008-0020	Rat Immunotox
N1872			2006-0010	Rat Fetal TK
			2006-0038	Rat Renal Fct
			2007-0021	Respiratory Safety
			2007-0023	Rat Pulmonary Fct
			2008-0020	Rat Immunotox
N2258				
N2259			2007-0003	Carcinogenicity Study
			2007-0019	Rabbit Skin Irritation Study
			2007-0022	Respiratory Study
			2007-0024	Rat Pulmonary Fct
			2007-0020	Immunotoxicity Study

2.6.7.4B Toxicology **Novel Excipient (FDKP) Analysis** **Test Article: Technosphere or Technosphere Insulin Inhalation Powder**

Novel Excipient (FDKP) TM5478 Data				
Lot No.	Assay Purity[a] (Wt %)	Specified Impurity (Area %) (RRT)[a]	Study No.[a]	Type of Study
		(b) (4)		
N2260		(b) (4)	2006-0034	Absorption, metabolism and elimination of C-FDKP
			2006-0035	
			2006-0036	
			2007-0005	
			2007-0009	
			2007-0010	
			2007-0011	
			2007-0012	
			2007-0013	

NT = Not Tested NA = Not Applicable ND = Not Detected RNA = Record Not Available
NR = None above reporting threshold of (b) (4)%
[a] Each study number is preceded by MKC-PC-

2.6.7.5 Toxicology: Single-Dose Toxicity

Species/ Strain	Method of Administration (Vehicle/ Formulation)	Doses (mg/kg)	Gender No./Grp	Observed Maximum Nonlethal Dose (mg/kg)	Approximate Lethal Dose (mg/kg)	Noteworthy Findings	Study Number
SD Rats	Oral Gavage: Technosphere in 0.1 M citric acid	T: 5000	5/M 5/F	5000 5000	> 5000	No significant findings	PDC-PC-1993-0001 (b) (4) #93-8117-21)
Beagle Dogs	Oral; gelatin capsules Technosphere	T: 5000	2/M 2/F	5000	> 5000	Moderate emesis 5 or 24 hours postdose; liquid feces	PDC-PC-1994-0002 (b) (4) #94452

2.6.7.6 Toxicology: Repeated-Dose Toxicity (Nonpivotal Studies)

Technosphere Insulin Inhalation Powder

Species/ Strain	Method of Administration (Vehicle/ Formulation)	Duration of Dosing	Doses (mg/kg)	Gender and No. per Group	NOAEL (mg/kg)	Noteworthy Findings	Study Number
Mouse/ TgrasH2	sc (PBS)	28 days	T: 10, 35, & 75 TI: 2.5, 5, 10, 15, & 35	10 M, 10 F	T: 75 T/I: 5	T: - well tolerated T/I: ≥10 mg/kg- ↑ mortality due to suspected hypo-glycemia	MKC-PC-2006-0011 AB29DV/AB29D-W.2S3R. (b) (4)
Beagle Dog	Inhalation (T)	5 days	T: 15.18 (M); T: 16.74 (F)	1 M, 1 F	15.18 (M) 16.74 (F)	Well tolerated; C_{max} ≈ 4.56 to 12.14 µg/mL of FDKP in plasma on Day 5	PDC-PC-1996-0001 (b) (4) No. I96009
Beagle Dog	Inhalation (TI)	5 days	T: 1.966 TI: 0.265, 0.894, & 1.966	1 M, 1 F	T: 1.966 TI: 0.265	≥ 0.894 mg/kg: marked, dose dependant ↓ in blood glucose	MKC-PC-2003-0022 (b) (4) 6031
Beagle Dog	Oral in saline (T)	30 days	T: 50 mg/kg	2 M, 2 F	T: 50	↑ incidence of emesis all Technosphere dose groups	PDC-PC-1994-0001 (b) (4) No. 94443

T = Technosphere
I = Insulin
TI = Technosphere Insulin
ND = Not determined

Species/ Strain	Method of Administration (Vehicle/ Formulation)	Duration of Dosing	Doses (mg/kg)	Gender and No. per Group	NOAEL (mg/kg)	Noteworthy Findings	Study Number
Mouse/ TgrasH2	sc (PBS)	28 days	T: 10, 35, & 75 TI: 2.5, 5, 10, 15, & 35	10 M, 10 F	T: 75 T/I: 5	T: - well tolerated T/I: ≥10 mg/kg- ↑ mortality due to suspected hypo-glycemia	MKC-PC-2006-0011 AB29DV/AB29D-W.2S3R (b) (4)
Beagle Dog	Inhalation (T)	5 days	T: 15.18 (M); T: 16.74 (F)	1 M, 1 F	15.18 (M) 16.74 (F)	Well tolerated; C_{max} ≈ 4.56 to 12.14 µg/mL of FDKP in plasma on Day 5	PDC-PC-1996-0001 (b) (4) No. I96009
Beagle Dog	Inhalation (TI)	5 days	T: 1.966 TI: 0.265, 0.894, & 1.966	1 M, 1 F	T: 1.966 TI: 0.265	≥ 0.894 mg/kg: marked, dose dependant ↓ in blood glucose	MKC-PC-2003-0022 (b) (4) 6031
Beagle Dog	Oral in saline (T)	30 days	T: 50 mg/kg	2 M, 2 F	T: 50	↑ incidence of emesis all Technosphere dose groups	PDC-PC-1994-0001 (b) (4) No. 94443

2.6.7.7A Toxicology: Repeated-Dose Toxicity (Pivotal Studies)

Report Title: A 13-Week Study of the Comparative Toxicokinetic Profile and of the Comparative Toxicity of Technosphere Particles and of Technosphere Insulin in CD-1 Mice

Test Article: Technosphere or Technosphere Insulin Inhalation Powder

Species/Strain: Mouse (CD-1)	**Duration of Dosing:** 13 weeks	**Study No.** MKC-PC-2005-0011
		(b) (4) No. 7679
Initial Age: 7-9 weeks	**Duration of Postdose:** None	**Location in CTD:** Vol. Page
Date of First Dose: 09 May 2005	**Method of Administration:** Subcutaneous	
Vehicle/Formulation: PBS	**GLP Compliance:** Yes	
No Observed Adverse Effect Level: Technosphere: 75 mg/kg/day; Technosphere Insulin: 10 mg/kg/day		

Daily Dose (mg/kg)	0 (Control)		Technosphere (10 mg/kg)		Technosphere (75 mg/kg)		Technosphere Insulin (10mg/kg)		Technosphere Insulin (35 mg/kg)	
Number of Animals	M:10	F:10	M:10	F:10	M:10	F:10	M:10	F:10	M:10	F:10
Toxicokinetics: AUC_{inf} (ng-min/mL)										
Day 1			495093	485712	3884375	4009813	494936	532182	1958241	1801102
Day 42			478301	372042	3096996	3036579	378957	331148	1438819	1446626
Day 91			495810	460799	3551454	4610297	340833	364224	1394130	1576578
Noteworthy Findings										
Died or Sacrificed Moribund	0	0	0	0	0	0	0	0	1	1
Body Weight [a] (%)										
Food Consumption/week[a] (%)	-	-	-	-	-	-	-	-	-	-
Clinical Observations	-	-	-	-	-	-	-	-	-	-
Hematology	-	-	-	-	-	-	-	-	-	-
Clinical Chemistry										
Terminal Blood Glucose	-	-	-	-	-	-	≥ 50% less than 0 or T groups	≥ 50% less than 0 or T groups	≥ 50% less than 0 or T groups	≥ 50% less than 0 or T groups

Daily Dose (mg/kg)	0 (Control)		Technosphere (10 mg/kg)		Technosphere (75 mg/kg)		Technosphere Insulin (10mg/kg)		Technosphere Insulin (35 mg/kg)	
Coagulation Parameters	-	-	-	-	-	-	-	-	-	-
Urinalysis	-	-	-	-	-	-	-	-	-	-
Organ Weights	-	-	-	-	-	-	-	-	-	-
Gross Pathology	-	-	-	-	-	-	-	-	-	-
Histopathology	-	-	-	-	-	-	-	-	-	-

Notes: - No noteworthy findings;

2.6.7.7B Repeat-Dose Toxicity

Report Title: Fumaryl-Technosphere (Powder): Four-Week Inhalation (Nose-Only) Toxicity Study in the Rat

Test Article: Technosphere Inhalation Powder

Species/Strain: Rat (Chbb:THOM)	**Duration of Dosing:** 28 Days	**Study No.** PDC-PC-1996-0002
		(b) (4) Study No. I96032
Initial Age: 6-8 weeks	**Duration of Postdose:** 6-week	**Location in CTD:** Vol. Page
Date of First Dose: 15 Jul 1996	**Method of Administration:** Inhalation	
Vehicle/Formulation: Neat	**GLP Compliance:** Yes	
Special Features: Nose-only exposure; 3 hours/day maximum		
No Observed Adverse Effect Level: 61.7 mg/kg/day		

Daily Dose (mg/kg)	0 (Control)		0.5		14.4		61.7	
Number of Animals	M:10	F:10	M:10	F:10	M:10	F:10	M:20	F:20
Toxicokinetics: AUC (µg-h/mL):								
Day 3	-	-	ND	ND	ND	ND	5.8	4.94
Day 24	-	-	ND	ND	ND	ND	6.36	6.69
Noteworthy Findings								
Died or Sacrificed Moribund	0	0	0	0	0	0	0	0
Body Weight [a] (%)	348 g	231 g	+0.4	+2.3	-2.8	-0.03	-0.9	+1.8
Food Consumption/week[a] (%)	139 g	102 g	-7.0	-3.2	-8.3	-6.0	-1.3	+1.8
Clinical Observations	-	-	-	-	-	-	-	-
Ophthalmoscopy	-	-	-	-	-	-	-	-
Hematology	-	-	-	-	-	-	-	-
Clinical Chemistry	-	-	-	-	-	-	-	-
Coagulation Parameters	-	-	-	-	-	-	-	-
Urinalysis	-	-	-	-	-	-	-	-

Notes: (-) No noteworthy findings;

[a] At end of dosing period; for controls, group means are shown. For treated groups, percent differences from controls are shown.

Daily Dose (mg/kg)	0 (Control)		0.5		14.4		61.7	
Number of Animals	M:10	F:10	M:10	F:10	M:10	F:10	M:20	F:20
Organ Weights	-	-	-	-	-	-	-	-
Gross Pathology								
Number examined	10	10	10	10	10	10	20	20
Histopathology								
Number examined	10 (all)	10 (all)	0	0	0	0	10 (all)	10 (all)
Postdose Evaluation								
Number evaluated	-	-	-	-	-	-	0	0

Notes: - = No noteworthy findings

2.6.7.7C Repeat-Dose Toxicity

Report Title: A 26-Week Inhalation Toxicity Study of Fumaryl-Technosphere (b) (4) **in the Albino Rat**

Test Article: Technosphere Inhalation Powder

Species/Strain: Sprague-Dawley Rat (SD Rat)	**Duration of Dosing:** 13/26 weeks	**Study No.** PDC-PC-1999-0002
		(b) (4) Study No. 91740
Initial Age: 6 weeks	**Duration of Postdose:** 4-week	**Location in CTD:** Vol. Page
Date of First Dose: 21 Dec 1999	**Method of Administration:** Inhalation	
Vehicle/Formulation: Neat	**GLP Compliance:** Yes	
Special Features: Nose-only exposure; 1 hour/day		
No Observed Adverse Effect Level: 5.3 mg/kg/day (M); 51.3 mg/kg/day (F)		

Daily Dose (mg/kg)	0 (Control)[a]		5.3		51.3	
Number of Animals	M:35	F:35	M:35	F:35	M:35	F35
Toxicokinetics: Not reported:						
Noteworthy Findings						
Died or Sacrificed Moribund[c]	0	0	1	0	0	2
Body Weight[a] (%)	562.4 g	300.8 g	-3.7	1.0	-10.3	-0.8
Clinical Observations	-	-	-	-	-	-
Ophthalmoscopy	-	-	-	-	-	-
Hematology	-	-	-	-	-	-
Clinical Chemistry	-	-	-	-	-	-
Coagulation Parameters	-	-	-	-	-	-
Urinalysis	-	-	-	-	-	-
Organ Weights	-	-	-	-	-	-
Gross Pathology	-	-	-	-	-	-
Histopathology	-	-	-	-	-	-

Notes: (-)No noteworthy findings

[a] At end of dosing period; for controls, group means are shown

[b] Deaths were procedural-related.

[c] For treated groups, percent differences from controls are shown.

2.6.7.7D Repeat-Dose Toxicity

Report Title: A 26-Week Inhalation Toxicity Study of a Powder Technosphere Insulin Formulation in the Albino Rat

Test Article: Technosphere or Technosphere Insulin Inhalation (With a 13-Week Interim Kill and a 30-Day Recovery Period) Powder

Species/Strain: Sprague-Dawley Rat (SD Rat)	**Duration of Dosing:** 13/26 weeks	**Study No.** MKC-PC-2003-0002
		(b) (4) Study No. 78235
Initial Age: 9-10 weeks	**Duration of Postdose:** 4-week	**Location in CTD:** Vol. Page
Date of First Dose: 22 Apr 2003	**Method of Administration:** Inhalation	
Vehicle/Formulation: Neat	**GLP Compliance:** Yes	
Special Features: Nose-only exposure; 1 hour/day		
No Observed Adverse Effect Level: Technosphere – 11.7 mg/kg/day; Technosphere Insulin – 1.91 mg/kg/day (males); -1.28 mg/kg/day (females)		

Daily Dose (mg/kg)	0 (Air Control)		Technosphere (11.7 mg/kg/day)		Technosphere Insulin (1.05 mg/kg/day or 2.94 IU/kg/day [M]; 0.404 mg/kg/day or 1.14 IU/kg/day [F])		Technosphere Insulin (1.91 mg/kg/day or 5.18 IU/kg/day [M]; 1.28 mg/kg/day or 3.46 IU/kg/day [F])	
Number of Animals	M:35	F:35	M30	F:30	M:30	F30	M:35	F:35
Toxicokinetics (n):	M:10	F:10	M:10	F:10	M:10	F:10	M:10	F:10
AUC_{last} (ng-min/ml):								
Day 1 (FDKP)	-	-	363087	682590	84627	145798	232266	129880
Day 182 (FDKP)	-	-	514362	703445	140457	97895	191492	176373
Noteworthy Findings								
Died or Sacrificed Moribund	0	0	0	3	0	0	1	1
Body Weight[a] (%)	620.4 g	344.6 g	-5.5 586.3 g	-8.0* 317.0 g	-1.2 +4.5**	-0.1 +8.5**	-3.5 +2.1**	-0.3 +8.3**

Daily Dose (mg/kg)	0 (Air Control)		Technosphere (11.7 mg/kg/day)		Technosphere Insulin (1.05 mg/kg/day or 2.94 IU/kg/day [M]; 0.404 mg/kg/day or 1.14 IU/kg/day [F])		Technosphere Insulin (1.91 mg/kg/day or 5.18 IU/kg/day [M]; 1.28 mg/kg/day or 3.46 IU/kg/day [F])	
Food Consumption/day[a] (%)	29 g	21 g	-6.9	+9.5	0	0	0	0
Clinical Observations	-	-	-	-	-	-	-	-
Ophthalmology	-	-	-	-	-	-	-	-
Hematology	-	-	-	-	-	-	-	-
Serum Chemistry	-	-	-	-	-	-	-	-
Urinalysis	-	-	-	-	-	-	-	-

Notes: (-)No noteworthy findings

[a] At end of dosing period; for controls, group means are shown. Group 2 food consumption occasionally statistically significant decrease compared to Group 1 in females. For treated groups, percent differences from controls are shown. Statistical significance is based on actual data (not on the percent differences). Both absolute and relative weights differed from controls in the direction indicated. Number indicates percent difference for the absolute organ weights.

[b] Versus Group 1 (Dunnett's Test [$p \leq 0.05$])

[c] Versus Group 2 (Dunnett's Test [$p \leq 0.05$])

Daily Dose (mg/kg)	0 (Air Control)		Technosphere (11.7 mg/kg/day)		Technosphere Insulin (1.05/2.94 IU/kg/day (M); 0.404/1.14 IU/kg/day (F)		Technosphere Insulin (1.91/5.18 IU/kg/day (M); 1.28/3.46 IU/kg/day (F)	
Number of Animals	M:35	F:35	M:30 (15)	F:30 (15)	M:30	F30	M:35	F:35
Organ Weights[b](%)								
Lung (g)	1.7195	1.2920	+6.8[b]	+5.0[b]	+6.5	-0.8	+6.0[b]	+1.0
Gross Pathology	-	-	-	-	-	-	-	-
No. Examined	30	30	30	30	30	30	30	30
Histopathology								
No. Examined (26 Wk)	10	10	10	10	10	10	10	10
Nasal Cavity								
Eosinophilic globules (olfact epi)	0	0	10	10	8	5	10	10
Eosinophilic globules (resp epi)	0	0	8	10	4	0	8	1
PCNA (% labeled bronchial cells)	2.38	2.02	3.21[b]	2.53	3.76[b]	3.03[b]	5.14[b]	4.55[b]
No. Examined (recovery)	5	5	0	0	0	0	5	5
Eosinophilic globules (olfact epi)	0	0					5	5
Eosinophilic globules (resp epi)	0	0					4	2
PCNA (% labeled bronchial cells)	1.99	1.79					3.58[b]	2.82[b]

Notes: (-) No noteworthy findings.

[a] Both absolute and relative weights differed from controls in the direction indicated. Number indicates percent difference for the absolute organ weights.

[b] Dunnett's Test = ($p \leq 0.05$) compared to controls.

[c] At end of dosing period; for controls, group means (grams) are shown. For treated groups, percent differences from controls are shown. (Statistical significance is based on actual data, not on the percent differences.)

2.6.7.7E Repeat-Dose Toxicity

Report Title: Fumaryl-Technosphere, about 5% Salmon Calcitonin, 4-Week Toxicity Study in Dogs, Inhalative and Subcutaneous Administration

Test Article: Technosphere; Technosphere/Calcitonin

Species/Strain: Beagle Dog	**Duration of Dosing:** 4 weeks	**Study No.** PDC-PC-1997-0001
		(b) (4) No. I97003
Initial Age: 9-13 months	**Duration of Postdose:** None	**Location in CTD:** Vol. Page
Date of First Dose: 17 Feb 1997	**Method of Administration:** Inhalation/Subcutaneous	
Vehicle/Formulation: Neat	**GLP Compliance:** Yes	
Special Features: Mouth-only exposure; between 8 and 63 minutes/day		
No Observed Adverse Effect Level: Technosphere – 40.3 mg/kg/day; AUC (combined M/F, Day 1 + 29) ≈ 55 µg-h/mL)		

Theoretical Achieved Daily Doses (mg/kg/day)	0 (Air Control) Inhalation		Technosphere 0.3 mg/kg/day Inhalation		Technosphere 4.0 mg/kg/day Inhalation		Technosphere 40.3 mg/kg/day Inhalation		Technosphere/Calcitonin 7.4 mg/kg/day Inhalation		Calcitonin 0.1/0.05 mg/kg/day sc	
Number of Animals	M:3	F:3	M:3	F:3	M:3	F:3	M:3	F:3	M:3	F:3	M:3	F:3
Toxicokinetics: (FDKP)												
AUC_{0-24h}(µg-h/mL)												
Day 1 (FKDP-1)			BLQ	BLQ	2.04	5.72	31.6	41.1	4.02	5.58	-	-
(FKDP-2)			BLQ	BLQ	1.61	4.52	22.2	34.9	3.15	4.45	-	-
Day 29 (FKDP-1)			BLQ	BLQ	3.19	3.54	23.6	29.6	4.80	4.35	-	-
(FKDP-2)			BLQ	BLQ	2.58	2.88	19.3	25.2	1.50	3.54	-	-
Noteworthy Findings												
Died or Sacrificed	-	-	-	-	-	-	-	-	-	-	-	-
Body Weight[a] (%)	13.84 g	13.41g	+1.7	+7.4	-0.7	3.1	2.6	-0.89	-4.3	-12.3	-13.4	-25.2
Food Consumption/week[a] (%)	2800 g	2260 g	0	+24	0	-5.7	0	8.8	0	-16.1	-21.9[c]	-74.2[c]
Ophthalmology	-	-	-	-	-	-	-	-	-	-	-	-
Electrocardiography	-	-	-	-	-	-	-	-	-	-	-	-

Theoretical Achieved Daily Doses (mg/kg/day)	0 (Air Control) Inhalation		Technosphere 0.3 mg/kg/day Inhalation		Technosphere 4.0 mg/kg/day Inhalation		Technosphere 40.3 mg/kg/day Inhalation		Technosphere/Calcitonin 7.4 mg/kg/day Inhalation		Calcitonin 0.1/0.05 mg/kg/day sc	
Number of Animals	M:3	F:3	M:3	F:3	M:3	F:3	M:3	F:3	M:3	F:3	M:3	F:3
Clinical Observations												
Emesis	-	-	-	-	-	-	-	+	+	+	-	-
Hematology	-	-	-	-	-	-	-	-	-	-	-	-
Platelets[b] (thous/µL)	308	328	316	366	347	282	305	324	379[d]	405[d]	406	433
Thrombin Time[b] (TT; sec)	15.6	17.2	16.7	16.8	16.6	14.4	17.0	15.9	13.6	15.7	13.9	15.6
Clinical Chemistry	-	-	-	-	-	-	-	-	-	-	-	-
Total Protein (g/L)	57.3	58.4	59.3	58.8	59.4	59.2	58.0	58.9	54.7	55.0	50.5[d]	54.2[d]
Total bilirubin (umol/L)	1.74	1.89	2.27	2.03	1.82	1.83	1.76	1.71	1.62	1.32	1.08[d]	1.10[d]
Alkaline phosphatase (U/L)	111	88	95	97	115	220	80.6	84	135	57	128	33
Urinalysis	-	-	-	-	-	-	-	-	-	-	-	-
Organ Weights	-	-	-	-	-	-	-	-	-	-	-	-
Gross Pathology	-	-	-	-	-	-	-	-	-	-	-	-
Histopathology												
No. Examined	3	3	3	3	3	3	3	3	3	3	3	3
Inj Site Inflammation	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	3	3

Notes:(-) no noteworthy findings; NA = not applicable

[a] At end of dosing period; for controls, group means in grams are shown. For treated groups, percent differences from controls are shown. Statistical significance is based on actual data, not on the percent differences.

[b] For hematology, statistics were conducted on pooled data only. Group 6 was only compared to Group 5; data is shown as discussed in report; however, no sex-related statistical comparisons were done;

[c] Statistically significant from Group (5) at Weeks 2 or 3; Tech/Calcitonin group means variably lower than Group 1 and statistically significant at Weeks 2 and 3 for males, and Weeks 1-3, for females; T-test with pooled variance (two sided).

[d] Pooled males + females statistics compared to Group 1

2.6.7.7F Repeat-Dose Toxicity

Report Title: A 13, 26 or 39-Week Inhalation Toxicity Study of Fumaryl-Technosphere (b) (4) or Insulin With Fumaryl-Technosphere (b) (4) Powder Formulation in the Beagle Dog

Test Article: Technosphere or Technosphere Insulin Inhalation Powder

Species/Strain: Beagle Dog	**Duration of Dosing:** 13/26/39 weeks	**Study No.** PDC-PC-2000-0003
		(b) (4) No. 91739
Initial Age: 6-7 months	**Duration of Postdose:** 4 weeks	**Location in CTD:** Vol. Page
Date of First Dose: 06 Apr 2000	**Method of Administration:** Inhalation	
Vehicle/Formulation: Neat	**GLP Compliance:** Yes	
Special Features: Nose-only exposure; 10-45 minutes/day		
No Observed Adverse Effect Level: Technosphere: 40.5 mg/kg; Technosphere Insulin: 1.9 mg/kg/day		

Theoretical Achieved Daily Dose (mg/kg/day)	Air Control (0)		Technosphere (5.0 mg/kg/day)		Technosphere (38.0 mg/kg/day)		Technosphere Insulin (1.9 mg/kg/day)	
Number of Animals	M:9	F:9	M:9	F:9	M:9	F9	M:8	F:8
Toxicokinetics: Not reported:								
Noteworthy Findings								
Died or Sacrificed Moribund	-	-	-	-	-	-	-	-
Body Weight[a] (%) Week 39	11.6	9.6	-6.8	-1.7	0	1.4	-	-
Body Weight[a] (%) Week 26	11.5	9.6	-4.8	-5.1	-0.2	0.1	-0.8	11.3[b]
Clinical Observations	-	-	-	-	-	-	-	-
Ophthalmoscopy	-	-	-	-	-	-	-	-
Electrocardiography	-	-	-	-	-	-	-	-
Hematology	-	-	-	-	-	-	-	-
Clinical Chemistry	-	-	-	-	-	-	-	-
Coagulation Parameters	-	-	-	-	-	-	-	-
Urinalysis	-	-	-	-	-	-	-	-
Organ Weights	-	-	-	-	-	-	-	-
Gross Pathology	-	-	-	-	-	-	-	-
Histopathology								

Notes: (-) No noteworthy findings

[a] At end of dosing period; for controls, group means are shown. For treated groups, percent differences from controls are shown.

[b] Insulin group dosed for 26 weeks only; female body weights were statistically increased ($p \leq 0.05$ by Dunnet's) over controls at Weeks 8-10 and 13.

2.6.7.7G Repeat-Dose Toxicity

Report Title: Technosphere Insulin: A 39-Week Inhalation Toxicity Study in Beagle Dogs Followed by a 8-Week Recovery Period

Test Article: Technosphere or Technosphere Insulin Inhalation Powder

Species/Strain: Beagle Dogs **Duration of Dosing:** 26 (High-dose Insulin) or 39 weeks **Study No.** MCK-PC-2003-0004

(b) (4) No. 2973

Initial Age: 7-8 months **Duration of Postdose:** 8 weeks **Location in CTD:** Vol. Page

Date of First Dose: 04 Nov 2003 **Method of Administration:** Inhalation

Vehicle/Formulation: Neat **GLP Compliance:** Yes

No Observed Adverse Effect Level: Technosphere 2.4 mg/kg/day; Technosphere Insulin: 1.92 mg/kg/day

Daily Dose (mg/kg)	0 (Control)		Technosphere (2.4 mg/kg/day)		Technosphere (10.9 mg/kg/day)		Technosphere Insulin (0.39 mg/kg/day)		Technosphere Insulin (1.92 mg/kg/day)	
Number of Animals	M:10	F:10	M:4	F:4	M:4	F:4	M:4	F:4	M:10	F:10
Toxicokinetics:										
FDKP – AUC_{inf} (ng-min/mL)										
Day 15			102506	102130	530619	534083	24092	24958	105142	115764
Day 22			66788	89670	536084	462019	16233	21373	67888	98597
Week 20			87981	97230	245681	372225	15897	21893	59681	83337
Week 39			102604	112946	395824	527344	16233	21373	72512	76284
Insulin – AUC_{last} (µU/min/mL)										
Day 15							2109	1853	6730	5713
Day 22							1427	1365	4635	4508
Week 20							488	853	5478	5757
Week 39							2682	1379	4278	3998
Noteworthy Findings										
Died or Sacrificed Moribund	-	-	-	-	-	-	-	-	-	-
Body Weight[a] (%)	-	-	-	-	-	-	-	-	-	-
Food Consumption/week[a] (%)	-	-	-	-	-	-	-	-	-	-

Daily Dose (mg/kg)	0 (Control)		Technosphere (2.4 mg/kg/day)		Technosphere (10.9 mg/kg/day)		Technosphere Insulin (0.39 mg/kg/day)		Technosphere Insulin (1.92 mg/kg/day)	
Number of Animals	M:10	F:10	M:4	F:4	M:4	F:4	M:4	F:4	M:10	F:10
Clinical Observations	-	-	-	-	-	-	-	-	-	-
Ophthalmoscopy	-	-	-	-	-	-	-	-	-	-
Electrocardiography	-	-	-	-	-	-	-	-	-	-
Hematology	-	-	-	-	-	-	-	-	-	-
Clinical Chemistry										
Serum Glucose[a] (mmol/L)	-	-	5.6→5.8	5.1→5.5	5.0→5.6	4.7→5.0	5.1→4.1	5.1→4.5	5.2→2.5	4.9→2.1
Wk 39 (pre →15 min post)	-	-	-	-	-	-	-	-	-	-
Coagulation Parameters	-	-	-	-	-	-	-	-	-	-
Urinalysis	-	-	-	-	-	-	-	-	-	-
Organ Weights	-	-	-	-	-	-	-	-	-	-
Gross Pathology	-	-	-	-	-	-	-	-	-	-
Histopathology (26 week)										
Histopathology (39 week)	4	4	4	4	4	4	4	4	4	4
No. Examined	0	0	0	0	3	3	0	1	0	0
Thymic Atrophy	0	0	0	0	1	2	0	0	0	0
minimal	0	0	0	0	2	1	0	1	0	0
mild	0	0	0	0	0	0	1	0	2	1
Lung –neutrophil infil[b]	0	0	0	0	0	0	0	0	1	1
minimal	0	0	0	0	0	0	1	0	1	0
mild	-	-	-	-	-	-	-	-	-	-
Histopathology – Recov (week 43)										

Notes: (-)No noteworthy findings;

[a] Serum Glucose values shown for predose and 15 minutes postdose at Week 39; other timepoints showed comparable declines; values returned to baseline by approx 240 minutes

[b] Lung neutrophil infiltrate in Insulin treated groups considered of minimal, if any toxicologic significance

2.6.7.8A Genotoxicity: In Vitro | **Report Title: Bacterial Reverse-Mutation Assay** | **Test Article: Technosphere or Technosphere Insulin Inhalation Powder**

Test for Induction of: Reverse mutation in bacterial cells	**No. of Independent Assays:** 2	**Study No. MKC-PC-2004-0002**
		AA89DG-DH503 (b)
Strains: S. typhimurium and E. coli	**No. of Replicate Cultures:** 2-3	**Location in CTD:** Vol. Page
Metabolizing System: Aroclor-induced rat liver S9, 7.1%	**No. of Cells Analyzed/Culture:** -	
Vehicles: **Test Article:** 100 mM PBS; **Positive Controls:** DMSO		**GLP Compliance:** Yes
Treatment: Plate incorporation; 48-hour incubation		**Treatment:** 27 May 2004
Cytotoxic Effects: None.		**Genotoxic Effects:** None.

Metabolic Activation	Test Article	Dose Level (µg/plate)	Assay #1 (Technosphere) Revertant Colony Counts (Mean ±SD)				
			TA 98	TA 100	TA 1535	TA 1537	WP2 uvrA
Without Activation	PBS	100 µL/plate	36 ± 6	135 ± 30	20 ± 3	7 ± 4	25 ± 10
	Technosphere	1.5	36 ± 1	164 ± 8	29 ± 5	8 ± 0	27 ± 0
		5.0	32 ± 4	167 ± 22	20 ± 4	6 ± 3	23 ± 2
		15	31 ± 4	152 ± 8	22 ± 6	7 ± 1	25 ± 6
		50	32 ± 2	147 ± 21	24 ± 4	6 ± 1	23 ± 4
		150	33 ± 4	165 ± 24	25 ± 4	5 ± 4	26 ± 7
		500	27 ± 6	172 ± 6	28 ± 0	6 ± 3	24 ± 2
		1500	31 ± 1	156 ± 17	28 ± 1	6 ± 1	26 ± 1
		5000	30 ± 1	162 ± 8	26 ± 2	12 ± 1	24 ± 1
	2-Nitrofluorene	1	276 ± 56				
	Sodium Azide	1		661 ± 4	471 ± 117		
	9-aminoacridine	1				1041 ± 73	
	MMS	1000					130 ± 2

Metabolic Activation	Test Article	Dose Level (µg/plate)	Assay #1 (Technosphere) Revertant Colony Counts (Mean ±SD)				
			TA 98	TA 100	TA 1535	TA 1537	WP2 uvrA
With Activation	PBS	100 µL/plate	31 ± 9	160 ± 36	26 ± 1	4 ± 1	18 ± 0
	Technosphere	1.5	43 ± 1	193 ± 31	22 ± 4	11 ± 1	25 ± 1
		5.0	45 ± 11	197 ± 8	20 ± 4	11 ± 2	28 ± 2
		15	33 ± 3	182 ± 7	28 ± 2	10 ± 2	26 ± 4
		50	30 ± 2	187 ± 8	22 ± 3	10 ± 4	21 ± 5
		150	34 ± 2	162 ± 4	20 ± 4	13 ± 1	28 ±4
		500	33 ± 3	157 ± 17	31 ± 6	9 ± 2	26 ± 6
		1500	33 ± 1	173 ± 8	24 ± 2	9 ± 4	18 ± 1
		5000	38 ± 1	152 ± 26	28 ± 3	9 ± 5	25 ± 1
	2-Aminoanthracene	1.0	1499 ± 344	1505 ± 35	248 ± 16	234 ± 66	
		10					766 ± 171

Metabolic Activation	Test Article	Dose Level (µg/plate)	Assay #2 (Technosphere) Revertant Colony Counts (Mean ± SD)				
			TA 98	TA 100	TA 1535	TA 1537	WP2 uvrA
Without Activation	PBS	100 µL/plate	17 ± 2	140 ± 13	22 ± 4	6 ± 2	13 ± 2
	Technosphere	50	17 ± 2	172 ± 12	23 ± 5	5 ± 3	19 ± 3
		150	17 ± 2	154 ± 17	19 ± 3	6 ± 1	15 ± 3
		500	14 ± 1	151 ± 12	24 ± 4	9 ± 3	17 ± 1
		1500	17 ± 2	149 ± 3	21 ± 3	4 ± 3	16 ± 3
		5000	18 ± 3	166 ± 13	22 ± 2	7 ± 4	16 ± 3
	2-Nitrofluorene	1	134 ± 25				
	Sodium Azide	1		554 ± 12	328 ± 46		
	9-aminoacridine	1				402 ± 19	
	MMS	1000					115 ± 7
With Activation	PBS	100 µL/plate	30 ± 8	161 ± 26	19 ± 4	5 ± 2	21± 3
	Technosphere	50	25 ± 6	173 ± 10	18 ± 3	8 ± 2	20 ± 2
		150	27 ± 3	175 ± 12	16 ± 5	7 ± 3	19 ± 4
		500	32 ± 3	181 ± 29	20 ± 6	9 ± 2	19 ± 6
		1500	24 ± 3	133 ± 13	19 ± 2	5 ± 2	18 ± 3
		5000	27 ± 8	159 ± 5	21 ± 5	8 ± 3	21 ± 2
	2-Aminoanthracene	1.0	845 ± 196	928 ± 96	114 ± 6	125 ± 30	
		10					454 ± 160

Metabolic Activation	Test Article	Dose Level (µg/plate)	Assay #1 (Technosphere Insulin) Revertant Colony Counts (Mean ± SD)				
			TA 98	TA 100	TA 1535	TA 1537	WP2 uvrA
Without Activation	PBS	100 µL/plate	19 ± 1	150 ± 6	17 ± 4	5 ± 3	14 ± 2
	Technosphere	1.5	23 ± 5	177 ± 11	26 ± 5	6 ± 1	17 ± 7
		5.0	18 ± 1	172 ± 11	27 ± 1	12 ± 1	19 ± 1
		15	17 ± 1	154 ± 1	22 ± 4	8 ± 2	18 ± 0
		50	19 ± 8	138 ± 10	21 ± 3	7 ± 1	20 ± 1
		150	19 ± 1	159 ± 25	18 ± 1	10 ± 3	21 ± 6
		500	21 ± 4	151 ± 18	20 ± 2	9 ± 1	19 ± 0
		1500	15 ± 1	161 ± 14	20 ± 1	10 ± 2	14 ± 4
		5000	20 ± 0	160 ± 9	21 ± 0	5 ± 1	17 ± 2
	2-Nitrofluorene	1	231 ± 23				
	Sodium Azide	1		780 ± 78	611 ± 2		
	9-aminoacridine	1				862 ± 135	
	MMS	1000					99 ± 1
With Activation	PBS	100 µL/plate	29 ± 0	183 ± 8	17± 0	9 ± 2	25 ± 1
	Technosphere	1.5	25 ± 0	174 ± 3	18 ± 2	9 ± 1	21 ± 3
		5.0	29 ± 0	180 ± 2	16 ± 1	7 ± 1	16 ± 1
		15	31 ± 5	201 ± 27	22 ± 1	5 ± 1	24 ± 1
		50	32 ± 3	154 ± 8	21 ± 4	11 ± 1	19 ± 3
		150	29 ± 1	174 ± 31	21 ± 0	8 ± 1	23 ± 4
		500	31 ± 4	183 ± 8	19 ± 1	8 ± 2	22 ± 1
		1500	24 ± 1	167 ± 25	18 ± 1	5 ± 0	20 ± 1
		5000	27 ± 1	169 ± 14	21 ± 1	9 ± 4	19 ± 1
	2-Aminoanthracene	1.0	1358 ± 266	1869 ± 38	256 ± 1	278 ± 18	
		10					841 ± 121

Metabolic Activation	Test Article	Dose Level (µg/plate)	Assay #2 (Technosphere Insulin) Revertant Colony Counts (Mean ±SD)				
			TA 98	TA 100	TA 1535	TA 1537	WP2 uvrA
Without Activation	PBS	100 µL/plate	13 ± 2	166 ± 9	18 ± 1	7 ± 2	14 ± 1
	Technosphere	50	13 ± 4	157 ± 30	17 ± 6	8 ± 6	18 ± 7
		150	13 ± 4	138 ± 16	17 ± 1	8 ± 6	15 ± 4
		500	14 ± 2	169 ± 16	23 ± 3	6 ± 3	18 ± 2
		1500	13 ± 5	136 ± 4	23 ± 5	5 ± 2	13 ± 2
		5000	10 ± 2	159 ± 5	23 ± 2	8 ± 2	15 ± 3
	2-Nitrofluorene	1	160 ± 42				
	Sodium Azide	1		553 ± 23	273 ± 15		
	9-aminoacridine	1				567 ± 151	
	MMS	1000					136 ± 17
With Activation	PBS	100 µL/plate	30 ± 1	181 ± 9	17 ± 1	7 ± 3	14± 3
	Technosphere	50	28 ± 2	184 ± 9	21 ± 4	7 ± 3	15 ± 3
		150	27 ± 3	152 ± 17	13 ± 3	8 ± 5	16 ± 3
		500	31 ± 5	183 ± 4	18 ± 3	8 ± 1	20 ± 3
		1500	26 ± 6	153 ± 14	20 ± 3	5 ± 3	15 ± 7
		5000	30 ± 6	175 ± 5	18 ± 3	6 ± 1	14 ± 2
	2-Aminoanthracene	1.0	524 ± 117	690 ± 135	123 ± 20	75 ± 34	
		10					373 ± 134

2.6.7.8B Genotoxicity: In Vitro

Report Title: In Vitro Mammalian Chromosome Aberration Test with Fumaryl- Technosphere (b) (4)

Test Article: Technosphere Inhalation Powder

Test for Induction of: Chromosome aberrations	**No. of Independent Assays:** 2	**Study No.** AA37RS.341 (b) (4) PDC-PC-2000-0006
Strains: Primary human lymphocytes	**No. of Replicate Cultures:** 2	**Location in CTD:** Vol. Page
Metabolizing System: Aroclor-induced rat liver S9	**No. of Cells Analyzed/Culture:** 100	
Vehicles: **Test Article:** DMSO; **Positive Controls:** DMSO		**GLP Compliance:** Yes
Treatment: Continuous treatment for 4 hours with or without S9; & 20 hours without S9		**Date of Treatment:** 21 Nov 2000
Cytotoxic Effects: Prelim assay – decreased mitotic indices: 4 hours > 50% at 450 µg/ml ± S9; 20 hours > 50% at 45 µg/ml without S9.		
Genotoxic Effects: None.		

4-hour Incubation:

Metabolic Activation	Test Article	Concentration (µg/ml)	% Mitotic Reduction (Mean Mitotic Index)	Aberrant Cells Mean %	Abs/Cell	Numerical[a] Abs/cell
Without Activation	DMSO	-	0 (5.4)	0	0	0
	Technosphere	281	31 (3.7)	0	0	0
		562.5	41(3.2)	0	0	0
		1125	61(2.1)	0	0	0
	Mitomycin	0.5	56 (2.4)	10.5[b]	0.11	0

[a] Includes polyploidy & endoreduplicated cells

[b] ($p < 0.01$) by Fisher's exact test

20-hour Incubation Without S9

Metabolic Activation	Test Article	Concentration (µg/ml)	% Mitotic Reduction (Mean Mitotic Index)	Aberrant Cells Mean %	Abs/Cell	Numerical[a] Abs/cell
Without Activation	DMSO	-	0 (5.8)	0	0	0
	Technosphere	35	38 (3.6)	0	0	0
		70	33 (3.9)	0	0	0
		140	55 (2.6)	0	0	0
	Mitomycin	0.25	67 (1.9)	7.5[b]	0.095	0

[a] Includes polyploidy and endoreduplicated cells

[b] ($p < 0.01$) by Fisher's exact test

2.6.7.8C Genotoxicity: In Vitro **Report Title: In Vitro Mammalian Chromosome Aberration Test** **Test Article: Technosphere Insulin**

		Inhalation Powder
Test for Induction of: Chromosome aberrations	**No. of Independent Assays:** 2	**Study No.** AA60RE.341 (b) (4)
		PDC-PC-2002-0003
Strains: Primary human lymphocytes	**No. of Replicate Cultures:** 2	**Location in CTD:** Vol. Page
Metabolizing System: Aroclor-induced rat liver S9	**No. of Cells Analyzed/Culture:** 100	
Vehicles: **Test Article:** PBS; **Positive Controls:** PBS		**GLP Compliance:** Yes
Treatment: Continuous treatment for 4 hours with or without S9; and 20 hours without S9		**Date of Treatment:** 07 Aug 2002

Cytotoxic Effects: Prelim assay – no significant decrease (≥50%) in mitotic index up to 5000 µg/ml for 4 hours with or without S9; and 20 hours without S9 .

Genotoxic Effects: None.

4-hour Incubation:

Metabolic Activation	Test Article	Concentration (µg/ml)	% Mitotic Reduction (Mean Mitotic Index)	Aberrant Cells Mean %	Abs/Cell	Numerical[a] Abs/cell
Without Activation	PBS	-	0 (9.2)	0	0	0
	Technosphere Insulin	1250	16 (7.7)	0	0	0
		2500	11 (8.2)	0	0	0
		5000	16 (7.7)	0	0	0
	Mitomycin	0.6	88 (1.1)	23.0[b]	0.24	0
With Activation	PBS	-	0 (8.1)	0	0	0
	Technosphere Insulin	1250	9 (7.4)	0	0	0
		2500	6 (7.6)	0	0	0
		5000	23(6.2)	0.5	0.005	0
	Cyclophosphamide	20	89 (0.9)	8.0[b]	0.08	0.5

[a] Includes polyploidy and endoreduplicated cells

[b] ($p < 0.01$) by Fisher's exact test

20-hour Incubation Without S9

Metabolic Activation	Test Article	Concentration (µg/ml)	% Mitotic Reduction (Mean Mitotic Index)	Aberrant Cells Mean %	Abs/Cell	Numerical[a] Abs/cell
Without Activation	PBS	-	0 (8.0)	0.5	0.005	0
	Technosphere Insulin	1250	1(7.9)	0.5	0.005	0
		2500	0(8.6)	0.5	0.005	0
		5000	0(8.8)	0	0	0
	Mitomycin	0.3	55(3.6)	25[b]	0.3	0

[a] Includes polyploidy and endoreduplicated cells

[b] ($p < 0.01$) by Fisher's exact test

2.6.7.9 Genotoxicity: In Vivo

Report Title: Mammalian Erythrocyte Micronucleus Test in Mice

Test Article: Technosphere Inhalation Powder

Test for Induction of: Bone marrow micronuclei	**Treatment Schedule:** Single dose	**Study No:** AA68EH.123 (b) (4)
		PDC-PC-2002-0011
Species/Strain: ICR Mice	**Sampling Time:** 24 and 48 hours after last dose.	**Location in CTD:** Vol. Page
Age: 6-8 weeks	**Method of Administration:** Intraperitoneal.	
Cells Evaluated: Polychromatic erythrocytes	**Vehicle/Formulation:** Aqueous solution.	**GLP Compliance:** Yes
No. of Cells Analyzed/Animal: 2000		**Date of Dosing:** 19 Nov 2002
Special Features: None.		
Toxic/Cytotoxic Effects: One male mouse died at 1800 mg/kg Day 2; clinical signs: ataxia and lethargy at doses ≥ 1400 mg/kg in males and females		
Genotoxic Effects: None.		
Evidence of Exposure: Mortality in 1/5 males at 1800 mg/kg; clin signs: piloerection in males at 1600 mg/kg and in females at 1800 mg/kg.		

Treatment	Sex	Time (h)	No of Mice	Mean % PCEs ± SD	Mean % Micronucleated PCEs ± SD[a]
Saline	M	24	5	0.529 ± .03	0.6 ± 0.55
	F	24	5	0.477 ± .06	0.5 ± 0.00
Technosphere					
400 mg/kg	M	24	5	0.484 ± 0.06	0.8 ± 0.45
450 mg/kg	F	24	5	0.480± 0.05	0.8 ± 0.67
800 mg/kg	M	24	5	0.467 ± 0.04	0.5 ± 0.35
900 mg/kg	F	24	5	0.470 ± 0.04	1.0 ± 1.12
1600 mg/kg	M	24	5	0.442 ± 0.08	0.4 ± 0.22
1800 mg/kg	F	24	5	0.460 ± 0.05	0.7 ± 0.76
Cyclophosphamide 50 mg/kg	M	24	5	0.459 ± 0.08	30.4 ± 4.63[b]
	F	24	5	0.361 ± 0.07	27.6 ± 5.85[b]
Saline	M	48	5	0.504 ± 0.05	0.6 ± 0.22
	F	48	5	0.517 ± 0.06	0.5 ± 0.35
Technoshpere					
1600 mg/kg	M	48	5	0.507 ± 0.08	0.4 ± 0.42
1800 mg/kg	F	48	5	0.506 ± 0.01	0.4 ± 0.42

[a] Number per 1000 PCEs

[b] ($p \leq 0.05$) Kastenbaum-Bowman Tables

2.6.7.10A Carcinogenicity

Report Title: 26-Week Subcutaneous Injection Oncogenicity Study with Technosphere Particles and Technosphere Insulin in Model 001178-T (hemizygous rasH2) Mice

Test Article: Technosphere or Technosphere Insulin Inhalation Powder

Species/Strain: Model 001178-T	**Duration of Dosing:** 26 weeks	**Study No.** MKC-PC-2007-0003
hemizygous rasH2 mice		(b) (4)No. 7260-107
Initial Age: 8 weeks	**Method of Administration:** Subcutaneous	**Location in CTD:** Vol. Page
Date of First Dose: 10 Jul 2007	**Vehicle/Formulation:** 10 mM PBS in Sterile Water for Injection	**GLP Compliance:** Yes
Treatment of Controls: N-methyl-N-nitrosurea (MNU) once via intraperitoneal injection		
Basis for High-Dose Selection: Dose levels were selected based on the results of MannKind Study MKC-PC-2006-0011. Due to early deaths in females treated with Technosphere Insulin at 2.5 or 5 mg/kg/day, the dose levels were reduced and females were treated at 0.6 or 1.25 mg/kg/day beginning on Dosing Day 77. A positive control group (Group 7) included for comparison purposes of survival and tumor data, received a single 75 mg/kg/day dose of N-methyl-N-nitrosurea (MNU) ip on Day 1.		
Special Features: NA		
Conclusion: There was no evidence of increased oncogenicity associated with the administration of Technosphere Particles to male and female hemizygous CB6F1/Jic-TgrasH2 mice via sc injection once daily for 26 weeks at 25 or 75 mg/kg/day, or with the administration of Technosphere Insulin at 2.5 and 5 mg/kg/day in males or 0.6 and 1.25 mg/kg/day in females. In females, Technosphere Insulin at dose levels of 2.5 and 5 mg/kg/day exceeded the maximum tolerated dose.		

Daily Dose:	Sham Control		Vehicle Control		Technosphere (25 mg/kg/day)		Technosphere (75 mg/kg/day)		Technosphere Insulin (2.5 mg/kg/day)[a]		Technosphere Insulin (5.0 mg/kg/day)[a]		Positive Control	
No. of Animals at Start:	M: 25	F: 25	M: 25	F: 25	M: 25	F: 25	M: 25	F: 25	M: 25	F: 25	M: 25	F: 25	M: 25	F: 25
Toxicokinetics:	-	-	18	18	38	38	38	38	38	38	38	38	-	-
AUC_{0-120} on Day 1 (ng•min/mL)	-	-	-	-	1175668	1153338	3699003	3241258	114684	107471	231447	166746	-	-
AUC_{0-120} on Week 26 (ng•min/mL)	-	-	-	-	1173006	962262	2968476	2753474	99695	22771	189459	44147	-	-
C_{max} on Day 1 (ng/mL)	-	-	-	-	38748	40803	134871	95992	4417	4008	8122	6195	-	-
C_{max} on Week 26 (ng/mL)	-	-	-	-	29623	27265	87892	80109	2237	648	4993	1325	-	-

Daily Dose:	Sham Control		Vehicle Control		Technosphere (25 mg/kg/day)		Technosphere (75 mg/kg/day)		Technosphere Insulin (2.5 mg/kg/day)[a]		Technosphere Insulin (5.0 mg/kg/day)[a]		Positive Control	
No. of Animals at Start:	M: 25	F: 25	M: 25	F: 25	M: 25	F: 25	M: 25	F: 25	M: 25	F: 25	M: 25	F: 25	M: 25	F: 25
Number of Animals:														
Died/Sacrificed Moribund	0	1	1	0	1	1	0	1	2	4	1	6	22	17
Terminal Sacrifice	25	24	24	25	24	24	25	24	23	21	24	19	3	8
Survival (%)	100	96	96	100	96	96	100	96	92	84	96	76	8	32
Body Weight (%) – Week 27[b]	30.6g	23.8g	31.7g	24.2g	↓3.2	↑1.2	↓1.6	0	↓3.5	↑1.2	↓4.4	↑0.8	27.4 g	24.8g
Food Consumption (%) – Week 26[b]	34.1g	32.1g	35.5g	31.2g	↑2.0	↑10.9	↑2.5	↑7.4	↑5.1	↑4.5	↓3.9	0	46.2 g	37.9g

Notes: (-) Not included in analysis

[a] Due to early deaths in females treated with Technosphere Insulin at 2.5 or 5 mg/kg/day, the dose levels were reduced and females were treated at 0.6 or 1.25 mg/kg/day beginning on Dosing Day 77.

[b] Body weight and food consumption percentages are based on the vehicle control values.

Daily Dose:	Sham Control		Vehicle Control		Technosphere (25 mg/kg/day)		Technosphere (75 mg/kg/day)		Technosphere Insulin (2.5 mg/kg/day)1		Technosphere Insulin (5.0 mg/kg/day)1		Positive Control	
No. of Animals at Start:	M: 25	F: 25	M: 25	F: 25	M: 25	F: 25	M: 25	F: 25	M: 25	F: 25	M: 25	F: 25	M: 25	F: 25
Animals With Neoplastic Lesions – Number of Animals examined	25	25	25	25	25	25	25	25	25	25	25	25	25	25
Lung														
Adenoma, Brochiolar-Alveolar	3	1	2	2	-	-	2	0	-	-	1	1	3	11
Carcinoma, Bronchiolar-Alveolar	1	0	0	0	-	-	0	0	-	-	1	0	0	0
Stomach, Non-glandular														
Carcinoma, Squamous Cell	0	0	0	0	-	-	0	1	-	-	0	0	6	7
Papilloma, Squamous Cell	0	0	0	0	-	-	0	0	-	-	0	0	12	15
Harderian Gland														
Adenoma	1	0	0	1	-	-	0	0	-	-	1	1	0	1
Carcinoma	0	0	0	1	-	-	0	0	-	-	0	0	2	0
Vagina														
Carcinoma, Squamous Cell	-	0	-	0	-	-	-	0	-	-		0		2
Papilloma, Squamous Cell	-	0	-	0	-	-	-	0	-	-	-	0	-	3
Urethra														
Papilloma, Transitional Cell	0	0	0	0	-	-	0	0	-	-	0	0	2	9
Body, Whole/CAV														
Hemangioma	2	1	0	1	-	-	0	0	-	-	0	1	2	1
Hemangiosarcoma	2	1	1	1	-	-	1	1	-	-	4	3	4	3
Lymphosarcoma	0	0	0	0	-	-	0	0	-	-	0	0	17	14
Myeloproliferative Neoplasia	0	1	0	1	-	-	0	0	-	-	0	0	2	3
Skin/Subcutis														
Papilloma, Squamous Cell	0	0	1	0	-	-	0	0	-	-	0	0	0	6
Carcinoma, Squamous Cell	0	0	0	0	-	-	0	0	-	-	0	0	1	3

Notes: (-) Not included in analysis. For neoplasm data, the dashes (-) indicate that the analysis was not performed since there were no target tissues identified.

[a] Due to early deaths in females treated with Technosphere Insulin at 2.5 or 5 mg/kg/day, the dose levels were reduced and females were treated at 0.6 or 1.25 mg/kg/day beginning on Dosing Day 77.

[b] Body weight and food consumption percentages are based on the vehicle control values.

2.6.7.10B Carcinogenicity

Report Title: Technosphere Insulin: A 104-Week Inhalation Carcinogenicity Study in Sprague-Dawley Rats

Test Article: Technosphere or Technosphere Insulin Inhalation Powder

Species/Strain: Rat/Sprague-Dawley	**Duration of Dosing:** 104 weeks	**Study No.** MKC-PC-2004-0001
		(b) (4) No. 5751
Initial Age: 6 weeks	**Method of Administration:** Inhalation	**Location in CTD:** Vol. Page
Date of First Dose: 16 Aug 2004	**Vehicle/Formulation:** Inhalation	
Treatment of Controls: Air/Technosphere Particles		**GLP Compliance:** Yes

Basis for High-Dose Selection: The Group 5 (High Dose) was selected based on minimal toxicity observed during a 13-week inhalation toxicity study in rats (an interim group of an ongoing 26-week inhalation toxicity study: Study No.. PDC-PC-2003-0002). In that study the high-dose for males was 5 IU/kg/day and 3 IU/kg/day for females. The dose difference was selected based on a previous dose-range finding study in which the dose of 5 IU/kg/day induced severe hypoglycemia in females. The Group 4 (Low Dose) was selected based on a multiple of the anticipated human therapeutic dose. Group 2 (Vehicle Control Low Dose) was exposed to aerosols of vehicle (Technosphere particles) at a mass concentration equal to that of Group 5 (High Dose), and Group 3 (Vehicle Control High Dose) was exposed to aerosols of vehicle (Technosphere particles) 25 times that of the anticipated human therapeutic dose.

Special Features: 12 additional males and 12 additional females per drug-treated group bled at 6 months for toxicokinetic monitoring and then removed from the study.

Conclusion: Inhalation administration of Technosphere Insulin or Technosphere particles once daily for 104 consecutive weeks was well tolerated by Sprague-Dawley rats and there were no indications that Technosphere Insulin or Technosphere particles had carcinogenic potential.

Group Designation	Air Control		Technosphere Particle Control Low Dose		Technosphere Particle Control High Dose		Technosphere Insulin Low Dose		Technosphere Insulin High Dose	
Estimated Achieved Dose of Technosphere particles (mg/kg/day):	M: 0	F: 0	M: 1.1041	F: 0.6784	M: 41	F: 46	M: 0.6572	F: 0.4413	M: 1.2313	F: 0.7608
Estimated Achieved Dose of Insulin (mg/kg/day):	M: 0	F: 0	M: 0	F: 0	M: 0	F: 0	M: 0.0787	F: 0.0529	M: 0.1457	F: 0.0900
Exposure Durations (minutes):	M: 60	F: 33	M: 60	F: 33	M: 60	F: 60	M: 60	F: 36	M: 60	F: 33
Achieved Aerosol Concentrations of Technosphere particles (mg/L)	0		0.03939		1.4493		0.0236		0.0447	

Group Designation	Air Control		Technosphere Particle Control Low Dose		Technosphere Particle Control High Dose		Technosphere Insulin Low Dose		Technosphere Insulin High Dose	
Achieved Aerosol Concentrations of Insulin® (mg/L)	0		0		0		0.0028		0.0053	
Toxicokinetics:										
C_{max} (FDKP ng/mL) Week 52: Week 78: Week 104:	- - -	- - -	M: 388 M: 175 M: 465	F: 203 F: 135 F: 262	M: 6308 M: 4577 M: 4488	F: 7458 F: 3062 F: 6260	M: 686 M: 94 M: 285	F: 223 F: 102 F: 133	M: 813 M: 186 M: 545	F: 509 F: 84 F: 160
C_{max} (Insulin µU/mL) Week 52: Week 78: Week 104:	- - -	- - -	BLQ BLQ BLQ	BLQ BLQ BLQ	BLQ BLQ BLQ	BLQ BLQ BLQ	M: 53.080 M: 7.037 M: 20.094	F: 4.483 F: 8.986 F: 15.352	M: 44.734 M: 18.713 M: 36.550	F: 27.881 F: 8.316 F: 13.511
BLQ = samples were below the limit of quantification										
Number of Animals:										
At Start: Main Study: Toxicokinetic Phase[a]	 M: 60 M: 6	 F: 60 F: 6	 M: 60 M: 6	 F: 60 F: 6	 M: 60 M: 6	 F: 60 F: 6	 M: 60 M: 6	 F: 60 F: 6	 M: 60 M: 6	 F: 60 F: 6
Died/Sacrificed Moribund	M: 19	F: 17	M: 22	F: 22	M: 17	F: 22	M: 19	F: 23	M: 16	F: 20
Survival (%)	M: 68	F: 72	M:63	F: 63	M: 72	F: 63	M: 68	F: 62	M: 73	F: 67
Mean survival (Days)	M: 674	F: 679	M: 699	F: 681	M: 693	F: 681	M: 690	F: 672	M: 692	F: 689
Body Weight Change (%)[b]	M : 254	F: 199	M: 238	F: 200	M: 224	F: 210	M: 219	F: 192	M: 214	F: 184
Noteworthy Findings: Ophthalmoscopy: Clinical Pathology: Gross Pathology:	 M: None M: None M: None	 F: None F: None F: None	 M: None M: None M: None	 F: None F: None F: None	 M: None M: None M: None	 F: None F: None F: None	 M: None M: None M: None	 F: None F: None F: None	 M: None M: None M: None	 F: None F: None F: None

Group Designation	Air Control		Technosphere Particle Control Low Dose		Technosphere Particle Control High Dose		Technosphere Insulin Low Dose		Technosphere Insulin High Dose	
Palpable Masses: Total No. of Animals Affected: Total No. of Palpable Masses: Mean Time of Onset (Week): Total No. Found At Necropsy:	 M: 15 M: 20 M: 71 M: 6	 F: 14 F: 28 F: 82 F: 18	 M: 18 M: 21 M: 68 M: 5	 F: 24 F: 35 F: 77 F: 24	 M: 9 M: 12 M: 66 M: 3	 F: 9 F: 10 F: 83 F: 7	 M: 24 M: 34 M: 66 M: 10	 F: 29 F: 50 F: 78 F: 38	 M: 19 M: 22 M: 61 M: 3	 F: 18 F: 27 F: 71 F: 19
Tumor Data: Adrenal Cortex:										
Adenoma, cortical	M: 1	F: 0	M: 0	F: 0	M: 2	F: 0	M: 0	F: 0	M: 0	F: 0
Carcinoma, cortical										
Overall Rate	M: 0	F: 0	M: 0	F: 0	M: 0	F: 0	M: 0	F: 0	M: 1	F: 3
Terminal Rate	-	0	-	F: 0	-	F: 0	-	F: 0	-	F: 3
Tests with Group 1 as Control										
Trend Test	-	-	-	-	-	-	-	F: $p = 1.000$	-	F: $p = 0.015$
Pairwise Comparisons	-	-	-	F: $p = 1.000$	-	-	-	F: $p = 1.000$	-	F: $p = 0.108$
Tests with Group 2 as Control										
Trend Test	-	-	-	-	-	-	-	-	-	F: $p = 0.040$
Pairwise Comparisons	-	-	-	-	-	-	-	F: $p = 1.000$	-	F: $p = 0.103$
Pheochromocytoma, malignant	M: 0	F: 0	M: 0	F: 2	M: 0	F: 0	M: 0	F: 0	M: 0	F: 0
Tumor Data: Brain:										
Oligodendroglimoma, benign	M: 0	F: 0	M: 0	F: 0	M: 0	F: 0	M: 1	F: 0	M: 0	F: 0
Oligodendroglimoma, malignant	M: 1	F: 0	M: 1	F: 0	M: 0	F: 0	M: 0	F: 0	M: 0	F: 0
Granular Cell Tumor, benign	M: 0	F: 0	M: 0	F: 0	M: 0	F: 0	M: 0	F: 1	M: 1	F: 0
Astracytoma, malignant										
Overall Rate	M: 1	F: 1	M: 4	F: 0	M: 4	F: 1	M: 3	M: 0	M: 1	F: 0
Terminal Rate	M:1	-	M: 2	-	M: 1	-	M: 3	-	M: 1	-

Group Designation	Air Control		Technosphere Particle Control Low Dose		Technosphere Particle Control High Dose		Technosphere Insulin Low Dose		Technosphere Insulin High Dose	
Tests with Group 1 as Control										
Trend Test	-	-	-	-	-	-	-	-	M: $p = 0.643$	-
Pairwise Comparisons	-	-	M: $p = 0.193$	-	-	-	M: $p = 0.308$	-	M: $p = 0.770$	-
Tests with Group 2 as Control										
Trend Test	-	-	-	-	-	-	-	-	M: $p = 0.947$	-
Pairwise Comparisons	-	-	-	-	-	-	M: $p = 0.786$	-	M: $p = 0.972$	-
Reticulosis, malignant	M: 0	F: 0	M: 0	F: 0	M: 0	F: 0	M: 0	F: 1	M: 0	F: 0
Non-Tumor Data										
Nasal Cavity										
Hyperplasia, goblet cell										
Level #1	M: 19	F: 16	M: 30*	F: 28	M: 22	F: 29*	M: 22	F: 19	M: 16	F: 19
Level #2	M: 2	F: 2	M: 2	F: 3	M: 2	F: 5	M: 2	F: 3	M: 1	F: 3
Level #3	M: 3	F: 2	M: 8	F: 7	M: 18**	F: 20**	M: 4	F: 4	M: 1	F:4
Level #4	M: 2	F: 1	M: 7	F: 6	M: 20**	F: 23**	M: 3	F: 3	M: 2	F: 4
Droplets, eosinophilic										
Level #1	M: 0	F: 0	M: 0	F: 2	M: 2	F: 3	M: 1	F: 2	M: 0	F: 2
Level #2	M: 23	F: 24	M: 22	F: 25	M: 39**	F: 46**	M: 20	F: 25	M: 18	F: 30
Level #3	M: 59	F: 58	M: 60	F: 57	M: 60	F: 60	M: 57	F: 58	M: 59	F: 60
Level #4	M: 57	F: 55	M: 59	F: 58	M: 59	F: 59	M: 56	F: 54	M: 57	F: 59
PCNA Activity:	M: Normal	F: Normal	M: Normal	F: Normal	M: Normal	F: Normal	M: Normal	F: Normal	M: Normal	F: Normal

[a] Toxicokinetic designated animals were used for blood sampling only and are not included in any other parameter calculations

[b] Presented as the percentage weight change at the end of study compared to Day 1

- Significantly different ($p \leq 0.05$) from air control group by the Fisher exact test.

** Significantly different ($p \leq 0.01$) from air control group by the Fisher exact test.

2.6.7.11 Reproductive and Developmental Toxicity

Nonpivotal Studies

Test Article: Technosphere Inhalation Powder

Species/ Strain	Method of Administration (Vehicle/ Formulation)	Dosing Period	Total Daily Doses mg/kg	No. per Group	Noteworthy Findings	Study Number
SD Rats	sc (PBS)	G6-17	T: 0, 10, 30, & 100	6 Males 6 Pregnant Females	All doses: Well-tolerated, no evidence of maternal toxicity or effects on embryo-fetal development.	PDC-PC-2002-0012 (b) (4) No. 96921
NZW Rabbit	sc (PBS)	G7-19	T: 0, 1, 10, 30, & 100	5 Pregnant Females	≥1: ↓ weight gain; scant feces ≥30: ↓ absolute/relative feed intake TK: Dose proportional FDKP exposure Recommended Doses: 0, 2, 10, 100	MKC-PC-2004-0003 (b) (4) 100001
SD Rats	sc to pregnant rats	G6-17	T: 0, 10, 30, & 100	10 Pregnant Females	Confirmed that fetal exposure occurs (dose proportional) as a result of treatment of the F0 generation dams with Technosphere particles	MKC-PC-2006-0010 (b) (4) 00009

G = Gestation day

T = Technosphere

2.6.7.12 Reproductive and Developmental Toxicity Fertility and Early Embryonic Development to Implantation (Pivotal)

Report Title: A Subcutaneous Injection Fertility Study of Fumaryl-Technosphere in Rats.

Test Article: Technosphere or Technosphere Inhalation Powder

Design similar to ICH 4.1.1? Yes	**Duration of Dosing:** M: 4 weeks prior to mating	**Study No.** PDC-PC-2001-0006
Species/Strain: Sprague-Dawley Rats **(SD Rat)**	**F:** 2 weeks prior to mating, through Day 7 of gestation	(b) (4) Study No. 98064
Initial Age: 9-11 weeks	**Day of Mating:** Day 0 **Location in CTD:** Vol. Page	
Date of First Dose: 15 Nov 2001	**Day of C-Section:** Day 16 of gestation	**GLP Compliance:** Yes
Special Features: No TK; BID dosing	**Method of Administration:** Subcutaneous	
No Observed Adverse Effect Level:	**Vehicle/Formulation:** Aqueous solution.	
F_0 Males: 100 mg/kg/day		
F_0 Females: 100 mg/kg/day		
F_1 Litters: 100 mg/kg/day		

Total Daily Dose (mg/kg)	**0 (Control)**	**10**	**30**	**100**
Males				
No. Evaluated	22	22	22	22
No. Died or Sacrificed Moribund	0	0	0	0
Clinical Observations:	-	-	-	-
Necropsy Observations	-	-	-	-
Body Weight (%[a])	-	-	-	-
Mean No. Days Prior to Mating	2.5	4.0	2.8	2.0
No. of Males that Mated	22	22	22	22
No. of Fertile Males	22	22	21	22
Spermatozoa per gram (millions)	689.12	659.53	673.62	687.26
Epididymal Sperm Motility (%)	63.0	67.4	63.0	64.0

- No noteworthy findings.
[a] After 4 weeks of dosing

Total Daily Dose (mg/kg)	**0 (Control)**	**10**	**30**	**100**
Females				
No. Evaluated	22	22	22	22
No. Died or Sacrificed Moribund	0	0	0	0
Clinical Observations	-	-	-	-
Necropsy Observations	-	-	-	-
Premating Body Weight (%[a])	-	-	-	-
Gestation Body Weight (%[a])	-	-	-	-
Premating Food Consumption (%[a])	-	-	-	-
Gestation Food Consumption (%[a])	-	-	-	-
Mean No. Days Prior to Mating	2.5	4.0	2.8	2.0
No. of Females Sperm Positive	22	22	21	22
No. of Pregnant Females	22	22	21	22
Mean No. Corpora Lutea	17.0	17.6	17.3	18.8
Mean No. Implantations	16.1	15.5	16.1	17.0
Mean % Preimplantation Loss	5.6	11.9	6.7	8.4
Mean No. Live Conceptuses	15.4	15.1	15.1	16.4
Mean No. Resorptions	0.7	0.4	0.9	0.6
No. Dead Conceptuses	0	0	0	0
Mean % Postimplantation Loss	4.5	2.5	6.2	3.2

Notes: (-) No noteworthy findings.
[a] At end of premating or gestation period.

2.6.7.13A Reproductive and Developmental Toxicity: Effects on Embryo-Fetal Development (Pivotal)

Report Title: Subcutaneous Injection Teratology Study of Fumaryl-Technosphere in Rats.

Test Article: Technosphere Inhalation Powder

Design similar to ICH 4.1.3? Yes	**Duration of Dosing:** G6-G17	**Study No.** PDC-PC-2001-0003
Species/Strain: Sprague-Dawley Rats **(SD Rat)**	**Day of Mating:** Day 0	(b) (4) Study No. 98061
Initial Age: 12-13 weeks	**Day of C-Section:** G20	**Location in CTD:** Vol. Page
Date of First Dose: 19 Nov 2001	**Method of Administration:** Subcutaneous	
Special Features: BID dosing	**Vehicle/Formulation:** Aqueous Solution	**GLP Compliance:** Yes
No Observed Adverse Effect Level:		
F_0 Females: 100 mg/kg/day		
F_1 Litters: 100 mg/kg/day		

Total Daily Dose (mg/kg)	**0 (Control)**	**10**	**30**	**100**
Dams/Does: AUC_{last} (ng-min/ml)[b]	-			
[after first of two daily doses]	-	374947	1098107	2840264
[extrapolated for twice daily dosing]	-	749894	2196214	5680527
No. Pregnant	21	22	21	20
No. Died or Sacrificed Moribund	0	0	0	0
No. Aborted or with Total Resorption of Litter	0	0	0	0
Clinical Observations	-	-	-	-
Necropsy Observations	-	-	-	-
Body Weight (%[a])	-	-	-	-
Food Consumption (%[a])	-	-	-	-
Mean No. Corpora Lutea	16.5	15.7	15.3	16.5
Mean No. Implantations	14.8	13.8	13.8	14.9
Mean % Preimplantation Loss	9.9	12.0	11.4	9.1

[a] At end of dosing period

[b] Taken from peri/postnatal study in rats (Study No. MKC-PC-2004-0008; (b) (4) No. LCL00003

Daily Dose (mg/kg)	**0 (Control)**	**10**	**30**	**100**
Litters: No. Litters Evaluated	21	22	21	20
No. Live Fetuses/litter	13.7	13.0	13.4	14.0
Mean No. Resorptions	1.1	0.7	0.4	1.0
No. Dead Fetuses	0	0	0	0
Mean % Postimplantation Loss	7.0	4.9	2.6	5.8
Mean Fetal Body Weight (g)	3.72	3.79	3.74	3.71
Fetal Sex Ratios (% males)	46.3	52.7	49.0	47.1
No. Fetuses Evaluated	288	287	281	279
Fetal Anomalies:				
Major Malformations (Total Fetuses)	0	0	0	0
Minor External and Visceral Anomalies (Total Fetuses)	5	4	6	3

Notes: (-) No noteworthy findings

2.6.7.13B Reproductive and Developmental Toxicity - Effects on Embryo-Fetal Development

Report Title: Subcutaneous Developmental Toxicity Study of Technosphere in Rabbits

Test Article: Technosphere Inhalation Powder

Design similar to ICH 4.1.3? Yes	**Duration of Dosing:** G7-G19	**Study No.** MKC-PC-2004-0004
Species/Strain: NZW Rabbit	**Day of Mating:** Day 0	(b) (4) No. LCL00002
Initial Age: 5 months	**Day of C-Section:** G29	**Location in CTD:** Vol. Page
Date of First Dose: 16 Aug 2004	**Method of Administration:** Subcutaneous	
Special Features: None	**Vehicle/Formulation:** Aqueous Solution	**GLP Compliance:** Yes
No Observed Adverse Effect Level:		
F_0 Females: 10 mg/kg		
F_1 Litters: 100 mg/kg		

Total Daily Dose (mg/kg)	**0 (Control)**	**2**	**10**	**100**
Dams/Does: Toxicokinetics: AUC_{last} (ng-min/mL) FDKP G19	-	482240	2689334	28737467
No. Pregnant	18	19	20	19
No. Died or Sacrificed Moribund	0	0	0	1
No. Aborted or with Total Resorption of Litter	0	0	0	0
Clinical Observations				
Scant feces	-	-	-	+++
Soft/liquid feces	-	-	+	+
Necropsy Observations	-	-	-	-
Body Weight (%[a])	4.05 Kg	-0.7	0.2	-2.5[b]
Food Consumption (%[a]) G 7-29	160.1 (g/day)	-4.6	-8.2	-22.5[b]
Mean No. Corpora Lutea	9.7	10.5	10.2	9.9
Mean No. Implantations	8.8	9.4	9.6	8.6

Notes: (-) No noteworthy findings; + = Mild; ++ = Moderate; +++ = Marked; G = Gestation day

[a] At end of gestation period. For controls, group means are shown. For treated groups, percent differences from controls are shown. Statistical significance for body weight is based on actual % body weight gain data through the dosing period (not on the percent differences. Likewise the percent difference in absolute food consumption is based on the actual data at various intervals throughout the dosing period, not specifically on G7-29.

[b] Dunnett's Test ($p < 0.05$)

Total Daily Dose (mg/kg)	**0 (Control)**	**2**	**10**	**100**
Litters: No. Litters Evaluated	18	19	20	19
No. Live Fetuses/litter	8.3	8.7	9.3	8.2
Mean No. Resorptions	0.6	0.6	0.4	0.4
No. Dead Fetuses	0	1	0	0
Mean % Postimplantation Loss	5.4	6.7	3.2	4.1
Mean Fetal Body Weight (g)	43.08	41.53	42.3	42.2
Fetal Sex Ratios (% males)	48.8	54.6	45.8	49.7
No. Fetuses Evaluated	149	166	186	155
Litters with Fetuses with any malformation/alteration	11	13	15	3
Fetuses with any malformation/alteration	21	26	27	3
% Fetuses with any malformation/alteration	14	15.1	15.0	1.5

2.6.7.14 Reproductive and Developmental Toxicity: Effects on Pre- and Post-natal Development, Including Maternal Function (Pivotal)

Report Title: Developmental and Perinatal-Postnatal Reproduction Toxicity Study of Subcutaneous Technosphere Particles in Rats, including a Postnatal Behavioral/Functional Evaluation

Test Article: Technosphere Inhalation Powder

Design similar to ICH 4.1.2? Yes	**Duration of Dosing:** G6 – L20	**Study No.** MKC-PC-2004-0008
Species/Strain: SD Rats	**Day of Mating:** Day 0	(b) (4) No. LCL00003
Initial Age: 9 weeks	**Method of Administration:** Subcutaneous	**Location in CTD:** Vol. Page
Date of First Dose: 27 Feb 2005	**Vehicle/Formulation:** Phosphate Buffer Saline	
Special Features: BID dosing	**Litters Culled/Not Culled:** Not culled during lactation period	**GLP Compliance:** Yes
No Observed AdverseEffect Level:		
F_0 Females: 100 mg/kg		
F_1 Males: 100 mg/kg		
F_1 Females: 100 mg/kg		

Total Daily Dose (mg/kg)	**0 (Control)**	**10**	**30**	**100**
F_0 Females: Toxicokinetics: (FDKP)				
AUC_{last} (ng-min/mL) Lactation D20				
[after first of two daily doses]	-	374947	1098107	2840264
[extrapolated for twice daily dosing]		749894	2196214	5680527
No. Pregnant	24	24	23	24
No. Died or Sacrificed Moribund	0	0	0	0
Clinical Observations	-	-	-	-
Necropsy Observations	-	-	-	-
Gestation Body Weight (%[a])	-	-	-	-
Lactation Body Weight (%[a])	-	-	-	-
Gestation Food Consumption (%[a])	-	-	-	-
Lactation Food Consumption (%[a])	-	-	-	-
Mean Duration of Gestation (days)	22.7	22.8	22.7	22.8
Abnormal Parturition	-	-	-	-

Total Daily Dose (mg/kg)		**0 (Control)**	**10**	**30**	**100**
F_1 Litters: (Preweaning)	No. Litters Evaluated	24	24	23	24
	Mean No. Pups/Litter	14.3	13.2	14.2	14.9
	Mean No. Liveborn Pups/Litter	14.2	13.1	14.1	14.9
	Mean No. Stillborn Pups/Litter	0.1	0.2	0.1	0
	Postnatal Survival to Day 4	-	-	-	-
	Postnatal Survival to Weaning	-	-	-	-
	Change in Pup Body Weights[a] (g)	-	-	-	-
	Pup Sex Ratios (% males) Day1 postpartum	49.1	51.7	50.3	47.3
	Pup Clinical Signs	-	-	-	-
	Pup Necropsy Observations	-	-	-	-
F_1 Males: (Postweaning)	No. Evaluated Postweaning	25	25	25	25
	No. Died or Sacrificed Moribund	-	-	-	-
	Clinical Observations	-	-	-	-
	Necropsy Observations	-	-	-	-
	Body Weight Change[b] (g)	-	-	-	-
	Food Consumption (%[b])	-	-	-	-
	Preputial Separation	-	-	-	-
	Sensory Function	-	-	-	-
	Motor Activity	-	-	-	-
	Learning and Memory	-	-	-	-
	Mean No. Days Prior to Mating	3.6	3.2	4.1	3.3
	No. of Males that Mated	24	24	23	25
	No. of Fertile Males	23	22	21	24

Notes: (-) No noteworthy findings.

[a] From weaning to mating.

[b] During post-weaning period.

Total Daily Dose (mg/kg)		0 (Control)	10	30	100
F_1 Females: (Postweaning)	No. Evaluated Postweaning	25	25	25	25
	No. Died or Sacrificed Moribund	0	0	0	0
	Clinical Observations	-	-	-	-
	Necropsy Observations	-	-	-	-
	Premating Body-Weight Change[a] (g)	-	-	-	-
	Gestation Body-Weight Change (g)	-	-	-	-
	Premating Food Consumption (%[b])	-	-	-	-
	Gestation Food Consumption (%[b])	-	-	-	-
	Mean Age of Vaginal Patency (days)	-	-	-	-
	Sensory Function	-	-	-	-
	Motor Activity	-	-	-	-
	Learning and Memory	-	-	-	-
	Mean No. Days Prior to Mating	3.7	3.2	4.3	3.3
	No. of Females Sperm Positive	25	25	25	25
	No. of Pregnant Females	24	23	23	24
	Mean No. Corpora Lutea	15.7	16.0	16.4	16.0
	Mean No. Implantations	15.1	14.9	15.6	15.0
F_2 Litters:	Mean No. Live Conceptuses/Litter	13.5	14.6	14.9	14.2
	Mean No. Resorptions	1.6	0.3	0.7	0.8
	No. Dead Conceptuses	0	0	0	0
	Mean % Postimplantation Loss	10.0	2.1	4.3	5.3
	Fetal Body Weights (g)	5.32	5.37	5.31	5.36
	Fetal Sex Ratios (% males)	52.1	50.4	50.1	53.9
	Fetal Anomalies	-	-	-	-

2.6.7.15 Local Tolerance

Test Article: Technosphere or Technosphere Insulin Inhalation System

Species/ Strain	Method of Administration	Doses	Gender and No./Group	Noteworthy Findings	Study Number
NZW Rabbit	Dermal: to intact & abraded skin	T: 0.5 g T/I: 0.5 g	Males: 6/group	Both were considered similar in irritancy potential and by FDA criteria were both considered non-irritants.	MKC-PC-2007-0019 (b) (4) No. LCL00021

T = Technosphere
TI = Technosphere Insulin

2.6.7.16 Other Toxicity Studies

Test Article: Technosphere or Technosphere Insulin Inhalation System

Species/ Strain	Method of Admin.	Duration of Dosing	Doses (mg/kg)	Gender and No./Grp	Noteworthy Findings	Study No.
Antigenicity						
Guinea Pigs	id	Single dermal inj & dermal application; 2 dermal challenges every 2 w	T, T/I:5% w/v dermal inj; T, T/I: 25% w/w dermal applic	20/M	Mild dermal irritation at 24 h, but did not reach criteria for positive dermal sensitization hazard.	PDC-PC-2001-0001 (b) (4) 7260-100
Immunotoxicity						
SD Rat	Inhalation; sc	28 d	Low T: 1.38 M; 0.82 F High T: 44.0 M; 47.0 F Low T/I: 0.77/0.10 M Low T/I: 0.50/0.067 F High T/I: 1.33/0.18 M High T/I: 0.79/0.11F S.C (T): 75 M & F	10/M 10/F	Inhalation route: No effects on lymphocyte subsets or T-cell dependent antigen response. sc:, ↓ NK cells; but of no clinical relevance & no concurrent sc control	MKC-PC-2008-0020 (b) (4) Study No. 75101

Species/ Strain	Method of Admin.	Duration of Dosing	Doses (mg/kg)	Gender and No./Grp	Noteworthy Findings	Study No.
Impurities						
SD Rat	sc	28 d	FDKP + Impurities: 0, 10, 35, & 75	10-15 M 10-15 F	≥ 35 mg/kg: local injection site reactions (with FDKP + known impurities (b) (4) (b) (4) - No systemic tox - Local inj site rxn: gross & microscopic path; reversible at 4 w - TK: exposure dose-proport. - NOAEL: 75 mg/kg/day	MKC-PC-2008-0006 (b). Study No. 70569 ( )
PS80						
SD Rat	Inhalation.	28 d	T: 12→16 & 45 PS80:0.29→0.40 & 1.11	10 M 10 F	NOAEL: High dose: 45 mg/kg Technosphere (actual) & 1.11 mg/kg PS80 (actual)	MKC-PC-2008-0005 (b). Study No. 75065
Phototoxicity – in vitro						
3T3 Mouse Fibroblast	In Vitro	20-60 min	T & T/I; 10, 50, 100, 150, 500, & 1000 mg/L with & without UVA exposure	48 wells of 96 well plate; 2 assays	No cytotoxicity with or without UVA exposure. Technosphere Particles & Technosphere Insulin were determined to be non-phototoxic	MKC-PC-2007-0004 (b) (4) No. LCL00016

Species/ Strain	Method of Admin.	Duration of Dosing	Doses (mg/kg)	Gender and No./Grp	Noteworthy Findings	Study No.
Ecotoxicity						
Daphnia magna	In culture medium	24 & 48 h	T:Nominal: 100 mg/L T:Actual: 97.2 mg/L	5/vessel 4 vessels	48-h EC50 : > 97.2 mg/L "No observed effect C" (NOEC) : ≥ 97.2 mg/L	MKC-2008-0001 (b) (4) No. MKC0002
Pseudokir-chneriella subcapitata	In culture medium	24, 48, & 72 h	T:Nominal: 100 mg/L T:Actual: 101 mg/L	6 flasks/ control & treated C	Area under the growth curve: EbC50 (72 h) : > 101 mg/L Average specific growth rate: ErC50 (0-72 h) : > 101 mg/L No observed effect C (NOEC) : 101 mg/L	MKC-PC-2008-0002 (b) (4) No. MKC0003
Intermediates						
NZW Rabbit	Topical Dermal	Single Dose Eval: 1, 24, 48, & 72 hours	Intermed 1, 2 & 3: 0.5 g Neat moistened with distilled water	3/group	(b) (4)	MKC-PC-2008-0016 (b) (4) No. 7260-112
Guinea Pig	Topical Dermal	3 weekly induction doses; challenge dose 2 weeks later	Intermed 1: 95% Intermed 2: 86% Intermed 3: 86%	10/group		MKC-PC-2008-0018 (b) (4) No. 7260-115

Species/ Strain	Method of Admin.	Duration of Dosing	Doses (mg/kg)	Gender and No./Grp	Noteworthy Findings	Study No.
SD Rat	Oral Gavage	Single; Limit Test	Intermed 1: 300, 2000 Intermed 2: 2000 Intermed 3: 2000	3/M 3/F	(b) (4)	MKC-PC-2008-0019 (b) (4) No. 7260-114
NZW Rabbit	Topical, Ocular	Single	Intermed 1: 0.0671 g Intermed 2: 0.0553 g Intermed 3: 0.0388 g	3/M		MKC-PC-2008-0023 (b) (4) No. 7260-113

Species/ Strain	Method of Admin.	Duration of Dosing	Doses (mg/kg)	Gender and No./Grp	Noteworthy Findings	Study No.
Chemico-Bio Properties						
NA	NA	NA	NA	NA	FDKP was determined to have $\log_{10}$Pow values of -2.4, < -3.4 & < -3.4 at pH 4, 7, & 9, respectively (equivalent Pow = 0.004, < 0.0004 & < 0.0004).	MKC-PC-2008-0021 (b) (4) No. MKC0004
NA	NA	NA	NA	NA	Technosphere powder was not considered readily biodegradable.	MKC-PC-2008-0024 (b) (4) No. MKC0006

OVERALL CONCLUSIONS AND RECOMMENDATIONS

Conclusions:

Afrezza™ is a rapid acting prandial insulin. It consists of Technosphere® Insulin Inhalation Powder pre-metered into unit dose cartridges (15 IU insulin and 30 IU insulin) and the MedTone® inhaler. Technosphere® Insulin (TI) has a medium diameter of 2.5 µm and is comprised of insulin, fumaryl diketopiperazine (FDKP, a novel excipient), and (b) (4) polysorbate 80. The MannKind Corporation intends to use Afresa™ for T1DM and T2DM patients. Note: MKC has since submitted a protocol to change its inhaler from MedTone® to Gen2® and to lower cartridge doses from 15/30 to 10/20 IU insulin under IND 61,729.

Clinical Exposure:

1 mg Technosphere Insulin = (b) (4) mg insulin (or 3 U) + (b) (4) mg Technosphere

1 U insulin = 0.0347 mg insulin

MRHD = 105 mg Technosphere Insulin (b) (4)

Projected human AUC = 46,000 ng•min/mL

FDKP AUC following 40 mg Technosphere without insulin: 1631.16 (ng*h/mL) (CV% 27.32)

FDKP AUC following 30 U Technosphere with insulin at Day 7 following 7 days administration: 20658 (min*ng/mL) (CV% 36.2)

***Pharmacology*:**

Inhalation studies in rats and dogs showed that TI could lower blood glucose levels in a dose-dependent manner whereas T alone had no effect on blood glucose levels.

Reviewer's comment: Inhaled Technosphere® insulin generated an expected pharmacological activity. The excipient, FDKP (Technosphere® particles), did not interfere the pharmacological activity of insulin.

***Safety Pharmacology*:**

Neither TI nor T produced adverse: ***i)*** neurological effects (i.e. FOB testing) in male rats by IV administration, ***ii)*** pulmonary effects (i.e. tidal volume, respiratory rate, airway resistance, arterial pressure, and heart rate) in male rats by IT or IV administration, ***iii)*** renal effects (i.e. electrolyte level, urine output, and creatinine clearance) in male and female rats by IV administration, and ***iv)*** cardiovascular effects (i.e. ECG and QTc intervals) *in vivo* (male dogs) by IV administration, as well as *in vitro* (hERG channel assay). Some findings such as an increase in motor activity in rats (high dose TI), hemodynamic changes with heart rate and blood pressure in dogs (PS80 contained T) were seen but were not considered treatment-related. In addition, PS80-enriched 28 day inhalation rat study showed that PS80-enriched Technosphere Powder (once daily for 28 days) did not produce any local or systemic effect.

Reviewer's comment: Based on the safety pharmacology findings, Technosphere® insulin or Technosphere® particles did not cause any treatment-related safety concerns.

***ADME*:**

TI (insulin+ FDKP) were absorbed rapidly in all species tested (mouse, minipigs, rat, and dog). PK profiles for both insulin and FDKP were consisted with the human PK profiles. The mean t_{max} for insulin and FDKP were about 15 min after dosing across all studies. Insulin levels returned to near baseline between 180 and 240 min after dosing. The mean t ½ ranged between 135 and 170 min. Insulin and FDKP exposure after TI administration were approx. dose linear.

T (FDKP) bioavailability in both rats and dogs was high by the IV administration, moderate by the inhalation administration, and poor following PO administration. Regardless of route of exposure, the primary route of excretion was through the kidney. FDKP could be distributed into lungs, trachea, kidney, thyroid/parathyroid glands, GI wall, and urinary bladder wall. In addition, FDKP could not be metabolized and was not a substrate for phase I and II biotransformation. Limited absorption of FDKP was detected in GI tract. Lastly, FDKP could also be detected in milk and it could also cross blood placental barrier in rats (1/10 peak plasma level).

Reviewer's comment: Animal findings suggested that Technosphere® insulin can be absorbed quickly and Technosphere® particles were not metabolized and primarily eliminated by the kidney. A similar PK profile of Technosphere® insulin was seen in humans.

***Repeated Dose Toxicology*:**

In 26 week inhalation **rat** study, there were increases in pulmonary organ weights, which correlated with T administration, in a dose-related manner at Week 26. Histopathological

findings, such as eosinophilic globules and epithelial degeneration in the nasal cavities, were observed in M and F given T and TI in non dose-dependent fashion, suggesting a local effect of T inhalation. These changes were diminished in females, but not in males, during the recovery period. In addition, the PCNA (proliferating cell nuclear antigen) analysis showed a slight change in epithelial cell activity in the upper airway bronchial cells in both T and TI groups at Week 26. However, no histopathological changes of epithelial hyperplasia were noted in any of the respiratory tree tissues. Carcinogenicity studies confirmed an absence of preneoplastic/neoplastic findings. Furthermore, there was minimal to slight myocardial degeneration/necrosis seen in 50% T-treated males at 11.7 mg/kg/day. Three deaths post-dosing were also found in T-treated group.

In 39 week inhalation **dog** study, there were minimal to mild alveolar and/or bronchial-interstitial neutrophil infiltrate of the lungs in both TI groups after 39 weeks of treatment. These findings regressed after 8 weeks of recovery. This lung irritation appeared to be in a T-dose dependent manner. Other findings, such as minimal to mild thymic atrophy (high-dose T), minimal to moderate hypocellularity of the seminiferous epithelium and minimal to mild germ cell degeneration (low-dose T and high-dose TI) were noted.

Species	***Duration***	***Route***	***Doses (mg/kg/day) NOAEL/NOEL***	***Findings***
Spraque-Dawley	26 wk w/ 30 d recovery, daily for 35-180 min	Inhalation Pivotal (nasal)	T: 11.7 TI: 1.05/0.04 (m/f), 1.91/1.28 (m/f) **$NOAEL_T$: < 11.7** **$NOAEL_{TI}$: 1.91(m)/1.28(f)**	▪ ↓ body wt in T control and HD TI groups in T dose-dependent manner ▪ ↓ glucose at HD TI groups ▪ ↑ pulmonary wt in T dose-related fashion ▪ Eosinophilic globules and epithelial degeneration were noted in the nasal cavities of both T and TI groups ▪ ↑ PCNA (bronchial cells) in TI groups, diminished during 4 wk recovery. ▪ 50% T-treated males: myocardial degeneration/necrosis, no functional effects seen.
Beagle	26/39 wk	Inhalation Pivotal (nose & mouth)	T: 2.4, 10.9 TI: 0.39, 1.92 **$NOAEL_T$: 2.4** **$NOAEL_{TI}$: 1.92**	▪ ↓ Serum glucose ▪ Neutrophil infiltrate of the lung in TI groups after 39 wk, regressed during 8 wk recovery ▪ Thymic atrophy in 6/8 high-dose T group at Week 29 ▪ Hypocellularity of the seminiferous epithelium and germ cell degeneration in ¼ low-dose T and 2/4 high-dose TI groups at Week 39

Reviewer's comment: Animal findings indicated that Technosphere® insulin and Technosphere® particles were well-tolerated in mice, rats, and dogs. There were inflammation observed in nasal cavities and lungs of T and/or TI treated animals, including eosinophilic globules and epithelial degeneration in the nasal cavities (rat), localized foci/inflammation sites of the respiratory tract (rat), and increased eosinophil counts and neutrophil infiltrates in the lungs (dog). These findings were apparent to be Technosphere® particles related in a dose-dependent manner.

Safety margin: The NOAELs for T in both rats and dogs are approx. 1.5X to 2X, based AUCs for FDKP. See calculations below.

105 mg TI – approx. (b) (4) Technosphere, with projected human AUC = 46,000 ng•min/mL
Assumed 60 kg as adult body weight = 1.57 mg/kg T
Therefore, 1 mg/kg T = 29,299 ng•min/mL

26 week rat study:

For NOAEL of T = 11.7 mg/kg/day in rats
AUC (FDKP) on Day 182 = 514362 ng•min/mL (male) and 703445 ng•min/mL (female)
Therefore, 1 mg/kg = 43,962 ng•min/mL (male) and 60,124 ng•min/mL (female)

Rat AUC vs. Projected human AUC - 1.5x (male) and 2x (female)

39-week dog study:

For NOAEL of T = 2.4 mg/kg/day in dogs
AUC (FDKP) on Day 182 = 102,604 ng•min/mL (male) and 112,946 ng•min/mL (female)
Therefore, 1 mg/kg = 42,752 ng•min/mL (male) and 47,060 ng•min/mL (female)

Dog AUC vs. Projected human AUC - 1.5x (male) and 1.6x (female)

In all species tested, safety margins for Technosphere® particles alone were considered underestimated since Technosphere NOAELs were tested the highest doses tested. Safety margins for Technosphere® insulin were modest because doses were limited by hypoglycemia, which was an expected pharmacological activity of insulin.

***Genetic Toxicology*:**

In vitro mammalian chromosome aberration and Ames assays (for T and TI) and mammalian erythrocyte micronucleus test (for T only) showed that there was no mutagenic potential and no apparent genetic toxicity with either T alone or TI. A micronucleus was not conduced with TI. This might be due to the facts that insulin genotoxicity is well established (negative).

***Carcinogenicity*:**

In rats, no pre-neoplasia and/or neoplasia were seen in the lung with both Technosphere® particles (46 mg/kg/day) and Technosphere® Insulin (up to 1.23 mg/kg/day). In mice, there was no evidence of increased oncogenicity associated with Technosphere® particles (25 or 75 mg/kg/day) or with Technosphere® Insulin (2.5 and 5 mg/kg/day in males or 0.6 and 1.25 mg/kg/day in females).

Species	*Duration*	*Route*	*Doses (mg/kg/day)*	*Findings*
Spraque-Dawley rats	104 wk	Inhalation Pivotal	T:1.1041/0.6784 (m/f), 41/46 (m/f) TI: 0.6572/0.4413 (m/f), 1.2313/0.7608 (m/f) Insulin: 0.0787/0.0529	▪ Adrenal cortical carcinoma (significant in high-dose TI females) ▪ Malignant astrocytoma (in low- and high-dose T males) ▪ Malignant schwannoma in nasal cavity (of low-dose T males) ▪ Fibroma in the skin/subcutis (in low-dose TI males and females) ▪ Pituitary adenoma/carcinoma (in low-dose TI males)

			(m/f), 0.1457/0.0900 (m/f)	*Lacks of dose-response and low incidences, within historical (background) rates, indicate not drug-related.*
rasH2 mice	26 wk	SC injection Pivotal	T: 25/75 TI: 2.5/0.6 (m/f), 5/1.25 (m/f)	▪ Bronchiolar-alveolar adenoma/carcinoma in the lung ▪ Hemangioma/hemangiosarcoma, primarily spleen ▪ Harderian gland adenoma/carcinoma ▪ Squamous cell neoplasms at multiple sites – stomach and skin/subcutis ▪ Myeloproliferative neoplasia *Found in the sham, vehicle control, high T, and high TI groups, not drug related.*

Reviewer's comment: Animal findings suggested that Technosphere® insulin and Technosphere® particles did not have carcinogenic potential. The carcinogenicity study findings were presented to ECAC on Sept 29th 2009 and ECAC concurred that these studies were adequate and were negative for carcinogenicity.

Safety margin: The NOAELs for T in both rats and mice are approx. 0.15X and 1.2X, based on Cmax (rat) and AUC (mouse), respectively. See calculations below.

104 week inhalation rat study:

Approx 20 mg Technosphere, with Cmax = 247 ng/mL (from MKC-T-131 clinical study)
Assumed 60 kg as adult body weight = 0.33 mg/kg T
Therefore, 1 mg/kg T = 748 ng/mL

For NOAEL of T = 41/46 mg/kg/day in M/F rats
Cmax on Week 104 = 4488 ng/mL (male) and 6260 ng/mL (female)
Therefore, 1 mg/kg = 109 ng/mL (male) and 136 ng/mL (female)

Rat Cmax vs. Projected human Cmax – 0.14x (male) and 0.18x(female)

26-week SC mouse study:

105 mg TI – approx. (b) (4) Technosphere, with projected human AUC = 46,000 ng•min/mL
Assumed 60 kg as adult body weight = 1.57 mg/kg T
Therefore, 1 mg/kg T = 29,299 ng•min/mL

For NOAEL of T = 75 mg/kg/day in mice
AUC on Week 26 = 2,753,474 ng•min/mL
Therefore, 1 mg/kg = 36713 ng•min/mL

Mouse AUC vs. Projected human AUC - 1.2x

***Reproductive Toxicology*:**

In rats, Technosphere® particles did not have effects on fertility or embryo/fetal development, but have an effect on pre/postnatal development (i.e. learning impairment at higher doses of T). In rabbits, Technosphere® particles seemed to produce maternal toxicity (i.e. weight loss at 2 mg/kg/day) and some malformations in fetuses (i.e. absent lung lobe and gallbladder in survived fetuses and cleft palate in dead fetus).

Species	*Duration*	*Route*	*Doses (mg/kg/day)*	*Findings*

			NOAEL/NOEL	
Spraque-Dawley rats	M: 4 wk prior to mating F: 2 wk prior to mating - GDs - 7, twice daily	SC injection	T : 10, 30, 100 Fertility NOAEL for F0 (M/F): 100 NOAEL for F1 (litters): 100	▪ No maternal toxicity ▪ No effect upon fertility ▪ No early embryonic developmental toxicity
Spraque-Dawley rats	GDs 6-17 (f) 21 d (m)	SC injection	T: 10, 30, 100 Pivotal Seg II NOAEL for F0 (F): 100 NOAEL for F1 (litters): 100	▪ No maternal toxicity ▪ Only minor anomalies (e.g. dilated ureters), but no difference between the control and treated groups
New Zealand White rabbits	GDs 7-19, once daily	SC injection	T: 2, 10, 100 Pivotal Seg II NOAEL for F0 (F): < 2 NOAEL for F1 (litters): < 2 *Sponsor NOAEL for F0 10* *Sponsor NOAEL for F1 (litters) 100*	▪ Maternal toxicity: One mortality at 100 mg/kg/day, Scant and soft/liquid feces, and Reduced body weigh at 2 mg/kg/day ▪ Malformations (i.e. cleft palate in one dead fetus, absence lung lobe and gallbladder in survived fetuses) at 2 mg/kg/day
Spraque-Dawley rats	GDs 7 to LDs 20, twice daily	SC injection	T: 10, 30, 100 Prei/postnatal NOAEL for F0 (F): 100 NOAEL for F1 (M): 10 NOAEL for F2 (F): 100 *Sponsor NOAEL for F1 100*	▪ F0: No maternal toxicity ▪ F1: Some pups failed to learn from at 30 mg/kg/day groups ▪ F1: Decreased male reproductive organ weights (testes and epididymides absolute weights) at 100 mg/kg/day ▪ F2: No T-treated gross external alternations observed in F2 generation.

Reviewer's comment: Rabbits seemed to be more sensitive to Technosphere® particles than rats, indicating specie difference.

Safety margin: FDPK safety margin is approx. 3x in rats and 8x in rabbits, based on AUC.

105 mg TI – approx (b) (4) Technosphere, with projected human AUC = 46,000 ng•min/mL
Assumed 60 kg as adult body weight = 1.57 mg/kg T
Therefore, 1 mg/kg T = 29,299 ng•min/mL

Seg II rat study [based on study pdc-pc-2001-0003]:

For NOAEL of T = 100 mg/kg/day in rats
AUC on GD 7 = 8335854 ng•min/mL
Therefore, 1 mg/kg = 84458.54 ng•min/mL

Rabbit AUC vs. Projected human AUC – 3x

Seg II rabbit study [based on study :

For NOAEL of T = 2 mg/kg/day in rabbits
AUC on GD 19 = 482240 ng•min/mL
Therefore, 1 mg/kg = 241,120 ng•min/mL

Rabbit AUC vs. Projected human AUC – 8x

***Special Toxicology*:**

T and/or TI was not considered as a dermal irritant (in rabbits and guinea pigs), immunotoxic (28-day inhalation/SC rat study), phototoxic (in mouse 3T3 fibroblasts cells), or exotoxic (in fresh fishes and algae). In addition, PS80, a component of TI, was not toxic in rats given up to 43 mg/kg/day T containing (b) (4) PS80.

Lastly, (b) (4) FDKP impurities (b) (4) were identified by the sponsor. These impurities were qualified in toxicology studies. Major impurities (b) (4) were further studied in 28-day SC rat study. Even though others, such as (b) (4), were not tested in this 28 day study, they were qualified in various studies (see tables below). (b) (4) was not tested because it could be removed during TI formulation. Lastly, even though individual FDKP impurities were not tested, FDKP containing the impurities was not mutagenic by Ames assay.

2.6.7.4B Toxicology **Novel Excipient (FDKP) Analysis** **Test Article: Technosphere or Technosphere Insulin Inhalation Powder**

Novel Excipient (FDKP) TM5478 Data																		
		Specified Impurity (Area %) (RRT)[a]																
Lot No.	Assay Purity[a] (Wt %)	(b) (4)															Study No.[a]	Type of Study
N1500		(b) (4)															2004-0001	Rat Inhalation Carc
N1501																	2004-0001	Rat Inhalation Carc
N1502																	2004-0001	Rat Inhalation Carc
N1871																	2004-0001 2006-0010 2006-0038 2007-0021 2007-0023 2008-0020	Rat Inhalation Carc Rat Fetal TK Rat Renal Fct Respiratory Safety Rat Pulmonary Fct Rat Immunotox
N1872																	2006-0010 2006-0038 2007-0021 2007-0023 2008-0020	Rat Fetal TK Rat Renal Fct Respiratory Safety Rat Pulmonary Fct Rat Immunotox
N2258																		
N2259																	2007-0003 2007-0019 2007-0022 2007-0024 2007-0020	Carcinogenicity Study Rabbit Skin Irritation Study Respiratory Study Rat Pulmonary Fct Immunotoxicity Study

Novel Excipient (FDKP) TM5478 Data																		
		Specified Impurity (Area %) (RRT)[a]																
Lot No.	Assay Purity[a] (Wt %)	(b) (4)															Study No.[a]	Type of Study
N2260		(b) (4)															2006-0034 2006-0035 2006-0036 2007-0005 2007-0009 2007-0010 2007-0011 2007-0012 2007-0013	Absorption, metabolism and elimination of C-FDKP

NT = Not Tested NA = Not Applicable ND = Not Detected RNA = Record Not Available

NR = None above reporting threshold of (b) (4) %

[a] Each study number is preceded by MKC-PC-

Reviewer's comment re: impurities: The sponsor provided their responses to two FDA questions with regards to impurities in the drug batches used in toxicology studies on 8/24/09 (see sponsor's response below). Three impurities ((b) (4)) were above (b) (4) %

level, which were further studied in 28 day rat study. No additional toxicity study needed for FDKP impurities.

1. *FDA: Please provide the levels of impurities in the drug batches used for toxicology testing.*

MannKind Response:

A complete list of FDKP process impurity levels and Lot information for all toxicology studies, including FDKP process impurity qualification studies is provided in Section 2.6.7.4A of the NDA. Table 1 lists the Lot numbers and FDKP process impurity levels qualified in three toxicology studies. .

Table 1. Levels of FDKP Process Impurities

FDKP Process Impurity	Area %	Lot Number of Test Article
MKC-PC-2008-0006; 28-Day Rat Tox Qualification Study		
(b) (4)	(b) (4)	FD 080.64
		FD 080.64
		FD 080.64
PDC-PC-1997-0001; 28-Day Dog Inhalation Tox Study		
(b) (4)	(b) (4)	PDC 102-002
		PDC 102-002
PDC-PC-1996-0002; 28-Day Rat Inhalation Tox Study		
(b) (4)	(b) (4)	PDC 098-012

Three drug batches were tested in toxicology studies to establish safety margins for FDKP process impurities. In one study, bulk FDKP was used (MKC-PC-2008-0006, a 28-day rat tox study with 3 impurities administered by subcutaneous injection) and, in the other two studies, two separate lots of Technosphere Inhalation Powder were used (PDC-PC-1997-0001, PDC-PC-1996-0002, 28-day dog and rat toxicology studies).

Levels of (b) (4) FDKP process impurities were (b) (4) (b) (4) respectively in Study MKC-PC-2008-0006 (Lot FD 080.64). In this study, the individually-prepared impurities were added to bulk FDKP and administered to rats by subcutaneous injection daily for 28 days. This study was used to qualify (b) (4) (b) (4)

Levels of FDKP process impurities (b) (4) were (b) (4) in a 28-day dog inhalation study (PDC-PC-1997-0001). The level of (b) (4) FDKP process impurity was (b) (4) in a 28-day rat inhalation toxicology study (PDC-PC-1996-0002. In these studies, Technosphere inhalation powder was administered to rats and dogs by inhalation daily for 28 days.

Impurity (b) (4) is a (b) (4) during the manufacture of Technosphere® particles because (b) (4) (b) (4) Since the level of the (b) (4) (b) (4) impurity is reduced to (b) (4) % during drug product manufacturing, qualification in toxicology studies was not necessary. Table 2 from Section 3.2 (3.2.A.3.3.2.1.1) is provided below and included in the NDA 22-472.

Table 2. Area% levels of (b) (4) impurity in FDKP and TI

Batch number (FDKP/TI)	% (b) (4) in FDKP	% (b) (4) in TI
N1872/Group A[a]	(b) (4)	
N2256/Group B[b]		
N2260/Group C[c]		
N3086/Group D[d]		
N3087/Group E[e]		
N3088/Group F[f]		

[a] Group A: Lots PM6317A, PM6325A, PM7030A, PM7031A
[b] Group B: Lots PM6338A, PM6339A, PM7032A, PM7033A
[c] Group C: Lots PM6340A, PM6341A, PM7036A, PM7037A
[d] Group D: Lots PPT2008.27, PPT2008.28
[e] Group E: Lots PPT2008.29, PPT2008.30
[f] Group F: Plots PPT2008.31, PPT2008.32

NR: not reportable ((b) (4) %)
ND: not detected (<(b) (4) %)

2. ***FDA: Did you do any toxicology studies with the aged product to qualify any degradants that are FDKP or drug related?***

MannKind Response:

MannKind has not conducted toxicology qualification studies with aged Technosphere Insulin Inhalation Powder. Toxicology qualification studies were not conducted on FDKP-related degradation products because (b) (4) Two FDKP-Insulin degradation products are observed after one month at room temperature storage. These FDKP-insulin degradation products were not qualified in toxicology studies because they are present at levels less than 0.5% (below the ICH qualification criterion).

Unresolved toxicology issues (if any):

None.

Recommendations:

From the pharmacology/toxicology point of view, this application is recommended for approval.

Suggested labeling:

The labeling is in general acceptable. However, following changes in the pregnancy and carcinogenicity section of the label are recommended.

Sponsor's proposed label:

2 Page(s) of Draft Labeling have been Withheld in Full as B4 (CCI/TS) immediately following this page

Executive CAC
Date of Meeting: June 1, 2004

Committee: Abby Jacobs, Ph.D., HFD-024, Acting Chair
David Jacobson-Kram, Ph.D., HFD-024, Member
Al DeFelice, Ph.D., HFD-110, Alternate Member
Jeri Elhage, Ph.D., HFD-510, Team Leader
Herman Rhee, Ph.D., HFD-510, Presenting Reviewer

Author of Draft: Herman Rhee, Ph.D., Presenting Reviewer

The following information reflects a brief summary of the Committee discussion and its recommendations. Detailed study information can be found in the individual review.

The committee did not address the sponsor's proposed statistical evaluation for the 2-yr carcinogen bioassays, as this does not affect the sponsor's ability to initiate the bioassays. The sponsor may seek guidance on the statistical evaluation of bioassay results from agency staff separately. Data files should be submitted electronically following section E of the 'Guidance for Industry, Providing Regulatory Submission in Electronic Format, New Drug Application.'

IND #: 61,729
Drug Name: rH-Insulin with Fumaryl Technosphere
Sponsor: MannKind Corporation, Danbury, CT
Sponsor contact name: George S. Goldstein, Vice President for Regulatory Affairs
Sponsor telephone and fax number: (203)796-3568; (203)796-3681

Background:

The drug product is a combination formulation of recombinant human insulin and Technosphere, which is a drug delivery system. Chemically technosphere is fumaryl diketopiperazine, whose carcinogenic potential has not been assessed. Technosphere can not be adequately evaluated in the combination formulation of fixed ratio insulin plus technosphere due to dose limiting hypoglycemic effects of insulin. Thus, the sponsor would like to test two doses of the combination formulation at the exposures representing 1X and the MTD for insulin while assessment of higher doses of technosphere only will be based on AUC ratio. According to the sponsor's study plan there are five groups: Air control, 2 doses of Technosphere alone and 2 doses of Technosphere plus insulin. Fumaryl Technosphere tested negative in the Ames assay, in vitro mammalian chromosomal aberration test and mouse micronucleus assay.

Rat Carcinogenicity Study Protocol and Dose Selection:

The combination drug product will be given by inhalation to Sprague Dawley male rats for 24 months. For males, the sponsor proposed the use of Technosphere at doses of 0(Air control), 0.74, and 1.33 mg/kg/day, which contain 0, 2.5, and 4.5 IU insulin, respectively. For the females, the doses of technosphere would be 0, 0.44 and 0.74 mg/kg/day, which contain 0, 1.5, and 2.5 IU insulin, respectively. The doses of insulin were selected by the sponsor based on the low dose representing daily clinical exposures and the high dose being the MTD for insulin-induced hypoglycemia based on a 6-month rat toxicology study. The basis for this selection was found to be adequate since the doses of insulin could not be increased due to the hypoglycemic action of insulin.

The sponsor proposed separate arms in the carcinogenicity study in rats because the carcinogenic potential of Technosphere (Fumaryl diketopiperazine) has not been assessed and the fixed ratio of insulin plus Technosphere combination does not allow the use of sufficiently high doses of Technosphere. The sponsor's choice for the highest doses for males and females were (b) (4) mg/kg/day, respectively, which were found to be grossly inadequate because the rats tolerated Technosphere at doses up to 50 mg/kg/day in the 6-month toxicology study. The high dose of 50 mg/kg/day in rats recommended by the Division should achieve AUC ratios > 25 X human exposure, based on a limited human pharmacokinetic study in Germany.

Executive CAC Recommendations and Conclusions:

The Committee agreed with the division's proposal, and recommended doses of 1.33 mg/kg/day Technosphere + 4.5 IU insulin and 0.74 mg/kg/day technosphere + 2.5 IU insulin for males and 0.74 mg/kg/day Technosphere + 2.5 IU insulin and 0.44 mg/kg/day Technosphere + 1.5 IU insulin for females. The study will include an air control, two Technosphere alone (LD and HD) groups, and two insulin plus Technosphere groups (LD and HD). For Technosphere alone, the Committee recommended doses of 1.33 and 50 mg/kg/day (3X, over 25X AUC) for males and 0.74 and 50 mg/kg/day (2X, over 25X) for females. The low dose of Technosphere alone was selected to provide a vehicle control for the high dose Technosphere plus insulin arms.

The Committee encourages the sponsor to generate toxicokinetics data in rats for Technosphere.

The sponsor has indicated in the proposed protocol that routine histopathological evaluation will be conducted for all animals euthanized *in extremis* or that die spontaneously, and for the control and high dose groups. Therefore, it is noted that the sponsor will also need to conduct histopathologic examination of other dose groups under any of the following circumstances:

(a) for any macroscopic findings in the low dose groups for a given tissue, they will need to look at that tissue for all of the dose groups
(b) for an increase in the incidence of tumors (rare or common) in the high dose group for a tissue, even if not statistically significant, they will also need to look at the next lower dose group
(c) for an increase in tumors in an organ for a tumor type that should be analyzed across tissue sites as well as by tissue site (e.g., hemangiosarcoma, lymphoma etc.; see McConnell et al, JNCI 76:283, 1986) they should look at all relevant tissues for that dose level and the next lower dose level,
(d) for an excessive decrease in body weight or survival in the examined dose group, they should examine lower dose groups.

Abigail Jacobs, Ph.D.
Acting Chair, Executive CAC

cc:\
/Division File, HFD-510
/Jeri Elhage, Team leader, HFD-510
/Herman Rhee, Reviewer, HFD-510
/Oluchi Elekwachi, PM, HFD-510
/ASeifried, HFD-024

ECAC Reviews for 6 mo carcinogen bioassay

Executive CAC
Date of Meeting: April 3, 2007

Committee: David Jacobson-Kram, Ph.D., OND IO, Chair
Joseph Contrera, Ph.D., OPS, Member
Abby Jacobs, Ph.D., OND IO, Member
Paul Brown, Ph.D., DDDP, Alternate Member
Todd Bourcier, Ph.D., DMEP, Team Leader & Presenting Reviewer

The following information reflects a brief summary of the Committee discussion and its recommendations.

The committee did not address the sponsor's proposed statistical evaluation for the 2-yr carcinogen bioassays, as this does not affect the sponsor's ability to initiate the bioassays. The sponsor may seek guidance on the statistical evaluation of bioassay results from agency staff separately. Data files should be submitted electronically following section E of the 'Guidance for Industry, Providing Regulatory Submission in Electronic Format, New Drug Application.'

IND # 61,729
Drug Name: Technosphere plus Insulin
Sponsor: MannKind Corporation

Background:
Technosphere plus insulin (T/I) is a formulation of human recombinant USP insulin adsorbed to Technosphere particles (Fumaryl diketopiperazine, FDKP) intended for inhalation delivery for treatment of Types 1 and 2 diabetes. T/I is currently in phase 3 studies of 12 months duration, titrated to a maximum daily exposure of 224 µg*h/ml FDKP and 60 IU of insulin. T/I tested negative in the standard battery of genotoxicity assays. The FDKP component of T/I is not metabolized by liver hepatocytes or microsomes, and is rapidly excreted intact via the urine.

Mouse Carcinogenicity Study Protocol and Dose Selection
Mannkind proposed a 6 month study in rasH2 transgenic mice, using a subcutaneous route of administration. The sponsor has completed a 2 yr rat study using the inhalation route intended clinically. The study would test sham, vehicle (PBS), FDKP at 10 and 75mg/kg, and T/I at 5 and 10mg/kg. Doses were selected based on a 1 month study in CByB16F1 mice. The 75mg/kg dose of FDKP is the maximum feasible dose (solubility and dose volume limitation), and the 10mg/kg dose of T/I is (or exceeds) the MTD (death related to insulin-induced hypoglycemia).

Executive CAC Recommendations and Conclusions:

The Committee recommended doses for Technosphere alone of 25 and 75 mg/kg/day by subcutaneous injection based on this being the maximum feasible dose, and recommended doses of 2.5 and 5 mg/kg/day for Technosphere + insulin based on this being a maximum tolerated dose (mortality at 10 and 35 mg/kg).

David Jacobson-Kram, Ph.D.
Chair, Executive CAC

cc:\
/Division File, DMEP
/Todd Bourcier, Ph.D., DMEP
/Herman Rhee, Ph.D., DMEP
/Haley Seymour, DMEP
/ASeifried, OND IO

Application Type/Number	Submission Type/Number	Submitter Name	Product Name
NDA-22472	ORIG-1	MANNKIND CORP	INSULIN HUMAN (RDNA ORIG)INH POWDER

This is a representation of an electronic record that was signed electronically and this page is the manifestation of the electronic signature.

/s/

MIYUN M TSAI-TURTON
12/08/2009

KAREN L DAVIS BRUNO
12/08/2009

45 Day Meeting Checklist
NONCLINICAL PHARMACOLOGY/TOXICOLOGY

NDA 22-472: This NDA is a 505(b)(1) application.
Submission date: March 16th 2009
Sponsor: MannKind Corporation (MKC), Paramus, NJ.
Drug: Afresa® (insulin monomer human [rDNA origin] inhalation powder) and Afresa® Inhaler

Introduction: Afresa® is a durg-device combination product. The Afresa® inhalation powder is premetered into a single unit dose cartridge, whereas the Afresa® inhaler is used as the delivery device for oral inhalation. The inhalation powder is formulated with absorbed insulin and Technosphere particles (fumaryl diketopiperazine, FDKP, and (b) (4) polysorbate 80).

Upon submission, a non-clinical development program was completed, under GLP and current FDA/ICH Guidelines. The program evaluated the pharmacology, safety pharmacology, pharmacokinetics, pharmacodynamics, toxicology/toxicokinetics, genotoxicity, reproductive and developmental toxicology, carcinogenicity of Afresa and/or the excipients, FDKP. In addition, a total of 44 clinical trials were conducted and completed by MKC. Currently, there are 5 ongoing trials.

ITEM	YES	NO	COMMENT
1) Does this section of the NDA appear to be organized (according to 21 CFR 314 and current guidelines for format and content) in a manner that would allow a substantive review to be completed?	Yes		The EDR submission has 5 modules – regional (forms), common technical document summary, quality (CMC), nonclinical study reports (pharm/tox), and clinical study reports (clinical trials).
2) Is this section of the NDA indexed and paginated in a manner to enable a timely and substantive review?	Yes		EDR submission
3) Is this section of the NDA sufficiently legible so that a substantive review can be done? Has the data been presented in an appropriate manner (consider tables, graphs, complete study reports, inclusion of individual animal data, appropriate data analysis, etc.)?	Yes		
4) Are all necessary and appropriate studies for this agent, including special studies/data requested by the Division during pre-submission communications/discussions, completed and submitted in this NDA? Please itemize the critical studies included and indicate any significant studies that were omitted from the NDA. (genotox, reprotox, adequate duration of chronic tox, carcinogenicity)	Yes		Have electronic files of the carcinogenicity studies been submitted for statistical review? Yes (Statistician: Min Min) Under 4.2.3.4 carcinogenicity that has data tabulation dataset (in XPT format) for: MCK-PC-2007-0003 – 26 week sc study with T/TI in rasH2 mice (CAC 1) MCK-PC-2004-0001 – 104 week inhalation study with TI in rats (CAC 2)

ITEM	YES	NO	COMMENT
5) Were the studies adequately designed (i.e. appropriate number of animals, adequate monitoring consistent with the proposed clinical use, state-of-the art protocols, etc.)?	Yes		GLP non-clinical development program was conducted by MKC.
6) If the formulation to be marketed is not identical to the formulation used in the toxicology studies (including the impurity profiles), has the sponsor clearly defined the differences and submitted reviewable supportive data (i.e. adequate repeat studies using the marketed product and/or adequate justification for why such repetition would not be necessary)?	Yes		FDKP impurities, including (b) (4) were tested under Afresa's non-clinical development program and were monitored during manufacturing development.
7) Does the route of administration used in animal studies appear to be the same as the intended human exposure route? If not, has the sponsor submitted supportive data and/or an adequate scientific rationale to justify the alternative route?	Yes		Oral inhalation route
8) Has the proposed draft labeling been submitted? Are the appropriate sections for the product included and generally in accordance with 21 CFR 201.577? Is information available to express human dose multiples in either mg/m2 or comparative serum/plasma AUC levels?	Yes		In Module 1.14. Labeling: 7 Drug Interactions: (7.1 Agents Affecting Glucose Concentrations:) 8 Use In Specific Populations: (8.1 Pregnancy: and 8.2 Nursing Mothers:) 13 Nonclinical Toxicology: (13.1 Carcinogenesis, Mutagenesis, and Impairment of Fertility:)

ITEM	YES	NO	COMMENT
9) From a pharmacology/toxicology perspective, is this NDA fileable? If not, please state in item # 10 below why it is not.	Yes		A full non-clinical development program was implemented.
10) Reasons for refusal to file: None			

Reviewing Pharmacologist: Miyun Tsai-Turton

Supervisory Pharmacologist: Karen Davis-Bruno

This is a representation of an electronic record that was signed electronically and this page is the manifestation of the electronic signature.

/s/

Miyun Tsai-Turton
5/18/2009 08:03:09 AM
PHARMACOLOGIST

Karen Davis-Bruno
5/18/2009 08:04:55 AM
PHARMACOLOGIST
P/T filing memo