

**CENTER FOR DRUG EVALUATION AND RESEARCH**

**APPROVAL PACKAGE FOR:**

**APPLICATION NUMBER**

**21-468**

**Pharmacology Review(s)**

NDA 21-468

**REVIEW AND EVALUATION OF NEW TOXICOLOGY DATA  
SUBMITTED WITH FOSRENOL<sup>®</sup> NDA RESUBMISSION**

**Xavier Joseph, D.V.M.  
June 8, 2004**

**NDA RESUBMISSION DATED:** January 26, 2004

**CENTER RECEIPT DATE:** January 26, 2004

**REVIEWER RECEIPT DATE:** January 28, 2004

**SPONSOR:** Shire Pharmaceutical Development Inc.  
1801 Research Blvd., Rockville, MD 20850

**DRUG:** Trade name – Fosrenol<sup>®</sup>  
Generic name – Lanthanum Carbonate Hydrate  
Code name – SPD 405  
CAS number – 54451-24-0  
Chemical formula –  $\text{La}_2(\text{CO}_3)_3 \cdot 4\text{H}_2\text{O}$   
Molecular weight –

**FORMULATION:** Chewable tablets containing 250, 500, \_\_\_\_\_ elemental lanthanum are formulated with lanthanum carbonate tetrahydrate (\_\_\_\_\_) (mg, respectively) and the following inactive components: dextrans (hydrated), colloidal silicon dioxide and magnesium stearate.

**PHARMACOLOGICAL CLASS:** Phosphate binding agent

**PROPOSED INDICATION:** C

3

**PROPOSED DOSAGE REGIMEN:** The recommended initial total daily dose is 750-1500 mg elemental lanthanum, given with meals in divided doses. The dose should be titrated weekly until an optimal serum phosphate level is reached. Lanthanum doses up to 3750 mg were evaluated in clinical studies. Most patients required a total daily dose of 1500-3000 mg lanthanum to reduce plasma phosphate levels to less than 6.0 mg/dl. Doses were generally titrated in increments of 750 mg/day.

**IND UNDER WHICH CLINICAL TRIALS WERE CONDUCTED:** IND 55054

**DISCLAIMER:** Tabular and graphical information is from sponsor's submission unless stated otherwise.

**PHARMACOLOGY/TOXICOLOGY REVIEW**

*Pharmacology/Toxicology studies conducted with lanthanum carbonate (Fosrenol) were reviewed earlier (Original NDA Review dated January 13, 2003). The following three additional toxicology studies are submitted with the Fosrenol NDA resubmission:*

1. *Four-week oral toxicity study in the rat – comparison of the carbonate and chloride of lanthanum*
2. *Intravenous bone marrow micronucleus test in the rat using lanthanum chloride, and*
3. *Unscheduled DNA synthesis in rat liver following 28 consecutive daily iv bolus injections of lanthanum chloride.*

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**Four-Week Oral (Gavage) Toxicity Study in the Rat – Comparison of the Carbonate and Lanthanum of Lanthanum**

The objective of the study was to compare the toxicity of lanthanum carbonate and lanthanum (a degradation product of lanthanum carbonate) when administered orally to the rat daily for 4 weeks, for the purpose of qualification of the degradant.

**Key Study Findings:** Oral administration of equivalent doses (in respect of the amount of elemental lanthanum delivered) of lanthanum carbonate for 4 weeks in rats produced histopathological lesions in the stomach (glandular and nonglandular epithelial hyperplasia, mineralized foci and submucosal inflammation). The incidences of the stomach lesions appeared to be slightly higher for the Lanthanum than for the carbonate salt. Plasma lanthanum exposure was comparable for the two salts.

**Study Number:** SPD0136

**Volume # and Page #:** 11 & 5-1

**Conducting Laboratory:** L

**Date of Treatment Initiation:** June 5, 2003

**GLP Compliance:** yes

**QA Report:** yes

**Drug Lot # and Purity:** Lanthanum carbonate – lot # F020010, purity – metal impurities – none detected  
Lanthanum – lot # LIMS 19859, purity – metal impurities – not provided

**Formulation:** formulated daily as a suspension in aqueous 0.5% carboxymethylcellulose.

**Methods**

Animals

*Species/strain:* Rat CD (SD) IGS BR VAF PLUS obtained from L

*No./Sex/Group:* 10 for main study and 9 for toxicokinetics

*Age:* 5–6 weeks

*Weight:* males – 157 to 221 g; females – 137 to 188 g

Animals were housed in groups of 3 or 5, by sex, in grid bottomed cages suspended over paper-lined trays. A pelleted diet L rodent diet L and tap water were available *ad libitum*.

Treatment

*Doses administered:* lanthanum carbonate – 0, 200 and 2000 mg/kg/day

lanthanum – 0, 160 and 1600 mg/kg/day

(elemental lanthanum doses of 103 and 1030 mg/kg/day were supplied by the low and

high doses, respectively, of each salt)  
*Route/Volume:* Oral gavage/10 ml/kg.

### Observations and Measurements

*Clinical Signs:* daily

*Mortality:* twice daily

*Body Weight:* one week before the initiation of treatment and then weekly thereafter up to and including the day of necropsy.

*Food Consumption:* week before the start of treatment and weekly during the treatment period (control and main study groups only)

*Ophthalmoscopy:* pre-dose (control and main study groups) and week 4 (control and two high dose groups only)

*Hematology:* week 4 [parameters evaluated – RBC, WBC (total and differential) and platelet counts, hemoglobin, PCV, MCV, MCH, MCHC, prothrombin and activated partial thromboplastin time (control and main study groups)]

*Clinical Chemistry:* week 4 [parameters evaluated - urea, creatinine, glucose, alkaline phosphatase, aminotransferases, total protein, albumin/globulin ratio, bilirubin, cholesterol, calcium, inorganic phosphate, sodium and potassium (control and main study groups)]

*Urinalysis:* week 4 (parameters evaluated – volume, specific gravity, glucose, pH, protein, color and appearance, phosphate and creatinine (control and main study groups))

*Toxicokinetics:* Blood samples were collected from the satellite groups on Day 26 at 0 (pre-dose) and 1, 2, 3, 4, 6, 9, 12 and 24 hours post-dose (3 rats/sex/group/time point).

*Postmortem Evaluation:* At the end of the treatment period, complete necropsies were performed on all animals, and adrenals, brain, heart, kidneys, liver, lungs, ovaries, pituitary, prostate, salivary gland (submandibular), seminal vesicles, spleen, testes, thymus, thyroids and uterus were weighed. The tissues listed in the Table below were fixed, and slides were prepared for microscopic examination. (The eyes, optic nerves and Harderian glands were fixed in Davidson's fluid, and testes and epididymides were first fixed in Bouin's fixative for 24 hours and then transferred to neutral buffered formaldehyde. All other tissues listed were fixed in neutral buffered formaldehyde.) All tissues from control and high dose animals were examined microscopically.

*Statistical Analysis:* Data were analyzed using analysis of variance and Dunnett's multiple comparison tests. Non-parametric analysis was done using Kruskal-Wallis

ANOVA and Wilcoxon's rank sum tests. Fisher's exact test was used for the analysis of urinalysis data.

Tissue	Weigh	Fix	Slide Preparation	Microscopic Examination
adrenal glands	✓	✓	✓	✓
aorta		✓	✓	✓
brain (3 levels examined)	✓	✓	✓	✓
caecum		✓	✓	✓
colon		✓	✓	✓
duodenum		✓	✓	✓
epididymides		✓	✓	✓
eyes (incl. optic nerves)		✓	✓	✓
femur and joint (incl. marrow)		✓	✓	✓
Harderian glands		✓	✓	✓
heart	✓	✓	✓	✓
Animal identification		✓		
Ileum (incl. Peyer's patch)		✓	✓	✓
jejunum		✓	✓	✓
kidneys	✓	✓	✓	✓
larynx		✓	✓	✓
liver	✓	✓	✓	✓
lungs (incl. mainstem bronchi)	✓	✓	✓	✓
mesenteric lymph node		✓	✓	✓
oesophagus		✓	✓	✓
ovaries	✓	✓	✓	✓
pancreas		✓	✓	✓
pituitary	✓	✓	✓	✓
prostate	✓	✓	✓	✓
rectum		✓	✓	✓
salivary gland (submandibular)	✓	✓	✓	✓
sciatic nerve		✓	✓	✓
seminal vesicles (incl. coagulating gland)	✓	✓	✓	✓
site of mammary gland		✓	✓	✓
skeletal muscle		✓	✓	✓
skin		✓	✓	✓
spinal cord (3 levels examined)		✓	✓	✓
spleen	✓	✓	✓	✓
sternum		✓	✓	✓
stomach		✓	✓	✓
submandibular lymph nodes		✓	✓	✓
testes	✓	✓	✓	✓
thymus	✓	✓	✓	✓
thyroids (incl. parathyroids)#	✓	✓	✓	✓
tongue		✓	✓	✓
trachea		✓	✓	✓
ureters		✓	✓	✓
urinary bladder		✓	✓	✓
uterus (incl. uterine cervix and oviducts)	✓	✓	✓	✓
vagina		✓	✓	✓
all gross lesions		✓	✓	✓

# weighed after fixation

## Results

*Mortality:* There were no deaths during the study.

*Clinical Signs:* There were increased incidences of hair loss in all male groups given lanthanum carbonate or lanthanum [ ] There were no other clinical signs that were considered to be related to drug treatment. (One low dose lanthanum [ ] group animal showed abnormal gait and reduced hind limb muscle tone during the study. This isolated occurrence was considered to be not treatment related.)

*Body Weights:* There were no treatment-related effects on body weight.

*Food Consumption:* Food consumption was slightly increased, compared to controls, for high dose females given lanthanum [ ]

*Ophthalmoscopy:* There were no treatment related findings.

*Hematology:* Higher than control total WBC counts were seen in high dose males and females given lanthanum carbonate [ ] This difference was more pronounced in males and was mainly due to higher absolute lymphocyte counts. Dose-dependent reductions in PCV values were noted in treated females for both carbonate and [ ]

*Clinical Chemistry:* Slightly higher than control alanine aminotransferase and potassium levels, and decreased total protein, albumin and globulin levels were observed in females given high dose lanthanum [ ] Inorganic phosphate levels were slightly higher, compared to control, for high dose males and females given lanthanum carbonate [ ]

*Urinalysis:* Urine phosphate levels were decreased at high dose levels for both carbonate [ ] except in females given carbonate, where the urine phosphate level was higher than control. Increased urinary pH was noted in high dose males given either salt.

*Organ Weights:* Reductions in absolute and relative thyroid weights were observed in high dose males given lanthanum carbonate and also in high and low dose males given [ ]

*Gross Pathology:* There were no treatment-related macroscopic findings.

*Histopathology:* Microscopically, treatment-related findings were observed only in the stomach. The incidences of these lesions are given below.

Test article	Control		Lanthanum carbonate		Lanthanum	
	Male	Female	Male	Female	Male	Female
Dose level (mg/kg/day)	0		2000		1600	
Number examined	10	10	10	10	10	10
Glandular epithelial hyperplasia	1	0	5	9	10	10
Mineralised foci	0	0	4	1	4	5
Submucosal inflammatory infiltrate	0	0	5	5	8	9
Marginal ridge hyperplasia/vacuolation	0	0	1	2	6	6

The incidences of submucosal inflammatory infiltrate and the marginal ridge hyperplasia/vacuolation were higher in lanthanum [ ] treated males and females than in lanthanum carbonate treated animals. The incidences of glandular epithelial hyperplasia in males and mineralized foci in females were higher in [ ] treated groups than in carbonate treated groups. In summary, although the overall responses of the stomach to both lanthanum salts are similar, the incidences of the stomach lesions were slightly higher for the [ ] than for the carbonate salt.

*Toxicokinetics:* The toxicokinetics data are given below.

Sex	Males				Females				
	Lanthanum carbonate		Lanthanum [ ]		Lanthanum carbonate		Lanthanum [ ]		
Dose level (mg/kg/day)	200	2000	160	1600	200	2000	160	1600	
Day 26	C <sub>max</sub> (ng/ml)	0.870	2.606	0.526	1.948	0.374	0.899	0.798	5.203
	T <sub>max</sub> (hours)	0	4	6	4	12	6	6	3
	AUC <sub>0-24</sub> (AUC 000-24.0) (ng h/ml)	6.084	21.460	5.180	26.161	5.847	15.476	7.827	17.226
	AUC <sub>0-inf</sub> (AUCinf) (ng h/ml)	NA	27.897	NA	42.513	NA	NA	NA	NA
	T1/2 (t1/2 z) (hours)	NA	10.6	NA	17.4	NA	NA	NA	NA
NA - not possible to calculate									

Lanthanum AUC values were generally similar for both salts in males and females, although these values at high dose levels were lower for females (both salts) than for males. The lanthanum  $C_{max}$  value for the high dose lanthanum  $\tau$  female group was higher than the corresponding value for the high dose lanthanum carbonate female group or either of the high dose male groups.

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**Intravenous Bone Marrow Micronucleus Test in the Rat using Lanthanum Chloride**

[This intravenous micronucleus study was conducted with a soluble salt of lanthanum in order to evaluate the clastogenic potential of lanthanum at higher plasma (and consequently bone marrow) exposure levels than obtained from oral studies.]

**Key Study Findings:** There were no significant increases in micronucleus frequency in the bone marrow cells of rats treated intravenously with lanthanum chloride at doses up to 0.1 mg/kg, a dose that produced a plasma lanthanum concentration about 2000 times the steady state plasma concentration in patients at the maximum recommended daily oral dose.

**Study Number:** — 7 (contract lab number)

**Volume # and Page #:** 12 & 5-323

**Conducting Laboratory:** [redacted]

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**Date of Treatment:** November 20, 2002

**GLP Compliance:** yes

**QA Report:** yes

**Drug lot # and Purity:** 0117352, purity not provided

**Formulation:** The test article was obtained [redacted] as a 10% aqueous solution of lanthanum as the chloride salt. This stock solution was diluted with physiological saline for injection to obtain necessary concentrations for dosing.

**Methods**

*Strain/Species:* young adult male Sprague Dawley rats from [redacted]  
(7 weeks-old and weighing 236-296 g)

1

*Doses used in Definitive Study:* 0.025, 0.05 and 0.10 mg/kg, iv (dose volume – 5 ml/kg)

*Basis of Dose Selection:* Doses were selected based on known clinical exposure levels and toxicokinetic data from previous rat studies. A dose of 0.10 mg/kg was selected as the high dose since this dose was shown to be the maximum iv dose associated with linear pharmacokinetics in the rat. Furthermore, it was estimated that this dose would provide a substantial multiple of plasma lanthanum exposure at steady state in patients receiving the maximum recommended daily oral dose of lanthanum carbonate. (The mean plasma lanthanum concentration achieved at this high dose in the current study was about 2000 times the steady state plasma concentration in patients.)

*Negative Control:* Saline

*Positive Control:* Cyclophosphamide dissolved in saline (2 mg/ml) given at 20 mg/kg.

*Study Design:* Groups of six male rats were given single iv bolus injections of saline, lanthanum chloride, or cyclophosphamide. Bone marrow samples (from one femur) from these animals were collected about 24 hours after dosing. From additional groups of rats given vehicle or lanthanum chloride at 0.1 mg/kg, bone marrow samples were collected 48 hours post-dose. The other femur (not used for the preparation of the bone marrow smears) was removed from vehicle and test article treated animals and frozen for future analysis of lanthanum content of bone marrow. (No analysis of these femurs was performed.)

For toxicokinetic analysis, satellite groups of five rats each were dosed with vehicle or each concentration of test article as described above and blood was collected about 2, 15 and 60 minutes after dosing. These animals were discarded after blood collection.

*Analysis:* Counting method – Bone marrow slides (2/animal) were stained with acridine orange. At least 2000 polychromatic erythrocytes (PCE) per animal were counted for micronucleated PCE evaluation and 1000 erythrocytes [PCE plus normochromatic erythrocytes (NCE)] were counted for the determination of relative proportion of PCE and NCE (PCE/NCE ratio).

Data were statistically analyzed using UKEMS guidelines (Lovell et.al. 1989. Statistical analysis of *in vivo* cytogenetic assays. In "Statistical Evaluation of Mutagenicity Test Data". UKEMS sub-committee on guidelines for mutagenicity testing. Report. Part III). Ed. D. J. Kirkland. Cambridge University Press.). For each group, inter-individual variation in the numbers of micronucleated PCE was estimated using a heterogeneity  $\chi^2$  test. The numbers of micronucleated PCE in each treated group were then compared with numbers in vehicle control groups using a 2 x 2 contingency table to determine  $\chi^2$ . A linear trend test was used to evaluate possible dose-response relationships. If the heterogeneity  $\chi^2$  test provided evidence of significant variability between animals within at least one group, then non-parametric analysis (Wilcoxon rank sum test) was performed.

The assay was considered valid if:

1. the incidence of micronucleated PCE (MNPCE) in the vehicle control group fell within or close to the historical control range
2. at least five animals out of each group were available for analysis, and
3. the positive control chemical induced a statistically significant increase in the frequency of MNPCE.

The test article was considered as positive if:

1. a statistically significant increase in the frequency of MNPCE occurred at least at one dose level, and
2. the frequency of MNPCE at such a point exceeded the historical vehicle control range.

## Results

The mean PCE/NCE ratios and the mean frequency of micronucleated PCEs for both time points are presented in the Table below. There were no significant treatment related differences, compared to concurrent control, in the PCE/NCE ratios or in the frequency of micronucleated PCEs at either 24 or 48 hr time points. It is noted that there was no evidence of any test article-induced toxicity to the bone marrow (indicated by a notable decrease in PCE/NCE ratio) even at the high dose (0.10 mg/kg), a dose that produced a plasma lanthanum concentration about 2000 times the steady state peak plasma concentration in patients. Although the mean frequency of MNPCE at the high dose for the 24 hr time point was slightly higher than the concurrent control value, the difference was not statistically significant. Moreover, it was within the laboratory's historical vehicle control range.

The positive control showed a significant increase in the frequency of MNPCEs ( $p \leq 0.001$ ), compared to concurrent control, and a marked decrease in PCE/NCE ratio.

Mean peak plasma lanthanum concentrations ( $C_{max}$ ) were 346, 905 and 1093 ng/ml for 0.025, 0.050 and 0.10 mg/kg dose groups, respectively. These represent substantial multiples of the steady state peak plasma level (1.06 ng/ml) measured in patients receiving the maximum recommended daily oral dose of lanthanum carbonate.

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## Summary of group mean data

## Lanthanum Chloride

## 24 hour time point

Treatment group (mg/kg)	Kill Time (hours)	Sex	Mean Ratio PCE/NCE	Group mean frequency of micronucleated PCE (per 1000 cells) per treatment group (±sd)
Vehicle (0)	24	M	1.48	0.42 ± 0.38
Lanthanum Chloride (0.025)	24	M	2.18	0.17 ± 0.26
Lanthanum Chloride (0.05)	24	M	1.57	0.33 ± 0.61
Lanthanum Chloride (0.10)	24	M	1.38	0.75 ± 0.61
Positive control, CPA (20)	24	M	0.46	7.50 ± 2.68

sd Standard deviation  
M Male

## 48 hour time point

Treatment group (mg/kg)	Kill Time (hours)	Sex	Mean Ratio PCE/NCE	Group mean frequency of micronucleated PCE (per 1000 cells) per treatment group (±sd)
Vehicle (0)	48	M	1.70	0.83 ± 0.52
Lanthanum Chloride (0.10)	48	M	1.42	0.25 ± 0.42

Sd Standard deviation  
M Male

**Unscheduled DNA Synthesis in Rat Liver Following 28 Consecutive Daily iv Bolus Injections of Lanthanum Chloride**

**Key study findings:** Rats treated daily for 28 consecutive days with iv bolus injections of lanthanum chloride at doses up to 0.1 mg/kg showed no induction of unscheduled DNA synthesis (UDS) in hepatocytes.

**Study Number:** — (contract lab number)

**Volume # and Page #:** 12 & 5-371

**Conducting Laboratory:** ☐

**Date of Treatment Initiation:** July 1, 2003

**GLP Compliance:** yes

**QA Report:** yes

**Drug Lot # and Purity:** 0117352, purity not provided

**Formulation:** The test article was obtained ☐ is a 10% aqueous solution of lanthanum as the chloride salt. This stock solution was diluted with physiological saline for injection to obtain necessary concentrations for dosing. Test article preparations were made weekly and stored at room temperature protected from light.

**Methods**

*Strain/Species:* male Han Wistar WI ☐ IGS BR rats from ☐  
(7-8 weeks-old and weighing 189-227 g)

*Doses:* 0.025, 0.05 and 0.10 mg/kg/day, given as an iv bolus injection once daily for 28 consecutive days (dose volume = 5 ml/kg)

*Basis of Dose Selection:* In a previous iv micronucleus study in rats, 0.1 mg/kg was selected as the high dose since it was the maximum iv dose associated with linear pharmacokinetics in this species and, further, it provided plasma lanthanum concentrations that were 2000 fold higher than the peak levels in patients given the maximum recommended daily dose. Hence, the same high dose (0.1 mg/kg) was considered appropriate for the current study. Two lower doses of 0.05 and 0.025 mg/kg were also selected.

*Negative Control:* Physiological saline

*Positive Control:* 2-acetamidofluorene (2-AAF), suspended in corn oil (7.5 mg/ml), was given orally by gavage at 75 mg/kg on day 28 (one day only) - dose volume = 10 ml/kg

*Study Design:* Groups of 4 male rats were given iv bolus injections of saline or test article for 28 days. A further group of four rats received 2-AAF and served as the positive control. About 12-14 hours after the final dose administration, animals were sacrificed and liver was perfused with collagenase. Primary cultures of hepatocytes were made from

three animals per group and treated with [<sup>3</sup>H] thymidine. Six slides from each animal were prepared with fixed hepatocytes, and of these, 3 slides were prepared for autoradiographic analysis.

Groups of five satellite animals were dosed for 28 days (as described above) with each concentration of test article or vehicle, for the determination of lanthanum levels in plasma and liver, evaluation of liver histopathology, and analysis of liver function markers (alanine and aspartate aminotransferases, alkaline phosphatase, gamma glutamyl transferase, triglycerides, cholesterol and bilirubin). Blood was collected from these animals about 2, 15 and 60 minutes after final dose administration. Samples of liver were collected from each animal and were frozen in liquid nitrogen for lanthanum level determination, or preserved in 10% neutral buffered formalin for histopathology evaluation.

*Analysis:* Autoradiographic slides were examined microscopically, nuclear and cytoplasmic grains were counted, and the net grains/nucleus (NNG; the number of grains present in the nucleus minus the mean number of grains in three equivalent areas of cytoplasm) was determined. One hundred cells per animal were analyzed using two of the three slides.

The study was considered valid if:

1. the negative control animals had a group mean NNG value that did not exceed the upper limit of the historical control range, and
2. the positive control treatments had group mean NNG values of 5 or more with 50% or more cells having NNG counts of five or greater.

The test article was considered positive if:

at any dose it yielded group mean NNG values greater than zero with 20% or more of cells in repair (mean NNG values  $\geq 5$ )

## Results

Mean net grain count values and the percent of cells in repair for each treatment group are summarized in Table 1, with individual animal values presented in Table 2. Treatment with lanthanum chloride at doses up to 0.1 mg/kg/day produced group mean net grain count values in the range -2.7 to -3.9, well below the threshold net grain count value of zero required for a positive response. At any dose level of lanthanum chloride tested, no more than 0.3% cells were seen in repair. The positive control produced a mean net grain count value of 36.4 with 100 % of cells in repair.

Mean peak plasma lanthanum concentrations were 702, 1332 and 3539 ng/ml, respectively, for the 0.025, 0.050 and 0.10 mg/kg dose levels, the concentration at the high dose being more than 3000 times the steady state peak plasma lanthanum concentration (1.06 ng/ml) observed in patients given the maximum recommended dose.

Mean liver lanthanum concentrations were 4.0, 5445.0, 12274.0 and 33974.0 ng/g, respectively, for the 0, 0.025, 0.050 and 0.10 mg/kg dose levels. The highest mean liver lanthanum concentration observed in the current study was about 27 times higher than the median liver concentration measured at the end of the oral carcinogenicity study performed with lanthanum carbonate in the rat.

There were no treatment related effects on markers of liver function or liver histopathology.

The assay is considered valid since the group mean net grain count for vehicle treated animals (-2.2) was less than the upper limit of the historical control range (-4.2 to 0.7) and the positive control chemical induced increases in group mean net grain counts of five or more (36.4) and more than 50% of cells (100%) had net grain counts of five or more. The results indicated that the test system was sensitive to a known DNA damaging agent requiring metabolism for its action.

*[Note: The criteria for a positive UDS test result for  $\square$  (where the present assay was performed), did not include a dose-related increase in group mean net grain counts, but only an increase in counts at least at one dose level. However, the group mean net grain counts (in the range of -2.7 to -3.9), obtained in the current study, were well below the threshold net grain count value of zero required for a positive response.]*

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**Table 1: Lanthanum chloride: group mean net grain count values**

Dose (mg/kg/day)	Compound	Net grain count (NNG)		Percent of cells in repair (NNG $\geq$ 5)		Net grain count of cells in repair	
		mean	SD	mean	SD	mean	SD
0	Saline	-2.2	0.4	0.3	0.6	6.0	-
0.025	Lanthanum chloride	-2.7	0.3	-	-	-	-
0.05	Lanthanum chloride	-3.4	0.2	0.3	0.6	8.0	-
0.1	Lanthanum chloride	-3.9	0.4	-	-	-	-
75	2-AAF	36.4	7.1	100.0	0.0	36.4	7.1

**Table 2: Lanthanum chloride: individual animal net grain count values**

Compound (mg/kg/day)	Animal number	Net grain count (NNG)		% cells in repair (NNG $\geq$ 5)	Net grain count of cells in repair		No. of cells scored
		mean	SD		mean	SD	
Saline 0	501	-1.68	0.14	0.00	-	-	100
	503	-2.44	0.42	0.00	-	-	100
	512	-2.42	0.34	1.00	6.00	-	100
Lanthanum chloride 0.025	506	-2.94	0.38	0.00	-	-	100
	516	-2.86	0.03	0.00	-	-	100
	517	-2.37	0.24	0.00	-	-	100
Lanthanum chloride 0.05	504	-3.19	0.80	0.00	-	-	100
	510	-3.44	0.38	0.00	-	-	100
	515	-3.47	0.25	1.00	8.00	-	100
Lanthanum chloride 0.1	507	-4.37	1.00	0.00	-	-	100
	508	-3.80	0.25	0.00	-	-	100
	513	-3.64	0.74	0.00	-	-	100
2-AAF 75	502	43.93	8.15	100.00	43.93	8.15	100
	509	29.88	2.78	100.00	29.88	2.78	100
	511	35.45	5.67	100.00	35.45	5.67	100

## SUMMARY AND EVALUATION

Lanthanum carbonate (Fosrenol) is being developed as a phosphate binding agent for the treatment of hyperphosphatemia in patients with end stage renal disease, since it inhibits the absorption of phosphate, thereby reducing the serum phosphate level. In the presence of HCl in the stomach, a greater portion of the administered lanthanum carbonate is converted to highly soluble chloride salt, with the release of carbon dioxide. The activity of lanthanum carbonate as a phosphate binder is dependent on the availability of soluble  $\text{La}^{3+}$  ions in the GI tract and the high affinity of  $\text{La}^{3+}$  for  $\text{PO}_4^{2-}$  ions that are released during the digestion of proteins. This binding results in the formation of highly insoluble lanthanum phosphate, a salt which is excreted in the feces, significantly reducing phosphate absorption.

The initial recommended total daily dose of Fosrenol is 750-1500 mg lanthanum, with weekly titration until an optimum serum phosphate level is reached (usually 1500-3000 mg lanthanum per day).

The nonclinical studies conducted with lanthanum carbonate were reviewed earlier (Original NDA Pharm/Tox Review dated January 13, 2003). Three additional toxicology studies were included and reviewed under this NDA resubmission.

A four-week oral toxicity study was conducted in rats to qualify a degradant [ ] that was identified since the original NDA submission. Groups of rats were orally dosed with vehicle or equivalent doses (in terms of elemental lanthanum delivered) of lanthanum carbonate [ ] daily for 28 days. The only histopathological lesions observed (for both salts) were in the stomach (glandular and nonglandular epithelial hyperplasia, mineralized foci and submucosal inflammation). Although the stomach lesions were similar for both salts, the incidences of these lesions appeared to be slightly higher for the [ ] than for the carbonate salt. Plasma lanthanum exposure for both salts was comparable.

The stomach lesions observed in the present study were similar to those observed in previous chronic studies in rodents with lanthanum carbonate. No stomach lesions were seen in the dog. It is believed that the longer duration of direct contact of lanthanum with the stomach wall (due to nocturnal feeding habits), together with the inability to vomit an irritant material, is likely to make rodents more susceptible than dogs to stomach lesions. It was shown that lanthanum carbonate is better tolerated if administered to dogs and humans with food.

For qualification of the impurity lanthanum [ ] a genotoxicity test was considered not necessary since, in the presence of hydrochloric acid, both [ ] and carbonate salt forms are converted to the same salt form (soluble chloride salt) upon dissolution in the stomach. Hence, irrespective of the salt form administered, only elemental lanthanum ions are absorbed, and since a full battery of

genotoxicity tests were conducted earlier with lanthanum carbonate, further testing with the [ ] was considered unnecessary.

Two new genotoxicity studies submitted with this NDA resubmission showed that there were no significant increases in micronucleus frequency in the bone marrow cells or any induction of unscheduled DNA synthesis in hepatocytes of rats treated intravenously with lanthanum chloride at doses up to 0.1 mg/kg, a dose that produced plasma lanthanum concentrations about 2000 - 3000 times the steady state peak plasma lanthanum concentration in patients (who received the drug via the oral route of administration at the maximum recommended dose), indicating that lanthanum chloride is not genotoxic. (It is noted that in animal oral toxicity studies, at maximum tolerated doses, steady state peak plasma lanthanum concentrations were only up to 11 fold higher than the peak plasma level in patients receiving the maximum recommended dose of 3 g elemental lanthanum per day.)

All studies appeared to be adequately performed.

In summary, as noted in the original NDA pharmacology/toxicology review, there are no approvability issues for lanthanum carbonate based on the non-clinical toxicity-testing program.

APPEARS THIS WAY  
ON ORIGINAL

**RECOMMENDATIONS**

The NDA is approvable with the following changes to the sponsor's proposed package insert (submitted on January 26, 2004).

- 1. The proposed **CLINICAL PHARMACOLOGY, Pharmacokinetics** section of the label should address significant tissue accumulation and slow clearance with treatment cessation in rats and dogs given lanthanum carbonate orally.

*The fifth paragraph presently reads as follows:*

[ ]

*We recommend that the above text be revised to read as follows:*

"Studies in mice, rats and dogs [ ] lanthanum concentrations in many tissues

[ ] There is no evidence from animal studies that lanthanum crosses the blood-brain barrier."

- 2. Under **PRECAUTIONS, General**, the first sentence of paragraph 2 should be deleted since the [ ] was addressed earlier under **CLINICAL PHARMACOLOGY, Pharmacokinetics**.

*The first sentence of paragraph 2 presently reads as follows:*

[ ]

- 3. Under **PRECAUTIONS, Carcinogenesis, Mutagenesis, Impairment of Fertility**, the doses used in animal studies should be compared to the human dose on a body surface area basis.

*The text of the above section presently reads as follows:*

[ ]

Lanthanum carbonate tested negative for mutagenic activity in an *in vitro* Ames assay using *Salmonella typhimurium* and *Escherichia coli* strains and in *in vitro* HGPRT gene mutation and chromosomal aberration assays in Chinese Hamster Ovary cells.

Lanthanum carbonate, at doses up to 2000 mg salt/kg/day (1.7 times the MRHD) did not affect fertility or mating performance of male or female rats.

*We recommend that the above text be revised to read as follows:*

“Oral administration of lanthanum carbonate to rats for up to 104 weeks, at doses up to 1500 mg of the salt/kg/day [2.5 times the maximum recommended daily human dose (MRHD) of 5725 mg on a mg/m<sup>2</sup> basis, assuming a 60 kg subject] revealed no evidence of carcinogenic potential. In the mouse, oral administration of lanthanum carbonate for up to 99 weeks, at doses up to 1500 mg of salt/kg/day (1.3 times the MRHD, 1500 mg/kg/day) was associated with glandular stomach adenoma in male mice.

Lanthanum carbonate tested negative for mutagenic activity in an *in vitro* Ames assay using *Salmonella typhimurium* and *Escherichia coli* strains and *in vitro* HGPRT gene mutation and chromosomal aberration assays in Chinese hamster ovary cells. Lanthanum carbonate also tested negative in an oral mouse micronucleus assay at doses up to 2000 mg salt/kg/day (1.7 times the MRHD) and in micronucleus and unscheduled DNA synthesis assays in rats given *iv* lanthanum chloride at doses up to 0.1 mg/kg, a dose that produced plasma lanthanum concentrations > 2000 times the peak human plasma concentration.

Lanthanum carbonate, at doses up to 2000 mg salt/kg/day (3.4 times the MRHD) did not affect fertility or mating performance of male or female rats.”

4. The text under **PRECAUTIONS, Pregnancy** presently read as follows:

“Pregnancy Category C. No adequate and well-controlled studies have been conducted in pregnant women — The effect of Fosrenol on the absorption of vitamins and other nutrients has not been studied in pregnant women. Fosrenol is not recommended for use during pregnancy —

*We recommend that the **Pregnancy** subsection be revised to read as follows, and also to include subsections on **Labor and Delivery** and **Nursing Mothers**, to read as follows:*

#### **Pregnancy**

*“Pregnancy Category C. No adequate and well-controlled studies have been conducted in pregnant women. The effect of Fosrenol on the absorption of vitamins and other nutrients has not been studied in pregnant women. Fosrenol is not recommended for use during pregnancy.”*

*“In pregnant rats, oral administration of lanthanum carbonate at doses as high as 2000 mg — kg/day (3.4 times the MRHD) resulted in no evidence of harm to the fetus. In pregnant rabbits, oral administration of lanthanum carbonate at 1500 mg/kg/day (5 times the MRHD) was associated with a reduction in maternal body weight gain and food consumption, increased post-implantation loss, reduced fetal weights and delayed fetal skeletal ossification.”*

*“Lanthanum carbonate administered to rats from implantation through lactation at 2000 mg — kg/day (3.4 times the MRHD) caused delayed eye opening, reduction in body weight gain and delayed sexual development (preputial separation and vaginal opening) of the offspring.”*

#### **Labor and delivery**

No lanthanum carbonate treatment-related effects on labor and delivery were seen in animal studies. The effect of lanthanum carbonate on labor and delivery in humans is unknown.

**Nursing mothers**

It is not known whether lanthanum carbonate is excreted in human milk. Because many drugs are excreted in human milk, caution should be exercised when Fosrenol is administered to a nursing woman."

|S|

Xavier Joseph, D.V.M.

Accepted by \_\_\_\_\_ on \_\_\_\_\_

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

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Xavier Joseph  
6/10/04 10:03:44 AM  
PHARMACOLOGIST

Charles Resnick  
6/10/04 11:08:45 AM  
PHARMACOLOGIST

**NDA 21-468**

**REVIEW AND EVALUATION OF PHARMACOLOGY  
AND TOXICOLOGY DATA**

**Xavier Joseph, D.V.M.  
January 13, 2003**

**ORIGINAL NDA DATED:** April 30, 2002  
**CENTER RECEIPT DATE:** April 30, 2002  
**REVIEWER RECEIPT DATE:** May 8, 2002

**SPONSOR:** Shire Pharmaceutical Development Inc.  
1901 Research Blvd., Rockville, MD 20850

**DRUG:** Trade name - FOSRENOL™  
Generic name - Lanthanum Carbonate Hydrate  
Code name - SPD 405  
CAS number - 54451-24-0  
Chemical formula -  $\text{La}_2(\text{CO}_3)_3 \cdot 4\text{H}_2\text{O}$   
Molecular Weight -

**FORMULATION:** Chewable tablets containing 250 or 500 mg lanthanum are formulated with the active ingredient lanthanum carbonate hydrate (477 or 954 mg, respectively) and the following inactive components: dextrans (hydrated), colloidal silicon dioxide, talc and magnesium stearate.

**PHARMACOLOGICAL CLASS:** Phosphate binding agent

**PROPOSED INDICATION:** □

1

**PROPOSED DOSAGE REGIMEN:** The recommended starting dose is 750 mg lanthanum (1431 mg lanthanum carbonate) daily, given with meals in divided doses. The dose is titrated weekly to a level [up to 3000 mg lanthanum (5725 mg lanthanum carbonate) per day] that achieves maintenance of acceptable serum phosphate levels.

**IND UNDER WHICH CLINICAL TRIALS WERE CONDUCTED:** IND 55054

**DISCLAIMER:** Tabular and graphical information is from sponsor's submission unless stated otherwise.

*Executive Summary*

I. Recommendations

A. Recommendation on Approvability

Lanthanum carbonate is approvable from a nonclinical perspective.

B. Recommendations for Nonclinical Studies

None

C. Recommendations on Labeling

The NDA is approvable with the following changes to the sponsor's proposed package insert (submitted on April 30, 2002).

L

J

1 pages redacted from this section of  
the approval package consisted of draft labeling

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.....

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## II. Summary of Nonclinical Findings

### A. Pharmacologic Activity

Lanthanum carbonate hydrate is being developed as a phosphate binding agent for the treatment of hyperphosphatemia in patients with end stage renal disease. Lanthanum ions act by binding with dietary phosphate in the stomach, forming insoluble lanthanum phosphate that is excreted in the feces, and thereby significantly reducing the absorption of dietary phosphate.

In *in vitro* studies, lanthanum carbonate showed high affinity for phosphate binding. When present in a two-fold molar excess, lanthanum carbonate removed more than 97% of the available phosphorus at pH 3 and 5, and more than 66% at pH 7.

In isolated perfused rat gut preparations, lanthanum carbonate (30.8 mg/kg) showed greater ability in inhibiting the transport of labeled phosphate across gut lumen (34.4% inhibition) than did an equimolar dose of aluminum hydroxide (17.5% inhibition).

In rats, single dose oral administration of lanthanum carbonate (1.5 or 2000 mg/kg) produced dose-dependent reductions (23-41%) in serum phosphate levels with no effects on serum calcium levels.

Studies in rats also showed that daily oral administration of lanthanum carbonate (1000 mg/kg) resulted in the excretion of about 56% of the administered labeled phosphate in the feces (versus 31% in controls).

In safety pharmacology studies, lanthanum carbonate had no significant effects on central nervous, cardiovascular and gastrointestinal systems.

#### B. Brief Overview of Pharmacokinetics

Lanthanum is minimally absorbed in rats and dogs after oral administration of lanthanum carbonate. Oral bioavailability was estimated to be 0.0007% in the rat and 0.00005% in the dog. Comparison of mean plasma levels and  $AUC_{(0-24h)}$  values after single and repeat dose administration of lanthanum carbonate showed no evidence of accumulation following repeated dosing in rats, mice and rabbits. In the dog, AUC values reached a steady state by week 13 of treatment. Plasma lanthanum exposures in animal toxicity studies, at maximum tolerated doses, exceeded the exposure in humans [at the maximum recommended human dose (MRHD) of 3000 mg lanthanum per day] by up to 7 fold.

The mean plasma protein binding ranged from 99.7% in humans to 99.9% in mouse, rat, rabbit and dog.

Animal studies showed that following administration of lanthanum carbonate, lanthanum is widely distributed in tissues, and the concentrations in many tissues (particularly gastrointestinal tract, bone and liver) greatly exceed the plasma concentration in mice, rats and dogs, and achieve steady state levels, at least in dogs, by approximately 26 weeks. Clearance of lanthanum from many tissues (stomach, femur, liver, spleen and sternum) is slow, with high lanthanum levels remaining 6 months after treatment cessation. Significant toxicity was not associated with these tissue lanthanum levels.

*In vitro* studies showed that lanthanum was not a significant inhibitor of any of the human liver cytochrome P450s (CYP 1A2, 2C9/10, 2C19, 2D6 and 3A4/5) examined, indicating a low potential for drug-drug interactions.

In the rat, an oral dose of lanthanum was shown to be excreted almost exclusively in the feces (99.3%). In the dog, about 93% of the administered dose was recovered either in the feces or vomit. Urinary excretion was minimal.

#### C. Brief Overview of Toxicology Findings

Chronic toxicity studies with lanthanum carbonate showed dose-related increased incidences of stomach lesions (epithelial hyperplasia of the limiting ridge and non-glandular region, sub-mucosal inflammation and inflammation of glandular epithelium) in rats and mice, but not in dogs. (The 52-week dog study did not reveal any significant

toxicity.) It was shown that lanthanum carbonate is better tolerated if administered to dogs and humans with food. Nocturnal feeding habits result in rodents receiving the drug (during the day) on an empty or partially-empty stomach. It is believed that the longer duration of direct contact of lanthanum with the stomach wall, together with the inability to vomit an irritant material is likely to make rodents more susceptible than dogs to stomach lesions.

Two years of oral administration of lanthanum carbonate at doses up to 1500 mg/kg/day (2.5 times the MRHD, on a mg/m<sup>2</sup> basis) did not produce any treatment-related tumors in the rat. In the mouse, oral administration of lanthanum carbonate for 99 weeks, at doses up to 1500 mg/kg/day (1.3 times the MRHD), produced a significant increasing trend for glandular stomach adenomas in males. Occurrence of these benign tumors was limited to the high dose treatment group (4/50 animals) and, on the basis of historical data, is considered to be a rare event in CD-1 mice.

In a fertility and embryonic development study in rats, lanthanum carbonate, at doses up to 2000 mg/kg/day (3.4 times the MRHD), did not affect fertility or mating performance, or produce any harm to the fetus. In a rabbit developmental toxicity study, oral administration of lanthanum carbonate (1500 mg/kg/day, 5 times the MRHD) was associated with maternal toxicity, increased post-implantation loss, reduced fetal weights and delayed skeletal ossification. In a study in which rats were dosed with lanthanum carbonate from implantation through lactation, 2000 mg/kg/day caused delayed eye opening, reduction in body weight gain, and delayed sexual development (preputial separation and vaginal opening) of the offspring. Mating performance and fertility of offspring were unaffected by the maternal treatment.

Lanthanum carbonate tested negative for genotoxicity in *in vitro* (bacterial reverse mutation assay, and mammalian cell gene mutation and cytogenetic assays in Chinese hamster ovary cells) and *in vivo* (mouse micronucleus assay) test systems.

In a chronic renal failure (CRF) rat model, oral administration of lanthanum carbonate (0, 100, 500, 1000 and 2000 mg/kg/day) for 12 weeks caused osteomalacia at 1000 (3 of 7 rats) and 2000 (1 of 4 rats) mg/kg/day doses. These bone effects were not seen in rats with normal renal function (NRF), given the same doses of lanthanum carbonate.

#### D. Nonclinical Safety Issues Relevant to Clinical Use

It was thought that the osteomalacia observed in CRF rats might be related to an excessive accumulation and/or direct toxic effect of lanthanum on the bone. However, bone analyses showed that there was no significant difference in the bone lanthanum concentrations between CRF (1.6 µg/g wet tissue) and NRF (1.2 µg/g wet tissue) rats, indicating a lack of direct relationship between bone lanthanum concentration and osteomalacia. It was also shown that higher bone lanthanum levels than observed in CRF rats were seen in chronic toxicity study animals (animals with normal renal function), in which no bone toxicity was observed.

It was observed that the doses of lanthanum that produced osteomalacia in renally impaired rats also caused marked reductions (78-86%) in urinary phosphate excretion, compared to NRF rats, indicating a state of phosphate depletion in CRF rats. The hypothesis that the osteomalacia in CRF rats was produced by phosphate depletion associated with high doses of a phosphate binder, and not by excessive accumulation or direct toxic effect of the compound on the bone, was tested using a non-absorbed, non-metallic phosphate binder (sevelamer), comparing its effects with that of lanthanum carbonate. Oral administration of sevelamer (1000 mg/kg/day) to CRF rats for 12 weeks produced impaired mineralization in 4 of 7 rats, while lanthanum carbonate produced these effects in 1 of 4 rats, indicating that the bone effects were secondary to phosphate depletion associated with high doses of a phosphate binder. Published literature also supports that hypophosphatemia, by any cause, can result in impaired bone mineralization.

At high doses of a phosphate binder, there is inadequate absorption of phosphate from the gut in CRF rats, requiring phosphorus mobilization from the bone, and thus causing a mineralization defect. In end stage renal disease patients, as hyperphosphatemia, rather than phosphate depletion, is a prerequisite for treatment with a phosphate binder, bone changes are unlikely to occur during treatment with lanthanum carbonate.

The glandular stomach adenomas observed in male mice were associated with proliferative changes due to gastric irritation induced by the very high stomach lanthanum concentration at the dose at which the tumors occurred in this species (2189 µg/g in mice vs 827 µg/g in rats and 349 µg/g in dogs). No neoplastic lesions (benign or malignant) were associated with lanthanum carbonate administration in the rat. In view of the above and in the absence of evidence of a genotoxic potential for lanthanum carbonate, the mouse tumor finding is not considered to be an approvability issue.

The apparently drug-related effects on rabbit embryo/fetal survival and development occurred at a relatively high dose, about 5 times the MRHD (on a mg/m<sup>2</sup>) basis, and may have been secondary to maternal toxicity observed at this dose. Moreover, the values observed in the study were within the sponsor's historical control range. Although in a study in which rats were dosed from implantation through lactation, 2000 mg/kg/day was associated with delayed eye opening and delayed sexual development of the offspring, mating performance and fertility of the offspring were unaffected by the maternal treatment. We do not consider the above findings to constitute an approvability issue.

Administrative

A. Reviewer signature: \_\_\_\_\_

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B. Supervisor signature: \_\_\_\_\_

Concurrence

/S/  
Charles A. Resnick, Ph.D

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## PHARMACOLOGY/TOXICOLOGY REVIEW

### I. PRIMARY PHARMACOLOGY

[Doses for all non-clinical studies are expressed on a salt basis (mg lanthanum carbonate/kg/day). 1000 mg lanthanum carbonate = 524 mg lanthanum.]

Sponsor's summaries of pharmacodynamic studies are provided below.

#### **Summary of *In Vitro* Studies to Identify a Suitable Phosphate Binding Agent for Preclinical and Clinical Evaluation (Report No. X00293-LAM-III; DIVO1054)**

The purpose of this investigation was to evaluate a number of different compounds for their relative ability to bind to phosphate and to assess the potential for absorption in to the systemic circulation. The aim was to identify the optimum compound for further development. Forty-five compounds were screened in the *in vitro* assay. Lanthanum carbonate removed >97% of phosphorus at pH 3 and 5 and >66% at pH 7, with a maximum element ion concentration of 302 ppm at pH 3 declining to 1 ppm at pH 7. The effects of lanthanum were comparable to aluminium hydroxide and superior to calcium salts. Lanthanum carbonate was chosen for further development.

The grinding of lanthanum carbonate demonstrated that the rate of binding could be altered by changes in particle size but that the final level of binding and element ion release was unchanged.

In conclusion, lanthanum carbonate was shown to be an effective phosphorus binder *in vitro* with low levels of released element ions at a pH range of 3-7.

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**A Comparative Study Between Lanthanum Carbonate  
and Aluminium Hydroxide as Inhibitors of Inorganic  
{<sup>32</sup>P} Phosphate Absorption from the Intestinal Tract  
(Report No. 97/06/SHIR/2)**

The purpose of this comparative study on isolated perfused rat gut was to measure the transport of phosphate across the gut lumen into the circulating perfusates and to compare the efficiency of lanthanum carbonate to that of aluminum hydroxide at binding and retaining the phosphate ( $^{32}\text{PO}_4^{2-}$ ) in the gut lumen inhibiting the transport of  $^{32}\text{PO}_4^{2-}$  into the circulation.

Three experiments were performed in which the vehicle and  $^{32}\text{PO}_4^{2-}$  were administered into the gut lumen. The amount of  $^{32}\text{PO}_4^{2-}$  absorbed into the perfusate accounted for approximately 60% of the administered dose for two of the three control experiments; the third experiment revealed that only 43.1% of the administered  $^{32}\text{PO}_4^{2-}$  transferred across.

The comparison between lanthanum carbonate and aluminum hydroxide at equimolar doses revealed that at the lowest dose level (lanthanum carbonate 9.2 and aluminum hydroxide 1.35 mg/kg) there was little difference between them in their ability to inhibit phosphate transport. At this dose, lanthanum carbonate showed no inhibition whereas aluminum hydroxide only reduced phosphate transport by 4.8%. At the highest dose (lanthanum carbonate, 30.8 and aluminum hydroxide, 4.56 mg/kg), lanthanum carbonate was more efficient at binding and retaining phosphate than aluminum hydroxide. At this level, there was an inhibition in the phosphate transport of 34.4% by lanthanum carbonate compared to 17.5% for an equimolar dose of aluminum hydroxide.

In conclusion, lanthanum carbonate administered into the small intestines at a dose of about 30 mg/kg body weight is capable of reducing the amount of  $^{32}\text{PO}_4^{2-}$  absorbed from the isolated perfused rat gut by approximately 30%. At this dose of lanthanum carbonate, the theoretical maximum would be 60%. By comparison, equimolar aluminum hydroxide is less efficient (18%) at preventing the transintestinal transport of  $\text{PO}_4^{2-}$  over 3 hours.

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ON ORIGINAL**

**Acute Oral (Gavage) Investigative Study in the Rat (Report  
No. SPD/82/96)**

The purpose of this study was to investigate the effects of a single oral (gavage) dose of lanthanum carbonate (1.5 and the 2000 mg/kg) on plasma and serum ionized calcium and phosphorus levels in male rats at 2 to 24 hours post dosing.

Seventy two male CD(SD)BR rats were divided into 12 groups of six. Two groups of rats were either not dosed or dosed with vehicle alone (0.5% w/v, carboxymethylcellulose) and served as controls. The remaining groups were administered lanthanum carbonate at a doses of 1.5 mg(salt)/kg or 2000 mg(salt)/kg. Blood samples were collected from the untreated control group and urine samples were collected from the control group given vehicle. Urine samples were collected from one group of treated rats each at the 1.5 and 2000 mg(salt)/kg doses. Blood samples were collected at 2, 4, 8, and 24 hours post dosing from 6 rats/time point at the 1.5 and 2000 mg(salt)/kg doses.

Ionized phosphorus in control rats averaged  $9.5 \pm 104$  mg% and  $9.3 \pm 104$  mg % in plasma and serum, respectively. Dose-dependent reductions in plasma and serum levels of ionized phosphorus were observed in rats treated with lanthanum carbonate (23 to 24% reductions at 8 and 24 hours after administration of the 1.5 mg/kg dose compared to controls, and 33 to 41% reductions at 8 and 24-hours after dosing at the 2000 mg/kg dose). Levels of ionized calcium in plasma or serum were not affected by lanthanum carbonate at either dose tested over the 24-hour sampling period.

The 2000 mg/kg dose caused a decrease in urinary volume and a reduction in urinary calcium levels over 0-6 hours. Urinary volume returned to normal over 6-24 hours when the animals had access to food and water. The 2000 mg/kg dose caused a decrease in urinary calcium levels over 0-6 hours.

Analysis of plasma samples indicated that minimal absorption of lanthanum carbonate occurred at a dose level of 2000 mg/kg. There was no evidence of any absorption at a dose level of 1.5 mg/kg.

In conclusion, the administration of lanthanum carbonate as a single dose of 2000 mg/kg by oral gavage did not cause any changes in the levels of plasma or serum calcium. However this dose caused a dose-dependent reduction in plasma and serum inorganic phosphate levels after 8 and 24-hours. There were no signs of further reduction after 24-hours. Urine volume was decreased over 0-6 hours but returned to normal when the animals were allowed access to food and water. Calcium levels in the urine were reduced over 0-6 hours as well. Analysis of plasma samples at this dose level indicated that minimal absorption of lanthanum had occurred.

**Studies to Compare the Efficacy of Lanthanum Carbonate and other Phosphate Binding Agents on the Gastrointestinal Absorption of  $^{32}\text{P}$ -labelled  $\text{H}_3\text{PO}_4$  in Rats (Report No. R00216-LAM-IIIIF; SPD/07)**

The study objective was to provide comparative information on the effects of phosphate-binding agents on the rates and routes of excretion of radioactivity, and thereby on the extent of absorption of phosphate from the gastrointestinal tract. This comparison was made on the assumption that essentially all the absorbed phosphate was excreted in the urine.

Groups of rats received daily oral doses of phosphate binding agents at a nominal dose level of either 100 or 1000 mg (salt)/kg. On Day 7, the rats received a single oral dose of 60 mg/rat of [ $^{32}\text{P}$ ]  $\text{H}_3\text{PO}_4$ .

After a single dose of [ $^{32}\text{P}$ ]  $\text{H}_3\text{PO}_4$  to five male rats, following the administration of lanthanum carbonate (100 mg (salt)/kg), a mean of 12.5% dose was excreted in the urine during 0-144 hours. Most of this radioactivity was excreted within the first 48 hours, representing 11.6% of the dose. During the 6 days after dosing, a mean of 29.5% of the dose was excreted in the feces with most (26.9%) occurring within the first 48 hours. Radioactivity remaining in the carcass accounted for 53.7% of which 36.4% was associated with the residual bone following digestion of the carcass. The total mean recovery of radioactivity after 144-hours was 95.8% of the dose.

After a single dose of [ $^{32}\text{P}$ ]  $\text{H}_3\text{PO}_4$  to five male rats, following the administration of lanthanum carbonate (1000 mg (salt)/kg), a mean of 2.4% of the dose was excreted in the urine during 0-144 hours. Most of this radioactivity (2.2%) was excreted in the urine after 48-hours. During the 6 days after dosing, a mean of 55.6% of the dose was excreted in the feces with most of this (53.4%) within the first 48 hours. Radioactivity remaining in the carcass accounted for 31.6% of which 21.1% was associated with the residual bone following digestion of the carcass. The total mean recovery of radioactivity after 144 hours was 89.6%.

In rats with normal renal function, lanthanum carbonate, aluminum hydroxide, calcium carbonate, and sevelamer increased fecal excretion, and reduced urinary excretion, of orally administered  $^{32}\text{P}$ -phosphorus at 1000 mg (salt)/kg. The increased fecal phosphorus excretion was higher for lanthanum carbonate, aluminum hydroxide, and calcium carbonate compared to sevelamer, whereas reduction in urinary phosphorus excretion was similar for all drugs. These findings are consistent with a reduction in the absorption of phosphorus due to the binding and precipitation of insoluble phosphate salts in the gastrointestinal tract. All drugs similarly reduce the urinary excretion of oxalate at 1000 mg (salt)/kg, but not at 100 mg (salt)/kg, consistent with binding and precipitation of insoluble oxalate salts in the gastrointestinal tract.

In conclusion, these results indicate that the phosphorus binding agents administered as 1000 mg(salt)/kg reduce the absorption of phosphorus in the gut.

## II. SAFETY PHARMACOLOGY

*Sponsor's summaries of safety pharmacology studies are provided below.*

### **Effects on Central Nervous System**

Single dose studies were performed with lanthanum carbonate in the mouse. Doses of 0, 200, 500, 1000 or 2000 mg lanthanum carbonate/kg were administered orally via gavage. Doses employed in these studies were selected on the basis of maximum tolerated doses determined in an acute toxicology study (SPD/42/96). These doses of lanthanum carbonate were 1.7, 4.4, 8.7, and 17.4 times, respectively, the anticipated maximum therapeutic dose (114.5 mg lanthanum carbonate/kg/day) for a 50 kg individual on the basis of body weight.

In addition, an Irwin Screen was conducted in mice during Week 62 of the mouse carcinogenicity study (SPD/88/C) and neurotoxicity assessments were conducted in dogs during Week 26 of the 52-week toxicity study (SPD/66/TK). No significant adverse effects were observed in either assessment.

### **Irwin Test in Mice Including Body Temperature Alterations (Report No. SPD/62/PH)**

This study was performed to investigate the effects of lanthanum carbonate in the Irwin Test and on body temperature in mice.

Four groups of six male CD-1 mice were fasted overnight prior to dosing. Thirty minutes prior to dosing, rectal body temperature of each mouse was recorded and the parameters of the Irwin Screen were recorded. Each mouse received an oral dose via gavage of either vehicle, or lanthanum carbonate (200, 500, and 1000 mg/kg). Mice were observed 1, 2, and 4 hours after dosing for changes in the parameters according to the method of Irwin. The body temperature of each animal was also measured at these intervals.

No animals died or were euthanized prematurely during the study. No clinical signs of reaction to treatment were observed in the Irwin Screen and the minor changes detected were not regarded as pharmacologically significant.

Lanthanum carbonate did not produce any effects on the Irwin Screen parameters or body temperature up to 4 hours after dosing. On the basis of lack of an adverse effects in this screen, the NOEL was determined to be >1000 mg (salt)/kg.

**Spontaneous Motor Activity in Mice (Report No.  
SPD/63/PH)**

This investigation assessed the effect of lanthanum carbonate on the spontaneous motor activity of mice.

Five groups of six male CD-1 mice were fasted overnight prior to dosing. Mice were orally administered via gavage, vehicle, lanthanum carbonate (200, 500, or 1000 mg/kg) or the positive control, chlorpromazine (5 mg/kg). Three hours after dosing, each animal was placed inside a perspex box in which the floor was divided and marked into four sections. The number of crossings from one section to another in a 3-minute interval was recorded.

No animals died or were sacrificed prematurely during the study. Lanthanum carbonate administration was not associated with any adverse clinical observations other than those recorded as spontaneous motor activity.

Mice administered the standard, chlorpromazine, had a statistically significant reduction ( $p < 0.001$ ) in mean line crossings. In comparison, the mean line crossings for mice receiving lanthanum carbonate were similar to control values.

On the basis of these findings, lanthanum carbonate had no effect on spontaneous activity in mice. The NOEL was determined to be  $>1000$  mg (salt)/kg.

**APPEARS THIS WAY  
ON ORIGINAL**

**Assessment of Proconvulsant Activity in Mice  
Following Oral Administration (Report No. SRU  
003/992850)**

The objective of this investigation was to assess the proconvulsant effects of orally administered lanthanum carbonate using both metrazol-induced and electroshock-induced convulsions in the mouse.

Mice were fasted overnight prior to conducting this study. Lanthanum carbonate was administered orally via gavage to male ICR CD-1 mice (10/group in the metrazol test and 12/group in the electroshock test) at doses of 500, 1000, and 2000 mg/kg. Control group animals for each test received vehicle (0.5% w/v carboxymethylcellulose) or amphetamine sulfate (30 mg/kg) in the metrazol test or bemegride (40 mg/kg) in the electroshock test. Employing amphetamine sulfate and bemegride as positive controls ensured validity of the test systems.

Forty-five minutes after oral administration of test treatments, each animal received either a subthreshold convulsive dose of metrazol (55 mg/kg, subcutaneous) or a subthreshold convulsive electroshock applied via ear electrodes. Mice were observed for the occurrence of convulsions for 45-minutes (metrazol test) or for one minute (electroshock test).

In the metrazol test, observations included the occurrence of generalized clonic seizures accompanied by loss of posture. The time to occurrence of this first seizure was recorded for each mouse. The presence or absence of tonic flexion or tonic extension of the hindpaws and the number of lethalties at the termination of the observation period was recorded. Signs of proconvulsant activity were also recorded.

In the electroshock test, the stimulus selected caused tonic extension of the hind limbs in a minority of vehicle-treated mice. Observations that were recorded included the presence or absence of tonic extension or tonic flexion of the hind paws and of clonic convulsions during a 1-minute-post shock period. Lethalties were also recorded.

In the metrazol test, lanthanum carbonate produced no significant proconvulsant activity (0/10) at oral doses of 500, 1000, and 2000 mg (salt)/kg. The positive control, amphetamine sulfate, produced marked and statistically significant proconvulsant activity (10/10) and decrease in mean time to first seizure.

In the electroshock test, lanthanum carbonate produced proconvulsant activity (i.e., tonic extension) in 6/12 animals at oral doses of 1000 and 2000 mg(salt)/kg that was not statistically significant in comparison to vehicle control group (3/12). The lanthanum carbonate dose of 500 mg/kg did not produce any statistically significant proconvulsant activity (4/12) in this test. In comparison, bemegride produced the expected statistically significant proconvulsant effect in 8/12 animals. Since lanthanum carbonate at the highest dose evaluated did not induce statistically significant proconvulsant activity in the metrazol and electroshock tests, the NOEL was determined to be >2000 mg (salt)/kg.

**Assessment of Anticonvulsant Activity in Mice  
Following Oral Administration (Report No. SRU  
005/992851)**

The purpose of this investigation was to assess the anticonvulsant effects of orally administered lanthanum carbonate using both metrazol-induced seizures and supramaximal electroshock-induced seizures in the mouse.

Lanthanum carbonate was orally administered via gavage to male ICR CD-1 mice (10/group) at doses of 500, 1000, and 2000 mg/kg. Additional control groups received either vehicle (0.5% w/v carboxymethylcellulose) or sodium phenobarbitone (30 mg/kg). Sodium phenobarbitone was included as positive control in both tests.

Forty-five minutes after dose administration, each animal received either metrazol (85 mg/kg, subcutaneous) or was subjected to a supramaximal shock applied via ear electrodes. In the metrazol test, all animals were continuously observed for 30 minutes for the occurrence of generalized clonic seizures accompanied by loss of posture. The presence or absence of tonic flexion or tonic extension of the hind paws and the number of deaths at the termination of the observation period were also noted. In the electroshock test, each mouse was observed for the presence or absence of tonic extension or tonic flexion of the hind paws and of clonic convulsions during the 1-minute post-shock period.

In the Metrazol Test, oral administration of lanthanum carbonate at doses of 500, 1000, and 2000 mg(salt)/kg produced no marked or statistically significant effects on the numbers of animals displaying clonic convulsions or on the mean time to the first convulsion when compared to the vehicle-treated group. In contrast, sodium phenobarbitone produced a statistically significant anticonvulsant effect in this test.

In the Electroshock Test, there were no statistically significant differences in the responses to supramaximal electroshock between mice dosed with any dosage of lanthanum carbonate and the vehicle-treated mice. In contrast, sodium phenobarbitone produced a statistically significant anticonvulsant activity in this test.

No signs of ataxia were observed following administration of lanthanum carbonate in either test.

Since lanthanum carbonate did not produce significant anticonvulsant activity in either the metrazol or supramaximal electroshock tests, the NOEL was determined to be >2000 mg (salt)/kg.

## Effects on Cardiovascular and Respiratory Function

### Cardiovascular and Respiratory Parameters in the Anaesthetised Dog (SPD/64/PH)

The object of this investigation was to assess the effect of intraduodenal administration of lanthanum carbonate on various cardiovascular and respiratory parameters in the anesthetized dog.

Doses employed in this study were selected on the basis of maximum tolerated doses determined in an acute and 14-day repeat oral dose range finding study in the Beagle dog (SPD/44/96). Doses evaluated in this study were 0, 200, 600, and 2000 mg (salt)/kg. These doses of lanthanum carbonate were 1.7, 5.1, and 17.4 times, respectively, the anticipated maximum therapeutic dose (114.5 mg lanthanum carbonate/kg/day) for a 50 kg human on the basis of body weight.

Four groups of three Beagle dogs were intraduodenally administered either vehicle (0.5% w/v carboxymethylcellulose) or lanthanum carbonate at dose of 200, 600, or 2000 mg/kg.

Each dog was anesthetized and surgically prepared to monitor blood pressure (femoral artery, systolic, diastolic and mean), heart rate, left ventricular pressure (end diastolic pressure, and dp/dt), ECG (QRS amplitude, P-R interval, and S-T interval), blood flow (mean and peripheral resistance), and respiration (rate, tidal volume, and minute volume).

No animals died or were euthanized prematurely during the study. No clinical signs were observed that were related to treatment.

Lanthanum carbonate at 200 mg/kg and higher did not cause any change in blood pressure, left ventricular pressure, femoral flow rate, peripheral resistance, and in respiration parameters (minute volume, rate, and tidal volume). Analysis of electrocardiographs revealed no effects on QRS, PR and ST intervals. All parameters remained stable throughout the 4-hour measurement period.

Since lanthanum carbonate at the highest dose tested did not adversely effect any cardiovascular or respiratory parameter, the NOEL was determined to be >2000 mg (salt)/kg.

**Analysis of Electrocardiograms Obtained from Study  
SPD/64/PH for the Assessment of Potential Effects on  
QT Intervals (Report No. SPD/64/a)**

The objective of this study was to re-analyze data from SPD/64/PH to determine potential effects on QT Intervals following intraduodenal treatment with lanthanum carbonate on anesthetized Beagle dogs.

Electrocardiogram traces were re-examined and the R-R interval was measured to calculate the heart rate. Q and T waves were verified and the QT interval measured. From this data the corrected QT (QTc) value was calculated.

No effects were observed on heart rate (calculated from R-R intervals) or QT interval after administration of the vehicle control or lanthanum carbonate at a dose of 600 mg(salt)/kg and higher. These parameters remained stable throughout the 4-hour observation period. There were no clear effects of treatment at any dose level upon the QTc value.

On the basis of this re-evaluation, the NOEL was determined to be >2000 mg (salt)/kg.

APPEARS THIS WAY  
ON ORIGINAL

## Effects on Gastrointestinal Function

### Charcoal Meal Transit in the Rat Small Intestine (Report No. SPD/47/PH)

This study was performed to identify any activity of lanthanum carbonate on gastrointestinal motility.

Five groups of six male CD rats were administered via gavage one of the following: vehicle (0.5% carboxymethylcellulose in water), lanthanum carbonate at doses of 200, 500, or 1000 mg/kg, or morphine used as standard at 20 mg/kg. Two hours after dosing with vehicle or lanthanum carbonate, or one hour after dosing with morphine, charcoal meal was orally administered. Rats were killed 30 minutes after charcoal meal administration, and the small intestine was removed. The distance traveled by the charcoal meal as a percent of the total length of small intestine was recorded.

No animals died or were euthanized prematurely during the study. Clinical observations included intestinal contents that were very fluid and some yellow liquid in all rats administered 500 and 1000 mg (salt)/kg. A minimal residue of intestinal charcoal meal was present in all animals given 1000 mg (salt)/kg. The mean percentage distances traveled by the charcoal meal in rats treated with lanthanum carbonate at dose levels of 200, 500 and 1000 mg/kg(salt)/day were 42, 43, and 39%, respectively. In comparison, the mean percentage distances traveled by the charcoal meal was 56% in the untreated control rats, and 29% in rats treated with morphine (20 mg/kg). On the basis of these findings, lanthanum carbonate was determined to have no significant effect on mean transit time in rats. However, the content of charcoal meal in the intestine was reduced at 1000 mg/kg lanthanum carbonate, as indicated by the presence of a yellow substance in the intestine that was probably the test article associated with mucus. These latter findings suggested that lanthanum carbonate inhibited gastric emptying at the 1000 mg(salt)/kg high dose.

On the basis of these observations, the NOEL for effects on gastric emptying was determined to be 500 mg (salt)/kg.

**Gastric Acid Secretion in Shay Rats (Report No.  
SPD/48/PH)**

This study was conducted in order to identify any effects of lanthanum carbonate on gastric function by analysis of gastric fluid secretion.

Five groups of six male CD rats were fasted prior to start of study. Approximately two hours before surgery, four groups of rats received, via gavage, vehicle or lanthanum carbonate doses of 200, 500, or 1000 mg/kg. Rats were anesthetized and the pylorus ligated. A fifth group of rats was intraduodenally administered the standard cimetidine at 60 mg/kg. Four hours after dose administration, rats were killed. Gastric fluid was collected and the volume, pH, and pepsin activity were measured.

No animal died or was euthanized prematurely during the study. There were no visible signs of reaction to treatment in any animal. At laparotomy the stomachs of the animal dosed with 1000 mg(salt)/kg lanthanum carbonate were observed to be partially distended. This may have been attributable to the test article remaining in the stomachs.

The standard, cimetidine (60 mg/kg), significantly reduced ( $p < 0.01$ ) the volume of secretion and the hydrogen ion concentration. Cimetidine reduced pepsin activity, but this reduction was not statistically significant.

Oral administration of lanthanum carbonate had no effect on the volume of secretion or pepsin activity, but the hydrogen ion concentration was reduced in a dose-dependent manner and this reduction was significant ( $p < 0.01$ ) at 1000 mg/kg.

In conclusion, lanthanum carbonate, when orally administered at dose levels of 200, 500, or 1000 mg(salt)/kg in the rat, had no effect on the volume of gastric fluid, or pepsin activity but the presence of lanthanum carbonate within the stomach of the rat reduced the acidity of the gastric contents by neutralization.

On the basis of these observations, the NOEL was determined to be 500 mg (salt)/kg.

### Urinary and Faecal Output in Rats (Report No. SPD/49/PH)

This study was designed to assess the effect of oral administration (gavage) of lanthanum carbonate on urinary and fecal output.

Five groups of six CD male rats were fasted overnight and deprived of water 2 hours prior to dosing. At the end of this two-hour interval, saline was administered. Thirty minutes later, each group of rats were orally administered via gavage one of the following: vehicle, lanthanum carbonate (200, 500, and 1000 mg/kg) or furosemide (20 mg/kg). Immediately after dosing, each rat was housed in a metabolic cage for the remainder of the day. Neither food nor water was available until 6 hours after dosing.

Urine and feces were collected at 3, 6, and 24 hours after dosing. Urine volume was measured and a sample taken for the determination of sodium, potassium, chloride, and phosphate concentrations. Fecal weights were also determined.

No animals were found dead or euthanized prematurely during the study. There were no visible signs of reaction to treatment with lanthanum carbonate in any animal. No episodes of diarrhea were recorded. These clinical observations suggested no disturbance of intestinal function.

The standard, furosemide, resulted in significant increases in urine volume and in sodium ( $p < 0.001$ ), chloride and potassium ( $p < 0.01$ ), and phosphate ( $p < 0.05$ ) concentrations in comparison to control values 3 hours post-dose. Furosemide produced an approximately 6-fold increase in urine volume and an approximately 10-fold loss in sodium in comparison to control values in this same time interval. No significant differences were observed for any parameters at 6 hours. Significant decreases in the concentrations of sodium ( $p < 0.05$ ) and chloride ( $p < 0.01$ ) and an increase in phosphate ( $p < 0.01$ ) in comparison to control values were observed at 24 hours.

No significant changes were observed in urine volume or electrolyte concentrations following treatment with lanthanum carbonate at 3 or 6 hours. The concentration of phosphate was reduced ( $p < 0.05$ ) approximately 5-fold following administration of 500 mg/kg lanthanum carbonate in comparison to control values at 24 hours. Less than 2-fold increases in urine volume ( $p < 0.05$ ) and sodium concentration ( $p < 0.01$ ) were observed following administration of 1000 mg/kg lanthanum carbonate. Phosphate concentration was decreased at this time interval, but this change was not statistically significant.

On the basis of the increases in urine volume and sodium concentration at 1000 mg/kg, and the lack of an affect on fecal weights at the highest dose of lanthanum carbonate, the NOELs for urine output and fecal output were determined to be 500 and 1000 mg (salt)/kg, respectively.

**Gastric Lesions in the Rat Stomach (Report No. SPD/50/PH)**

The objective of this study was to assess whether lanthanum carbonate produces lesions in the rat stomach.

Five groups of six male CD rats were administered orally via gavage, vehicle, lanthanum carbonate at doses of 200, 500, or 1000 mg/kg, or the positive control, aspirin, at 150 mg/kg. All animals were sacrificed 3 hours after dose administration. Stomachs were opened and examined for macroscopic gastric damage as indicated by hemorrhagic lesions.

No animals were found dead or sacrificed prematurely during the study. There were no clinical observations related to treatment in any animal prior to examination of the gastrointestinal tract.

Examination of the gastrointestinal tract revealed bloated appearance of the stomach for animals in the 200 (1/6), 500 (2/6) and 1000 (4/6) mg(salt)/kg dose groups. These observations were correlated to the presence of test article in the stomachs of these animals. The observations of retained fluids and test article in stomachs were attributed to the physical character of the formulation delivered to the rats.

In contrast to the positive control, aspirin, which showed gastric lesions in all treated animals (6/6), the incidence of gastric lesions from lanthanum carbonate at doses as high as 1000 mg/kg was identical to the incidence in the control group (0/6).

In conclusion, lanthanum carbonate was shown not to cause gastric damage in the rat. Therefore, the NOEL was determined to be 1000 mg (salt)/kg.

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### **The Effect of Lanthanum Carbonate on Aspirin Induced Gastric Damage in Rat (Report No. SPD/51/PH)**

The objective of this investigation was to assess whether lanthanum carbonate affects aspirin-induced gastric damage in the rat.

Six groups of six male CD rats were fasted overnight prior to dosing. Groups 1 and 3-6 received aspirin (150 mg/kg) following treatment with vehicle, sodium chloride (6%), or lanthanum carbonate (200, 500, or 1000 mg/kg). Group 2 rats received a lanthanum carbonate dosage of 1000 mg/kg alone. Administration of all substances was orally via gavage. Three hours after dose administration, all animals were sacrificed, stomachs opened, and examined for macroscopic gastric damage.

No animals died or were killed prematurely during the study. There were no clinical observations related to reaction to treatment in any animal prior to examination of the gastrointestinal tract.

Examination of the gastrointestinal tract revealed the presence of test article and retained clear fluids in the stomach for animals given either lanthanum carbonate (1000 mg/kg) alone or lanthanum carbonate (200 mg/kg and higher) in the presence of aspirin. A bloated appearance of the stomach generally correlated with these observations. This bloated appearance was attributed to an effect of the test article on gastric emptying and was considered related to the physical character of the formulation delivered to the stomach of the rat (see Study Number SPD/50/PH).

Gastric lesions were observed in all animals (6/6) given the positive control, aspirin. Sodium chloride (6%) was gastroprotective. This was considered attributed to its hypertonicity. Sodium chloride in the presence of aspirin produced a statistically significant decrease ( $p < 0.01$ ) in mean number of gastric lesions in comparison to the incidence with aspirin alone (3 versus 7). Lanthanum carbonate in the presence of aspirin was also gastroprotective. Lanthanum carbonate produced statistically significant decreases ( $p < 0.05$  to  $p < 0.01$ ) in the mean number of gastric lesions at all dose levels in comparison to animals receiving aspirin alone.

On the basis of the lack of gastric irritancy at the highest dose and the gastroprotective action at the lowest dose of lanthanum carbonate, the NOELs for gastric irritancy and gastric protection were determined to be  $> 1000$  mg(salt)/kg and  $< 200$  mg (salt)/kg, respectively.

## Effects on Bone

### **The Effects of Lanthanum Carbonate on the Activity and Differentiation of Bone Cells In Vitro (Report No. V00008-00011-LAM-IIIIF; SPD/LA/01-04)**

The purpose of this investigation was to evaluate the effects of lanthanum carbonate on the activity and differentiation of bone cells *in vitro*. Four assays were performed which included (1) bone resorption assay, (2) osteoclast differentiation assay, (3) osteoblast differentiation assay, and (4) bone formation assay

The bone resorption assay cultures osteoclasts on bone slices, which are allowed to resorb bone. The amount of bone resorbed during the culture period is determined by measuring the amount of collagen cross-links released into culture medium.

Osteoclast differentiation is investigated by employing bone marrow cultures. Osteoclast precursors in bone marrow can be induced to form multinucleated osteoclast-like cells (MNC) in the presence of vitamin D<sub>3</sub> or parathyroid hormone. MNC express high levels of tartrate-resistant acid phosphatase (TRAP) and calcitonin receptors. The amount of TRAP released from osteoclasts in culture is measured and has been shown to correlate with the number of osteoclasts formed.

The osteoblast differentiation assay monitors the following three distinct periods of osteoblast development: cell proliferation and secretion of extracellular matrix (ECM), ECM maturation and ECM mineralization. During these developmental periods there is a sequential expression of osteoblast phenotypic markers. These markers are alkaline phosphatase expression during maturation of the osteoblast and the deposition of calcium into mature organic matrix with the onset of mineralization.

The bone formation assay monitors the activity of mature osteoblasts by following their ability to form mineralized bone matrix. This is accomplished by demineralizing the formed bone matrix, and determining the amount of calcium released.

Positive control substances were employed to show that the assays were capable of detecting the desired response. Specifically, inhibition was demonstrated by bafilomycin A1 in the bone resorption assay and by 17- $\beta$  estradiol in the osteoclast differentiation assay, and activation was demonstrated by vitamin D in the osteoblast differentiation assay and by 17- $\beta$  estradiol in the bone formation assay.

Lanthanum carbonate was added to cultures to give final concentrations of 100, 500, 1000, 5000, and 15000 ng of elemental lanthanum per mL of medium.

In the bone resorption assay, there was no significant effect of lanthanum either on the amount of  $\text{Ca}^{2+}$  released into the medium or on osteoclast number. The positive control, bafilomycin A1, completely inhibited ( $p < 0.001$ ) bone resorption. A non-statistically significant concentration-dependent inhibition of bone resorption was

observed at 100 and 500 ng/mL. The slight non-statistically significant decrease observed at 15000 ng/mL did not appear to be due to slight toxic effects at this high concentration. Microscopic analysis of the morphology of the osteoclasts did not reveal any toxic effects. Therefore, the NOEL was determined to be >15000 ng/mL.

In the osteoclast differentiation assay, there was a concentration-dependent inhibitory response with lanthanum carbonate concentrations of 500 ng/mL and higher. This inhibitory effect was statistically significant at 100 ng/mL ( $p < 0.01$ ) and from 1000 ng/mL to 15000 ng/mL ( $p < 0.001$ ). On the basis of this inhibitory activity, the NOEL was determined to be <100 ng/mL.

In the osteoblast differentiation assay, lanthanum showed a concentration-dependent response. Test concentrations of 5000 and 15000 ng/mL inhibited ( $p < 0.001$ ) while 100 ng/mL significantly activated ( $p < 0.05$  to  $p > 0.01$ , respectively) osteoblast differentiation. No significant response was observed at lanthanum concentrations of 500 and 1000 ng/mL. Therefore, the NOEL was determined to be 1000 ng/mL.

In the bone formation assay, all concentrations of lanthanum showed statistically significant ( $p < 0.001$  and  $p < 0.01$  to  $p > 0.001$ ) activation of bone formation activity of mature osteoblasts. Lanthanum concentrations of 5000 and 15000 ng/mL may also have cytotoxic effects on osteoblast precursor cells, which may moderate the activation of mature osteoblasts *in vivo* (see osteoblast differentiation discussion below). On the basis of these effects, the NOEL was determined to be <100 ng(base)/mL.

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### III. PHARMACOKINETICS/TOXICOKINETICS

*Sponsor's summaries of pharmacokinetic/toxicokinetic studies are provided below.*

#### Absorption

Endogenous (background) systemic levels of lanthanum (i.e. those systemic concentrations estimated following administration of placebo were generally close to or below the limit of quantification of the assay ( $\sim$  ng/g). In general, oral administration of increasing doses of lanthanum carbonate (200 to 2000 mg/kg/day) resulted in dose-dependent, but less than proportional, increased levels of systemic exposure. At the highest dose levels tested in mice, rats, rabbits and dogs, exposure was 2- to 10-fold greater than that observed in placebo-treated animals. In general, systemic exposure in female animals was not significantly different than that seen in males.

As lanthanum is not metabolized, the fraction of an oral dose that is absorbed will approximate the drug's bioavailability. Oral bioavailability was estimated to be 0.0007% in the rat and 0.0005% in the dog (Study Nos. SPD0102 and SPD0104). However, the true oral bioavailability may be greater, since the intravenous doses used for calculating the oral bioavailability, 0.03 and 0.003 mg(salt)/kg/day for the rat and dog, respectively, were in the linear part of the dose/exposure curve, whereas exposures after oral doses of 1500 and 2000 mg(salt)/kg/day in rat and dog, respectively, were less than proportional. Nevertheless the data indicate that lanthanum is minimally absorbed when administered as the carbonate salt.

The time to peak plasma exposure ( $T_{max}$ ) after first administration was variable, consistent with a poorly absorbed drug. The mean in healthy human subjects ranged from approximately 3 to 6 hours (Study No. LAM-IV-108) compared to 1-6 hours in mice (Study Nos. SPD/85/C; SPD0098; SPD/86/C), 2 to 8 hours in rat (Study Nos. SPD/61/W; SPD0099), 8 hours in rabbit (Study No. SPD0097) and 1 to 9 hours in dog (Study No. SPD/78/W; SPD0100; SPD/46/96).

Comparison of mean plasma levels and  $AUC_{(0-24h)}$  values after single and repeat dose administration of lanthanum carbonate showed no evidence of accumulation following repeated dosing in rats (Study No. SPD0099) and mice (Study Nos. SPD/85C, SPD00098, SPD/86/C) and Rabbits (Study No. SPD0097). However, in dogs, exposure ( $AUC_{(0-24h)}$ ) was significantly increased after 4-weeks of repeated oral dosing at a dose of 2000 mg/kg compared to Week 1 values (Study No. SPD0100-TK). Comparison of  $AUC_{(0-24h)}$  values in dogs at doses of 200, 600 and 2000 mg/kg across studies at weeks 4 (Study No. SPD/46/96), 13 (Study No. SPD59/96), and 25 (Study No. SPD/66/C) showed that exposure at Week 13 was increased relative to values at Week 4, but were comparable or higher than respective values at Week 25, suggesting that exposure had attained steady state levels by Week 13 in dogs. This is also supported by comparable plasma lanthanum concentrations during Weeks 26 and 51 of the 52-week oral toxicity study in dogs (Study No. SPD/66/C).

### Comparison of Plasma Exposure in Animals and Man

At steady state, plasma lanthanum exposure in animal toxicology studies ( $AUC_{(0-24h)}$ ) exceeded exposure in humans at the maximum therapeutic dose of 3 g by up to 7 fold in oral studies and by up to 6,679 fold in intravenous studies (Table 5-3).

**Table 5-3 Comparison of the Maximal Steady State Plasma Lanthanum Exposures During Oral Toxicology Studies to the Average Human Plasma Exposure at the Maximum Intended Clinical Dose of 3 g/day**

Species	Oral Dose g(salt)/kg/day)	$C_{max}$ (ng/ml)	$AUC_{(0-24h)}$ (ng·h/ml)	Multiple of Patient $AUC_{(0-24h)}$ and ( $C_{max}$ )	Study Reports
Man (healthy subjects)	114.5 <sup>a</sup>	0.53	10.0	0.3 (0.5)	LAM-IV-109
Man (dialysis patients)	114.5 <sup>a</sup>	1.06	31.1	1 (1)	LAM-IV-111
Mouse <sup>b</sup>	1500	1.8	25.96 <sup>c</sup>	0.8 (1.8)	SPD0098
Rat	1500	0.79	14.6	0.5 (0.7)	SPD0099
Rabbit	1500	2.57	38.0	1.2 (2.4)	SPD0097
Dog	2000	11.4	207	6.7 (10.8)	SPD0100
<b>Intravenous</b>					
Rat	0.3	1,588	2,790	90 (1498)	SPD0102
Dog <sup>b</sup>	1.0	22,065	207,722	6,679 (20816)	SPD0104

<sup>a</sup> Assuming a 50kg patient taking 3g elemental lanthanum per day

<sup>b</sup> Females

<sup>c</sup> values = average plasma concentrations (ng/mL)

Tabulated summaries of the pharmacokinetic and toxicokinetic data from nonclinical studies are provided in Appendix II. Briefly, these data show that plasma exposure to lanthanum generally increased with increasing oral doses of lanthanum carbonate in animals and man, although the increases were less than proportional to the dose increment, especially at maximum tolerated doses. For example, mice treated for 4 weeks at 100, 500 or 1500 mg(salt)/kg/day (dose ratio 1: 5: 15) had  $AUC_{0-24h}$  ratios of 1: 2.2: 4.0 (males) and 1: 6.0: 11.9 (females) (Study No. SPD0098); and dogs treated for 13 weeks at 200, 600 or 2000 mg(salt)/kg/day (doses ratio 1: 3: 10) had  $AUC_{0-24h}$  ratios of 1: 3.7: 4.2 (males) and 1: 1.8: 3.1 (females) (Study No. SPD/59/96). This is consistent with the low aqueous solubility and poor oral bioavailability of lanthanum.

In contrast to oral dosing, exposure after intravenous administration of lanthanum chloride to rats and dogs, increased approximately proportionately with dose, except at maximum tolerated doses, where it was greater than proportional. Rats dosed for 4 weeks at 0.003, 0.03 and 0.3 (dose ratio 1: 10: 100) had  $AUC_{0-24h}$  ratios of 1: 11: 259 (males) and 1: 7: 224 (females); and dogs dosed at 0.003, 0.05 and 1.0 mg(salt)/kg/day for the same period (dose ratio 1: 17: 333) had  $AUC_{0-24h}$  ratios of 1: 18: 2387 (males) and 1: 33: 3279 (females). These findings suggest that plasma clearance mechanisms may become saturated at exposure levels substantially above those occurring in patients at therapeutic doses.

The plasma elimination half-life ( $t_{1/2}$ ) of lanthanum after oral administration was estimated to be approximately 36 hours in man (Study No. LAM-IV-109), 9 hours in mouse (Study No. SPD0098), 13 to 20 hours in rat (SPD0099), 20 hours in rabbit (Study No. SPD0097) and 20 to 26 hours in dog (Study No. SPD0100). In view of the retention of lanthanum in tissue compartments (see below), the  $t_{1/2}$  values possibly relate to an initial, albeit major, plasma clearance phase and underestimate the true terminal elimination half-life of the drug. The initial clearance phase reflects the transfer of lanthanum in to tissue pools as well as excretory processes.

Tissue lanthanum concentrations in animals were higher than plasma concentrations, sometimes by several orders of magnitude. Whilst tissue concentrations may be a more relevant indicator of overall body burden, such data are not available for man and so could not be used for safety margin calculations. As plasma and tissue concentrations are in equilibrium at steady state, it is reasonable to use plasma lanthanum concentrations for cross-species exposure comparisons although, in so doing, it is assumed that plasma:tissue concentration ratios are the same between species.

Safety margins for systemic toxicities observed in animal studies were therefore calculated by comparing the steady state human  $AUC_{0-24h}$  at the expected maximum therapeutic dose of 3g lanthanum/day with the animal  $AUC_{0-24h}$  at the no effect dose for the relevant toxicity. In healthy volunteers, oral administration of 3g lanthanum/day for 5 days resulted in a steady state  $C_{max}$  of 0.53 ng/ml and  $AUC_{0-24h}$  of 10 ng.h/ml (Study No. LAM-IV-109). Regular monitoring of plasma lanthanum throughout a Phase III clinical study showed that mean concentrations after 3g lanthanum/day ranged from 0.4860 to 0.5986 ng/ml (Study No. LAM-IV-301), but was somewhat higher and considerably more variable at steady state in renal dialysis patients ( $C_{max}$ :  $1.06 \pm 98.3$  ng/ml;  $AUC_{0-24h}$ :  $31.1 \pm 130.3$  ng.h/mL; Study No. LAM-IV-111). A human  $AUC_{0-24h}$  of 31.1 ng.h/ml (Study No. LAM-IV-111) was therefore used for safety margin calculations.

### Distribution

#### Plasma Protein Binding

The extent of protein binding in patients could not be accurately determined owing to the low levels of lanthanum measured in patients (approximately 0.5 ng/ml) and limitations of the assay sensitivity. With a lower limit of quantification of — ng/ml, it is theoretically impossible to quantify levels of protein binding above 90%.

However, *in vitro* studies using blood samples from mouse, rat, rabbit dogs and humans (Study No. V00117-LAM-III, SRU002) and an *ex vivo* study in rats (Study No. R00185-LAM-III, SRU059) suggest that lanthanum binds extensively to plasma proteins.

Results from *in vitro* analysis of lanthanum over a concentration range of 0.1 to 250 ng/mL to plasma proteins in human, mouse, rat, rabbit and dog plasma are presented in Table below.

### Binding of Lanthanum to Plasma Proteins

Study No. V00117-LAM-IIIIG, SRU/002	Mean Protein Binding (%)						
	Lanthanum Conc. (ng/mL)	0.1 <sup>a</sup>	0.5 <sup>a</sup>	2.5 <sup>a</sup>	10 <sup>a</sup>	50	250
Human Plasma Proteins		58.5	89.3	97.9	99.0	99.3	99.7
Mouse Plasma Proteins		47.4	88.8	97.6	99.4	99.8	99.9
Rat Plasma Proteins		22.5	85.2	97.5	99.3	99.9	>99.9
Rabbit Plasma Proteins		11.5	85.6	97.3	99.4	99.9	99.9
Dog Plasma Proteins		84.4	90.2	98.2	99.6	99.9	99.9
Human $\alpha_1$ -acid glycoprotein		73.3	75.5	95.0	98.8	99.8	99.8
Human Serum Albumen		95.3	96.8	98.7	99.6	99.9	99.9
Human Transferrin		40.0	57.4	96.9	99.4	99.7	>99.9

<sup>a</sup> Lower % binding at this concentration is an artefact of the assay quantification limit

Briefly, at 250 ng lanthanum/ml, which represents approximately 470 times the peak plasma concentration in man after a dose of 3 g elemental lanthanum, lanthanum was shown to be extensively bound to plasma proteins. *In vitro* binding ranged from 99.7 to >99.9% in human, mouse, rat, rabbit and dog plasma (Study No. V00117-LAM-IIIIG SRU/002). These results suggest a high capacity for protein binding of lanthanum in all species.

Lanthanum was similarly highly bound to human serum albumin (99.9%), transferrin (>99.9%) and  $\alpha_1$ -glycoprotein (99.8%) at physiological concentrations of the isolated proteins (Study No. V00117-LAM-IIIIG, SRU/002). However, these may not be the main carrier proteins *in vivo* due to competition from higher-affinity endogenous ligands. For example, the affinity of transferrin for  $Fe^{3+}$  exceeds its affinity for lanthanide ions and spectroscopic studies have failed to detect the presence of transferrin-lanthanide ion complexes in blood (Evans (f), 1990).

The results of these *in vitro* assays are also supported by *ex vivo* results in rats following i.v. dosing (Study No. R00185 LAM-IIIIG, SRU 059/013227). In the latter study, the distribution ratio of lanthanum in whole blood to plasma was calculated to be 0.55 (based on calculations using AUC values), suggesting that lanthanum is distributed almost entirely to the plasma compartment with negligible amounts bound to the cellular components of blood

Collectively, the results suggest that there is a high capacity for plasma protein binding of lanthanum in all species. According to Evans (Evans (c), 1990), the major ligand on proteins for lanthanide elements is the carboxyl group and, in many cases, calcium-binding sites are occupied with varying affinities relative to calcium (depending on the protein).

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### Tissue Concentrations of Lanthanum

The distribution of lanthanum in tissues following single and repeated oral dosing for 4-weeks was studied in rats and dogs.

Following administration of a single oral dose of lanthanum carbonate (600 mg/kg) in rats, greatest concentrations of lanthanum occurred in cecum and stomach followed by jejunum or colon (Study Nos. SPD/091 and SPD/093).

Similarly, after 4-weeks of repeated oral administration of lanthanum carbonate (1500 mg(salt)/kg) in rats (Study No. SPD0099) highest concentrations were observed in the gastrointestinal tract and associated lymph nodes. The median concentration of lanthanum in most tissues, including femur, teeth, liver, and kidneys, was  $<1 \mu\text{g/g}$  and was lower at 4 and 26 weeks of drug free withdrawal than at the end of dosing. Clearance of lanthanum was tissue dependent with the slowest elimination observed in femur, teeth, trachea, kidney, glandular stomach and mesenteric lymph nodes (Study No. SPD0099) (see Section 5.5.5.4.4).

In dogs, repeated daily oral administration of lanthanum carbonate (2000 mg(salt)/kg) for 4 weeks resulted in increased exposure ( $C_{\text{max}}$  and AUC) compared to that observed on day 1 of dosing (Study No. SPD0100-TK; see discussion above). At the end of the 4-week dosing period medium concentrations of lanthanum in most tissues were  $<1 \mu\text{g/g}$ . In particular, levels in the brain and other neuronal tissues were below the limit of detection for the assay ( $\sim 1 \mu\text{g/g}$ ). Cerebrospinal fluid concentrations were also close to the limit of quantification ( $\sim 1 \text{ ng/mL}$ ). Tissues which exhibited median levels  $>1 \mu\text{g/g}$  included stomach (corpus, fundus, and pylorus), rectum, teeth, duodenum, liver, and ileum (Study No. SPD0100-TK). Clearance appeared slow or absent from liver, femur, and fundic stomach.

#### Repeated Oral Dosing in Chronic Toxicity Studies

Additionally, tissue levels were determined in the long-term toxicity and carcinogenicity studies after dosing for 80 weeks in the mouse, 78 weeks in the rat and 52 weeks in the dog (Study Nos. SPD/88/C, SPD/87/C, and SPD/66/TK). For ease of discussion, tissues from these studies have been arbitrarily categorized into those that had generally low ( $\sim 1 \mu\text{g/g}$  or less), intermediate ( $10 \mu\text{g/g}$  or less) or high (greater than  $10 \mu\text{g/g}$ ) median lanthanum concentrations at the highest doses administered (See Table 5-5 below).

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Table 5-5

Species:	Range of median concentrations ( $\mu\text{g/g}$ wet tissue)		
	Mouse	Rat	Dog
Weeks dosed:	80 weeks	78 weeks	52 weeks
Maximum dose:	1500 mg (salt)/kg/day	1500 mg (salt)/kg/day	2000 mg (salt)/kg/day
Study Reference:	SPD/88C	SPD/87/C	SPD/66/TK
<b>LOW CONCENTRATION TISSUES (&lt;LLOQ to 1.0 <math>\mu\text{g/g}</math> wet tissue)</b>			
Aorta	0.481 - 0.482	1.09 - 1.48	nd
Adrenals	<0.642 - <1.46	0.216 - 0.544	nd
Brain	0.031 - 0.059	<0.007 - 0.059	0.046 - 0.056
Epididymides	0.087	0.089	nd
Eyes	0.145 - 0.146	0.094 - 0.428	nd
Heart	0.115 - 0.139	0.052 - 0.077	0.130 - 0.455
Kidney	0.197 - 0.291	0.784 - 1.54	0.503 - 0.659
Lacrimal glands	nd	0.099 - 0.140	nd
Mammary gland	0.141 - 0.234	0.707 - 0.782	nd
Ovaries	0.312	0.278	nd
Pituitary	<3.35 - <3.86	0.961 - 1.85	nd
Prostate	<0.175	0.177	0.086
Salivary gland	0.185 - 0.193	0.079 - 0.144	0.171 - 0.227
Sciatic nerve	<0.460 - <0.532	0.038 - 0.267	nd
Seminal vesicles	0.050	0.097	nd
Skeletal muscle	0.057 - 0.071	0.023 - 0.076	0.787 - 0.905
Spinal cord	3.05 - 6.90 †	2.88 - 3.10 †	0.243 - 0.313
Spleen	0.820 - 1.43	0.892 - 2.12	0.086 - 0.115
Submandibular LN	0.992 - 1.06	0.694 - 1.09	nd
Testes	0.113	0.201	0.798
Thymus	<0.320 - 0.226	0.034 - 0.230	0.197 - 0.814
Thyroids	<1.524 - 1.475	0.285 - 0.418	nd
Urinary bladder	<0.197 - 0.292	0.087 - 0.135	0.374 - 1.95
Uterus	0.203	0.126	3.87
Vagina	0.313	0.102	nd
<b>INTERMEDIATE CONCENTRATION TISSUES (&gt;1.0 to 10 <math>\mu\text{g/g}</math> wet tissue)</b>			
Bone marrow	nd	nd	1.06 - 8.13
Femur - plate	5.63 - 8.15	3.39 - 4.45	3.23 - 3.89
Femur - shaft	3.60 - 5.08	2.23 - 4.24	1.84 - 2.51
Liver	2.34 - 3.59	1.26 - 1.90	7.25 - 11.1
Sternum	3.49 - 6.71	2.48 - 2.78	nd

Table 5-5 (Continued)

HIGH CONCENTRATION TISSUES (10µg/g wet tissue)			
Cecum	716-2,337	467-534	nd
Colon	222 - 506	128 - 158	24.8 - 122
Duodenum	79.8 - 190	96.4 - 123	11.4 - 13.1
Ileum	370 - 3,696	989 - 1,354	nd
Jejunum	38.4 - 87.2	537 - 583	nd
Mesenteric LN	24.0 - 53.8	86.1 - 767	1.37 - 1.54
Oesophagus	22.4 - 23.6	174 - 214	nd
Rectum	23.3 - 90.9	6.06 - 19.5	35.1 - 60.6
Stomach	2,024 - 2,189	578 - 827	248 - 349
NOT CATEGORIZED (results possibly influenced by contamination)			
Fat	nd	0.066 - 0.839	1.10 - 6.56
Lungs	0.207 - 0.214	0.197 - 105	2.58 - 4.00
Pancreas	27.0 - 27.5	1.16 - 4.98	0.121 - 0.175
Skin	0.423 - 0.612	2.83 - 6.26	nd
Tongue	0.320 - 1.42	4.24 - 4.45	nd
Trachea	60.4 - 181	134 - 177	nd

nd = not determined

† comparison with intravenous data suggests higher levels due to contamination from surrounding bone

#### Tissues with Low Concentrations

Briefly the data in Table 5-5 above show that lanthanum concentrations were low (defined as ~1µg/g wet tissue or less), in the majority of tissues in mice, rats and dogs after chronic oral administration, even with chronic treatment at maximal doses. Tissues that generally had low median concentrations (1 µg/g wet tissue or less), irrespective of route, dose or treatment duration were: brain, epididymides, mammary gland, ovaries, pituitary, prostate, sciatic nerve, seminal vesicles, skeletal muscle, thymus and thyroids. Additionally, adrenals, aorta, heart, kidney, lacrimal glands, salivary glands, spleen, submandibular lymph node, urinary bladder, uterus and vagina generally had low concentrations in oral studies, although concentrations above 1 µg/g were observed after intravenous dosing. Other tissues that may have fallen into this category include the lungs/trachea, pancreas and skin, all of which have a high potential for contamination from misplaced dose, surrounding tissues or feces.

Considering the CNS toxicity (encephalopathy) that has been associated with exposure of renal dialysis patients to aluminum, distribution of lanthanum to the brain and cerebrospinal fluid is discussed in detail below.

#### Brain, Cerebrospinal Fluid and Blood: Tissue Barriers

Table 5-6 below shows the distribution of lanthanum to brain and cerebrospinal fluid. As is shown, tissue levels of lanthanum in brain and cerebrospinal fluid (CSF), were consistently below or around the lower limit of quantification for the assay (LLoQ = ~ 1 ng/g for brain tissue and ~ 1 ng/mL for CSF), except after very high intravenous doses.

**Table 5-6 Brain and Cerebrospinal Fluid Lanthanum Concentrations at Maximum Tolerated Doses in Animals**

Species (Report)	Route	Duration of Dosing	Dose mg(salt)/kg/day	Range of Medians ( $\mu\text{g/g}$ wet tissue)	Overall Range ( $\mu\text{g/g}$ wet tissue)
<b>Brain</b>					
Rat (SPD0099)	po	4 weeks	1500	<0.014	<0.014 – 0.025
Rat (SPD/87/C)	po	78 weeks	1500	<0.007 – 0.059	<0.007 – 0.123
Rat (SPD0102)	iv	4 weeks	0.3	<0.007 – 0.009	<0.007 – 0.013
Rat - Uremic (LAN-02)	po	13 weeks	1000 (NRF)	0.018	<0.007 – 0.255
			1000 (CRF)	0.023	0.010 – 0.072
			2000 (NRF)	0.018	<0.008 – 0.025
			2000 (CRF)	0.053	0.030 – 0.144
Dog (SPD0100)	po	4 weeks	2000	<0.011 – 0.016	<0.011 – 0.605
Dog (SPD/66/TK)	po	52 weeks	2000	0.045 – 0.046	0.016 – 0.139
Dog (SPD0104)	iv	4 weeks	1.0	0.035 – 0.162	0.032 – 0.224
Mouse (SPD/88/C)	po	80 weeks	1500	0.031 – 0.059	0.015 – 0.369
<b>Cerebrospinal Fluid (CSF)</b>					
Dog (SPD0100)	po	4 weeks	2000	-	<0.05 – 0.190
Dog (SPD0104)	iv	4 weeks	0.05 1.0	-	<0.05 – 0.07 0.07 – 0.860

CRF – chronic renal failure  
NRF – normal renal function

Table 5-6 above shows that even at the maximum tolerated intravenous dose in dogs (1mg (salt)/kg/day), which was associated with peak plasma lanthanum exposure more than 20,000 times higher than in patients, the range of median concentrations of lanthanum in cerebellum, cerebrum and mid brain was 0.035 to 0.162  $\mu\text{g/g}$  wet tissue and 0.22 to 0.85 ng/ml in CSF. Concentrations in the CSF were more than 3 orders of magnitude lower than  $C_{\text{min}}$  plasma concentrations (plasma: CSF ratio 2954 to 7459) (Study No. SPD0104). Although, lanthanum levels in spinal cord were higher in mice and rats following chronic oral dosing compared to dogs, comparison of oral and intravenous data, as well as data from other neuronal tissue, suggests that spinal cord has a low potential for accumulation of lanthanum, and that contamination from surrounding tissues may be responsible for elevated concentrations in some oral studies.

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Tissues Containing Intermediate (10 µg/g or less) or High (greater than 10 µg/g) Levels of Lanthanum

Lanthanum concentrations in bone, liver and gastrointestinal tract showed the clearest relationship to dose, route and treatment duration. Median concentrations in bone and liver generally remained intermediate (defined as 10 µg/g wet tissue or less), even after chronic oral administration at maximal doses, although they exceeded this concentration after intravenous administration. The highest concentrations after chronic oral administration occurred in the gastrointestinal tract where, at maximal doses, they exceeded 100 or even 1000 µg/g wet tissue. Although, it is likely that residual lanthanum from the lumen of the gastrointestinal tract contributed in part to these high levels, relatively high levels in glandular stomach following intravenous dosing suggest the possibility of selective distribution in this tissue. Given the patterns of distribution in bone, liver, and gastrointestinal tract; these tissues are discussed individually below in relation to toxicological findings observed in each tissue.

Bone

Bone lanthanum concentrations measured at maximum doses in animal studies are summarized in Table 5-7 below in comparison with the concentrations measured in dialysis patients receiving lanthanum carbonate.

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**Table 5-7 Femur Lanthanum Concentrations at Maximum Tolerated Doses in Animals**

Species (Report)	Route	Duration of Dosing	Dose (mg (salt)/kg/day)	Range of Medians ( $\mu\text{g/g}$ wet tissue)	Overall Range ( $\mu\text{g/g}$ wet tissue)
Man (dialysis patient) (LAM-IV- 303)	po	Up to 52 weeks	Up to 143 <sup>a</sup>	1.8	
Rat (SPD0099)	po	4 weeks	1500	0.35 - 0.58	
Rat (SPD/87/C)	po	78 weeks	1500	2.2 - 4.4	
Rat (SPD0102)	iv	4 weeks	0.3	6.9 - 8.2	
Rat - Uremic (LAN-02)	po	13 weeks	2000 (NRF) 2000 (CRF)	1.2 1.6 <sup>Ost</sup>	
Dog (SPD0100)	po	4 weeks	2000	0.33 - 0.77	
Dog (SPD/66/TK)	po	26 weeks	2000	2.0 - 3.3	
Dog (SPD/66/TK)	po	52 weeks	2000	1.8 - 3.9	
Dog (SPD0104)	iv	4 weeks	1.0	26.0 - 54.5	
Mouse (SPD/88/C)	po	80 weeks	1500	3.6 - 8.1	

CRF - chronic renal failure

NRF - normal renal function

Ost - associated with osteomalacia

a - range of patient doses 500 to 3750mg/day, ie 19 to 143 mg (salt)/kg/day for a 50kg patient.

The data in Table 5-7 above show that highest levels of femur lanthanum concentrations were observed following 4-weeks of repeated daily intravenous dosing, with median concentrations ranging from 6.9 - 8.2  $\mu\text{g/g}$  wet tissue in rats (Study No. SPD0102) and from 26.0 - 54.5  $\mu\text{g/g}$  wet tissue in dogs (Study No. SPD0104).

Chronic repeated oral administration of lanthanum carbonate at maximal doses of 1500 and 2000 mg(salt)/kg/day, in rats and dogs, respectively, was associated with an increased range of median concentrations of lanthanum in femur compared to respective values observed following 4-weeks of dosing. In dogs, bone lanthanum concentrations were comparable between 26 and 52 weeks, suggesting the achievement of steady state and the lack of continued accumulation in this tissue after 26 weeks of repeated oral administration (Study Nos. SPD0100 and SPD/66/TK).

Bone concentrations in long - term animal studies exceeded those measured in renal dialysis patients in bone biopsies taken after one year of lanthanum treatment.

A study using [ ]

[ ] investigated any morphological localization of lanthanum in the bone of rats treated

with 1500 mg(salt)/kg/day for 4 weeks (Study A00068-LAM-IIIIG). No association with specific morphological features such as the growth plate were observed, but there was a general co-localisation of lanthanum with calcium.

Median femur concentrations in renally impaired rats dosed for 12 weeks were up to 1.6 µg/g wet weight compared to 4.4 µg/g wet weight in rats dosed for 78 weeks (Study No. SPD/87/C), 8.1 µg/g wet weight in mice dosed for 80 weeks (Study No. SPD/88/C) and 3.9 µg/g wet weight in dogs dosed for 52 weeks (Study No. SPD/66/TK).

In renally impaired rats, some of which developed osteomalacia (Study Nos. LAN-01 and LAN-02), bone concentrations were substantially below those measured in longer-term studies in normal animals, in which no bone toxicity was observed. In addition, there was no substantial rise in bone lanthanum concentrations in the renally impaired, compared to renally competent, rats. Collectively these data suggest that the lesions observed in renally impaired rats were unlikely to be a direct response to the lanthanum present in bone.

#### Liver

Liver lanthanum concentrations measured at maximum doses in animal studies are summarized in Table 5-8 below.

**Table 5-8 Liver Lanthanum Concentrations at Maximum Tolerated Doses in Animals**

Species (Report)	Route	Duration of Dosing	Dose (mg (salt)/kg/day)	Range of Medians (µg/g wet tissue)	Overall Range (µg/g wet tissue)
Rat (SPD0099)	po	4 weeks	1500	0.61 - 0.86	
Rat (SPD/87/C)	po	78 weeks	1500	1.3 - 1.9	
Rat (SPD0102)	iv	4 weeks	0.3	53.2 - 82.4	
Rat - Uremic (LAN-02)	po	13 weeks	2000 (NRF) 2000 (CRF)	0.98 3.5	
Dog (SPD0100)	po	4 weeks	2000	1.5	
Dog (SPD/66/TK)	po	26 weeks	2000	6.2 - 7.4	
Dog (SPD/66/TK)	po	52 weeks	2000	7.3 - 11.1	
Dog (SPD0104)	iv	4 weeks	1.0 0.05	408 - 453 <sup>Hep</sup> 18.0 - 20.7	
Mouse (SPD/88/C)	po	80 weeks	1500	2.4 - 3.6	

CRF - chronic renal failure  
NRF - normal renal function  
Hep - associated with hepatitis

Median liver lanthanum concentrations after oral administration generally remained below 10 µg/g wet tissue in all species (Table 5-8). Concentrations increased between 4 and 26 weeks of treatment in dogs, but there was little further increase between 26 and 52 weeks, indicating achievement of steady state after approximately 26 weeks. In intravenous studies, extremely high concentrations of 309 to 535 µg/g wet tissue were associated with hepatitis in dogs (Study No. SPD0104). No toxicity occurred at concentrations of 17.8 to 23.7 µg/g (0.05 mg (salt)/kg/day) in the dog, or 41.7 to 92.6 µg/g (0.3 mg (salt)/kg/day) in the rat (Study Nos. SPD0104 and SPD0102).

In contrast to bone, renally impaired rats had significantly higher liver lanthanum concentrations compared to sham-operated control rats (Study No. LAN-02) although they were 100 times less than those associated with toxicity in the dog.

#### Gastrointestinal Tract

Stomach lanthanum concentrations measured at maximum doses in animal studies are summarized in Table 5-9 below.

**Table 5-9 Stomach Lanthanum Concentrations in Animals**

Species (Report)	Route	Duration of Dosing	Dose (mg (salt)/kg/day)	Range of Medians (µg/g wet tissue)	Overall Range (µg/g wet tissue)
Rat (SPD0099)	po	4 weeks	1500	46.3 - 90.1	
Rat (SPD/87/C)	po	78 weeks	1500	82.7 - 113 558 - 827 <sup>inf</sup>	
Rat (SPD0102)	iv	4 weeks	0.3	1.4 - 6.0	
Dog (SPD0100)	po	4 weeks	2000	7.2 - 57.6	
Dog (SPD/66/IK)	po	26 weeks	2000	8.9 - 78.3	
Dog (SPD/66/TK)	po	52 weeks	2000	248 - 349	
Dog (SPD0104)	iv	4 weeks	1.0	46.8 - 49.9	
Mouse (SPD/88/C)	po	80 weeks	1500	69.7 - 83.4 2024 - 2189 <sup>Inf/Ade</sup>	

Inf - associated with epithelial proliferation and inflammation

Ade - associated with adenoma formation

High lanthanum concentrations occurred throughout the gastrointestinal tract in all species after chronic oral dosing, and were generally highest in the stomach. In rodents, glandular stomach had higher concentrations than non-glandular stomach, for example, after 4 weeks of gavage dosing in rats, median concentrations were 46.3 to 90.1 µg/g and 3.8 to 19.8 µg/g in glandular and non-glandular stomach, respectively (Study No. SPD0099). The highest median wet weight stomach concentrations achieved in long-

term studies were 2189  $\mu\text{g/g}$  in mouse (Study No. SPD/88/C), 827  $\mu\text{g/g}$  in rat (Study No. SPD/87/C) and 349  $\mu\text{g/g}$  in dog (Study No. SPD/66/TK).

In repeat-dose studies, these very high concentrations were associated with gastric mucosal and submucosal inflammation as well as epithelial cell hyperplasia in rodents (Study Nos. SPD/87/C and SPD/88/C). In the mouse carcinogenicity study, there was progression to benign neoplasia (adenomas) in some animals (Study No. SPD/88/C). No significant gastric pathology occurred after oral doses of 100 mg (salt)/kg/day, at which median wet weight stomach concentrations were 69.7 to 83.4  $\mu\text{g/g}$  in mice and 82.7 to 113  $\mu\text{g/g}$  in rats. No gastric pathology occurred in dogs treated chronically with 2000 mg (salt)/kg/day, even though median stomach lanthanum concentrations of up to 248  $\mu\text{g/g}$  to 349  $\mu\text{g/g}$  were recorded (Study No. SPD/66/TK).

Electron microscopy of the full thickness of the stomach wall was conducted on samples from mice, rats and dogs taken at the end of the chronic toxicity and carcinogenicity studies (Study No. X00223-LAM-IIA). This revealed electron dense inclusions in occasional macrophages, which were assumed to be lanthanum. No degenerative changes were present in the macrophages and no inclusions were found in other cell types.

The better gastric tolerance in dogs may be related to several factors. Lanthanum carbonate is better tolerated if administered with food in man and dogs (Study No. SPD/44/96 and Study No. SPD0100). Rodents are nocturnal feeders and, as they were routinely dosed during the day, received lanthanum into an empty or part-empty stomach. Furthermore, dogs are able to vomit to expel an irritant material, whereas rodents are not. The combination of greater direct contact of lanthanum with the stomach wall and an inability to expel lanthanum is a likely explanation for the specific occurrence of histopathological changes in the rodent stomach.

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### Clearance of Lanthanum from Tissues

The clearance of lanthanum from tissues was studied in rats and dogs dosed for 4 weeks and allowed a further 4-week or 26-week period off-dose (Study Nos. SPD0099 and SPD0100).

In the 4-week tissue distribution and clearance study in rats (Study No. SPD 0099-TK), after 4 weeks of oral administration of the 1500 mg(salt)/kg dose, tissues in male and female rats that showed highest concentrations of lanthanum (median levels of 134 to 90060 ng/g) included: stomach (glandular), cecum, colon, rectum, duodenum, stomach (nonglandular), mesenteric lymph nodes, teeth, pituitary, ileum, liver, jejunum, femur, sternum, thyroids, trachea, skin, esophagus, kidney. The patterns of distribution and clearance of lanthanum in male and female rats were generally similar, with no significant sex-related differences observed.

Rates of clearance in the rat differed greatly between tissues. After 4 weeks off-dose, more than 99% of drug was cleared from the lower bowel (cecum, colon and rectum), more than 90% from the non-glandular stomach and ileum, and more than 75% from the liver. In contrast, there was little or no appreciable clearance from glandular stomach and upper small intestine (duodenum and jejunum) over this period. Clearance from bone, cartilage and teeth was slow, with more than 66% (sternum), 82% (femur), 60% (trachea) and 75% (teeth) of drug still present in these tissues after 4 weeks off-dose. Tissues in rats that exhibited >100 ng/g and that retained >50% of the levels observed at the end of the dosing period included: stomach (glandular), duodenum, stomach (nonglandular), mesenteric lymph nodes, teeth, pituitary, liver, jejunum, femur, sternum, thyroids, trachea, kidney, and submandibular lymph node.

At the end of 26-weeks off drug, tissues in rats that exhibited >100 ng/g and that retained >50% of the levels observed at the end of the dosing period included: stomach (glandular), duodenum, mesenteric lymph nodes, jejunum, femur, sternum, and trachea. Pituitary, thyroids and nonglandular stomach (females) also exhibited levels >100 ng/g at the end of the 26-week off drug period, but were  $\leq$  50% of the levels observed at the end of dosing (Study No. SPD 0099-TK).

In the 4-week tissue distribution and clearance study in dogs (Study No. SPD 0100), after 4 weeks of oral administration of the 2000 mg(salt)/kg dose, tissues in male and female dogs with highest concentrations of lanthanum (median levels of 115 to 57623 ng/g) included: stomach (corpus), stomach (fundus), stomach (pylorus), rectum, duodenum, submandibular lymph nodes, liver, ileum, teeth, femur (growth plate), mesenteric

lymphnodes, colon, lungs, testes, femur (shaft), caecum, thyroids, skin, and lacrimal glands.

The dog showed a similar pattern of tissue clearance compared to the rat, except that drug was cleared more rapidly from upper small intestine (duodenum) and teeth, and more slowly from liver, with respectively, 91%, 60% and 29% of drug cleared after 4 weeks off-dose. More than 95% of drug was cleared from colon and rectal tissue, more than 75% from corpus stomach and ileum, and 66% was cleared from pyloric stomach after 4 weeks. In contrast, 83 to 100% of drug was retained in femur and fundic stomach over the same period. Tissues in both sexes that exhibited >100 ng/g and that retained >50% of the levels observed at the end of the dosing period included: stomach (fundus), submandibular lymph nodes, liver, femur (growth plate), lungs, femur (shaft), cecum, thyroids, skin. Stomach (corpus), stomach (pylorus), duodenum, ileum, teeth, mesenteric lymph nodes also exhibited levels >100 ng/g, but were less than 50% of the levels observed at the end of dosing.

At the end of 26-weeks off drug, tissues in dogs, that exhibited >100 ng/g and that retained >50% of the levels observed at the end of the dosing period included: stomach (fundus), liver, and femur (growth plate and shaft). Stomach (corpus), stomach (pylorus), teeth ileum and lungs also exhibited levels of lanthanum that were >100 ng/g, but were less than 50% of the levels observed at the end of dosing (Study No. SPD0100).

These studies demonstrate that lanthanum is cleared steadily from most tissues, but very slowly from stomach, parts of the upper small intestine, and bone. In bone, the slow clearance may reflect binding to extracellular bone mineral and organic matrix (Evans (f), 1990). There appear to be no adverse toxicological consequences of the retention of lanthanum in tissues other than at the very high concentrations that were achieved in the upper gastrointestinal tract of rodents after oral administration and in the liver of dogs after intravenous administration.

### Metabolism

Traditional drug metabolism studies were unnecessary for lanthanum carbonate because of its elemental and inorganic form. Moreover, extensive published literature indicates that biochemical interactions with lanthanides are almost exclusively ionic (Evans (c), (d), 1990) and therefore the formation of covalently bound organic metabolites *in vivo* is extremely unlikely.

Lanthanide ions have an overwhelming preference for oxygen donor atoms and therefore the most common biological ligands are carboxyl, phosphate and hydroxyl groups. As the carbonate, phosphate and hydroxide salts of lanthanum are insoluble at physiological pH, chemical considerations alone might predict that lanthanum would precipitate out in plasma. There is abundant evidence that this does not occur, because of the rapid formation of soluble complexes with the organic ligands present in plasma, including proteins (see Section 5.5.5.4.1, Plasma Protein Binding), amino acids, nucleotides,

phospholipids and numerous other endogenous biochemicals containing oxygen donor atoms.

Within tissues, lanthanum is likely to reside predominantly in the extracellular compartment because of its inability to pass through the plasma membrane of healthy cells. Within this compartment, lanthanum has been shown to be associated with the outer surface of cell membranes. This is consistent with the high affinity of lanthanides for ionic binding to surface ligands, including membrane proteins, the sialic acid residues of glycoproteins and phospholipid bilayers.

In an *in vitro* study on the effects of lanthanum carbonate on a range of isoenzyme-specific cytochrome P450 substrates in human liver microsomes, lanthanum carbonate did not significantly inhibit the metabolism of any of the isoenzyme-specific cytochrome P450 substrates in human liver microsomes tested, suggesting a low potential to affect the metabolism of other drugs (Study No. SRU/006). Consistent with this, lanthanum carbonate did not prolong hexobarbital sleeping time in mice (Study No. SRU/004).

### Excretion

As expected for a minimally absorbed drug, the great majority of an oral dose of lanthanum carbonate was excreted in the feces. In rats, 99.3% of the dose was recovered in feces (Study No. SPD/60/W) and in the dog, 93.4% was recovered either in feces or vomit (Study No. SPD/78/W) within 7 days. Recovery of the dose from urine was very low, representing less than 0.004% in rats and 1.14% in dogs over the same time period. In a separate study, 0.007% of the dose was eliminated in the urine in 48 hours in rats (Study No. SRU/001). These values probably over-estimate true urinary excretion, because in animal studies there is a strong likelihood that urine is contaminated with trace amounts of feces owing to collection methods. In man, urinary excretion in healthy subjects represented 0.00031% of the administered dose (Study No. LAM-IV-109).

In end-stage renal disease patients, renal function is negligible, and it is important to consider other routes of excretion for the small fraction of the dose that is systemically absorbed. The lack of appreciable concentration of lanthanum in plasma and tissues in renally impaired rats compared to rats with normal renal function (LAN-01 and LAN-02) and in plasma over time in chronic renal failure patients (Study No. LAM-IV-301) indicates significant excretion via non-renal routes.

This is exemplified by a study in bile-duct cannulated rats following administration of a single intravenous dose of the more soluble lanthanum chloride salt (Study No. R00185-LAM-IIIIG, SRU 059/013227). In this study, the mean total recovery over a 42-day period, was 76.39% of the administered dose, and nearly 97% (74.13% of the administered dose) of this total was recovered in the feces. In a separate phase of this investigation, conducted in bile duct-cannulated rats, 79% of the total dose recovered over the first 120 hours after intravenous administration, was recovered in the bile. These findings show that biliary excretion is the predominate route of elimination for circulating lanthanum in rats (R00185-LAM-IIIIG, SRU 059/013227).

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In another study in bile duct-cannulated rats, 0.00007% of an orally administered dose was eliminated in bile (Study No. R00118-LAM-IIIIG, SRU/001). If it is assumed, based on a comparison of the oral and intravenous pharmacokinetics of lanthanum, that only 0.0007% of an oral dose is absorbed in the rat, then biliary excretion is responsible for eliminating approximately 10% of the absorbed dose over a period of 48 hours. Biliary excretion has been demonstrated also for other lanthanide elements in the rat.

Some absorbed lanthanum is also excreted directly across the gastrointestinal tract wall into the lumen, although in rats the proportion excreted via this route was small and difficult to quantify relative to that excreted in the bile (R00185-LAM-IIIIG, SRU 059/013227).

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#### IV. GENERAL TOXICOLOGY

##### Acute Oral Toxicity Studies in Rats and Mice

Single dose oral (gavage) administration of lanthanum carbonate to Sprague-Dawley rats or CD-1 mice at doses up to 2000 mg/kg resulted in no treatment-related mortalities, clinical signs or macroscopic findings (14-day observation period). Based on these findings, the maximum tolerated dose was determined to be greater than 2000 mg/kg.

##### Chronic Oral Toxicity Studies in Rats and Mice

##### 1. Twenty-six Week Oral (Gavage) Toxicity Study in the Rat with a Four Week Treatment-free Period

**Key findings:** Dose-related increased incidences of stomach lesions (hyperplasia of fundus epithelium and mucus cells, sub-mucosal inflammation and hyperplasia at the limiting ridge) were seen in treated males and females, especially at the 600 and 2000 mg/kg/day dose levels.

**Study number:** SPD/81/C

**Volume #:** 1.13 – 1.14

**Conducting laboratory and location:** ☐

**Date of study initiation:** December 16, 1996

**GLP compliance:** yes

**QA report:** yes

**Drug lot# and purity:** Lanthanum carbonate lot #s 6268/960502 (12-16-1996 to 1-26-1997) and B1066-960802 (1-27-1997 to 6-20-1997), purity – ☐ total metal impurities – ☐ (specifications – ☐ total metal impurities – not more than ~ 1 ppm)

**Formulation:** formulated daily as a suspension in 0.5% aqueous carboxymethyl-cellulose)

##### **Animals:**

*Species/strain:* Rat, CD (SD) BR strain (VAF plus) obtained from ☐

*#/sex/group:* 20 + 5\* (\*5 rats/sex/group were used for the recovery phase.)

*Satellite groups used for toxicokinetics:* All dose groups (6 rats/sex/group)

*Age:* 5 weeks

*Weight:* males – 135 to 205 g

females – 115 to 161 g

Animals were housed in groups of five, by sex, in grid-bottomed stainless steel

cages suspended over cardboard-lined trays. A pelleted diet — rat and mouse maintenance diet — and tap water were available *ad libitum*.

**Dosing:**

*Doses administered:* 0 (vehicle), 100, 600 and 2000 mg/kg/day – once daily

*Route/volume:* Oral gavage/10 ml/kg

**Observations and measurements:**

*Clinical signs:* daily

*Mortality:* twice daily

*Body weights:* pre-dose, on the first day of dosing and then weekly thereafter

*Food consumption:* pre-dose, and then weekly

*Ophthalmoscopy:* pre-dose (all rats from main study groups), and weeks 13 and 26 (control and high dose groups only)

*Clinical pathology:* weeks 13 and 26 (parameters evaluated – RBC, WBC (total and differential), platelet and reticulocyte counts, hemoglobin, PCV, MCH, MCHC, MCV, cell morphology, prothrombin time, activated partial thromboplastin time, fibrinogen, alkaline phosphatases, aminotransferases, albumin, globulin, A/G ratio, BUN, cholesterol, triglycerides, creatinine, glucose, total bilirubin, total protein, sodium, potassium, calcium, inorganic phosphorus and chloride. Ten main study rats/sex/group. Blood was also collected during week 30 for aminotransferase evaluations in recovery phase animals (control and high dose females only).

*Urinalysis:* weeks 13 and 26. Urine samples were obtained from 10 rats/sex/group (main study). In addition, urine samples were obtained from the same animals during weeks 12 and 25 for inorganic phosphate, calcium and volume determinations. From recovery phase animals (males and females from all groups), urine samples were collected during week 29 for inorganic phosphate, calcium (females only) and volume determinations.

*Postmortem evaluation:* At necropsy, blood samples were collected for PTH and calcitonin level determinations and adrenals, brain, heart, kidneys, liver, lungs, ovaries, pituitary, prostate, salivary glands, seminal vesicles, spleen, testes, thymus, thyroids and uterus (all animals) were weighed. The following tissues were preserved for histopathological examination: All tissues from the control and high dose animals that were killed at the end of the study (including recovery animals), tissues of all animals that died or were killed in extremis, stomachs from all animals of all groups, and all gross lesions were examined microscopically. Since microscopic examination of HD animals revealed treatment-related findings in the lungs, spleen and kidneys, these tissues from the lower groups were also examined.

## Tissues Preserved for Histopathological Examination

adrenals	pituitary
aorta	prostate
blood smear*	rectum
brain (3 sections)	salivary gland
caecum	sciatic nerve
colon	seminal vesicles
duodenum	site of mammary gland
epididymides	skeletal muscle
eyes (incl. optic nerves)	skin
femur (incl. marrow)	spinal cord (3 levels)
harderian glands	spleen
heart	sternum
ileum	stomach
jejunum	submandibular lymph node
kidneys	testes
lacrimal glands	thymus
liver	thyroids (incl. parathyroids)
lungs (incl. mainstem bronchi)	tongue
mesenteric lymph node	trachea
oesophagus	urinary bladder
ovaries	uterus (incl. cervix)
pancreas	vagina
all gross lesions	

*Toxicokinetics:* on the first day of dosing, and on one occasion each during weeks 13 and 26 (at 2 and 20 hours post-dose; 3 rats/sex/group/time-point). [Note: After completion of blood collection during week 26, some of the satellite animals were discarded without necropsy, but others were used to collect tissues (liver, brain, femur and kidneys) for lanthanum level determinations. The femur samples were also used for total calcium and phosphorus level analyses.]

*Statistical analysis:* Analysis of variance (ANOVA) was performed on all data. Residuals from this preliminary analysis were examined for heterogeneity of variance using Levene's test. If the Levene's test was significant at the 1% level, then a non-parametric analysis using Kruskal-Wallis ANOVA followed by Shirley's non-parametric version of Williams' test was performed. If the Levene's test showed no significance, then pairwise tests of all treated groups versus control were performed using William's test.

### Results:

*Mortality:* There were no deaths considered to be related to the toxicity of the test drug. Most of the deaths were attributed to dosing injury. The mortality data are presented below.

Dose (mg/kg/day)	Sex	Day of death / euthanesia	Cause of death
-----			
Main study			
100	M	31	dosing error
100	M	144	ND (died at dosing)
2000	M	126	dosing error
0	F	113	dosing error
100	F	25	ND (cannibalized)
600	F	123	accidental death
TK study			
2000	M	22	ND
100	F	8	ND

ND = not determined

*Clinical signs:* Throughout the treatment period, white feces were noted in high dose animals and, on a few occasions, in some mid dose animals. No other clinical signs were observed.

*Body weights:* Body weight data are presented in Figures 1 and 2. During most of the treatment period, slightly higher body weight gains, compared to controls, were noted for high dose main study females. For satellite animals, increased

body weight gains were seen in the mid and high dose female groups during the first 14 weeks of the study. During the recovery period, higher than control body weight gains were observed for both high dose males and females. Body weight gains in other treated groups were similar to that of controls.

*Food consumption:* Food consumption of high dose females was generally higher than control, especially during the first 13 weeks of the study. Food consumption values in other treated groups (main and satellite studies) were similar to control.

*Ophthalmoscopy:* There were no treatment-related findings.

*Hematology:* Slight reductions in hemoglobin and packed cell volume values (not dose-dependent) were seen in mid and high dose males at 13 weeks, but not at 26 weeks. Reductions in MCV values were noted in mid and high dose males at week 13, and in all treated female groups at weeks 13 and 26. MCH levels were reduced in high dose males and females at both time points.

*Clinical chemistry:* Slightly higher than control inorganic phosphate levels, were seen in high dose males at weeks 13 and 26.

*Urinalysis:* A dose-related increase in urine pH was noted in all treated male groups and in the high dose female group at weeks 13 and 26. At week 13, there was a high incidence of blood pigments in the urine from high dose males, which was associated with the appearance of RBCs in a few animals. In females, the above effects were less pronounced. Increased incidence of leukocytes was noted in all treated male groups and in mid and high dose female groups. Females treated at 600 or 2000 mg/kg/day had increased urine volumes with reduced specific gravities. There were no significant urinary findings after the recovery period.

At week 25, reduced inorganic phosphate levels in urine were seen in all treated male and female groups (dose-related). A non-dose related reduction in urine calcium levels was noted in treated female groups. After the recovery period, reduced calcium and phosphorus levels were still observed in mid and high dose females, although differences from control did not achieve statistical significance.

*Parathyroid hormone (PTH) and calcitonin levels:* After 26 weeks of treatment, higher than control PTH and calcitonin levels were observed in high dose males and high dose females, respectively.

Figure 1. Group Mean Bodyweights (g) - Males

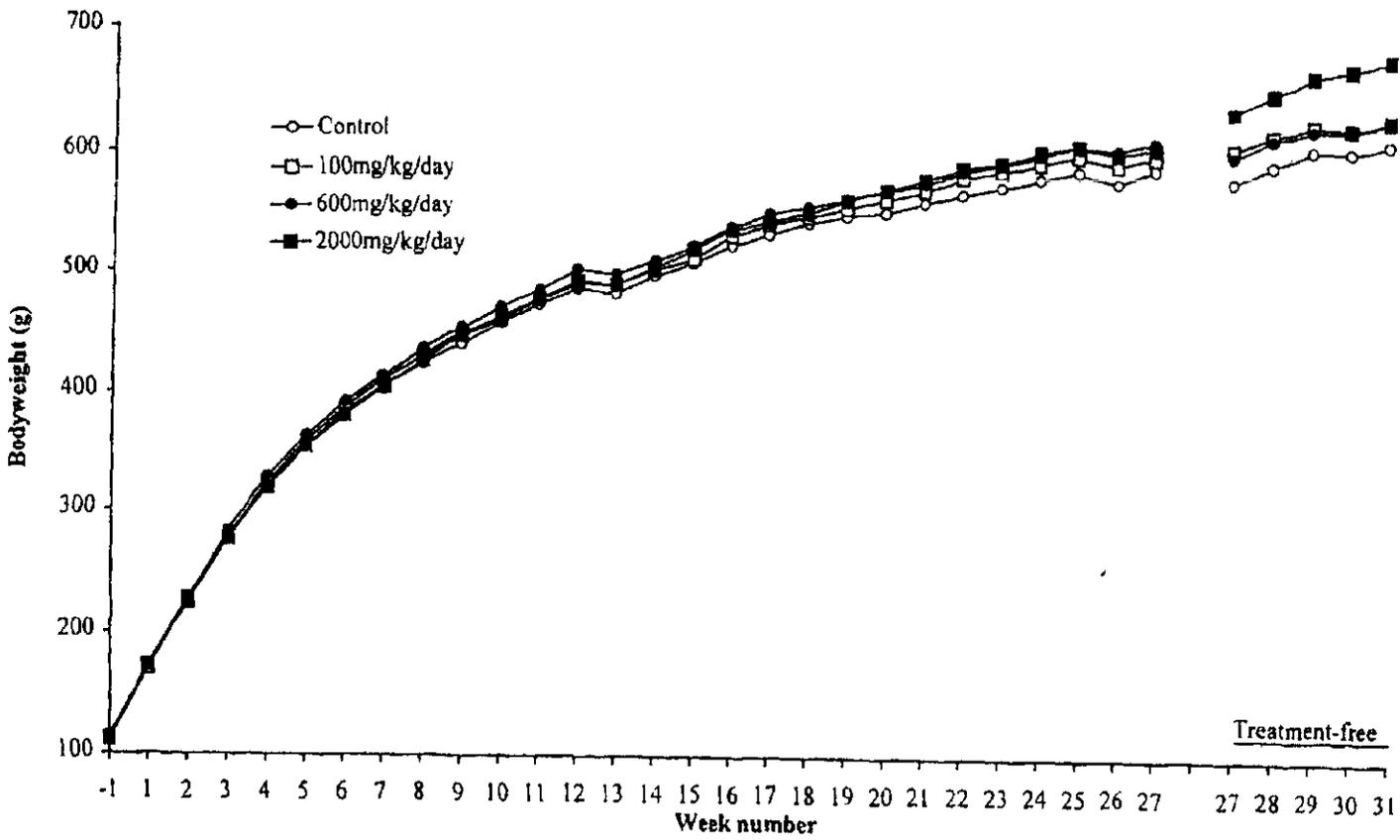
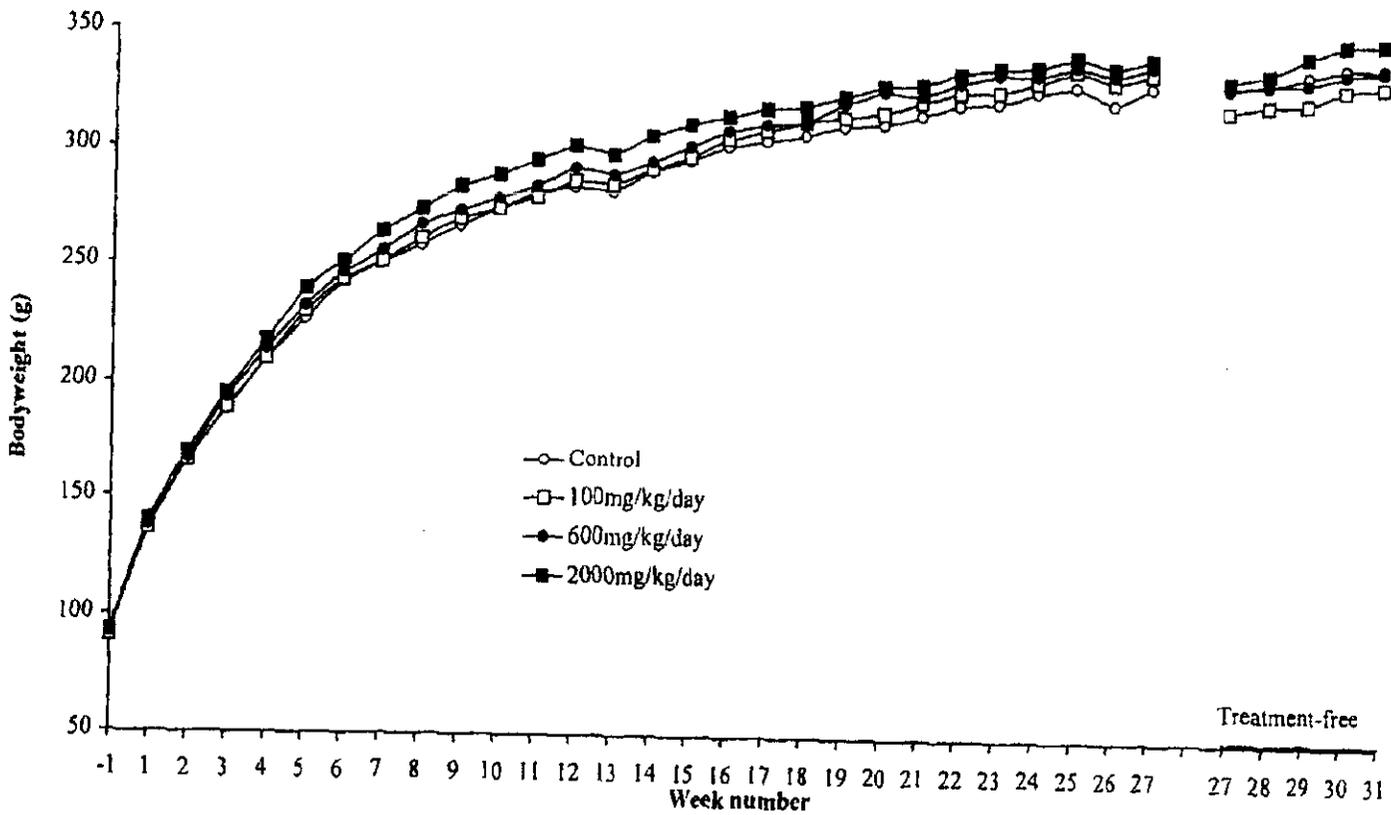


Figure 2. Group Mean Body Weights – Females



*Organ weights:* Treatment-related reductions in absolute (8-9%) and relative (9-13%) adrenal weights were observed in mid and high dose females. Non dose-related increases in absolute (8-17%) and relative (25%) thyroid weights were seen in treated males.

*Gross pathology:* Macroscopically, thickened pale colored areas in the glandular region of the stomach were seen in a large number of high dose animals and also in some mid dose animals. Two high dose females had prominent limiting ridge in the stomach. After the recovery period, the macroscopic stomach lesions were observed to be less severe than those seen at the terminal kill.

*Histopathology:* Treatment-related findings were observed in the stomach, kidney, spleen and lungs.

The incidence and the severity of the stomach lesions are presented in Table 1. Increased incidences of mineralization with hyperplasia of the foveolar epithelium (fundus and pylorus), hyperplasia of the mucus cells, presence of eosinophilic chief cells, hyperplasia at the limiting ridge, and sub-mucosal inflammation were noted in mid and high dose males and females. A dose-relationship for the incidence and severity of the above lesions was noted except for the mineralization and hyperplasia of the pylorus foveolar epithelium. Mineralization with hyperplasia of the foveolar epithelium (fundus) and an increased incidence of sub-mucosal inflammation were also seen in low dose animals.

After the recovery period, focal mineralization, focal sub-mucosal inflammation and hyperplasia at the limiting ridge appeared to persist at levels similar to those seen at the terminal kill; however, hyperplasia of the foveolar epithelium and eosinophilic chief cells appeared to have regressed and were less prominent (Table 1).

A decrease in the incidence and severity of mineralization (calcification) in the outer stripe of the medulla of the kidney in mid and high dose females, and a decrease in the incidence of brown pigment (hemosiderin) deposition in the spleen in high dose animals were noted.

In the lung, an increased incidence of perivascular inflammation was noted in treated males (not dose-dependent).

Table 1. The Incidence and Severity of Stomach Lesions

Dosage level Lanthanum carbonate (mg/kg/day)	0	100	500	2000	0	100	500	2000
Sex	Male				Female			
Number examined	20	18	20	19	19	19	19	20
<b>Mineralisation, focal</b>								
Minimal	0	8	17	12	0	1	14	20
Slight	0	0	1	5	0	0	2	0
Total	0	8	18	17	0	1	16	20
<b>Hyperplasia, foveolar epithelium, fundus</b>								
Minimal	0	3	6	6	0	0	12	8
Slight	0	0	2	6	0	0	5	9
Moderate	0	0	0	5	0	0	0	3
Total	0	3	8	17	0	0	17	20
<b>Hyperplasia, foveolar epithelium, pylorus</b>								
Minimal	0	0	1	1	0	0	3	3
Slight	0	0	3	0	0	0	1	1
Moderate	0	0	0	1	0	0	0	0
Total	0	0	4	2	0	0	4	4
<b>Hyperplasia, mucous cells</b>								
Minimal	0	0	4	8	0	0	3	4
Slight	0	0	2	3	0	0	2	6
Moderate	0	0	0	2	0	0	0	1
Total	0	0	6	13	0	0	5	11
<b>Eosinophilic chief cells</b>								
Minimal	0	0	3	8	0	0	3	6
Slight	0	0	0	0	0	0	1	0
Total	0	0	3	8	0	0	4	6
<b>Inflammation, sub-mucosal, focal</b>								
Minimal	2	6	11	15	0	2	12	14
Slight	0	0	1	0	0	0	0	1
Total	2	6	12	15	0	2	12	15
<b>Hyperplasia, limiting ridge</b>								
Minimal	0	0	3	14	0	0	3	9
<b>Hyperkeratosis, limiting ridge</b>								
Minimal	0	0	0	3	0	0	0	0

Table 1. (contd.). The Incidence and Severity of Stomach Lesions – Recovery Groups

Dosage level Lanthanum carbonate (mg/kg/day)	0	100	600	2000	0	100	600	2000
Sex	Male				Female			
Number examined	5	5	5	5	5	5	5	5
Mineralisation, focal								
Minimal	0	0	4	4	0	0	5	2
Slight	0	0	0	1	0	0	0	3
Total	0	0	4	5	0	0	5	5
Hyperplasia, foveolar epithelium, fundus								
Minimal	0	0	0	1	0	0	0	0
Eosinophilic chief cells								
Minimal	0	0	0	1	0	0	0	1
Inflammation, sub-mucosal, focal								
Minimal	0	0	2	3	0	0	1	4
Hyperplasia, limiting ridge								
Minimal	0	0	0	3	0	0	0	1
Hyperkeratosis, limiting ridge								
Minimal	0	0	0	1	0	0	0	0

*Toxicokinetics:* Plasma lanthanum levels were higher at 2 hr than at 20 hr, and a dose related increase in plasma levels was generally observed. Lanthanum levels in weeks 13 and 26 were generally similar to those seen on day 1 of the study, indicating no significant accumulation with time.

*Tissue lanthanum levels:* A dose-related increase in lanthanum levels was seen in all four tissues (brain, femur, liver and kidney) analyzed. It is noted that the sample size for the assay was small (n=3), and there were wide variations between individual values (particularly for femur values).

*Bone calcium and phosphorus levels:* An apparent marginal reduction in both total bone calcium and phosphorus levels was noted in treated animals (not dose dependent) compared to controls.

## 2. Thirteen-Week Oral (Gavage) Toxicity Study in the Mouse

**Key findings:** Increased incidences of stomach lesions (epithelial hyperplasia of the limiting ridge and non-glandular region, and mucosal inflammatory cell infiltration in the glandular region) at 1500 & 2000 mg/kg/day

**Study number:** SPD/86/C

**Volume #:** 1.10

**Conducting laboratory and location:** □

**Date of study initiation:** March 27, 1997

**GLP compliance:** yes

**QA report:** yes

**Drug lot # and purity:** Lanthanum carbonate lot # B1066-960802, purity : □  
 □ % , total metal impurities – none detected. (specifications:  
 □ , total metal impurities – not more than  
 ppm)

**Formulation:** formulated daily as a suspension in 0.5% carboxymethylcellulose

### Methods

#### Animals:

*Species/strain:* Mouse ~ CD-1 (ICR) BR strain (VAF plus) obtained from □

*#/sex/group:* 10

*Satellite groups used for toxicokinetics:* 0, 500 and 2000 mg/kg/day (30 mice/sex/group)

*Age:* 5-6 weeks

*Weight:* males – 29 to 36 g  
 females – 23 to 27 g

Animals were housed in groups of five, by sex, in grid-bottomed stainless steel cages suspended over cardboard-lined trays. A pelleted diet ( — rat and mouse maintenance diet — ) and tap water were available *ad libitum*.

#### Dosing:

*Doses:* 0 (vehicle control), 500, 1500 and 2000 mg/kg/day – once daily

*Route/volume:* oral (gavage)/10 ml/kg

#### Observations and Measurements:

*Clinical signs:* daily

*Mortality:* twice daily

*Body weights:* pre-dose, on the first day of dosing and weekly thereafter

*Food consumption:* pre-test and then weekly

*Ophthalmoscopy:* pre-test (all mice from main study groups) and week 13 (control and high dose groups only)

*Hematology:* week 13 [parameters evaluated – RBC, WBC (total and differential), and platelet counts, cell morphology, hemoglobin, PCV, MCH, MCHC and MCV. A blood smear was also prepared.]

*Clinical chemistry:* week 13 (parameters evaluated – alkaline phosphatase, amino-transferases, BUN, total protein, albumin, globulin, A/G ratio, total bilirubin, creatinine, glucose, cholesterol, triglycerides, calcium, inorganic phosphorus, sodium, potassium and chloride)

[5 animals/sex/group (main study) were used for hematological evaluations and the remaining 5 animals/sex from each group were used for clinical chemistry evaluations.]

*Post-mortem examination:* All animals grossly examined. Weights determined for adrenals, brain, epididymides, heart, kidneys, liver, lungs, ovaries, pituitary, prostate, spleen, testes, thymus and uterus. The following tissues were preserved for histopathological examination.

adrenals	rectum
aorta	salivary gland
brain (3 sections)	sciatic nerve
caecum	seminal vesicles
colon	site of mammary gland
duodenum	skeletal muscle
epididymides	skin
eyes (incl. optic nerves)	spinal cord (3 levels)
femur (incl. marrow)	spleen
heart	sternum
ileum	stomach
jejunum	submandibular lymph node
kidneys	testes
liver + gall bladder	thymus
lungs (incl. mainstem bronchi)	thyroids (incl. parathyroids)
mesenteric lymph node	tongue
oesophagus	trachea
ovaries	urinary bladder
pancreas	uterus (incl. cervix)
pituitary	vagina
prostate	

all gross lesions

(Though blood smears were prepared from all animals, these smears were not examined.)

All tissues from control and high dose animals, tissues from all animals that died or killed in extremis, and all gross lesions from all animals were examined microscopically. Since the high dose animals showed stomach and liver lesions, these tissues from the lower groups were also examined.

*Toxicokinetics:* on the first day of dosing and on one occasion during week 13, at the following time points: pre-dose and 2, 4, 6 and 8 hours post-dose (3 animals/sex/group/time point (Animals were not re-used. After blood collection, majority of animals were discarded without necropsy, but some were used to collect samples for tissue lanthanum analysis.)

*Tissue lanthanum analysis:* Liver, brain, femur and kidneys (3 animals/sex/group) were collected on day 1 and during week 13 for tissue lanthanum analysis. In addition, the femur sample was used for total calcium and phosphorus level determinations.

#### **Statistical Analysis:**

An analysis of variance (ANOVA) was performed on all data. The residuals from this preliminary analysis were examined for heterogeneity of variance using Levene's test. If Levene's test was significant at 1% level, then a non-parametric analysis of the data was performed using Shirley's non parametric equivalent of the Williams' test. If Levene's test showed no significance, then pairwise tests of all treated groups versus control were performed using Williams' test.

#### **Results**

*Mortality:* One main study mid dose female (animal # 61) was found dead on day 7 of the study. The clinical signs observed prior to death included hunched posture, hypoactivity, piloerection, rapid breathing and weight loss. Macroscopic examination revealed ruptured esophagus, adhesions to lung lobes and abnormal white material in the thoracic cavity, suggestive of a dosing error.

Two satellite animals, a control male (# 110) and a high dose male (#164), were killed in weeks 12 and 11, respectively, due to swollen, scabbed urogenital area/ penis lesions with discharge.

Three other satellite males [a control (# 101) and 2 high dose (#s 159 and 163) males] were found dead in weeks 6, 5 and 10 of treatment, respectively. The causes of these deaths were not determined.

*Clinical signs:* There were no treatment-related clinical signs.

*Body weights:* Body weight data are presented graphically in Figures 3 & 4. A non-dose related body weight gain was noted in treated main study males. Body weight gains for treated main study females were similar to those of controls. Compared to controls, the overall body weight gains were slightly lower in satellite high dose males and slightly higher in high dose females. The body weight gains in the other satellite group (low dose) were similar to those of control.

*Food consumption:* Total food consumption values for the main study treated males were slightly lower (about 6% lower at the high dose) than those of controls (not dose related). In main study treated females, and also in satellite males and females, food consumption values were generally similar to those of controls.

*Ophthalmoscopy:* There were no treatment-related findings.

*Hematology:* Slightly higher hemoglobin values (compared to controls), in high dose males and higher WBC counts in mid and high dose females (not dose related) were noted.

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Figure 3. Group Mean Body Weights (g) - Males

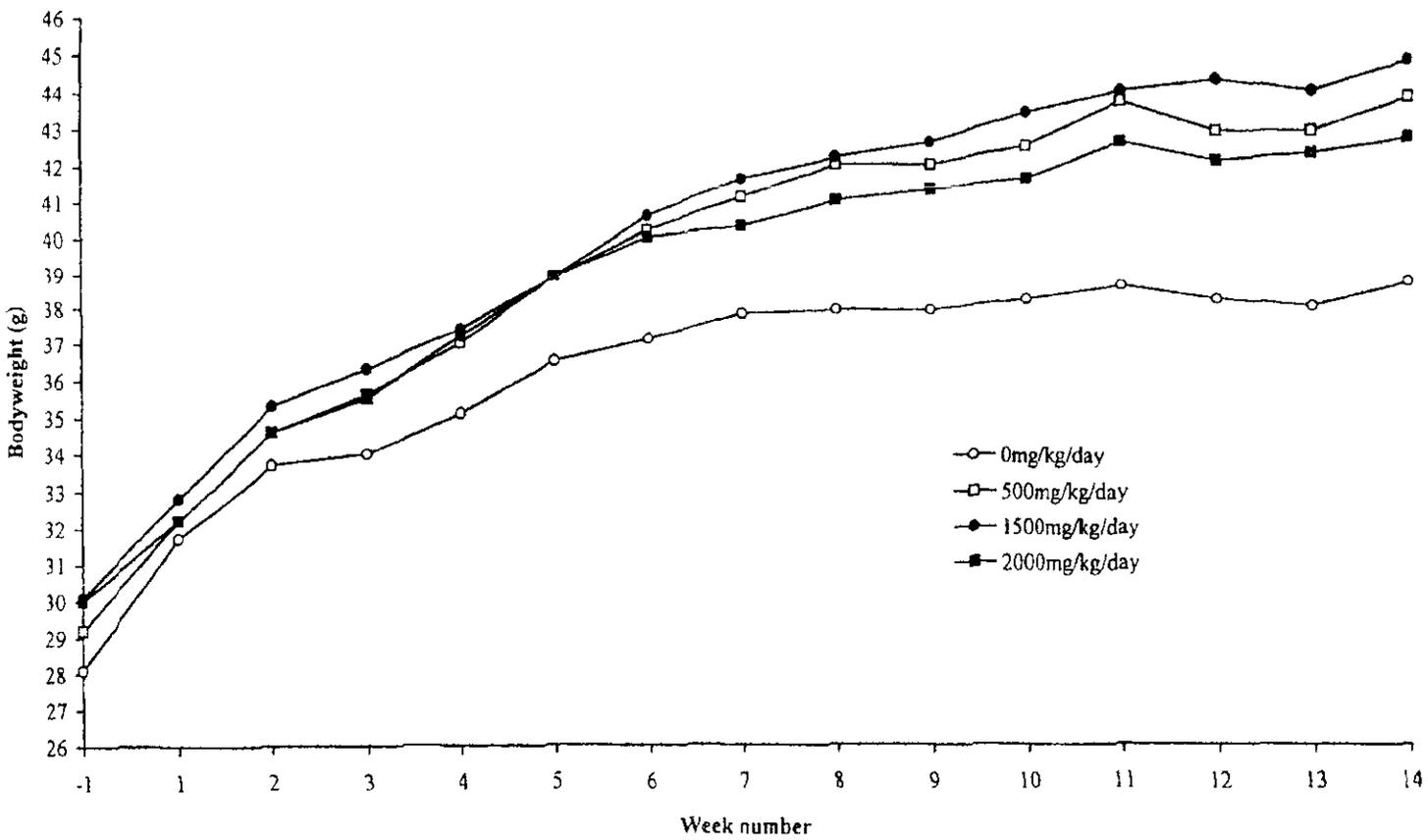
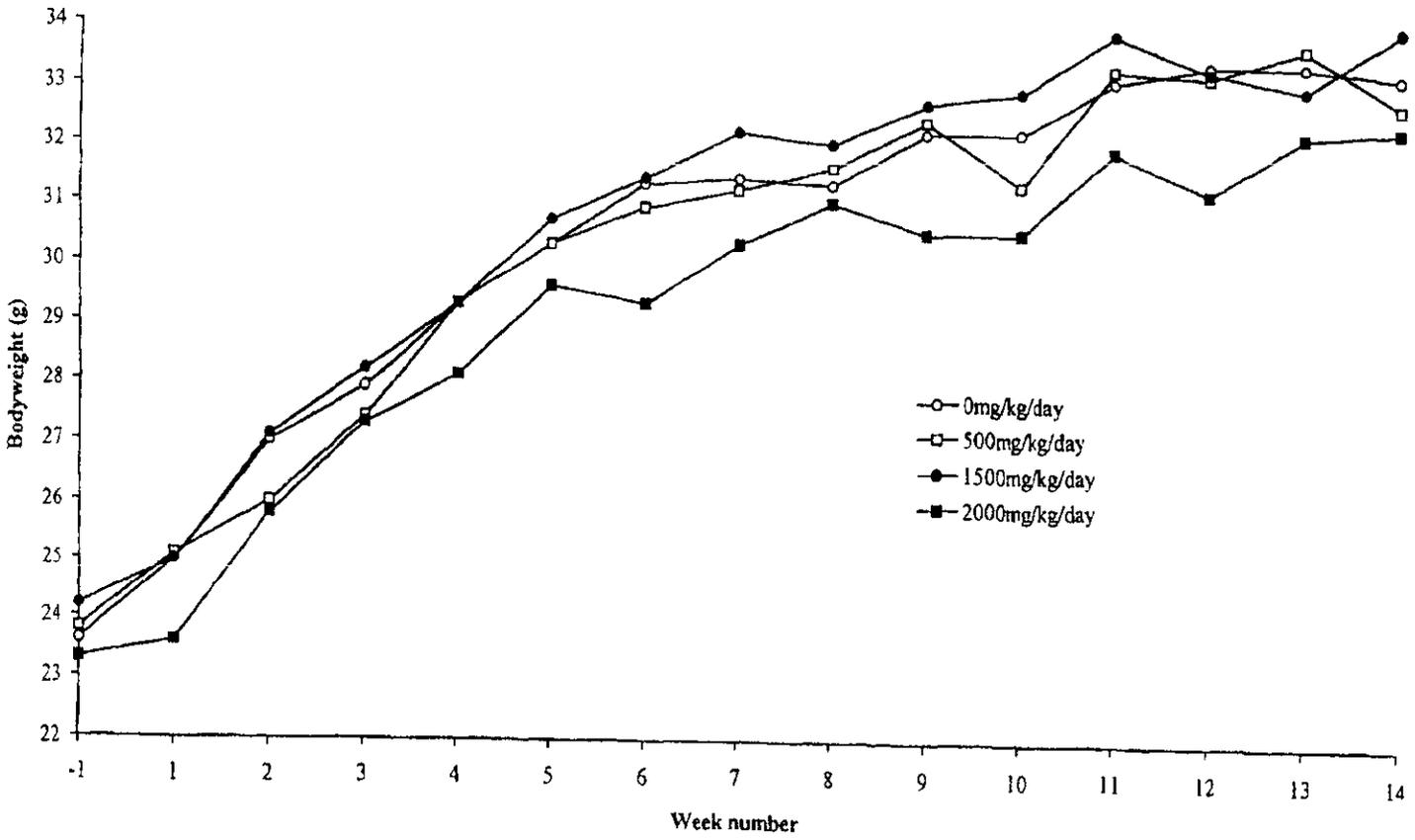


Figure 4. Group Mean Body Weights - Females



*Clinical chemistry:* A dose-related reduction in BUN levels was observed in treated females.

*Organ weights:* Non dose-related increases in absolute kidney weights (10 to 18%), but not in relative kidney weights, were seen in mid and high dose males.

*Gross pathology:* Macroscopically, prominence of the limiting ridge and thickening of the glandular and nonglandular regions of the stomach were observed in mid and high dose animals. An accentuated lobular pattern of the liver was noted in treated animals, the incidence being higher in males than in females.

*Histopathology:* Treatment-related findings were seen in the stomach and liver. The stomach lesions included increased incidences of epithelial hyperplasia and/or hyperkeratosis of the limiting ridge, epithelial hyperplasia of the non-glandular region, and mucosal inflammatory cell infiltration in the glandular region of stomach of the mid and high dose animals. The incidence and the severity of these lesions are given below.

Findings	Males				Females			
	Dose Groups							
	1	2	3	4	1	2	3	4
-----								
Hyperkeratosis -- limiting ridge								
minimal	0	1	4	3	0	0	3	3
slight	0	0	0	0	0	0	1	1
moderate	0	0	0	0	0	0	0	1
Epithelial hyperplasia - limiting ridge								
minimal	0	0	5	6	0	0	3	3
slight	0	0	0	1	0	0	2	4
moderate	0	0	1	0	0	0	1	2
Epithelial hyperplasia non-glandular								
minimal	0	0	3	0	0	0	3	0
slight	0	0	1	0	0	0	0	0
Sub-mucosal inflammation								
slight	0	0	0	0	0	0	0	1
Mucosal inflammatory cell infiltration -glandular								
minimal	1	0	6	7	1	1	2	7
slight	0	0	0	0	0	0	1	2
Glandular dilatation								
minimal	0	0	2	0	0	1	1	0
-----								

N=10 mice/sex/group Dose groups (mg/kg/day): 1 = control; 2 = 500, 3 = 1500 & 4 = 2000

Although no significant differences in the incidences of these lesions were seen between mid (1500 mg/kg/day) and high (2000 mg/kg/day) dose males, the incidences of epithelial hyperplasia of the limiting ridge and mucosal inflammatory cell infiltration in the glandular region in females were higher at the high dose than at the mid dose level.

Minimal or slight centrilobular hepatocyte hypertrophy was seen in all treated male groups (not dose dependent).

*Toxicokinetics:* A dose-related increase in plasma lanthanum levels was generally observed, the levels being higher at the 2 hour time point than at other times. The plasma levels at week 13 of treatment were generally similar to those on day 1 of the study.

*Tissue lanthanum levels:* Higher levels of lanthanum, compared to controls, were seen in all four tissues analyzed (brain, femur, liver, and kidney), especially in the femur. Absorption of lanthanum was apparent after a single administration of test article and appeared to be dose related. There appeared to be more of a trend towards higher concentrations in the liver and femur during week 13 than on day 1 of treatment, but the pattern was not consistent.

*Total bone calcium and phosphorus levels:* On day 1, there was an apparent marginal reduction in both calcium and phosphorus levels, compared to controls, in high dose animals. This was mainly due to low values for one high male and one high dose female. In week 13, the levels of calcium and phosphorus for treated animals were similar to those of controls, and the values were generally higher than those obtained on day 1.

[Note: In an amendment dated June 19, 2002 (submission #005), the sponsor stated that additional sections of stomach from animals in this study were cut, stained with proliferating cell nuclear antigen (PCNA) and examined microscopically. The results of this investigation are provided below.

Examination of Stomachs from 13-week mouse study stained for PCNA

Dose Level: (mg/kg/day)	Males				Females			
	0	500	1500	2000	0	500	1500	2000
No. Examined:	10	10	10	10	10	10	10	10
Hyperplasia of the glandular stomach*	1	0	8	9	3	3	5	6
Mineralised foci -								
<i>minimal</i>	0	1	6	5	0	0	6	4
<i>slight</i>	0	0	2	0	0	0	0	1
<b>Total</b>	0	0	8	5	0	0	6	5

\* As assessed by positive PCNA staining.

Increased incidences of hyperplasia of the glandular stomach and mineralized foci in the superficial epithelium of the stomach mucosa were seen at 1500 and 2000 mg/kg/day. Increasing the dose from 1500 to 2000 mg/kg/day did not appear to have any significant effect on the incidences of the above lesions. ]

#### Toxicity Studies in Dogs

These studies are summarized by Dr. John Koerner in his review of canine toxicology and toxicokinetic studies, attached.

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## V. GENETIC TOXICOLOGY

### 1. Bacterial Reverse Mutation Assay (Ames Test)

**Key findings:** In the Ames test, lanthanum carbonate produced dose-related increases in revertant colonies in the tester strain *E. coli* WP2uvrA pKM101. However, these increases were not equal to or greater than two times the mean negative control value. No such response was noted in a *Salmonella* tester strain (TA102) that has a similar sensitivity and reversion profile to the *E. coli* strain.

**Study no:** M/AMES/42075

**Study type:** *In vitro* bacterial reverse mutation assay (Ames test)

**Conducting laboratory and location:** ☐

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**Study dates:** Not provided (Report date -- August 1996)

**GLP compliance:** Yes (UK, OECD, USA and Japanese codes of GLP)

**QA report:** Yes (x) No. ( )

**Drug batch # and % purity:** 6268/951001, % purity -- not provided

**Formulation/vehicle:** Test article was dissolved in water (50 mg/ml)

**Method:** Plate incorporation test

**Bacterial strains:** *Salmonella typhimurium* tester strains TA1535, TA1537, TA1538, TA98, TA100 & TA102, and *Escherichia coli* strains WP2 uvrA and WP2 uvrA pKM101.

**Dose selection criteria:** A preliminary toxicity range finding study was conducted using strains TA98 and WP2 uvrA pKM101 at concentrations of 1.6, 8, 40, 200, 1000 and 5000 µg/plate, in the presence and absence of S-9. After 24-hr incubation at 37°C, the plates were examined for bacterial lawn density. Results are given below.

Strain	% S-9	Concentration of test substance (µg/plate)						
		0	1.6	8	40	200	1000	5000
TA98	0	+	+	+	+	+	+c	+c
TA98	10	+	+	+	+	+	+c	+c
WP2 uvrA pKM101	0	+	+	+	+	+	+c	+c
WP2 uvrA pKM101	10	+	+	+	+	+	+c	+c

+ = Normal Growth c = Compound Precipitation (not expected to interfere with colony counting)

Precipitation was noted at concentrations of 1000 and 5000 µg/plate. Based on the above results, the following doses were selected for the first experiment: 8, 40, 200, 1000 and 5000 µg/plate (with and without S-9) for all *Salmonella* strains, and 50, 250, 500, 2500 and 5000 µg/plate (with and without S-9) for *E. coli* strains. For the second experiment,

doses of 8, 40, 200, 1000 and 5000 µg/plate were used for both Salmonella and E. coli strains.

*Test agent stability:* Test agent was shown to be stable at room temperature.

*Metabolic activation system:* The liver microsomal fraction (S-9) was prepared from Fischer 344 rats induced with β-naphthoflavone and sodium phenobarbitone.

*Controls:* Vehicle – water; Negative control – water; Positive controls without S-9 – sodium azide, 1 µg/plate (TA1535 and TA100), 9-aminoacridine, 50 µg/plate (TA1537), 2-nitrofluorene, 0.5 µg/plate (TA1538 and TA98), cumene hydroperoxide, 100 µg/plate (TA102) and 4-nitroquinoline –1-oxide, 1 µg/plate (E. coli strains); Positive controls with S-9 – 2-aminoanthracene, 2 µg/plate for all salmonella strains, and 4 µg/plate for E. coli strains.

*Exposure conditions:* 100 µl each of test article solution, solvent control (water) or appropriate positive control, S-9 mix or 0.2 M phosphate buffer (500 µl) and bacterial culture (100µl, ~ 10<sup>8</sup> cells) were added into respective tubes containing molten top agar. After mixing, the mixture was poured onto Vogel Bonner minimal agar plates. When the overlay had solidified, the plates were inverted and incubated for about 65 hours at 37°C.

*Analysis:* The numbers of revertant colonies on each plate were counted using an automatic colony counter. Mean, standard deviation and Dunnett's t-statistic were calculated for each concentration and bacterial strain. (All concentrations were plated in triplicate and the experiment was repeated using fresh cultures.)

*Criteria for positive results:* A test compound is considered to be positive for mutagenic effect if it produces a dose response with statistically significant increases in revertant numbers.

**Summary of study findings:** Results of the two experiments and the statistical analyses of the data are presented in Tables 2 to 5. Increases in revertant colonies, compared to control cultures (except at the highest dose of 5000 µg/plate), were seen only with the E. coli WP2 uvrA pKM 101 strain in experiment 1 with metabolic activation (increases in revertant colonies without S-9 were not dose-dependent) and in experiment 2 (with and without metabolic activation). These increases attained statistical significance at concentration levels of 500 and 2500 µg/plate with and without S-9 in experiment 1 and at 200 and 1000 µg/plate without S-9 in experiment 2. No other statistically significant increases in revertant colonies were seen with any tester strains, including TA102 which has a similar sensitivity and reversion profile to E. coli WP2 uvrA pKM101.

**Study validity:** The assay is considered valid since negative control data were within the historical control range, and all positive controls induced significant increases in revertant colonies within expected ranges, demonstrating the sensitivity of the assay and the metabolizing activity of the S-9.

[Note: The final report on the bacterial reverse mutation test, issued in August 1996, concluded that "lanthanum carbonate has shown some mutagenic potential under the conditions employed in this test" since statistically significant increases in revertants were seen with the E. coli WP2 uvrA pKM101 strain. It was stated in that report that "a positive result is indicated by a dose response with statistically significant increases in revertant numbers." In an amendment to the final report (dated November 17, 2000 and submitted to the agency under NDA 21-468 on April 30, 2002), a re-appraisal of the results of the study was made using a revised set of criteria, "combining a fold-increase in revertants, statistical analysis and linearity of response." Treatment data sets that show responses  $\leq 1.5$  times the concurrent negative control value are considered negative and responses  $\geq 3$  times are considered positive. Assays in which responses fall between 1.5 and 3 times the control value are subjected to further analysis including statistics. A one-sided Dunnett's test (using rank transformed data and Bonferroni correction) and a linear regression analysis showed that the observed increases in E. coli revertants were not statistically significant. Moreover, the mean numbers of revertants were not greater than two times the mean negative control value for the E. coli strain. Although dose-dependent increases in revertant colonies were seen in the E. coli WP2 uvrA pKM101 strain in experiments 1 and 2, since numbers of revertants were not equal to or greater than two times the mean negative control value, and also because the revertant counts in treated groups were within the historical control range, these increases are considered to be not biologically significant. Moreover, a Salmonella strain, TA102, which has a similar sensitivity and reversion profile to the E. coli strain, showed no mutagenic response to lanthanum carbonate. ]

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Table 2.

MEAN NUMBER OF REVERTANTS PER PLATE								
EXPERIMENT 1								
Strain	% S-9	Concentration of test substance ( $\mu\text{g}/\text{plate}$ )						PC
		0	8	40	200	1000	5000	
TA1535	0	13.3	12.7	12.0	12.0	9.7	7.7	347.7
TA1537	0	9.3	11.3	7.0	13.3	7.3	5.0	211.3
TA1538	0	21.7	19.3	23.7	25.0	25.0	20.7	99.0
TA98	0	26.7	29.0	29.7	18.7	21.0	17.3	122.3
TA100	0	89.0	92.0	96.3	95.5	83.3	101.7	388.0
TA102	0	241.0	264.3	235.0	215.3	239.0	198.3	451.3
TA1535	10	10.0	10.7	12.7	14.0	9.7	9.3	24.3
TA1537	10	9.0	15.7	9.3	7.0	14.3	7.7	250.3
TA1538	10	27.7	30.7	25.7	29.3	23.7	29.3	1281.0
TA98	10	38.0	36.7	39.0	35.3	41.7	35.7	1358.3
TA100	10	84.0	94.0	86.3	93.0	93.3	85.0	1412.0
TA102	10	278.3	312.3	288.7	229.0	298.3	190.0	402.0
Strain	% S-9	0	50	250	500	2500	5000	PC
WP2 uvrA	0	27.0	19.5	22.0	18.3	17.7	20.7	901.7
WP2 uvrA pKM101	0	192.7	229.7	209.7	262.7	236.7	173.0	1150.7
WP2 uvrA	10	22.3	25.0	26.7	25.7	20.0	14.0	106.3
WP2 uvrA pKM101	10	125.3	172.5	175.5	199.7	239.0	124.7	492.3

PC = Positive Control

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Table 3.

t-statistic from Dunnett's test in Experiment 1								
Strain	% S-9	Concentration of test substance (µg/plate)					Degrees of freedom	
		8	40	200	1000	5000	N	D
TA1535	0	-0.18	-0.32	-0.33	-1.06	-1.79	5	12
TA1537	0	0.63	-0.91	1.06	-0.75	-1.92	5	12
TA1538	0	-0.87	0.61	1.14	1.13	-0.40	5	12
TA98	0	0.42	0.63	-1.70	-1.17	-2.06	5	12
TA100	0	0.44	1.10	0.81	-0.92	1.89	5	11
TA102	0	1.32	-0.32	-1.48	-0.10	-2.57	5	12
TA1535	10	0.12	0.83	1.27	-0.23	-0.37	5	12
TA1537	10	2.36	0.07	-0.88	1.91	-0.54	5	12
TA1538	10	0.76	-0.59	0.44	-1.17	0.44	5	12
TA98	10	-0.31	0.20	-0.62	0.73	-0.48	5	12
TA100	10	0.81	0.09	0.74	0.77	0.06	5	12
TA102	10	2.45	0.76	-3.88	1.45	-7.22	5	12
Strain	% S-9	50	250	500	2500	5000	N	D
WP2 uvrA	0	-1.13	-0.96	-1.71	-1.79	-1.13	5	11
WP2 uvrA pKM101	0	2.98	1.41	5.42*	3.52*	-1.68	5	12
WP2 uvrA	10	0.60	0.98	0.77	-0.48	-1.91	5	12
WP2 uvrA pKM101	10	2.86	3.10	5.42*	7.87*	-0.06	5	10

p<0.01

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Table 4.

MEAN NUMBER OF REVERTANTS PER PLATE								
EXPERIMENT 2								
Strain	% S-9	Concentration of test substance (µg/plate)						PC
		0	8	40	200	1000	5000	
TA1535	0	8.0	8.3	10.7	16.3	7.0	11.3	285.0
TA1537	0	8.3	9.3	8.7	9.3	10.3	5.3	222.0
TA1538	0	16.0	17.3	17.7	16.3	19.7	18.0	74.0
TA98	0	25.7	27.7	24.7	33.7	26.3	23.0	80.3
TA100	0	98.0	78.7	94.7	99.0	103.3	83.7	383.3
TA102	0	252.7	274.0	250.0	269.7	267.0	169.7	494.0
WP2 uvrA	0	30.7	25.7	31.3	33.0	29.0	30.7	647.7
WP2 uvrA pKM101	0	168.7	204.7	211.7	229.3	238.3	121.7	752.7
TA1535	10	12.3	11.7	13.3	10.3	13.3	13.3	104.7
TA1537	10	15.0	16.3	13.7	14.7	12.7	12.0	269.0
TA1538	10	32.7	36.7	40.3	31.7	40.7	27.7	1126.7
TA98	10	32.0	34.7	39.3	33.0	33.0	31.0	1013.3
TA100	10	104.3	93.7	92.0	97.3	84.0	85.7	908.3
TA102	10	242.3	268.7	267.0	255.7	221.5	189.3	507.7
WP2 uvrA	10	43.3	45.0	31.3	38.3	43.3	43.3	128.0
WP2 uvrA pKM101	10	177.5	183.0	216.0	217.3	230.0	127.3	858.3

PC= Positive Control

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Table 5.

t-statistic from Dunnett's test in Experiment 2								
Strain	% S-9	Concentration of test substance (µg/plate)					Degrees of freedom	
		8	40	200	1000	5000	N	D
TA1535	0	-0.01	0.70	1.78	-0.16	0.59	5	12
TA1537	0	0.49	0.17	0.54	0.91	-1.97	5	12
TA1538	0	0.38	0.56	0.09	1.17	0.59	5	12
TA98	0	0.51	-0.19	1.78	0.16	-0.70	5	12
TA100	0	-2.74	-0.42	0.16	0.75	-2.03	5	12
TA102	0	1.27	-0.18	1.02	0.85	-5.62	5	12
WP2 uvrA	0	-0.98	0.09	0.42	-0.31	-0.11	5	12
WP2 uvrA pKM101	0	2.41	2.84	3.88*	4.40*	-3.39	5	12
TA1535	10	-0.18	0.40	-0.55	0.30	0.35	5	12
TA1537	10	0.27	-0.35	-0.25	-0.41	-0.50	5	12
TA1538	10	0.56	1.06	-0.13	1.15	-0.76	5	12
TA98	10	0.54	1.44	0.28	0.28	-0.10	5	12
TA100	10	-1.14	-1.33	-0.73	-2.25	-2.09	5	12
TA102	10	1.25	1.18	0.66	-0.77	-2.62	5	11
WP2 uvrA	10	0.39	-2.70	-1.10	0.05	0.05	5	12
WP2 uvrA pKM101	10	0.28	1.91	2.41	3.10	-3.42	5	9

\*p<0.01

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Historical control values for the bacterial reverse mutation assay are given below.

NEGATIVE CONTROL						
STRAIN	-S9			+S9		
	Mean	SD	n	Mean	SD	n
TA1535	10.83	4.41	210	13.18	4.95	208
TA1537	6.92	3.73	216	11.99	5.14	218
TA1538	19.55	7.45	153	31.76	10.41	156
TA98	20.21	7.39	222	33.01	11.61	219
TA100	94.91	25.63	243	98.00	20.12	237
WP2 <i>uvrA</i>	21.59	13.36	98	23.41	11.09	87
WP2 <i>uvrA</i> pKM101	181.88	98.69	80	196.44	108.64	66

POSITIVE CONTROL								
STRAIN	Compound	-S9			+S9			
		Mean	SD	n	Compound	Mean	SD	n
TA1535	SA	266.2	81.1	182	2AA	114.6	44.3	189
TA1537	9AA	248.3	125.9	179	2AA	147.4	76.5	179
TA1538	2NF	124.4	55.9	119	2AA	792.8	270.9	125
TA98	2NF	130.3	53.9	185	2AA	752.4	318.2	192
TA100	SA	399.0	82.1	200	2AA	821.5	399.5	206
WP2 <i>uvrA</i>	4NQO	551.8	203.0	79	2AA	114.5	67.8	81
WP2 <i>uvrA</i> pKM101	4NQO	741.3	308.6	51	2AA	421.9	199.1	60

[Note: In a letter dated October 28, 2002, the sponsor stated that the above historical control data were generated by *ℓ* who conducted the study) during the 2-year period immediately preceding the study (1994-1996). The 'n' value shown in the table represents the number of control plates examined for each strain.]

## 2. In Vitro Mammalian Cell Gene Mutation Test

**Key Findings:** Lanthanum carbonate was found not to induce mutations at the HGPRT locus of Chinese hamster ovary cells either in the presence or absence of metabolic activation.

**Study number:** M/PMC/42078

**Study type:** HGPRT forward mutation assay in cultured Chinese hamster ovary cells

**Conducting laboratory and location:**  $\square$

**Study dates:** Not provided (Report date -- November 1996)

**GLP compliance:** Yes (UK, OECD, USA and Japanese codes of GLP)

**QA report:** Yes (x) No. ( )

**Drug batch # and % purity:** 6268/951001, % purity -- not provided

**Formulation/vehicle:** Lanthanum carbonate was formulated in culture medium (McCoy's 5A medium supplemented with glutamine, antibiotics and 10% fetal calf serum) at desired concentrations.

### Methods

*Cell line:* Chinese hamster ovary cells obtained from the  $\square$

*Dose selection criteria:* In a preliminary toxicity range finding study, cells (200 per culture) were treated for 3 hours at 37°C with lanthanum carbonate at doses up to 5000 µg/ml in the presence and absence of S-9 mix. At the end of the treatment period, cells were washed twice with phosphate buffered saline to remove the test substance, and allowed to grow in fresh culture medium for 7 days. The results are given below.

Survival as % of Control		
Dose (µg/ml)	-S9	+S9
0	100.0%	100.0%
5	117.6%	96.7%
25	122.5%	90.5%
50	132.9%	80.1%
250	76.8%	78.7%
500	53.0%	49.7%
2500	91.4%	40.0%
5000	3.7%	24.8%

The top dose, 5000 µg/ml, in the absence of S-9, produced toxicity resulting in less than 10% survival. Based on the above results, the following doses were selected for the two independent experiments: 500, 1000, 1500 and 2000 µg/ml – without S-9  
250, 500, 2500 and 5000 µg/ml – with S-9

*Test agent stability:* Test agent was shown to be stable (about 2 years) at room temperature.

*Metabolic activation system:* The S-9 fraction was prepared from the livers of sodium phenobarbitone and beta-naphthoflavone induced male Fischer 344 rats.

*Controls:* Vehicle control – culture medium (McCoy's 5A); Positive controls – ethylmethanesulphonate (without S-9) and benz(a)-pyrene (with S-9) were dissolved in DMSO and then diluted in culture medium.

*Exposure conditions:* Log phase cells were trypsinised, counted and appropriate volumes of culture were added to respective sterile plastic flasks ( $1.9 \times 10^6$  cells/flask). After allowing time for attachment of cells to the tissue culture flask, the culture media of duplicate cultures were replaced with media containing appropriate concentrations of lanthanum carbonate, vehicle or positive control, with and without S-9 mix. After 3 hours of treatment, cells were washed twice with phosphate buffered saline, trypsinised and fresh cultures were made with a cell concentration of  $10^5$  cells/flask. These were allowed to grow for 7 days. In addition, 200 cells/dose were seeded in respective flasks (in triplicate) and allowed to grow for 7 days to assess the Day 0 survival.

Mutations at the HGPRT locus were assessed by seeding  $2 \times 10^5$  cells/dose/per plate in a medium containing 6-thioguanine (6-TG, 2 µg/ml) as the selective agent and the plates were incubated at 37°C for 7 days. (Resistance to 6-TG indicates that a mutation, induced by the test compound, had occurred at the HGPRT locus, and cells can proliferate in the presence of 6-TG. 6-TG is cytotoxic to non-mutated cells.)

*Analysis:* At the end of the incubation period, colonies were fixed, stained and counted. Plating efficiency and mutation frequency were calculated as follows:

Plating efficiency = colony count/cells plated

Mutation frequency = plating efficiency in selective media/plating efficiency in non-selective media

Data were statistically analyzed using methods described in "Statistical Evaluation of Mutagenicity Test Data" (Ed. D.J. Kirkland, Cambridge University Press, 1989).

*Criteria for positive results:* A test compound is considered to be positive for mutagenic effect if the following conditions are met:

- 1) Statistically significant increases in mutation frequency of treated groups over control - to be repeated in an independent experiment
- 2) a dose response in mutation frequency – to be repeated in an independent experiment

**Summary of study findings:** The Day 0 survival data for both experiments are presented below.

Experiment 1.

Without S-9

Dose ( $\mu\text{g/ml}$ )	0	50	250	500	1000	1500	2000
Mean colony number	130.0	124.7	117.3	128.3	104.3	83.7	21.3
% of control	100.00%	95.90%	90.26%	98.72%	80.26%	64.36%	16.41%

With S-9

Dose ( $\mu\text{g/ml}$ )	0	25	250	500	2500	5000
Mean colony number	115.33	123.67	97.33	91.50	144.83	129.00
% of control	100.00%	107.23%	84.39%	79.34%	125.58%	111.85%

Experiment 2.

Without S-9

Dose ( $\mu\text{g/ml}$ )	0	50	250	500	1000	1500	2000
Mean colony number	163.0	147.0	110.7	121.7	100.3	92.3	101.3
% of control	100.00%	90.18%	67.91%	74.66%	61.53%	56.63%	62.15%

With S-9

Dose ( $\mu\text{g/ml}$ )	0	50	250	500	2500	5000
Mean colony number	108.7	83.7	101.0	102.0	74.7	104.7
% of control	100.00%	77.00%	92.92%	93.84%	68.72%	96.32%

The top dose of lanthanum carbonate, without S-9, produced about 16% survival in Experiment 1 and 62% survival in Experiment 2.

[

]

Based on the results of the Day 0 survival only the top 4 doses were plated for mutation assessment.

Mutation frequency data presented below. There were no statistically significant increases in mutation frequency in drug treated groups compared to negative control either in the presence or absence of metabolic activation. Both positive controls produced significant increases in mutation frequencies ( $p < 0.05$ ) when compared to negative controls.

Mutant frequencies ( $\times 10^{-6}$ )

Experiment 1	-S9	Dose ( $\mu\text{g/ml}$ )	0	500	1000	1500	2000	PC
		Mutation frequency	2.473	16.532	8.546	0.902	11.430	574.338
		t		1.739	1.127	-0.511	1.383	7.903*
	+S9	Dose ( $\mu\text{g/ml}$ )	0	250	500	2500	5000	PC
		Mutation frequency	10.603	15.987	9.183	6.795	7.512	89.619
		t		0.753	-0.234	-0.730	-0.575	5.416*
Experiment 2	-S9	Dose ( $\mu\text{g/ml}$ )	0	500	1000	1500	2000	PC
		Mutation frequency	15.510	0.897	2.018	14.061	14.983	540.884
		t		-2.679	-2.318	-0.191	-0.068	10.606*
	+S9	Dose ( $\mu\text{g/ml}$ )	0	250	500	2500	5000	PC
		Mutation frequency	5.046	1.810	1.673	12.374	1.450	65.611
		t		-0.429	-0.460	0.664	-0.506	2.848*

\* $p < 0.05$

**Study validity:** The assay is considered valid since both positive control chemicals induced statistically significant increases in mutation frequency in both studies, indicating that the cells are sensitive to the mutagenic effects of known genotoxicants and that the S-9 used in the study was capable of metabolizing the inactive precursor to a genotoxic intermediate.

[Note: A separate study conducted in October 2001 at the [ ] testing facility, [ ] (study report submitted to the agency under NDA 21468 on April 30, 2002) showed that there were no effects on osmolality or pH of the culture medium for the Chinese hamster ovary cells at lanthanum carbonate concentrations tested in the *in vitro* mammalian cell gene mutation and cytogenetic assays. It is stated that lanthanum carbonate solutions showed visible precipitation upon standing (all preparations of 200  $\mu\text{g/ml}$  and above and one preparation at 100  $\mu\text{g/ml}$ ).]

### 3. In Vitro Mammalian Cell Cytogenetics Test

**Key Findings:** Significant increases in the incidence of chromosome aberrations, associated with cytotoxicity, were seen with and without metabolic activation.

**Study number:** M/CCA/42077

**Study type:** Cytogenetic assay in Chinese hamster ovary (CHO) cells

**Conducting laboratory and location:** [ ]

**Study dates:** Not provided (Report date – September 1996)

**GLP compliance:** Yes (UK, OECD, USA and Japanese codes of GLP)

**QA report:** Yes (x) No. ( )

**Drug batch # and % purity:** 6268/951001, % purity – not provided

**Formulation/vehicle:** Lanthanum carbonate was suspended by sonication in culture medium (McCoy's 5A medium supplemented with glutamine, 10% fetal calf serum and antibiotics) at desired concentrations immediately prior to use.

#### Methods

*Cell line:* A CHO cell line, obtained from the [ ]  
[ ] was grown in McCoy's 5A medium. This line has [ ]

*Dose selection criteria:* In a toxicity range-finder study, logarithmically growing cell cultures ( $3 \times 10^5$  cells/flask) were treated with the test drug (0, 50, 100, 250, 500, 1000, 2500 and 5000  $\mu\text{g/ml}$ ) or positive control solutions in the presence and absence of S-9 at 37°C. After 3 hours of treatment, the cells treated with S-9 were washed and incubated again in fresh medium until harvesting after about 1.5 cell cycle time. Treatment of cells without S-9 continued until harvesting. Cells were arrested in metaphase with colcemid (at a final concentration of 0.2  $\mu\text{g/ml}$ ), fixed, stained and scored for chromosome damage. The results are given below.

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Dose/ $\mu\text{g}/\text{mL}$	Without S-9	
	Mean % Mitotic Index	Relative MI %
0	5.63	100
50	5.09	90
100	5.16	92
250	3.43	61
500	1.71	30
1000	NS	
2500	NS	
5000	NS	

NS = not scored

Dose-dependent reductions in mitotic indices, compared to negative control, were noted in treated groups. At 500  $\mu\text{g}/\text{ml}$ , a 70% reduction in mitotic index was seen. The slides from cultures dosed with 1000  $\mu\text{g}/\text{ml}$  and above, without S-9, were not scorable due to lanthanum carbonate deposits on the slides. Based on the above results, doses up to 500  $\mu\text{g}/\text{ml}$  were chosen for treatment in the absence of S-9 for the first cytogenetic experiment. With S-9, the same doses as in the range-finder study were used.

*Test agent stability:* Test agent was shown to be stable at room temperature.

*Metabolic activation system:* The S-9 fraction was prepared from the livers of beta-naphthoflavone and sodium phenobarbitone induced male Fischer 344 rats.

*Controls:* Vehicle control – culture medium (McCoy's 5A)

Positive controls – mitomycin C, 0.3  $\mu\text{g}/\text{ml}$  – without S-9

cyclophosphamide, 15  $\mu\text{g}/\text{ml}$  – with S-9

*Exposure conditions.* The first experiment was conducted in the same way as the range-finder study. The following doses were used without S-9: 50, 100, 200, 350 and 500  $\mu\text{g}/\text{ml}$ . All slides, except those from the 100  $\mu\text{g}/\text{ml}$  concentration, were scored for aberrations. Slides prepared from the range-finder study cultures, treated with S-9, were scored in the first cytogenetic experiment (500, 2500 and 5000  $\mu\text{g}/\text{ml}$  doses).

In the second experiment, a second harvest time, 24 hours later than the first, was included. The treatment doses were as follows: 500, 2500 and 5000  $\mu\text{g}/\text{ml}$  with S-9 and 20, 50, 100, 200, 350 and 500  $\mu\text{g}/\text{ml}$  without S-9. Cultures used for the second harvest time were seeded at  $1.5 \times 10^5$  cells per culture. The treatment of the second harvest cultures without S-9 was terminated at 1.5 cell cycles by removing the treatment medium,

washing with PBS, and reincubating in fresh culture medium until harvesting. Slides of all treatment doses were scored for aberrations.

A third cytogenetic experiment was performed at a single harvest time in the absence of S-9 to determine clone forming ability for the assessment of toxicity. The doses used were 50, 200, 350, 400, 450, 500 and 550  $\mu\text{g/ml}$ . A concurrent set of duplicate cultures was dosed in the same way as the cytogenetics cultures. At harvest time, viable cell counts were performed and 100 cells per flask were used for assessment of clone forming ability. All slides were scored for chromosome aberrations except that only negative controls and top dose levels were scored at the second harvest time.

*Analysis:* One hundred cells were counted from each pair of slides (2 slides/culture). Only well spread metaphases with a chromosome count of 18-22 were scored for chromosome damage. Cells with more than this number of chromosomes were recorded as numerical aberrations. Scoring was done using UKEMS test procedures [Basic Mutagenicity Tests. UKEMS recommended test procedures. (1990) ed. D.J. Kirkland, Cambridge University Press]. Only 25 metaphase spreads were scored from each positive control culture. The mitotic index for each negative control and lanthanum carbonate treated culture was determined from a minimum of 1000 cells. A test article was considered to be positive if it produced a dose response relationship with at least one dose level having significantly more chromosome aberrations ( $p < 0.05$ ) than the solvent control.

**Summary of study findings:** Results of the experiments are summarized in Tables 6 to 8. In the absence of S-9, significant increases in the incidence of chromosome aberrations were seen at dose levels of 200  $\mu\text{g/ml}$  and above. These increases were not dose related in experiments 1 and 3, but a dose relationship was seen in experiment 2. It is noted that the increases in aberrations were associated with cytotoxicity (decreased mitotic index and loss of clone forming ability). In experiment 3, doses of 350  $\mu\text{g/ml}$  and above caused more than 80% cytotoxicity as measured by clone forming capacity. There were no aberrations at non-cytotoxic doses or at the second harvest time. In the presence of metabolic activation, non-dose related increases in aberrations were seen at 500  $\mu\text{g/ml}$  and above. These dose levels produced reductions in mitotic indices by more than 50% compared to negative controls. [According to the sponsor, "these increases were not consistent or above the level expected for this cell line (Table 8A)."] Positive control chemicals produced significant increases in the incidence of chromosomal aberrations in all 3 experiments.

**Study validity:** The assay is considered valid since both positive control chemicals induced statistically significant increases in chromosomal aberrations, indicating that the cells are sensitive to the mutagenic effects of known clastogens, and that the S-9 used in the study was capable of metabolizing the inactive precursor to a genotoxic intermediate.

(Note: The sponsor has concluded that "lanthanum carbonate has not shown clastogenicity, under the conditions of this test, which is believed to be of biological significance." According to the sponsor, "the observed increases in chromosome

aberrations have the characteristics of an artifact of high cytotoxicity. This is supported by the inconsistency of response with respect to dose and between replicate cultures.”)

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ON ORIGINAL**

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Table 6. Experiment 1

Dose (µg/mL)	Total cells	Aberrant cells	Mitotic Index	Chromatid			Chromosome			Multiple aberrations	Numerical aberrations			% cells with aberrations	
				Gaps	deletion	exchange	deletion	exchange	Poly		Endo	Hyper	with gaps	without gaps	
Without Metabolic Activation 1st harvest															
50	200	6	6.20	2	2	1	0	1	0	10	0	7	3	2	
200	200	16	6.25	7	9	0	0	2	0	17	0	11	8	5	
350	200	19	6.00	10	8	1	3	1	0	17	0	2	9.5	5.5	
500	200	13	3.85	4	6	1	2	1	0	13	0	12	6.5	4.5	
Control	400	11	11.40	4	6	0	0	1	0	38	0	12	2.8	1.8	
MMC	50	35		4	30	20	1	0	3	4	0	3	70	70	
With Metabolic Activation 1st harvest															
500	200	12	9.70	6	3	0	3	1	0	3	0	1	6	3.5	
2500	200	8	6.45	5	6	0	0	0	0	1	0	3	4	3	
5000	200	15	5.75	6	5	0	4	0	0	2	0	0	7.5	4.5	
Control	400	13	9.05	7	4	0	5	0	0	34	0	5	3.3	1.8	
CPA	50	31		4	27	24	3	1	1	3	0	9	62	62	

Table 7. Experiment 2

Dose ( $\mu\text{g/ml}$ )	Total cells	Aberrant cells	Mitotic Index	Gaps	Chromatid		Chromosome		Multiple aberrations	Numerical aberrations			% cells with aberrations	
					deletion	exchange	deletion	exchange		Poly	Endo	Hyper	with gaps	without gaps
Without Metabolic Activation 1st harvest														
20	200	5	3.45	3	2	0	0	0	0	9	1	4	2.5	1
50	200	4	2.15	3	0	0	0	1	0	7	0	2	2	0.5
100	200	6	2.05	3	2	0	0	1	0	10	0	0	3	1.5
200	200	11	1.60	4	6	0	0	1	0	4	0	1	5.5	3.5
350	200	11	0.80	4	6	1	1	0	0	4	0	1	5.5	4
500	116	13	1.20	3	9	1	0	2	0	0	0	1	11.2	9.5
Control	400	12	3.03	4	4	1	2	1	0	15	0	5	3	2
MMC	50	18		6	10	6	2	1	0	1	0	0	36	30
With Metabolic Activation 1st harvest														
500	200	8	4.45	8	1	0	3	0	0	3	0	3	4	1
2500	200	15	3.40	7	5	0	4	0	0	4	0	3	7.5	4.5
5000	200	12	3.25	6	3	0	1	2	0	5	0	3	6	3
Control	400	8	9.30	6	0	0	1	1	0	9	0	3	2	0.5
CPA	50	26		8	22	14	1	2	1	1	0	0	52	44
Without Metabolic Activation 2nd harvest														
500	200	6	2.53	0	4	1	0	2	0	4	0	4	3	3
Control	400	17	2.79	4	5	1	4	3	0	8	0	6	4.3	3.3
With Metabolic Activation 2nd harvest														
5000	200	13	4.85	4	7	0	2	1	0	7	0	7	6.5	4.5
Control	400	14	8.50	7	3	1	5	0	0	13	0	7	3.5	1.8

Table 8. Experiment 3

Dose (µg/mL)	Total cells	Aberrant cells	Mitotic Index	Chromatid		Chromosome		Multiple aberrations	Numerical aberrations			% cells with aberrations		cfu/culture	
				Gaps	deletion	exchange	deletion		exchange	Poly	Endo	Hyper	with gaps		without gaps
Without metabolic activation 1st harvest															
50	182	5	6.9	3	2	0	0	0	0	1	0	0	2.7	1.1	104.56%
200	200	3	6.25	2	1	0	0	0	0	2	0	0	1.5	0.5	33.30%
350	200	8	5.05	4	4	0	0	0	0	5	0	0	4	2	19.61%
400	142	10	5.1	3	7	0	1	1	0	5	0	1	7	5.6	17.61%
450	93	6	5.3	4	3	0	0	0	0	3	0	0	6.5	3.2	17.08%
500	97	2	3.85	1	1	0	1	0	0	4	0	1	2.1	2.1	10.38%
550	120	10	4.6	5	5	0	2	1	0	1	0	1	8.3	5.8	10.48%
Control	400	5	9.28	3	2	0	0	0	0	3	0	0	1.3	0.5	100.00%
MMC	50	23		6	19	12	2	0	0	3	0	0	46	42	

Historical Control Data collected from studies conducted at [ ]  
during the period from 1993 to 1995.

1

	Mean cells analysed per culture	Results per 100 cells scored											% cells with aberrations				
		Aberrant cells	Mitotic Index	Gaps	Chromatid		Chromosome		Multiple aberrations	Numerical aberrations			with gaps		without gaps		
					deletion	exchange	deletion	exchange		Poly	Endo	Hyper	Range	Range			
Negative Control without S-9 <sup>1</sup>	Mean SD	271.6 99.45	3.05 2.10	5.02 3.22	2.05 1.45	0.41 0.57	0.05 0.13	0.60 0.95	0.31 0.46	0.00 0.00	3.79 4.96	0.01 0.05	1.26 2.96	3.05 2.10	0 - 6.8	1.20 1.33	0 - 4.8
Negative Control with S-9 <sup>1</sup>	Mean SD	265.09 110.20	3.35 2.46	4.91 2.57	2.10 1.62	0.51 0.57	0.13 0.26	0.44 0.48	0.39 0.56	0.02 0.07	2.13 2.03	1.55 5.01	0.85 1.28	3.96 3.43	0 - 12	1.63 1.61	0 - 5.8
Positive Control without S-9 <sup>2</sup>	Mean SD	50.00 0.00	57.86 14.33	Not available	11.57 7.07	32.29 15.27	44.71 23.07	9.43 18.52	2.71 2.43	5.71 4.63	3.00 6.01	0.14 0.53	2.14 3.63	57.86 14.33	36 - 88	54.86 15.04	30 - 88
Positive Control with S-9 <sup>2</sup>	Mean SD	50.00 0.00	56.77 26.49	Not available	10.00 9.35	38.31 25.77	39.69 30.40	16.15 38.53	1.54 2.18	7.54 10.99	5.08 8.39	0.15 0.55	2.46 4.56	55.57 25.84	14 - 94	53.29 25.72	12 - 94

Table 8A

<sup>1</sup> This data comprises of 4 studies (n=22), all of which used aqueous solvents (growth medium, phosphate buffered saline or ultra-high purity water).

<sup>2</sup> This data comprises of 6 studies (n=14).

Please note this testing facility now [ ]

[ ] during the period covered by these data

#### 4. Mouse Micronucleus Test

**Key Findings:** Significant increases in micronucleus frequency were noted in males treated at 1250 and 2000 mg lanthanum carbonate/kg and sacrificed after 48 hours. However, these increases did not exceed the historical control range, and also did not meet other criteria for a positive test.

**Study number:** M/MMN/42354

**Study type:** *In vivo* bone marrow micronucleus assay

**Conducting laboratory and location:** [ ]

**Study dates:** Not provided (Report date – July 1997)

**GLP compliance:** Yes (UK, OECD, USA and Japanese codes of GLP)

**QA report:** Yes (x) No. ( )

**Drug batch # and % purity:** 6268/951201, % purity – not provided

**Formulation/vehicle:** Lanthanum carbonate was formulated in 0.5% carboxymethyl-cellulose

#### **Methods**

*Strain/species:* Young adult CDI mice

*Dose selection criteria:* In a dose range finding study, groups of male and female mice were dosed with 2000 mg lanthanum carbonate/kg (limit dose; ICH Guideline S2A, 1995) or vehicle alone. Animals were observed for signs of toxicity for 96 hours post dose. Blood was collected for drug level determinations 2 hours after dosing and at the end of the observation period. No mortality or signs of toxicity were seen. On the basis of the above results, 2000 mg/kg was chosen as the top dose for the main study, and 1250 and 800 mg/kg were selected as the mid and low doses, respectively.

*Controls:* Vehicle – 0.5% carboxymethyl cellulose; Positive control – Mitomycin C – dissolved in 0.9% sterile saline at concentration of 0.4 mg/ml

*Study design:* Groups of male and female mice (15/sex/group) were given single doses of lanthanum carbonate (800, 1250 and 2000 mg/kg) or vehicle solution by oral gavage. Positive control animals (5/sex) received a single ip injection of mitomycin C (4 mg/kg).

*Sampling times:* Five male and female animals each from the treated and control groups were sacrificed at 24, 48 and 72 hours after treatment. Positive control animals were sacrificed 24 hours post dose. Bone marrow cells were harvested from both femurs of each animal and slides were prepared.

*Analysis:* Counting method – Slides were stained using the method of Gollapudi and Kamra (Mut. Res., 64, 1979, pp 45-46). A minimum of 2000 polychromatic erythrocytes

(PCE) per animal were counted for micronucleated PCE (MN-PCE) evaluation, and 1000 erythrocytes per animal were counted for determination of polychromatic to normochromatic erythrocyte (NCE) ratio.

Data were statistically analyzed using the UKEMS guidelines (Kirkland, D., 1989. 'Statistical Evaluation of Mutagenicity Test Data', UKEMS sub-committee on guidelines for mutagenicity testing. Report, Part III).

The study was considered acceptable if:

1. negative control animals (both sexes at all sacrifice times) had a mean micronucleus count of not more than 4 micronuclei per animal
2. positive control animals had a minimum micronucleus count of approximately 10 micronuclei per animal

The test drug was considered to have produced a positive response if :

1. a dose-dependent increase in micronucleus frequency was noted in drug-treated groups
2. statistically significant increase in micronucleus frequency, compared to negative control, was noted in any test drug group.
3. a minimum mean value of 5 micronuclei per animal was seen in one treated group

**Summary of study findings:** The mean counts of MNPCEs and the mean PCE/NCE ratios are presented in Tables 9 & 10, respectively. Significant increases in the frequency of MNPCEs, compared to control, were noted in males treated at 1250 and 2000 mg/kg ( $p < 0.05$  in both cases) and sacrificed at 48 hours. Significant increases in PCE/NCE ratios were found for females at 48 hours at 2000 mg/kg ( $p < 0.01$ ) and at 72 hours at 800 and 1250 mg/kg ( $p < 0.05$  and  $p < 0.001$ , respectively)

The positive control showed a marked increase in the number of MNPCEs, compared to control, but no significant difference was seen in the PCE/NCE ratio.

According to the sponsor, the statistically significant increases in micronucleus frequency observed in treated males were "due to the very low control values (0 MNPCE count for the males sacrificed at 48 hours) rather than any real increase in micronucleus frequency due to treatment". It is noted that these increases did not meet the criteria for a positive test of exceeding the minimum mean value of 5 micronuclei per animal in a treated group. Moreover, since the increases did not exceed the historical control range (Table 11), and also because the mean micronucleus frequency value in control females was much higher than the mean values observed in treated males, the increases are not considered biologically significant. It is noted that no significant bone marrow toxicity (reduction in PCE:NCE ratio) was seen at the top dose of 2000 mg/kg (limit dose).

**Study validity:** The assay is considered valid since the positive control chemical significantly increased the micronucleus frequency ( $p < 0.001$ ) and the negative control values were within the historical control range.

Table 9.

**Adjusted Mean Count of MNPCE (/2000)  
Group Effects by Sex (Excl. +ve Control)**

**At 24 hours**

Variable	Group	Adjusted Means				
		Control	800mg/kg	1250mg/kg	2000mg/kg	+ve control
Males	Geometric mean	1.49	0.97	1.35	0.25	-
	95% CI	(0.58, 2.93)	(0.25, 2.11)	(0.49, 2.71)	(0.00, 0.96)	-
Females	Geometric mean	3.28	2.13	1.93	0.89	-
	95% CI	(1.72, 5.75)	(0.98, 3.93)	(0.86, 3.62)	(0.20, 1.98)	-
Males	Geometric mean	1.49	-	-	-	88.7###
	95% CI	(0.09, 4.69)				(38.2, 203.9)
Females	Geometric mean	3.28	-	-	-	75.1###
	95% CI	(0.87, 8.78)				(32.3, 172.9)

# $p < 0.05$  ## $p < 0.01$  ### $p < 0.001$  (1-sided increase Students t-test)

**At 48 hours**

Variable	Group	Adjusted Means			
		Control	800mg/kg	1250mg/kg	2000mg/kg
Males	Geometric mean	0.00	0.64	1.05*	1.17*
	95% CI	(0.00, 0.61)	(0.02, 1.65)	(0.27, 2.30)	(0.34, 2.50)
Females	Geometric mean	2.10	1.93	1.70	1.86
	95% CI	(0.92, 4.01)	(0.82, 3.73)	(0.68, 3.36)	(0.77, 3.62)

\* $p < 0.05$  \*\* $p < 0.01$  \*\*\* $p < 0.001$  (1-sided increase Dunnett's test)

**At 72 hours**

Variable	Group	Adjusted Means			
		Control	800mg/kg	1250mg/kg	2000mg/kg
Males	Geometric mean	0.64	1.61	1.40	1.27
	95% CI	(0.02, 1.65)	(0.62, 3.19)	(0.49, 2.87)	(0.41, 2.65)
Females	Geometric mean	0.15	1.17	0.52	1.17
	95% CI	(0.00, 0.85)	(0.35, 2.49)	(0.00, 1.44)	(0.35, 2.49)

Table 10

**Mean PCE/NCE Ratio (%)**  
**Group Effects by Sex (Excl. +ve Control)**

At 24 hours

Variable	Group	Means				
		Control	800mg/kg	1250mg/kg	2000mg/kg	+ve control
Males	Mean	115	81	92	98	-
	95% CI	(91, 138)	(58, 104)	(68, 115)	(75, 121)	-
Females	Mean	91	119	97	114	-
	95% CI	(68, 114)	(96, 142)	(74, 120)	(91, 137)	-
Males	Mean	115	-	-	-	87
	95% CI	(80, 149)	-	-	-	(52, 122)
Females	Mean	91	-	-	-	91
	95% CI	(56, 126)	-	-	-	(56, 125)

At 48 hours

Variable	Group	Means			
		Control	800mg/kg	1250mg/kg	2000mg/kg
Males	Mean	101	77	134	50
	95% CI	(66, 136)	(42, 112)	(99, 169)	(15, 85)
Females	Mean	114	130	121	204**
	95% CI	(80, 150)	(95, 164)	(86, 156)	(169, 239)

\*p<0.05 \*\*p<0.01 \*\*\*p<0.001 (2-sided Dunnett's test)

At 72 hours

Variable	Group	Means			
		Control	800mg/kg	1250mg/kg	2000mg/kg
Males	Mean	89	125	96	91
	95% CI	(60, 118)	(96, 155)	(67, 125)	(62, 120)
Females	Mean	145	208*	229***	181
	95% CI	(115, 174)	(179, 237)	(200, 258)	(152, 210)

\*p<0.05 \*\*p<0.01 \*\*\*p<0.001 (2-sided Dunnett's test)

Table 11.

## Summary of Negative Control Micronucleated PCE Frequency

Number (year)	Geometric Mean	Arithmetic Mean	SD	Upper Range (95%)
120 (1994-1995)	1.02	1.41	1.51	4.43
120 (1996)	0.90	1.23	1.30	3.84

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## VI. CARCINOGENICITY

**104-Week Oral (Gavage) Carcinogenicity Study in the Rat**

**Key findings:** Two-year oral administration of lanthanum carbonate at 0, 0, 100, 500 and 1500 mg/kg/day did not increase the incidence of tumors in the rat.

**Study number:** SPD/87/C

**Volume #:** 1.27 – 1.34

**Conducting laboratory and location:**  $\tau$

3

**Date of study initiation:** April 10, 1997

**GLP compliance:** yes

**QA report:** yes

**Drug lot# and purity:** Lanthanum carbonate, lot #s B1066-960802 (weeks 1-42), B1066-960902 (weeks 43-65), B1066-960904 (weeks 66-106) and B1066-980601 (weeks 106-107), purity – within test article specifications

**CAC concurrence on protocol:** No

**Species/strain:** Rat CD (SD) BR VAF PLUS strain obtained from  $\tau$

J

**Number and age at start of study:** 60/sex/main study group (groups 1 to 5); additional 5/sex at 0, 100 and 1500 mg/kg/day dose levels (groups 6 to 8) for plasma and tissue lanthanum investigations during week 78. All tissues listed in Table 1 were collected for lanthanum analysis; 5 to 6 weeks of age

**Animal housing:** Groups of 5 (same sex)/cage

**Formulation:** Formulated daily as a suspension in 0.5% aqueous carboxymethylcellulose

**Drug stability:** Stable for about 2 years (inorganic salt) – stored at room temperature, in the dark, when not in use

**Methods**

*Doses:* 0, 0, 100, 500 and 1500 mg/kg/day (2 identical vehicle controls)

*Basis of dose selection:* “The high dose was considered to represent the maximum tolerated dose on the basis of findings in a 6 month toxicity study in rats.” (Basis of dose selection is described under the ‘adequacy of the carcinogenicity study’ subsection under **Evaluation** section.)

*Route of administration:* Oral (gavage)

*Frequency of drug administration:* Once daily, 7 days a week for up to 104 weeks until the day before necropsy

Interim sacrifices: None

*Deviations from original study protocol:* Major deviations include: a) during week 8, on one occasion (6-1-1997), six low dose (100 mg/kg/day) females (animal #s 421 to 426) were given the control formulation as well as the correct 100 mg/kg/day formulation, b) during week 69, on 8-1-1998, all surviving Group 2 control males, except animal #s 116, 118 and 120, received a single administration of the low dose (100 mg/kg/day) formulation, and c) during week 78, because of deaths in the satellite high dose male group during blood collection, one high dose male (#300) from the main study was transferred to the satellite high dose group. (Tissues were not collected from this animal for histopathologic examination.) Other deviations are considered not significant.

*Statistical methods:* Analysis of variance (ANOVA) was performed on all continuous or semi-continuous parameters. The residuals from this preliminary analysis were examined for heterogeneity of variance using Levenes test. If the Levenes test was significant at the 1% level, then a non-parametric analysis using Kruskal-Wallis ANOVA test was performed to assess overall differences between treatment groups, followed by Shirley's non-parametric version of Williams test, which was based on mean ranks rather than arithmetic means. If Levenes was not Gsignificant, then Williams test was performed.

Survival analysis was performed by the Kaplan-Meier technique.

All statistical tests on tumor incidence were performed using two-sided trend tests. The two vehicle control groups were combined for statistical analysis. All analyses were adjusted for mortality and analyses of fatal and non-fatal findings were combined. Analysis of the incidence of a particular tumor depended on the frequency of occurrence. If there were two or less occurrences for a particular sex, then no analysis was performed. If there were between 3 and 9 occurrences, then the data were analyzed using an exact permutational test. If the number of a particular tumor (within a given sex) exceeded 9, then the incidence was analyzed using chi-squared tests.

Non-neoplastic findings were analyzed using an exact permutational test (Fishers Exact) and Cochran-Armitage Trend test.

## Observations and Measurements

*Clinical signs:* daily – from week 27 onwards, weekly palpation of all animals for the detection of superficial swellings

*Mortality:* twice daily

*Body weights:* weekly for the first 16 weeks and then every fourth week

*Food consumption:* weekly for the first 16 weeks and then for one week in every 4 weeks

*Hematology:* during week 103 for all surviving animals or before sacrifice for those killed in extremis – [parameters evaluated – RBC, WBC (total and differential) and platelet counts]

*Clinical chemistry:* not performed

*Organ weights:* at sacrifice (Organs specified in Table 1 from 10 rats/sex/group were weighed.)

*Gross pathology:* at sacrifice

*Histopathology:* Organs/tissues listed in Table 12 (from all animals in all groups) were examined.

*Toxicokinetics:* on one occasion each during weeks 52 and 78 (blood collected 2 hours post-dose)

## Results

*Mortality:* The survival data over the course of the study are presented graphically in Figures 5 and 6, and the percent survival at the termination of the study is given below.

Sex :	Males					Females				
Group :	1	2	3	4	5	1	2	3	4	5
Dose level (mg/kg/day) :	0	0	100	500	1500	0	0	100	500	1500
% survival up to Week 104 :	48	32	42	43	38	45	33	45	40	48

There were no significant differences in the survival rates between control and treated groups of either sex.

*Clinical signs:* No treatment-related clinical signs were noted.

*Body weights:* Body weight data are presented graphically in Figures 7 to 10. Administration of lanthanum had no effects on body weight or body weight gain.

*Food consumption:* There were no treatment-related effects on food consumption.

*Hematology:* No treatment-related hematology findings.

*Organ weights:* Dose-related decreased absolute liver weights (mean weights 9 to 17% lower than control) were seen in treated males, with no effects on relative liver weights. Treatment-related reductions in absolute (26-29%) and relative (31-38%) adrenal weights were seen in treated females.

Table 12. Necropsy Procedures

Tissue	Weigh	Fix	Slide Preparation	Microscopic Examination
adrenal glands	✓	✓	✓	✓
aorta		✓	✓	✓
brain (3 sections)	✓	✓	✓	✓
caecum		✓	✓	✓
colon		✓	✓	✓
duodenum		✓	✓	✓
epididymides	✓	✓	✓	✓
eyes (incl. optic nerves)		✓	✓	✓
femur (incl. marrow)		✓	✓	✓
heart	✓	✓	✓	✓
Harderian glands*		✓	✓	✓
ileum		✓	✓	✓
jejunum		✓	✓	✓
kidneys	✓	✓	✓	✓
lacrimal glands		✓	✓	✓
liver	✓	✓	✓	✓
lungs (incl. mainstem bronchi)	✓	✓	✓	✓
mesenteric lymph node		✓	✓	✓
oesophagus		✓	✓	✓
ovaries	✓	✓	✓	✓
pancreas		✓	✓	✓
pituitary	✓	✓	✓	✓
prostate	✓	✓	✓	✓
rectum		✓	✓	✓
salivary gland	✓	✓	✓	✓
sciatic nerve		✓	✓	✓
seminal vesicles	✓	✓	✓	✓
site of mammary gland		✓	✓	✓
skeletal muscle		✓	✓	✓
skin		✓	✓	✓
spinal cord (3 levels)		✓	✓	✓
spleen	✓	✓	✓	✓
sternum		✓	✓	✓
stomach		✓	✓	✓
Submandibular lymph nodes		✓	✓	✓
testes	✓	✓	✓	✓
thymus	✓	✓	✓	✓
thyroids (incl. parathyroids) +	✓	✓	✓	✓
tongue		✓	✓	✓
trachea		✓	✓	✓
urinary bladder		✓	✓	✓
uterus (incl. cervix)	✓	✓	✓	✓
vagina		✓	✓	✓
all gross lesions		✓	✓	✓
all tumours and masses		✓	✓	✓

+weighed after fixation ==

\* omitted from original protocol list in error

Figure 5. % Survival – Males

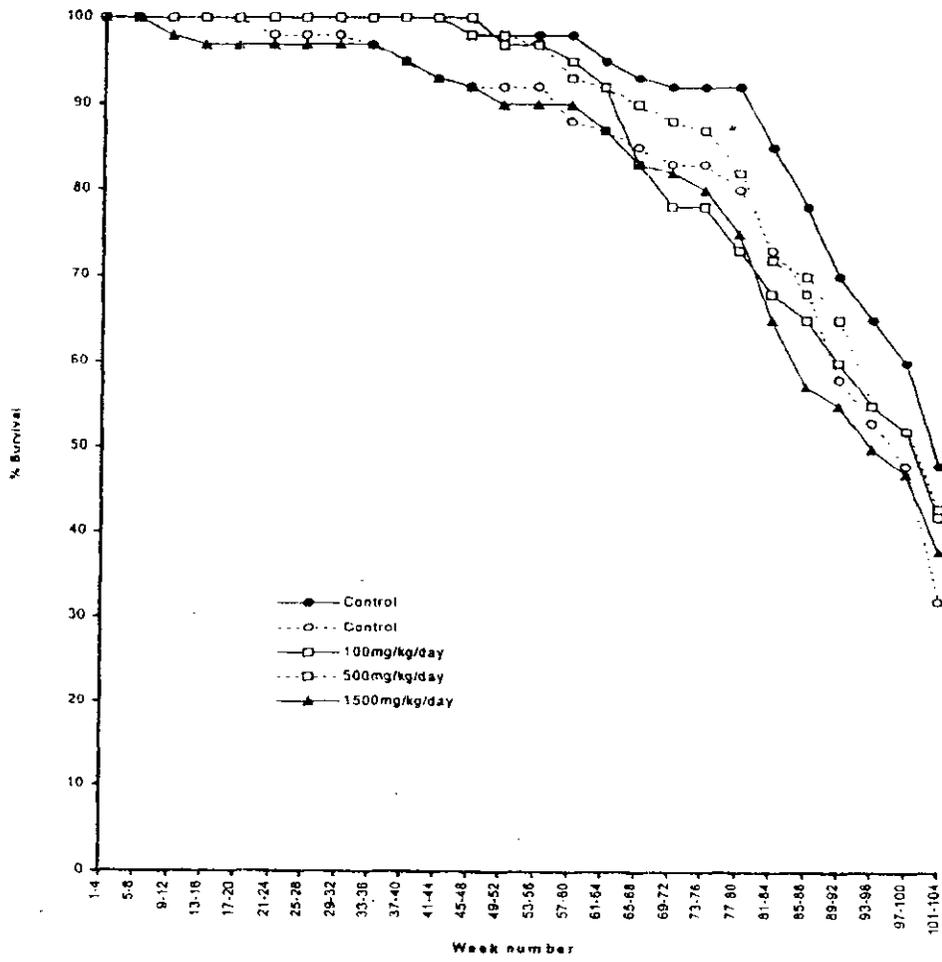


Figure 6. % Survival - Females

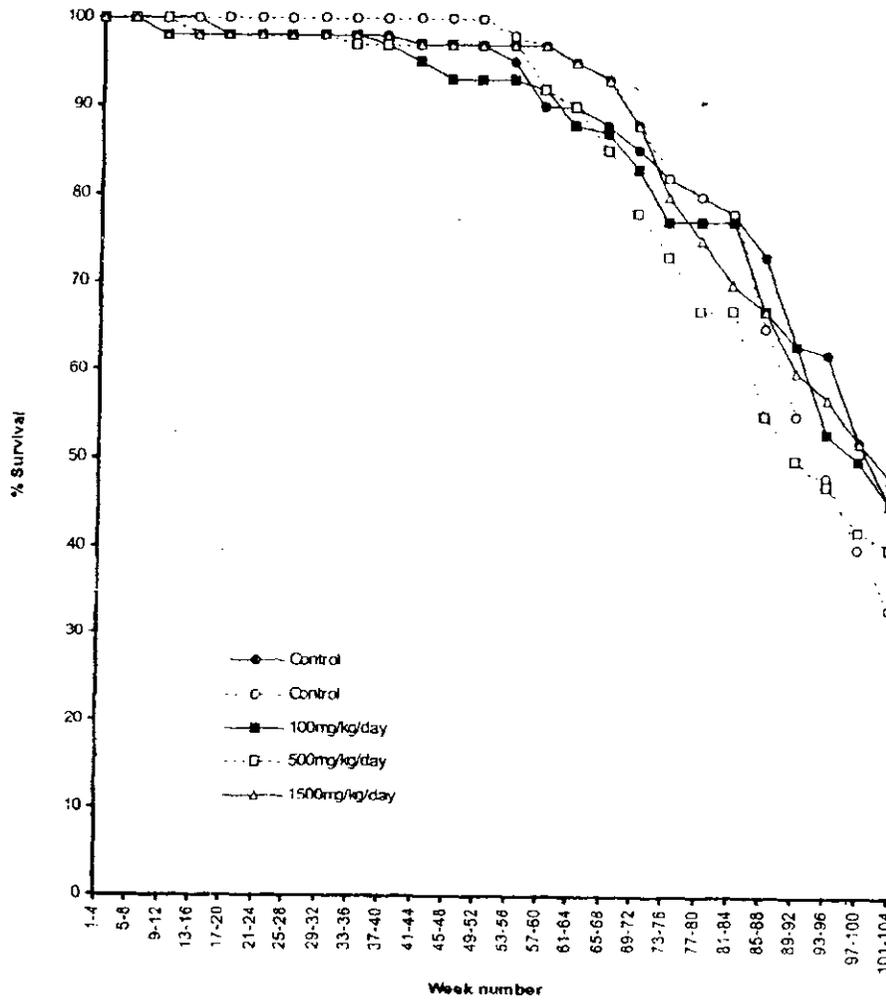


Figure 7. Mean Body Weights – Weeks 1 to 16 - Males

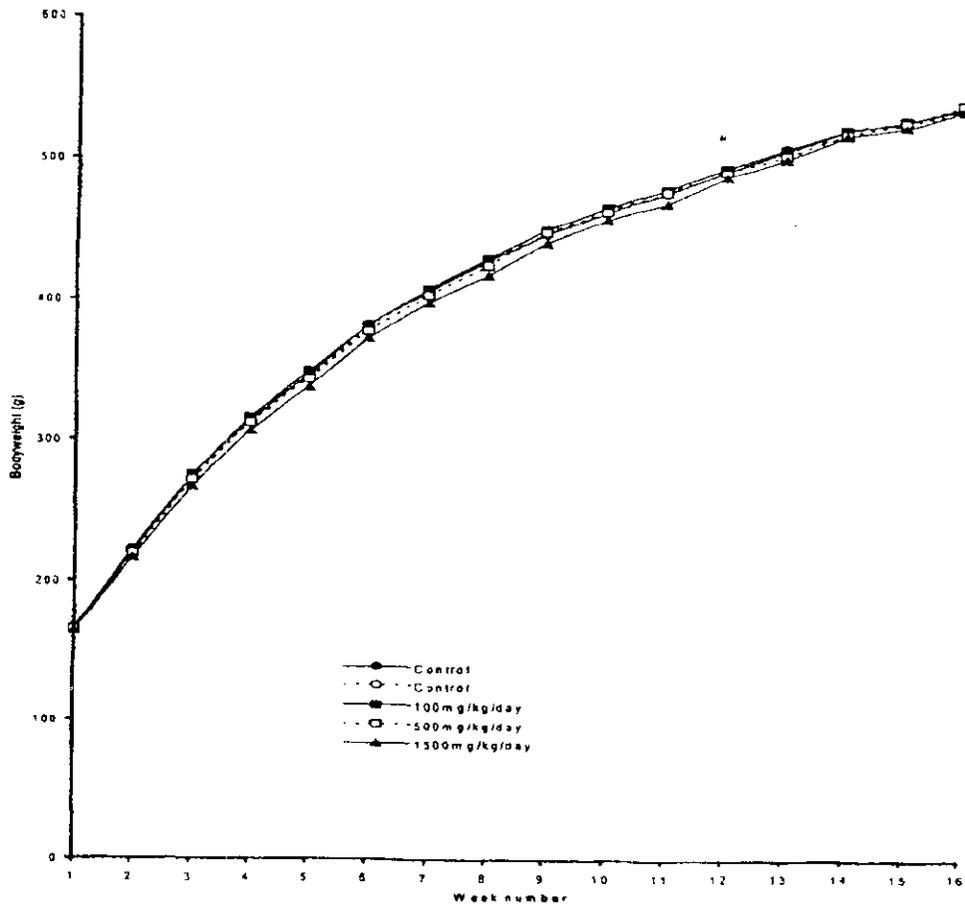


Figure 8. Mean Body Weights – Weeks 16 to 104 - Males

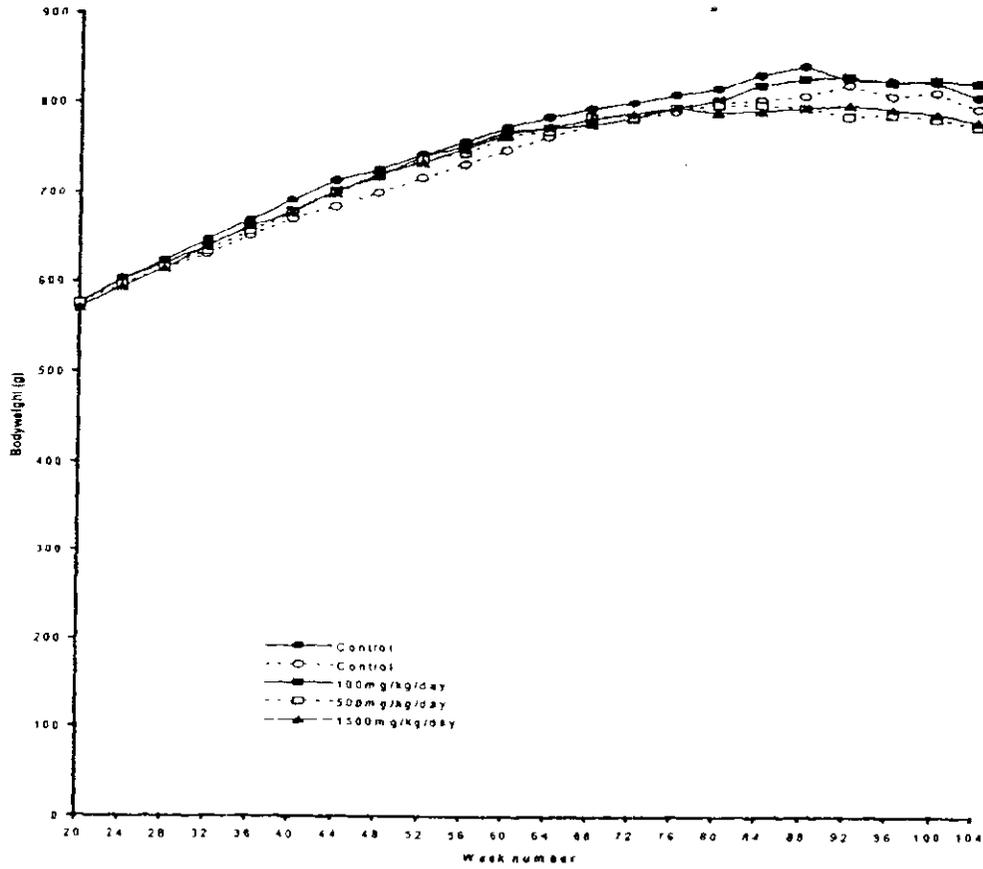


Figure 9. Mean Body Weights – Weeks 1 to 16 - Females

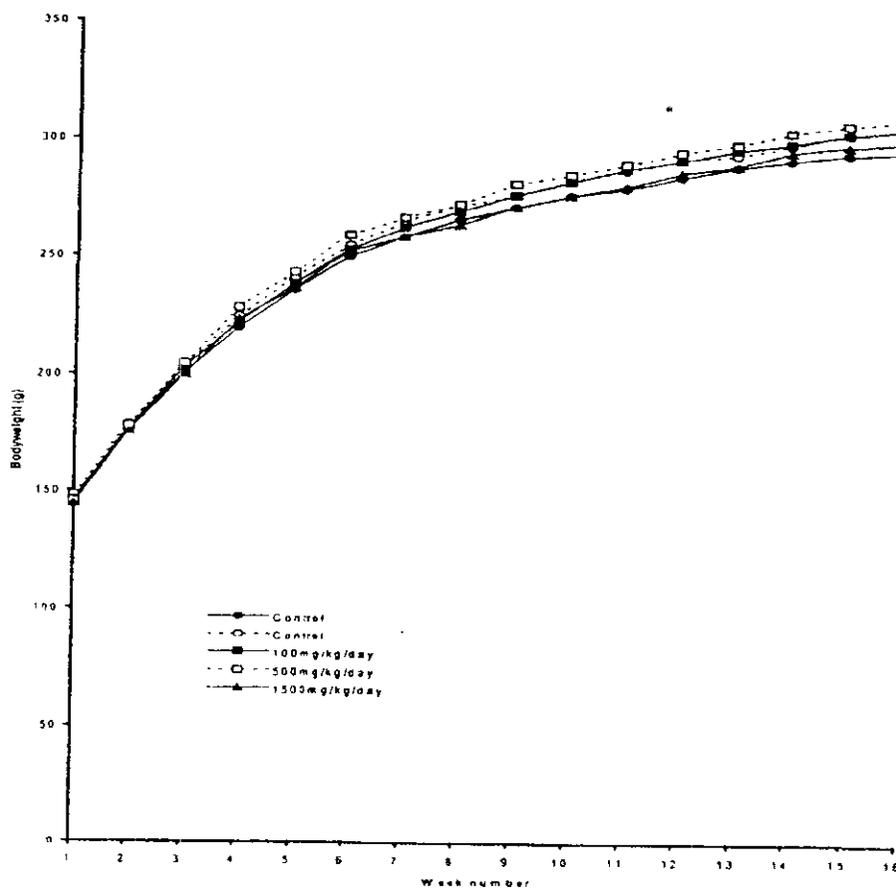
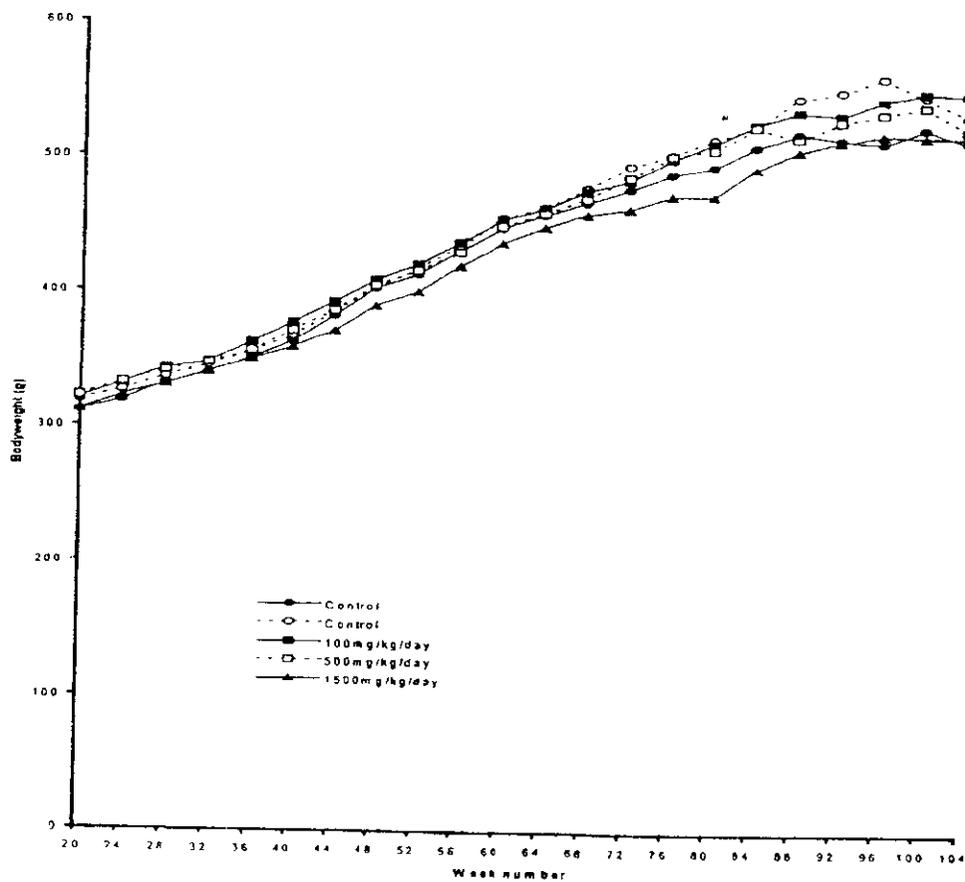


Figure 10. Mean Body Weights – Weeks 16 to 104 – Females



*Gross pathology:* There were treatment-related macroscopic stomach findings for the two highest dose groups of both sexes. Increased incidences of abnormal shape of the glandular region and the limiting ridge of the stomach were noted in the above groups (Table 2). No other remarkable treatment-related gross lesions were noted.

*Histopathology: Non-neoplastic findings* – Increased incidences of epithelial hyperplasia (limiting ridge), focal antral hyperplasia and focal glandular epithelial hyperplasia of the stomach were seen in male and female animals given lanthanum carbonate at dose levels of 500 and 1500 mg/kg/day (Table 13). At 100 mg/kg/day, the incidences of these stomach lesions were similar to those of controls.

Table 13. Summary of Macroscopic and Microscopic Findings in the Stomach

	MALES					FEMALES				
	60	60	60	60	59	60	60	60	60	60
Number examined	60	60	60	60	59	60	60	60	60	60
Dose in mg/kg/day	0	0	100	500	1500	0	0	100	500	1500
<b>Macroscopic Findings</b>										
Abnormal Contents	0	0	1	3*	7***	1	0	2	5*	4*
Abnormal Shape (Glandular Region)	4	1	8*	14***	20***	5	1	4	17***	12**
Abnormal Shape (Limiting Ridge)	1	0	2	9***	13***	0	2	0	9***	13***
<b>Microscopic Findings</b>										
Epithelial Hyperplasia (Limiting Ridge)	3	3	4	8	12**	5	5	5	8	15**
Focal Antral Hyperplasia	0	0	0	4*	8***	0	0	0	3*	11***
Occasional degenerate foci	0	0	0	8***	15***	0	0	0	5**	8***
Focal Glandular Epithelial Hyperplasia	0	1	1	2	4*	0	0	1	3*	6**

Groups 1 and 2 combined for statistical analysis

\* = 5% level, \*\* = 1% level, \*\*\* = 0.1% level

In the kidney, the incidences of pelvic/papillary mineralization, transitional cell hyperplasia and chronic progressive nephropathy were markedly reduced in females at 500 and/or 1500 mg/kg/day. The incidences of these kidney findings are given below.

Number examined	MALES					FEMALES				
	60	60	60	60	59	60	60	60	60	60
Dose in mg/kg/day	0	0	100	500	1500	0	0	100	500	1500
Pelvic/ papillary mineralisation	2	1	2	1	0	28	33	21	3***	1***
Transitional cell hyperplasia	9	13	14	9	8	39	36	29	14***	4***
Chronic progressive nephropathy	32	23	20	23	24	13	17	17	14	7*

Groups 1 and 2 combined for statistical analysis \* = 5% level, \*\* = 1% level, \*\*\* = 0.1% level

*Neoplastic findings* – The incidences of various types of tumors observed in the study are presented in Table 14.

Sponsor's analyses showed statistically significant increased incidences for the following tumors:

- male - hemopoietic system: histiocytic sarcoma (trend). Incidence = 1/60 (control), 1/60 (control), 1/60 (LD), 0/60 (MD) and 4/59 (HD)
- male – liver: histiocytic sarcoma (control vs HD, and trend). Incidence = 0/60 (control), 0/60 (control), 1/60 (LD), 0/60 (MD) and 4/59 (HD)
- female -- skin and sc tissue: adenocarcinoma (control vs low dose). Incidence = 7/53 (control), 8/49 (control), 16/54 (LD), 8/46 (MD) and 6/46 (HD)
- female – skin and sc tissue: fibroma (control vs HD, and trend). Incidence = 4/53 (control), 2/49 (control), 2/54 (LD), 3/46 (MD) and 4/46 (HD)
- female -- skin and sc tissue: lipoma (trend). Incidence = 2/53 (control), 0/49 (control), 0/54 (LD), 2/46 (MD) and 3/46 (HD)
- combined sexes – liver: histiocytic sarcoma (control vs HD, and trend) Incidence = 0/120 (control), 1/120 (control), 1/120 (LD), 0/120 (MD) and 4/119 (HD)
- combined sexes – skin and sc tissue: lipoma (trend). Incidence = 6/88 (control), 2/77 (control), 4/88 (LD), 4/79 (MD) and 7/69 (HD)

(Note: Control groups were combined for the statistical analyses.)

Table 14. Neoplastic Findings

Daily Dose (mg/kg/day)		Males					Females				
		0	0	100	500	1500	0	0	100	500	1500
Number examined		60	60	60	60	59	60	60	60	60	60
Adrenals	Large granular lymphocyte leukaemia	0	0	0	0	0	1	0	0	0	0
	Malignant lymphoma	0	0	0	1	0	0	0	1	0	0
	Cortical adenoma	1	3	0	0	1	2	6	0	1	1
	Phaeochromocytoma	6	5	4	3	2	1	2	0	0	2
Brain	Oligodendroglioma	0	0	0	0	1	0	0	1	0	0
	Adenocarcinoma	1	0	0	0	0	4	3	1	2	0
	Anaplastic glioma	0	0	1	0	0	0	0	0	0	0
	Astrocytoma	0	0	0	0	0	2	1	1	0	0

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Table 14. (continued)

Daily Dose (mg/kg/day)		Males					Females				
		0	0	100	500	1500	0	0	100	500	1500
Number examined		60	60	60	60	59	60	60	60	60	60
Brain (cont)	Malignant lymphoma	0	0	0	2	0	0	0	0	0	0
	Granular cell tumour	0	1	0	1	0	0	0	0	0	1
	Meningeal sarcoma	0	1	0	0	0	0	0	0	0	0
	Pineal gland tumour	0	1	0	0	0	0	0	0	0	0
Caecum	Malignant lymphoma	0	0	0	0	0	0	0	1	0	0
Number examined		60	60	60	60	59	60	60	59	60	60
Colon	Malignant lymphoma	0	0	0	0	0	0	0	1	0	0
Number examined		60	60	60	60	59	60	60	60	60	60
Duodenum	Leiomyoma	0	0	0	0	0	0	0	1	0	0
Epididymides	Sarcoma	0	0	1	0	0	-	-	-	-	-
Number examined		60	59	60	60	59	60	60	59	60	60
Femur	Adenocarcinoma	1	0	0	0	0	0	0	0	0	0
	Large granular lymphocyte leukaemia	0	0	0	0	0	1	0	0	0	0
	Malignant lymphoma	0	0	0	1	0	0	0	0	0	0
	Histiocytic sarcoma	0	1	0	0	1	0	0	0	0	0
Number examined		60	60	60	60	59	60	60	60	60	60
Haemopoietic system	Large granular lymphocyte leukaemia	0	1	1	0	0	1	0	0	0	0
	Lymphoma	0	0	1	2	0	0	0	1	0	0
	Histiocytic sarcoma	1	1	1	0	4	0	1	0	0	0
Number examined		60	58	60	60	59	60	60	60	60	60
Hartmanian glands	Adenoma	0	1	1	0	0	0	0	0	0	0
	Malignant lymphoma	0	0	0	0	0	0	0	1	0	0
Number examined		60	60	60	60	59	60	60	60	60	60
Heart	Malignant lymphoma	0	0	0	1	0	0	0	0	0	0
	Malignant schwannoma	1	0	0	0	0	0	0	0	0	0
Ileum	Malignant lymphoma	0	0	0	1	0	0	0	1	0	0
Jejunum	Malignant lymphoma	0	0	0	1	0	0	0	1	0	0
	Adenocarcinoma	0	0	0	0	0	0	1	0	0	0
	Leiomyosarcoma	0	0	1	0	0	0	0	1	0	0
Kidneys	Histiocytic sarcoma	0	0	0	0	1	0	0	0	0	0
	Mesenchymal tumour	0	0	1	0	0	0	0	0	0	0
	Nephroblastoma	0	0	0	0	0	1	0	0	0	0
	Malignant lymphoma	0	0	0	0	0	0	0	1	0	0
Number examined		60	59	60	60	58	59	59	58	58	60
Lacrimal glands	Malignant lymphoma	0	0	0	1	0	0	0	1	0	0

Table 14. (continued)

Daily Dose (mg/kg/day)		Males					Females				
		0	0	100	500	1500	0	0	100	500	1500
Number examined		60	60	60	60	59	60	60	60	60	60
Liver	Large granular lymphocyte leukaemia	0	1	1	0	0	1	0	0	0	0
	Adenoma	1	1	2	0	1	2	3	2	0	0
	Malignant lymphoma	0	0	1	2	0	0	0	1	0	0
	Histiocytic sarcoma	0	0	1	0	4	0	1	0	0	0
Lungs	Adenocarcinoma	0	2	1	0	0	0	0	0	0	0
	Malignant lymphoma	0	0	0	1	0	0	0	1	0	0
	Pulmonary adenoma	1	0	0	2	1	0	0	0	0	0
	Histiocytic sarcoma	0	0	2	0	1	0	0	0	0	0
	Adenocarcinoma	2	0	0	0	0	0	0	1	1	0
	Large granulocytic leukaemia	0	1	0	0	0	0	0	0	0	0
Mesenteric lymph nodes	Sarcoma	0	0	0	0	0	0	0	0	0	1
	Large granular lymphocyte leukaemia	0	0	0	0	0	1	0	0	0	0
	Malignant lymphoma	0	0	0	2	0	0	0	1	0	0
	Histiocytic sarcoma	0	0	0	0	2	0	0	0	0	0
Ovaries	Haemangioma	4	1	3	3	0	1	0	0	0	1
	Luteoma	-	-	-	-	-	0	1	0	0	0
	Granulosa cell tumour	-	-	-	-	-	0	1	0	0	0
Number examined		-	-	-	-	-	0	0	0	0	1
Number examined		60	60	60	60	58	60	60	60	60	60
Pancreas	Malignant lymphoma	0	0	0	0	0	0	0	1	0	0
	Islet cell adenoma	6	3	3	3	1	3	0	1	1	2
	Islet cell carcinoma	0	1	1	0	0	0	0	0	0	0
	Exocrine cell adenoma	0	1	0	0	0	0	0	0	0	0
	Exocrine cell carcinoma	0	0	1	0	0	0	0	0	0	0
Number examined		52	59	56	58	55	56	56	51	57	56
Parathyroid glands	Adenoma	1	0	0	1	0	0	0	0	0	2
Number examined		59	59	60	59	59	60	59	60	58	59
Pituitary gland	Adenoma pars distalis	20	18	21	17	16	36	36	39	35	39
	Adenocarcinoma	1	0	0	0	0	4	3	1	2	0
	Adenoma pars intermedia	0	1	0	1	0	2	0	0	0	0
	Malignant lymphoma	0	0	0	1	0	0	0	0	0	0
Number examined		60	60	60	60	59	60	60	60	60	60
Prostate gland	Liposarcoma	1	0	0	0	0	-	-	-	-	-
	Adenoma	1	1	1	0	0	-	-	-	-	-
Rectum	Adenoma	0	0	0	0	1	0	0	0	0	0
	Malignant lymphoma	0	0	0	0	0	0	0	1	0	0



Table 14. (continued)

Daily Dose (mg/kg/day)		Males					Females				
		0	0	100	500	1500	0	0	100	500	1500
Number examined		60	60	60	60	59	60	60	60	60	60
Stomach	Papilloma	0	1	0	0	0	0	0	0	0	0
	Basal cell adenoma	0	1	0	0	0	0	0	0	0	0
Number examined		59	57	60	57	59	60	59	58	57	60
Submandibular lymph node	Malignant lymphoma	0	0	0	1	0	0	0	0	0	0
	Number examined	60	60	60	60	59	59	60	60	60	60
Testes	Interstitial cell adenoma	4	2	1	0	3	-	-	-	-	-
	Interstitial cell carcinoma	0	1	0	0	0	-	-	-	-	-
Thymus	Histiocytic sarcoma	0	0	0	0	1	0	0	0	0	0
	Malignant schwannoma	0	0	1	0	0	0	0	0	0	0
	Malignant lymphoma	0	0	0	1	0	0	0	0	0	0
	Thymoma	0	0	1	0	0	0	0	0	0	0
	Thymic lymphoma	0	0	0	0	1	1	0	1	0	0
Number examined		60	60	60	60	59	60	60	60	59	60
Thyroid gland	Follicular cell adenoma	2	0	0	1	2	1	1	1	0	0
	C cell adenoma	8	5	3	6	6	5	3	6	1	2
	C cell carcinoma	0	1	1	0	0	0	0	2	0	0
	Follicular cell carcinoma	0	0	0	1	0	0	0	0	0	0
Number examined		60	58	60	60	59	60	59	59	58	60
Tongue	Squamous cell carcinoma	0	0	0	0	0	0	0	0	1	0
Number examined		60	60	60	60	59	59	60	60	60	60
Urinary bladder	Papilloma	1	0	0	0	0	0	0	0	0	0
Number examined		-	-	-	-	-	60	60	60	60	60
Uterine cervix	Polyp	-	-	-	-	-	1	1	1	2	3
	Sarcoma	-	-	-	-	-	1	0	0	0	0
	Basal cell adenoma	-	-	-	-	-	0	0	1	0	0
	Leiomyoma	-	-	-	-	-	0	0	1	0	1
	Mesenchymal tumour	-	-	-	-	-	0	0	0	1	0
Uterus	Polyp	-	-	-	-	-	4	6	2	4	4
	Leiomyoma	-	-	-	-	-	1	0	0	0	0
	Leiomyosarcoma	-	-	-	-	-	1	0	0	0	0
Vagina	Lipoma	-	-	-	-	-	0	1	0	0	0
	Fibroma	-	-	-	-	-	0	0	1	0	0

Table 14. (continued)

Daily Dose (mg/kg/day)	Males					Females				
	0	0	100	500	1500	0	0	100	500	1500
Number examined	1	0	1	0	2	1	1	0	0	0
Abdominal fat	Liposarcoma	1	-	0	-	0	0	0	-	-
	Lipoma	0	-	0	-	2	1	1	-	-
	Histiocytic sarcoma	0	-	0	-	1	0	0	-	-
	Sarcoma (from skin tumour)	0	-	1	-	0	0	0	-	-
Number examined	0	1	0	0	0	-	-	-	-	-
Abdominal cavity	Sarcoma	-	1	-	-	-	-	-	-	-
Number examined	0	0	0	1	1	-	-	-	-	-
Abdominal wall	Histiocytic sarcoma	-	-	-	0	1	-	-	-	-
	Leiomyosarcoma	-	-	-	1	0	-	-	-	-
Number examined	0	0	1	1	0	-	-	-	-	-
Cranium	Metastasis malignant lymphoma	-	-	0	1	-	-	-	-	-
	Benign osteoma	-	-	1	0	-	-	-	-	-
Number examined	0	0	0	0	1	-	-	-	-	-
Diaphragm	Histiocytic sarcoma	-	-	-	-	1	-	-	-	-
Number examined	1	0	0	0	1	-	-	-	-	-
Epididymal fat	Histiocytic sarcoma	0	-	-	-	1	-	-	-	-
	Benign lipoma	1	-	-	-	0	-	-	-	-
Number examined	0	0	1	1	0	0	0	0	0	1
Fore limbs	Papilloma	-	-	1	1	-	-	-	-	0
Hind limbs	Benign papilloma	-	-	0	0	-	-	-	-	1
Number examined	2	1	1	1	2	-	-	-	-	-
Lymph nodes	Histiocytic sarcoma	1	0	1	0	2	-	-	-	-
	Malignant lymphoma	0	0	0	1	0	-	-	-	-
	Haemangioma	0	1	0	0	0	-	-	-	-
	Chromaffin cell adenoma	1	0	0	0	0	-	-	-	-
Number examined	0	0	0	0	1	-	-	-	-	-
Mesenteric fat	Histiocytic sarcoma	-	-	-	-	1	-	-	-	-
Number examined	1	0	0	0	0	1	1	0	0	0
Pinnae	Benign papilloma	1	-	-	-	-	0	0	-	-
	Benign neural crest tumour	0	-	-	-	-	1	1	-	-

Table 14. (continued)

Daily Dose (mg/kg/day)		Males					Females				
		0	0	100	500	1500	0	0	100	500	1500
Number examined		0	0	0	1	2	0	0	3	2	0
Subcutaneous fat	Benign adenolipoma	-	-	-	0	0	-	-	1	0	-
	Benign lipoma	-	-	-	0	2	-	-	1	2	-
	Malignant mammary adenocarcinoma	-	-	-	0	0	-	-	1	0	-
	Metastasis malignant lymphoma	-	-	-	1	0	-	-	0	0	-
Number examined		1	1	2	0	2	0	1	0	0	0
Tail	Papilloma	1	1	2	-	1	-	1	-	-	-
	Benign basal cell tumour	0	0	0	-	1	-	0	-	-	-
Number examined		1	0	0	0	0	-	-	-	-	-
Ileo-caecal junction	Malignant leiomyosarcoma	1	-	-	-	-	-	-	-	-	-
Number examined		0	0	0	1	0	-	-	-	-	-
Pancreatic fat	Leiomyosarcoma	-	-	-	1	-	-	-	-	-	-
Number examined		0	0	0	1	0	0	0	1	0	0
Stomach fat	Leiomyosarcoma	-	-	-	1	-	-	-	0	-	-
	Malignant mesothelioma	-	-	-	0	-	-	-	1	-	-
Number examined		1	1	0	0	0	0	2	2	0	0
Subcutaneous tissue	Benign fibroadenoma	0	0	-	-	-	-	1	0	-	-
	Lipoma	0	1	-	-	-	-	0	2	-	-
	Malignant adenocarcinoma	0	0	-	-	-	-	1	0	-	-
	Squamous cell carcinoma	1	0	-	-	-	-	0	0	-	-

According to the sponsor, "all incidences were considered to be within normal background variation, and the differences were without any biological significance." (Historical control data for the statistically significant tumors are not provided.)

The FDA statistical analyses of the rat tumor data showed that there were no statistically significant increased incidences of tumors for either sex in the 2-year bioassay. The differences in the results obtained between the FDA and the sponsor's analyses were attributed to the lower significance levels (p values of 0.005 for common tumors and 0.025 for rare tumors) used in the FDA analyses. The sponsor used a significance level of 0.05 for all tumors. Furthermore, FDA uses the asymptotic test when fatal and incidental tumors occur in the same time interval if the number of tumors across groups is 4 or more. The sponsor used the asymptotic test only when the number of tumors was 10 or more across groups.

[Note: The sponsor was asked to clarify the discrepancy in the numbers of liver histiocytic sarcomas reported in male rats. The number of animals with this tumor in the electronic data set (0, 0, 0 and 1 for C, LD, MD and HD, respectively) was not consistent with the number in the study report (0, 1, 0 and 4 for the respective groups). According to

the sponsor (submission # 023, dated October 22, 2002), the electronic data set numbers (0, 0, 0 and 1) represent the incidences of primary histiocytic sarcomas, while the study report numbers included both primary and secondary (metastatic) histiocytic sarcomas of the liver. The FDA statistician used the electronic data set (primary tumors) for the analysis.]

*Toxicokinetics:* Mean plasma levels of lanthanum, about 2 hours after drug administration (on one occasion each in weeks 52 and 78), are given below.

Group and sex		6M	7M	8M	6F	7F	8F
Dose level (mg/kg/day)		0	100	1500	1	100	1500
La ng/ml (ppb) Week 52	Mean	<0.05	0.23	1.254	<0.05 - 0.08	0.15	0.868
	SD	0	0.128	0.181		0.049	0.576
La ng/ml (ppb) Week 78	Mean	<0.05	0.2125	1.405	<0.05	0.158	1.2075
	SD	0	0.058	0.388	0	0.085	0.497

LLoQ = <0.05

(LLoQ = Lower limit of quantitation)

A dose related increase in plasma lanthanum levels was noted. Plasma levels at 1500 mg/kg/day were about 5 to 7 times greater than the levels at 100 mg/kg/day. There were no significant differences in plasma levels between sexes or between weeks 52 and 78.

*Tissue lanthanum levels:* High levels of lanthanum (> 10 µg/g) were present throughout the GI tracts of animals receiving lanthanum carbonate. Levels in the cecum, colon, duodenum, ileum, jejunum, stomach and esophagus were up to 4 orders of magnitude greater than those found in the control animals [background systemic levels, close to the limit of quantification of the assay, — g/g) were generally seen following placebo administration; thought to be of endogenous origin]. Levels of lanthanum detected in the bones of treated animals (>1 - ≤10 µg/g) were greater than the levels in controls (maximum control value = 0.193 µg/g). The lanthanum content in the brains of treated animals was found to be minimal (up to 0.1 µg/g).

Lanthanum concentrations in various tissues are presented below.

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Lanthanum Tissue Distribution - 1500 mg/kg/day

Median tissue concentration (ug/g wet weight)	Tissue			
LLoQ or up to 0.1ug/g	Abdominal fat (M) Brain Epididymides	Eyes + optic nerves (F) Heart Lacrimal glands (F)	Seminal vesicles Salivary glands (F) Sciatic nerves (F)	Skeletal muscle Thymus (F) Urinary bladder (F)
>0.1 - 1 ug/g	Abdominal fat (F) Adrenals Eyes + optic nerves (M) Harderian glands Kidney (F) Lacrimal glands (M)	Lungs (F) Mammary gland Ovaries Pituitary (M) Prostate	Salivary glands (M) Sciatic nerves (M) Spleen (F) Submandibular lymph nodes (F), Testes	Thymus (M), Thyroids Urinary bladder (M) Uterus Vagina
>1 - 10 ug/g	Aorta Femur - growth plate Femur - shaft Kidney (M)	Liver Pancreas Pituitary (F) Rectum (M)	Skin Spinal cord Spleen (M) Sternum	Submandibular lymph nodes (M) Tongue
>10 - 100 ug/g	Duodenum (F)	Mesenteric lymph nodes (F)	Rectum (F)	
>100 - 1000 ug/g	Caecum Colon Duodenum (M)	Ileum (F) Jejunum Lungs (M)	Mesenteric lymph nodes (M) Oesophagus	Stomach Trachea
>1000 - 10000 ug/g	Ileum (M)			

Lanthanum Tissue Distribution - 100 mg/kg/day

Median tissue concentration (ug/g wet weight)	Tissue			
LLoQ or up to 0.1ug/g	Abdominal fat Adrenals Brain Epididymides Eyes + optic nerves Harderian glands Heart	Kidneys (F) Lacrimal glands Lungs (F) Ovaries Pancreas (F) Pituitary (F) Prostate	Salivary glands Sciatic nerve (F) Seminal vesicles Skeletal muscle Submandibular lymph nodes (F)	Testes Thymus Thyroids (F) Urinary bladder Uterus Vagina
>0.1 - 1 ug/g	Aorta Femur - growth plate Femur-shaft Kidneys (M) Liver	Mammary gland Mesenteric lymph nodes (M) Pancreas (M) Pituitary (M)	Rectum (F) Sciatic nerve (M) Skin Spinal cord Spleen	Sternum Submandibular lymph nodes (M) Thyroids (M) Tongue
>1 - 10 ug/g	Duodenum (F) Ileum (F)	Jejunum (F) Lungs	Mesenteric lymph nodes (F)	Oesophagus Rectum (M)
>10 - 100 ug/g	Caecum (M) Colon	Duodenum (M)	Jejunum (M)	Stomach (M) Trachea (F)
>100 - 1000 ug/g	Caecum (F)	Ileum (M)	Stomach (F)	Trachea (M)

## Evaluation

*Adequacy of the carcinogenicity study:* It is considered that the study was adequately performed. Survival to study termination ranged from 32 to 48% for males and from 33 to 48% for females (non-dose dependent). The high dose was selected based on the results of a twenty-six week oral toxicity study in rats (0, 100, 600 and 2000 mg/kg/day). In that study, dose-related increased incidences of stomach lesions (hyperplasia of the fundus epithelium, presence of eosinophilic chief cells, hyperplasia of the mucus cells, sub-mucosal inflammation and hyperplasia at the limiting ridge) were seen, especially in mid and high dose (600 and 2000 mg/kg/day) animals, the severity of lesions being higher at the high dose. In view of the potential for progression to excessive

inflammation and gastric ulceration over the course of the lifetime study, a dose of 1500 mg/kg/day was selected as the top dose for the 2-year rat bioassay.

*Evaluation of tumor findings:* Two-year oral administration of lanthanum carbonate at 0, 0, 100, 500 and 1500 mg/kg/day did not increase the incidence of tumors in the rat. The highest dose employed in the study (1500 mg lanthanum carbonate/kg/day or 786 mg lanthanum/kg/day) is about 3 times the maximum recommended human dose (MRHD) of 3000 mg lanthanum/day (50 mg lanthanum/kg/day for a 60 kg subject) on a mg/m<sup>2</sup> basis.

[Note: In an amendment dated June 19, 2002 (submission # 005), the sponsor informed us that stomach sections from the chronic studies in different species were re-examined microscopically by a single pathologist to standardize the terminology used in describing the mineralisation findings in the stomach, since different pathologists previously described the same lesions differently. In the two-year rat study, the mineralized foci were previously described as "occasional degenerate foci". Results of the re-evaluation of these lesions are presented below.

Incidence of mineralised foci in the 104-week rat study (SPD/87/C)

Dose Level: (mg/kg/day)	Males					Females				
	0	0	100	500	1500	0	0	100	500	1500
No. Examined:	60	60	60	60	60	60	60	60	60	60
Mineralised foci -										
<i>minimal</i>	0	0	8	26	24	0	0	2	15	24
<i>slight</i>	0	0	2	8	10	0	0	0	5	12
<i>moderate</i>	0	0	0	0	1	0	0	0	0	1
<b>Total</b>	<b>0</b>	<b>0</b>	<b>10</b>	<b>34</b>	<b>35</b>	<b>0</b>	<b>0</b>	<b>2</b>	<b>20</b>	<b>37</b>

It is stated that the foci of mineralization appeared to be well contained within giant cells in the superficial gastric glandular mucosa of treated rodents. There was no apparent inflammatory response, nor any hyperplastic response of the adjacent epithelium. According to the sponsor, these findings suggest that lanthanum carbonate alters local mineral balance in the gastric mucosa when administered as a large gavage dose, and these lesions are rodent-specific since they were not seen in the chronic dog study.]

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**99-Week Oral (Gavage) Carcinogenicity Study in the Mouse**

**Key findings:** Oral administration of lanthanum carbonate (at 0, 100, 500 and 1500 mg/kg/day) for 99 weeks produced significant increasing trends (with  $\alpha$  levels based on concurrent control incidence rates) for adenoma of the glandular stomach, hemangiosarcoma of the liver and for the combined incidences of hemangioma and hemangiosarcoma of the liver (but not for all organs) in males. However, when  $\alpha$  levels were selected on the basis of historical control incidence rates, the liver tumor findings did not attain statistical significance.

**Study number:** SPD/88/C

**Volume #s:** 1.22 – 1.26

**Conducting laboratory and location:** [ ]

**Date of study initiation:** October 10, 1997

**GLP compliance:** yes

**QA report:** yes

**Drug lot# and purity:** Lanthanum carbonate, lot #s B1066-960901 (weeks 1-26), B1066-960902 (weeks 26-44), B1066-960904 (weeks 44-79) and B1066-980601 (weeks 80-99), purity: [ ]

[ ] total metal impurities – [ ] specifications:  
[ ] total metal impurities – not more than

**CAC concurrence on protocol:** No

**Species/strain:** Mouse/ — CD-1 (ICR) BR VAF PLUS strain obtained from [ ]

**Number and age at start of study:** 50/sex/main study group; additional 10/sex at 0, 100 and 1500 mg/kg/day for plasma and tissue lanthanum level determinations; 5 to 6 weeks old

**Animal housing:** Males were housed singly and females in groups of 5, in grid bottomed cages

**Formulation:** Formulated daily as a suspension in 0.5% aqueous carboxymethylcellulose

**Drug stability:** Stable for about 2 years (inorganic salt) – stored at room temperature, in the dark, when not in use

**Methods**

*Doses:* 0 (vehicle control), 100, 500 and 1500 mg/kg/day

*Basis of dose selection:* “The high dose level was considered to be the maximum tolerated dose on the basis of findings from a preliminary 3 month study in mice.” (Basis of dose selection is described under the *adequacy of the carcinogenicity study* subsection under **Evaluation**)

*Route of administration:* Oral (gavage)

*Frequency of drug administration:* once daily for up to 99 weeks

*Interim sacrifices:* None

*Deviations from original study protocol:* The deviations are as follows: a) on January 17, 1998, the automated data capture system failed in the middle of a dosing session. Of the 5 control females in a cage (animal #s 211 to 215), 2 mice (#s 212 and 214) were dosed before the system went down. Of the 3 remaining mice, only one animal had received dose solution when the system was restored. Since it was not possible to determine the identity of that dosed animal, the remaining 2 mice were not dosed on that day. b) on June 19, 1998, a control female animal (# 431 – satellite TK group) was anesthetized to remove the damaged tip of the tail, c) on November 19, 1998, 5 low dose females (#s 446-450, TK group) received mid dose formulation in error, and d) on March 7, 1999, a high dose male (# 164) was not dosed. Other deviations are considered minor.

*Statistical methods:* Analysis of variance (ANOVA) was performed on all continuous and semi-continuous parameters. Residuals from this preliminary analysis were examined for heterogeneity of variance using Levenes test. If the Levenes test was significant at the 1% level, then a non-parametric analysis using Kruskal-Wallis ANOVA was performed to assess overall differences between treatment groups, followed by Shirley's non-parametric version of Williams test, which was based on mean ranks rather than arithmetic means. If the Levenes test was not significant, then Williams test was used for further analyses.

The survival data were analyzed using the Peto method (chi-squared analysis with time adjustment). A test for trend across all groups and pairwise tests of control against treated groups were performed using two-sided 5% tests.

Histopathological lesion analysis was performed for both sexes separately, and for sexes combined for all neoplastic findings that had incidence greater than 3 for a particular sex. The analysis included a one-sided test for increasing trend followed by pair-wise comparisons for increasing incidence of all treated groups against control. Analysis was normally carried out using procedures that adjusted for age at death or age at onset.

If the incidence of a particular finding in a given sex was 10 or more (when all groups considered), then the analysis was performed combining fatal and incidental lesions using the asymptotic chi-squared test, adjusted for mortality. If the incidence was between 3 and 9 for a particular sex, then the exact test was used (for trend and pairwise tests) for the fatals and incidentals combined.

## **Observations and Measurements**

*Clinical signs:* daily; from study week 27 onwards, weekly palpation of all animals for the detection of superficial swellings

*Mortality:* twice daily

*Irwin screen:* During week 62, all surviving satellite animals were subjected to Irwin test.

*Body weights:* pre-test, weekly for the first 16 weeks, and then every fourth week

*Food consumption:* pre-test, weekly for the first 16 weeks and then for one week in every 4 weeks

*Hematology:* during week 99 for all surviving animals (where possible from decedents also) -- [parameters evaluated – RBC, WBC (total and differential) and platelet counts]

*Clinical chemistry:* not performed

*Post mortem examination:* All animals were grossly examined. Organs specified in Table 15 from 10 mice/sex/group were weighed. Organs from animals found dead or killed in extremis were also weighed, but the data were not used for statistical analyses. All organs/tissues listed in Table 1 (from all animals) were microscopically examined.

*Toxicokinetics:* during week 52 (blood collected from 5 animals/sex/satellite group - 2 hours post dose)

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Table 15. Necropsy Procedures

Tissue	Weigh	Fix	Slide Preparation	Microscopic Examination
adrenal glands	✓	✓	✓	✓
aorta		✓	✓	✓
blood smear (if appropriate)		✓	✓	✓
brain (3 sections)	✓	✓	✓	✓
caecum		✓	✓	✓
colon		✓	✓	✓
duodenum		✓	✓	✓
epididymides	✓	✓	✓	✓
eyes (incl. optic nerves)		✓	✓	✓
femur and joint (incl. marrow)		✓	✓	✓
Harderian glands*		✓	✓	✓
heart	✓	✓	✓	✓
ileum		✓	✓	✓
jejunum		✓	✓	✓
kidneys	✓	✓	✓	✓
liver (incl. gall bladder)	✓	✓	✓	✓
lungs (incl. mainstem bronchi)	✓	✓	✓	✓
mesenteric lymph node		✓	✓	✓
oesophagus		✓	✓	✓
ovaries	✓	✓	✓	✓
pancreas		✓	✓	✓
pituitary+	✓	✓	✓	✓
prostate	✓	✓	✓	✓
rectum		✓	✓	✓
salivary gland		✓	✓	✓
sciatic nerve		✓	✓	✓
seminal vesicles		✓	✓	✓
site of mammary gland		✓	✓	✓
skeletal muscle		✓	✓	✓
skin		✓	✓	✓
spinal cord (3 levels)		✓	✓	✓
spleen	✓	✓	✓	✓
sternum		✓	✓	✓
stomach		✓	✓	✓
submandibular lymph nodes		✓	✓	✓
testes	✓	✓	✓	✓
thymus	✓	✓	✓	✓
thyroids (incl. parathyroids)		✓	✓	✓
tongue		✓	✓	✓
trachea		✓	✓	✓
urinary bladder		✓	✓	✓
uterus	✓	✓	✓	✓
vagina		✓	✓	✓
all gross lesions		✓	✓	✓
all tumours and masses		✓	✓	✓

+ weighed after fixation

\*omitted from the original protocol list in error

## Results

*Mortality:* The survival data over the course of the study are presented graphically in Figures 11 and 12, and the percent survival at the termination of the study is given below.

Males				
Group No.	1	2	3	4
Dose level (mg/kg/day)	(0)	(100)	(500)	(1500)
Number Found Dead	4	3	8	4
Number Euthanased	18	17	19	22
% survival	56	60	46	48

Females				
Group No.	1	2	3	4
Dose level (mg/kg/day)	(0)	(100)	(500)	(1500)
Number Found Dead	4	4	4	3
Number Euthanased	28	23	27	19
% survival	36	46	38	56

There were no significant differences in the survival rates between control and treated groups of either sex.

*Clinical signs:* There were no treatment-related clinical signs.

*Body weights:* Body weight data are presented graphically in Figures 13 and 14. Administration of lanthanum carbonate had no significant effects on body weight or body weight gain.

*Food consumption:* No treatment-related effects.

*Hematology:* Dose-related increases in neutrophil numbers were noted in mid and high dose females, and a dose-related increase in eosinophils was seen in mid and high dose males and also in high dose females.

*Organ weights:* Administration of lanthanum carbonate had no significant effects on either absolute or relative organ weights.

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Figure 11. % Survival - Males

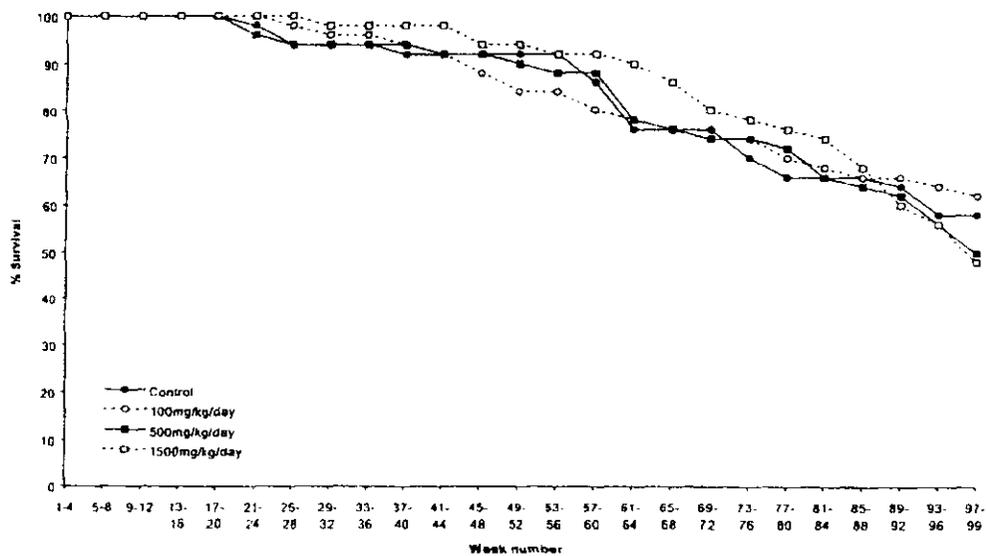


Figure 12. % Survival - Females

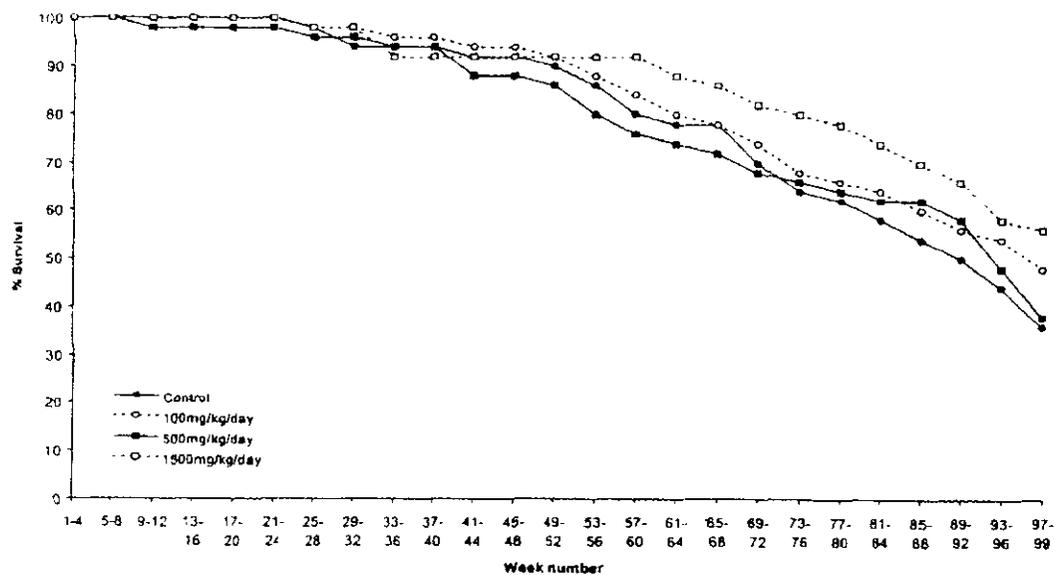
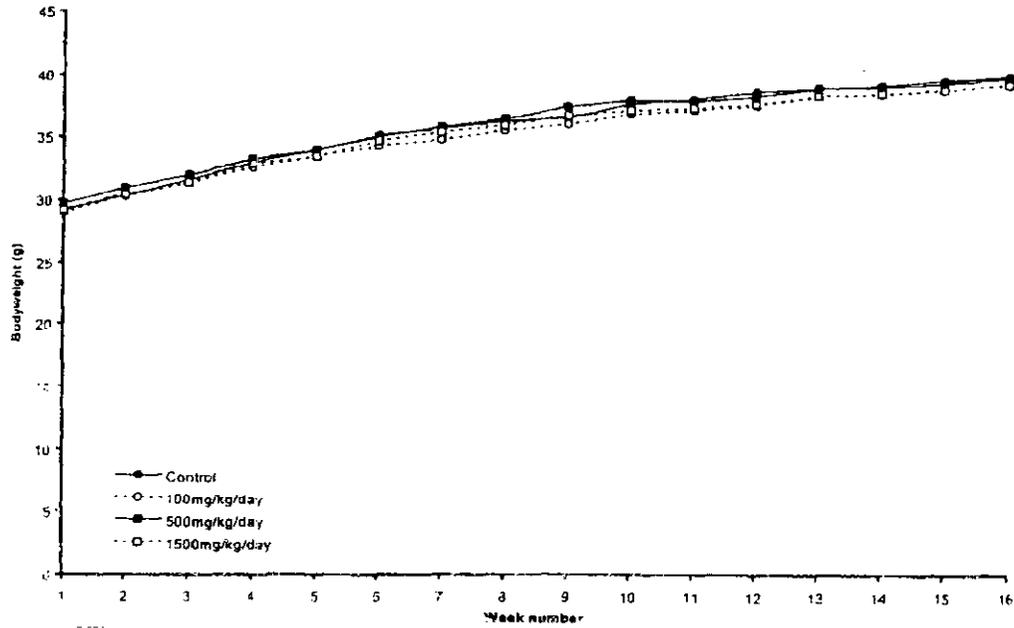


Figure 13. Mean Body Weights - Weeks 1 to 16 - Males



Mean Body Weights - Weeks 16 to 99 - Males

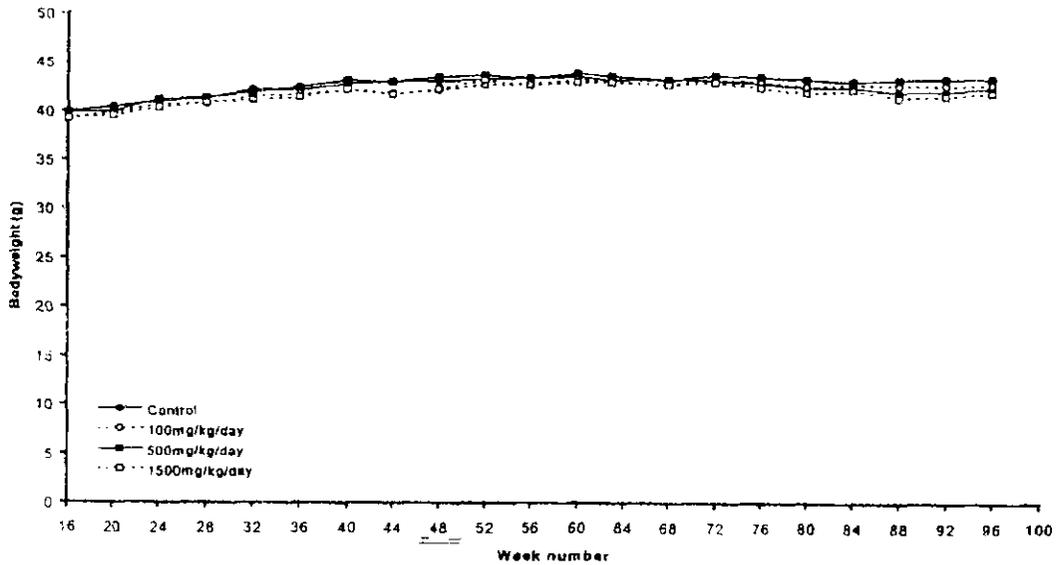
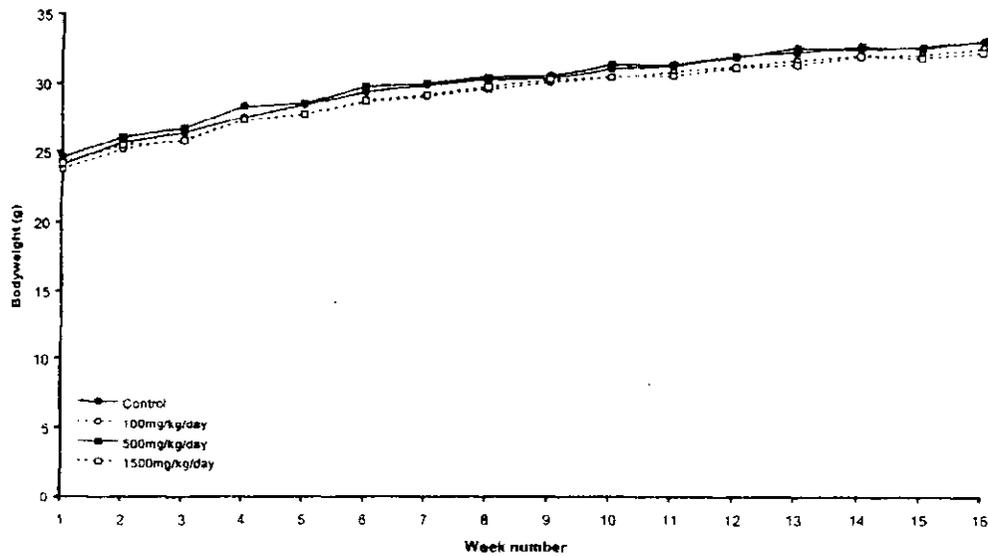
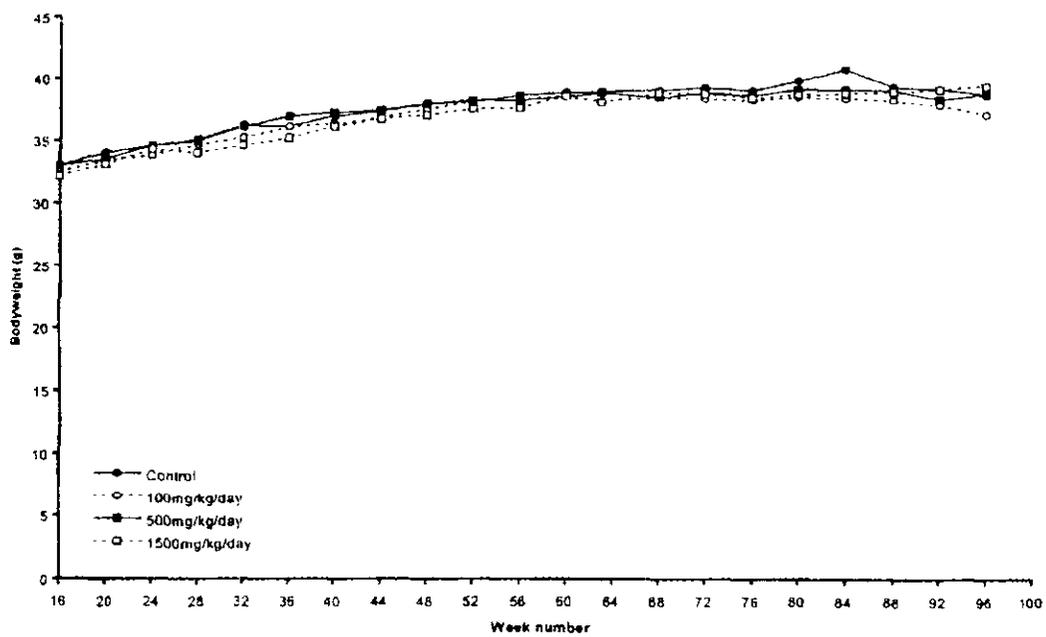


Figure 14. Mean Body Weights – Weeks 1 to 16 - Females



Mean Body Weights – Weeks 16 to 99 - Females



*Gross pathology:* There were treatment-related macroscopic findings in the two highest dose groups (500 and 1500 mg/kg/day) of both sexes, including changes in the color of the mucosa of the stomach, abnormal shape of the limiting ridge, and presence of nodules or masses in the stomach. The incidences of these findings are given below. There were no other significant treatment-related gross findings in the study.

Daily Dose (mg/kg/day)		0	100	500	1500	0	100	500	1500
Macroscopic Findings		Incidence of findings							
		Males				Females			
Number Examined		50	50	50	50	50	50	50	50
Stomach	Abnormal shape of limiting ridge	0	0	1	1	0	0	0	13
	Abnormal colour	4	3	3	6	3	5	10	10
	Nodules	0	0	0	3	0	0	1	1

*Histopathology: Non-neoplastic findings:* Treatment-related non-neoplastic findings were limited to the stomach of both sexes. Increased incidences of glandular hyperplasia, mucosal and/or submucosal inflammation of the glandular stomach and squamous epithelial hyperplasia of the limiting ridge were seen in both sexes given 1500 mg/kg/day. The incidence and the severity of these lesions, presented below, were more pronounced in females than in males, and a dose relationship was noted for mucosal and submucosal inflammation in females.

Dosage level (mg/kg/day)	Males				Females			
	0	100	500	1500	0	100	500	1500
Total no: examined	50	50	50	49	50	50	50	50
Glandular hyperplasia Total	13	11	12	18	7	9	6	19
Minimal	5	4	7	3	3	4	2	4
Slight	0	2	2	3	2	3	4	5
Moderate	4	2	0	9	2	0	0	3
Marked	4	2	3	3	0	2	0	4
Severe	0	1	0	0	0	0	0	3
Squamous epithelial hyperplasia (limiting ridge) Total	1	4	1	9	5	5	4	19
Minimal	1	1	0	2	1	3	1	7
Slight	0	2	1	6	1	1	2	5
Moderate	0	1	0	1	3	1	1	7
Mucosal and Sub mucosal inflammation Total	1	2	1	5	0	1	5	18
Minimal	0	0	1	2	0	0	2	7
Slight	0	2	0	3	0	0	3	5
Moderate	0	0	0	0	0	1	0	5
Marked	1	0	0	0	0	0	0	1

*Neoplastic findings:* The incidences of tumors observed in the study are presented in Table 16.

Neoplastic lesions were observed in the stomach and/or proximal small intestine of high dose males and females, and in a mid dose female.

A carcinoma of the glandular stomach (animal # 377) and a squamous cell carcinoma of the non-glandular stomach (animal # 391) were seen in two high dose females that were killed in extremis (on study days 495 and 596, respectively). Adenoma of the glandular stomach was noted in four high dose males and in one high dose and one mid dose female. Sponsor's analysis on the incidence of adenoma of the glandular stomach showed a statistically significant dose-related trend in males.

Adenocarcinoma of the jejunum was seen concurrently with gastric adenoma in a high dose female (# 395), and adenoma of the duodenum was seen in two high dose males (#s 152 & 155) concurrently with gastric adenoma.

Sponsor's analyses showed that there were no other treatment-related increased incidences of tumors in the study.

FDA's statistical analyses of the mouse tumor data revealed significant positive trends for adenoma of the stomach, hemangiosarcoma of the liver and for the combined incidences of hemangioma and hemangiosarcoma of the liver in males [all tumors were considered as rare tumors ( $\alpha$  level=0.025) based on concurrent control incidence rate]. The incidences of these tumors are as follows:

- stomach adenoma – 0/50 (C), 0/50 (LD), 0/50 (MD) and 4/50 (HD)  $p = 0.0041$
- liver hemangiosarcoma – 0/50 (C), 0/50 (LD), 1/50 (MD) and 3/50 (HD)  
 $p = 0.0149$
- liver hemangiosarcoma + hemangioma – 0/50 (C), 0/50 (LD), 2/50 (MD) and 3/50 (HD)  $p = 0.0182$

The incidences of combined hemangiosarcoma and hemangioma for all organs did not attain statistical significance (based on concurrent control incidence rate); 1/50 (C), 0/50 (LD), 3/50 (MD) and 4/50 (HD)  $p = 0.022$  ( $>0.005$ )

Historical control incidence rates from 46 studies (data compiled by  $\Gamma$   $\uparrow$ ) showed that the liver hemangiosarcoma is a common tumor (incidence rate  $>1\%$ ) for the  $\text{— CD-1 (ICR) BR VAF PLUS}$  mouse. When tested at an  $\alpha$  level of 0.005, hemangiosarcoma of the liver and the combined incidence rates of hemangioma and hemangiosarcoma of the liver as well as of all organs, across groups, did not reach statistical significance. [Historical control data (12 studies) from the contract laboratory also showed that the liver hemangiosarcoma (incidence rates ranging from 0 to 6%) is a common tumor (submission serial # 024 dated October 24, 2002).]

Pairwise comparisons between high dose and control groups were not statistically significant for either stomach adenoma (p=0.0672) or liver hemangiosarcoma (p=0.0819).

It is noted that there were no carcinomas of the stomach in males in this study.

Table 16. Neoplastic Findings

Daily Dose (mg/kg/day)		0	100	500	1500	0	100	500	1500
Neoplastic Findings		Males				Females			
Number Examined		50	50	50	50	50	50	50	50
Abdominal cavity	Carcinoma	0	0	0	0	0	0	0	1
Number Examined		50	50	50	49	50	50	50	50
Adrenals	Cortical adenoma	9	6	10	8	1	1	0	0
	Pheochromocytoma	0	0	0	2	0	1	0	0
	Subcapsular cell adenoma	6	7	7	4	3	4	4	3
Number Examined		50	50	50	50	50	50	50	50
Brain	Sarcoma, meningeal	0	1	0	0	0	0	0	0
Number Examined		-	-	-	-	2	-	1	1
Cervical gland	Haemangioma	-	-	-	-	0	-	1	0
Number Examined		50	50	50	50	50	50	50	50
Colon	Adenocarcinoma	1	0	0	0	0	0	0	0
Number Examined		-	-	-	1	2	1	-	-
Diaphragm	Lymphoma	0	0	0	0	1	0	-	-

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Table 16. (continued)

Neoplastic Findings		Males				Females			
Daily Dose (mg/kg/day)		0	100	500	1500	0	100	500	1500
Number Examined		50	50	50	50	50	50	50	50
Duodenum	Adenoma	0	0	0	2	0	0	0	0
Number Examined		50	50	50	50	-	-	-	-
Epididymides	Sarcoma	0	0	0	1	-	-	-	-
Number Examined		50	50	49	50	50	50	50	50
Femur	Mast cell sarcoma	0	0	0	1	1	0	0	0
	osteoma	0	0	0	0	2	0	0	0
Number Examined		10	6	6	9	13	16	14	7
Haematopoietic tumor	Lymphoma	8	6	6	8	12	14	11	7
	Histiocytic sarcoma	2	0	0	1	1	2	3	0
Number Examined		11	8	8	8	18	13	11	16
Harderian gland	Adenoma	3	2	2	0	0	3	0	2
	Adenocarcinoma	0	0	0	0	1	1	0	1
Number Examined		50	50	50	50	50	50	50	50
Jejunum	Adenocarcinoma	0	0	0	0	0	0	0	1
Kidneys	Tubular adenoma	0	1	1	0	0	1	0	0
Liver	haemangiosarcoma	0	0	1	3	0	0	1	1
	haemangioma	0	0	1	0	0	0	0	0
	Hepatocellular carcinoma	2	1	8	4	1	0	0	1
	Hepatocellular adenoma	7	4	7	7	0	2	0	2
	Sarcoma	0	0	1	0	0	0	0	0
Lungs	Mass cell sarcoma	0	0	0	1	1	0	0	0
	Bronchoalveolar adenoma	9	9	8	8	5	9	1	5
	Bronchoalveolar carcinoma	5	1	7	9	2	2	3	3
	Carcinoma	0	0	0	0	0	0	1	0
Number Examined		9	8	6	7	14	9	12	8
Lymph nodes	Lymphoma	0	0	1	0	0	0	0	0
Number Examined		50	48	49	50	50	50	50	50
Mesenteric lymph nodes	Haemangioma	0	0	0	0	0	1	1	0
Number Examined		-	-	-	-	50	49	50	50
Ovaries	Papillary cystadenoma	-	-	-	-	0	1	0	1
	Luteoma	-	-	-	-	1	0	1	0
	Tubulostromal adenoma	-	-	-	-	0	2	0	1
Number Examined		49	48	50	50	49	50	49	50
Pituitary gland	Adenoma - pars anterior	0	0	3	0	3	5	7	2
	Adenocarcinoma	0	0	0	1	0	0	0	0
	Sarcoma, meningeal	0	1	0	0	0	0	0	0
	Adenoma - pars intermedia	0	0	0	0	1	0	0	2
Number Examined		50	50	50	50	-	-	-	-
Seminal vesicles	Adenoma	0	0	0	1	-	-	-	-

Table 16 (continued)

Neoplastic Findings		Males				Females			
		0	100	500	1500	0	100	500	1500
Daily Dose (mg/kg/day)		50	50	50	50	50	50	50	50
Number Examined		50	50	50	49	50	50	50	50
Skin	Haemangiosarcoma	0	0	1	0	0	0	0	0
	Adenocanthoma	0	0	0	0	1	1	0	0
	Adenocarcinoma	0	0	0	0	0	0	0	1
	Sebaceous adenoma	0	0	0	0	0	1	0	0
	Mammary adenoma	0	0	0	0	1	0	0	0
	Sarcoma	0	0	0	0	0	1	0	0
	Fibrosarcoma	0	0	0	0	0	0	0	1
	Osteosarcoma	0	0	0	0	1	0	0	0
	Chondroma	0	0	0	0	0	0	1	0
	Rhabdomyosarcoma	0	0	0	0	0	1	0	0
	Mammary adenocarcinoma	0	0	0	0	1	1	1	2
	Lymphoma	0	0	0	1	0	0	0	0
	Papilloma	1	0	0	0	0	0	0	0
	Squamous cell carcinoma	0	0	0	1	0	0	0	0
Hibernoma	0	0	1	0	0	0	0	0	
Spinal cord	Mast cell sarcoma	0	0	0	1	1	0	0	0
Spleen	Haemangiosarcoma	1	0	0	1	0	0	0	0
	Mast cell sarcoma	0	0	0	1	1	0	0	0
Sternum	Mast cell sarcoma	0	0	0	1	1	0	0	0
	Osteoma	0	0	0	0	0	0	1	0
Number Examined		50	50	50	49	50	50	50	50
Stomach	Adenoma	0	0	0	4	0	0	1	1
	Squamous cell carcinoma	0	0	0	0	0	0	0	1
	Carcinoma	0	0	0	0	0	0	0	1
Number Examined		50	50	50	50	-	-	-	-
Testes	Interstitial cell adenoma	1	1	0	1	-	-	-	-
	Haemangioma	0	0	0	1	-	-	-	-
Number Examined		-	1	1	-	-	1	-	-
Tail	Fibrous histiocytoma	0	0	0	0	-	1	-	-
Number Examined		49	49	48	47	50	50	49	49
Thymus	Thytioma	0	0	0	0	1	1	0	0
Number Examined		50	50	50	50	49	50	50	50
Thyroid gland	Follicular cell adenoma	0	0	0	0	1	1	0	0
Number Examined		50	50	50	50	50	50	50	50
Tongue	Papilloma	0	0	0	1	0	0	0	0
Number Examined		-	-	-	-	50	49	49	50
Uterine cervix	Leiomyosarcoma	-	-	-	-	1	1	2	0
	Leiomyoma	-	-	-	-	0	2	1	0
	Polyp	-	-	-	-	0	1	1	0

Table 16 (continued)

Neoplastic Findings		Males				Females			
Daily Dose (mg/kg/day)		0	100	500	1500	0	100	500	1500
Number Examined		-	-	-	-	50	50	50	50
Uterus	Leiomyoma	-	-	-	-	1	0	0	2
	Leiomyosarcoma	-	-	-	-	1	1	2	0
	Endometrial adenocarcinoma	-	-	-	-	0	0	2	0
	Adenoma	-	-	-	-	1	0	0	0
	Polyp	-	-	-	-	4	3	4	3
	Haemangiosarcoma	-	-	-	-	1	0	1	1
	Haemangioma	-	-	-	-	0	0	2	1
	Histiocytic sarcoma	-	-	-	-	0	1	0	0
Number Examined		-	-	-	-	50	49	50	50
Vagina	Squamous cell carcinoma	-	-	-	-	1	0	0	0
	Leiomyosarcoma	-	-	-	-	1	0	0	0
	Polyp	-	-	-	-	1	0	0	0

*Toxicokinetics:* Dose dependent increases in plasma lanthanum concentrations were seen.

With the exception of one control female (animal # 437), which had a plasma level of — ng lanthanum/ml, all control animals had plasma levels <0.05 ng/ml.

At 100 mg/kg/day, plasma levels at 2 hr post dose ranged from  $\bar{L}$   $\bar{J}$  ng/ml, and at 1500 mg/kg/day, the values ranged from  $\bar{L}$   $\bar{J}$  ng/ml. No gender differences in plasma lanthanum levels were noted.

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*Tissue lanthanum levels:* Median tissue concentrations are given below.

Median tissue concentration (ug/g wet weight)	Tissue			
	100 mg/kg/day		1500 mg/kg/day	
LLoQor upto 0.1µg/g	Adrenals Aorta Brain Epididymides Eyes and optic nerves  Heart Kidneys Lungs Mammary gland (M) Mesenteric LN Ovaries Pituitary Prostate Salivary gland	Sciatic nerve Seminal vesicles Skeletal muscle Skin (M) Spleen Submandibular LN (M) Testes Thymus Thyroids Tongue Urinary bladder Uterus/cervix Vagina	Adrenals Brain Epididymides Pituitary Sciatic nerve	Seminal vesicles Skeletal muscle Thymus (M) Thyroids (F) Urinary bladder (F)
>0.1 to 1µg/g	Femur (growth plate)  Femur (shaft) Liver Mammary gland (F) Oesophagus Pancreas (M)	Skin (F) Spinal cord Spleen (F)  Stomum Submandibular LN (F)	Aorta Eyes and optic nerves Heart Kidneys Lungs Mammary glands Ovaries Salivary gland Skin	Spleen (M) Submandibular LN (M) Testes Thymus (F) Tongue (F) Urinary bladder (M) Uterus/cervix Vagina
>1 to 10 µg/g	Duodenum (M) Jejunum (M) Pancreas (F)	Rectum Trachea (F)	Femur (growth plate) Femur (shaft) Liver Spinal cord Spleen (F)	Stomum Thyroids (M) Tongue (M) Submandibular LN (F)
>10 to 100 µg/g	Caecum Colon Duodenum (F) Ileum	Jejunum (F) Stomach Trachea (M)	Duodenum (F) Jejunum Mesenteric LN Oesophagus	Pancreas Rectum Trachea (M)
>100 to 1000 µg/g			Caecum (F) Colon Duodenum (M)	Ileum (F) Trachea (F)
>1000 to 10000 µg/g			Caecum (M) Ileum (M)	Stomach

At 1500 mg/kg, the median lanthanum concentration in the majority of tissues was less than 1 µg/g. Intermediate concentrations (>1 µg/g to 10 µg/g) were found in bone, spinal cord, liver and spleen. The highest concentrations (above 10 µg/g) were found in the GI tract.

[Note: In an amendment dated June 19, 2002 (submission # 005), the sponsor stated that in order to further characterize the neoplastic stomach lesions (adenomas), and to examine the point of attachment of the neoplasm to the stomach wall, serial sections of stomach neoplasms were made (from 4 high dose males, and one high dose and one mid dose female each), stained with H & E, and examined microscopically. The point of attachment demonstrated the proliferative neoplastic lesion arising within existing areas of hyperplasia. The lesions in high dose animals were all pedunculated, whereas the lesion in the mid dose female had a more sessile appearance. Examination of additional sections from this mid dose female revealed an absence of tortuosity of glands that was a feature of the adenomas in the high dose animals. Hence, according to the sponsor,

“hyperplasia could be considered a more appropriate pathological term than adenoma (as it was originally described) for the change seen in this intermediate dose group animal.” Additionally, previously prepared stomach sections of all animals from the 99-week mouse study were re-examined to standardize the terminology used in describing the mineralisation findings in the stomach. The results are presented below.

Incidence of mineralised foci in the 99-week mouse study (SPD/88/C)

Dose Level: (mg/kg/day)	Males				Females			
	0	100	500	1500	0	100	500	1500
No. Examined:	50	50	50	49	50	50	50	48
Mineralised foci -								
<i>minimal</i>	0	0	13	17	1	0	10	14
<i>slight</i>	0	0	1	18	0	0	0	18
<i>moderate</i>	0	0	0	8	0	0	0	3
<b>Total</b>	<b>0</b>	<b>0</b>	<b>14</b>	<b>43</b>	<b>1</b>	<b>0</b>	<b>10</b>	<b>35</b>

Dose-dependent incidences of mineralised foci were seen in both sexes at mid and high dose levels.]

### Evaluation

*Adequacy of the carcinogenicity study:* It is considered that the study was adequately performed. Survival to study termination ranged from 46 to 60% for males and from 36 to 56% for females (non-dose dependent). The doses were selected based on the results of a thirteen-week oral toxicity study in the mouse (0, 500, 1500 and 2000 mg/kg/day). In that study, increased incidences of stomach lesions (epithelial hyperplasia of the limiting ridge and non-glandular region, and mucosal inflammatory cell infiltration in the glandular region) were seen at 1500 and 2000 mg/kg/day. Proliferating cell nuclear antigen staining of the stomach sections showed that the incidences of the glandular stomach hyperplasia were mostly similar at 1500 and 2000 mg/kg/day dose levels. Inflammation of the glandular epithelium was more prevalent and severe at 2000 mg/kg/day than at 1500 mg/kg/day, particularly in females. In order to reduce the risk of excessive inflammation and eventual gastric ulceration compromising the lifetime studies, a dose of 1500 mg/kg/day was selected as the top dose for the 99-week mouse bioassay.

*Evaluation of tumor findings:* Oral administration of lanthanum carbonate (at 0, 100, 500 and 1500 mg/kg/day) for 99 weeks produced significant positive trends (with  $\alpha$  levels based on concurrent control incidence rates) for adenoma of the glandular stomach, hemangiosarcoma of the liver and for the combined incidences of hemangioma and hemangiosarcoma of the liver (but not for all organs) in males. However, when  $\alpha$  levels were selected on the basis of historical control incidence rates, the liver tumor findings did not attain statistical significance. The high dose (1500 mg/kg/day) employed in the study is about 1.3 times the maximum recommended human dose of 3000 mg lanthanum/day, on a mg/m<sup>2</sup> basis.

## VII. REPRODUCTIVE AND DEVELOPMENTAL TOXICOLOGY

### 1. Fertility and Embryonic Development Study in Rats

**Key findings:** Oral administration of lanthanum carbonate to male and female rats during pre-mating, mating and up to gestational day 17, at dose levels up to 2000 mg/kg/ day, did not affect fertility or mating performance, or produce any harm to the fetus.

**Study number:** SPD/54/97

**Volume # and page #:** 1.38, & 5-054

**Conducting laboratory and location:** [ ]

**Date of study initiation:** October, 1996

**GLP compliance:** Yes (UK and OECD GLP regulations)

**QA report:** yes (x) no ( )

**Drug, lot #s, and % purity:** Lanthanum carbonate, lot #s 6268/960404, 6268/960405 & 6268/960501, [ ] metal impurities (total) - [ ] (within specifications)

**Formulation:** Test article was suspended in 0.5% aqueous carboxymethyl-cellulose on the day of use.

#### Methods

**Animals:** Sprague-Dawley derived rats of the — (SD) IOPS-Caw strain, males weighing 188-199 g (42-44 days old) and females 60-79 g (25-28 days old), were obtained from [ ] Males were acclimatized for one week and females for eight weeks before the initiation of dosing.

**Number/sex/group:** 25

**Doses employed:** 0 (vehicle), 200, 600 and 2000 mg/kg/day. [It is stated that the doses were selected based the existing toxicity data and also on the basis of the results from a preliminary study in rats conducted at [ ] at dose levels of 200, 600, 1000 or 2000 mg/kg/day. In that study, males (6/group) were dosed from 14 days prior to mating, through the mating period until the day before necropsy after the end of the mating period. Females were dosed from 14 days prior to mating, through mating and pregnancy, until the day before necropsy on day 7 post-partum. No significant treatment related effects were seen in the parental or F1 generations except that the F1 pup bodyweights were slightly reduced in the group treated at 2000 mg/kg/day. Based on these findings, 2000 mg/kg/day was selected as the high dose for the reproductive toxicity studies. A low dose level of 200 mg/kg/day was selected as the no-effect level, with an intermediate dose level of 600 mg/kg/day.]

**Route of administration:** oral (gavage)

*Study design:* Males were dosed for at least 63 days before mating with treatment continued throughout the mating period until the day before necropsy. Females were dosed for 14 days before mating with treatment continued through the mating period till day 17 of pregnancy. The animals were dosed once daily at a constant volume of 10 ml/kg.

*Parameters and endpoints evaluated:* clinical signs – daily; mortality – twice daily; body weights – twice weekly for males and females during the pre-mating period (daily during pregnancy); food consumption – weekly (during pregnancy over days 0 to 6, 6 to 12, 12 to 17 and 17 to 20)

Males were killed after the end of the mating period and females were sacrificed on day 20 of pregnancy. Animals were subjected to complete necropsies. Organs or tissues showing macroscopic abnormalities were fixed in neutral buffered formaldehyde.

Testes and epididymides were removed and the testes were weighed. The testes were fixed in Bouins fluid for about 24 hours and then transferred to neutral buffered formaldehyde. The epididymides were fixed in neutral buffered formaldehyde.

For females, pregnancy status was determined, and numbers of corpora lutea, implantation sites, resorptions, and dead and live fetuses were recorded. The implantations were numbered separately for the right and left horns. The live fetuses and their placenta were removed, and the uterus and ovaries were fixed in neutral buffered formaldehyde. Fetal and placental weights, fetal sex and external fetal abnormalities were recorded.

Copulation and fertility indices and pre- and post-implantation losses were calculated.

One half of the live fetuses were fixed in Bouins fluid for subsequent examination for visceral abnormalities using a combined sectioning/dissection technique. The remaining fetuses were briefly fixed in 70% alcohol, viscera examined and eviscerated. The carcasses were then cleared in Alizarin red S to visualize the ossified skeleton and examined for skeletal variants and abnormalities.

Structural congenital abnormalities that impair or potentially impair the survival of the fetus were classified as major abnormalities. Other defects were classified as minor abnormalities. Commonly observed variations in the degree of ossification from that expected of a day 20 gestation fetus together with common variations in the extent of renal pelvic cavitation and ureter dilatation were recorded as variants.

Analysis of variance (ANOVA) was performed on all parameters. Residuals from this preliminary analysis were examined for heterogeneity of variance using Levene's test. If the Levene's test was significant at the 1% level, then the particular variable concerned was subjected to a non-parametric analysis. Otherwise, William's test was performed to compare the high dose with control at the two-sided 5% level. If this test was statistically

significant, then comparisons of the subsequent doses against control were performed at the one-sided 5% level until a non-significant difference was found. If Levene's test indicated that there were significant differences in the treatment group variances, or if a parametric analysis was deemed to be inappropriate, then a Kruskal-Wallis ANOVA was performed to assess overall differences between the treatment groups, followed by Shirley's non-parametric version of Williams' test, which is based on mean ranks rather than the arithmetic means. Nominal data were analyzed using the Fisher's Exact Test.

## Results

*In-life observations:* Two males were found dead during the study. One male (animal # 60; 600 mg/kg/day group) was found dead on day 25 of treatment. At necropsy, the thoracic cavity was found to contain red fluid with white contents, thickened pericardium and reddened lungs, suggestive of dosing injury. A second male (animal # 85; 2000 mg/kg/day group) was found dead on day 63 of treatment. Since that animal was cannibalized and the remaining tissues were partly autolysed, the cause of death was not determined. There were no other deaths in the study.

There were no treatment-related effects on clinical signs, body weight gain, food consumption, mean number of days taken to mate, and on the fertility and mating performance (copulation and fertility indices) of animals.

There was no effect of treatment on the numbers of females pregnant in each group. Pregnancy rates were 92, 96, 100, and 92% at 0, 200, 600 and 2000 mg/kg/day, respectively.

*Necropsy findings:* There were no treatment-related macroscopic findings observed at the necropsy of males or females.

The absolute and relative testes weights were similar in treated and control animals.

*Developmental toxicity:* The mean numbers of corpora lutea, implantations, live fetuses, and the mean percent pre- and post-implantation losses were unaffected by drug treatment (Table 17). Total numbers of live fetuses per group, mean number of fetuses per dam, numbers of male and female fetuses, mean litter weight, and mean fetal and placental weights are given in Table 18. The percentage of male fetuses was significantly higher for the high dose group than for the control group. According to the sponsor, this increase was within the background range and there was no dose response, although the effect was only seen at the high dose. All other parameters were similar in control and treated groups.

The fetal and the litter incidences of external, visceral and skeletal malformations and variations are summarized in Table 19. Although there was no statistically significant increase in the incidence of any one major abnormality, the overall number of major fetal abnormalities were increased at mid and high doses, the incidence being higher at the mid

dose than at high dose. The percentage increase of major abnormalities was within the sponsor's historical control range.

Statistically significant increased incidences of malformations/variations are described below. Increased incidences of cervical rib, a minor skeletal malformation, were observed at the low (200 mg/kg/day) and high (2000 mg/kg/day) doses, but not at the mid (600 mg/kg/day) dose. The fetal incidence of cervical rib was as follows: 0/175 @ 0 mg/kg/day, 5/182 @ 200 mg/kg/day, 0/192 @ 600 mg/kg/day and 6/172 @ 2000 mg/kg/day. The litter incidences were 0/23, 1/24, 0/25 and 3/23 at 0, 200, 600 and 2000 mg/kg/day, respectively. Although the incidences at the low dose (2.7%) and high dose (3.4%) were outside the sponsor's historical control range (0-1.9%), there was no dose relationship; a 10-fold increase in dose from 200 to 2000 mg/kg/day did not significantly increase the percent incidence of this malformation.

Dose-related increased incidences of increased pelvic cavitation of kidneys, a variation, were noted in treated groups (statistically significant at the high dose). The fetal incidences were 14/341, 22/352, 30/371 and 40/336 at 0, 200, 600 and 2000 mg/kg/day, respectively, and the litter incidences were 8/23, 9/24, 15/25 and 15/23 at 0, 200, 600 and 2000 mg/kg/day, respectively. It is noted that the incidences of increased pelvic cavitation observed in treated groups (5.9-11.5%) were within the historical control range of 0.0 to 41.5%.

**Evaluation:** Oral administration of lanthanum carbonate to male rats for 63 days prior to mating and through the mating period until necropsy, and to the females for 14 days prior to mating, during mating and up to day 17 of pregnancy, at dose levels up to 2000 mg/kg/day (about 3 times the maximum recommended human dose, on a mg/m<sup>2</sup> basis) did not affect fertility or mating performance, or result in evidence of injury to the fetus. Although statistically significant increased litter incidence of fetal cervical rib, a minor skeletal malformation, was noted at the highest dose, there was an absence of a dose relationship for this abnormality. The incidences of pelvic cavitation, a variation observed at a higher than control rate in the kidneys of fetuses from treated animals, were within the historical control range. The study appeared to be adequately performed and is consistent with the ICH Guidelines.

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## Group mean uterine / implantation data

Group	:	1	2	3	4
Test article	:	Control	Lanthanum Carbonate		
Dose level (mg/kg/day)	:	0	200	600	2000

	Group 1	Group 2	Group 3	Group 4
Number of females with implantations at scheduled kill	23	24	25	23
Number of corpora lutea	386	420	420	376
Mean number per female	16.8	17.5	16.8	16.3
Standard deviation	1.9	1.6	2.2	2.4
Number of implantations	363	380	393	352
Mean number per female	15.8	15.8	15.7	15.3
Standard deviation	2.0	2.2	2.2	3.7
Mean % pre-implantation loss	5.7	8.9	6.3	6.9
Number of early embryo/foetal deaths	21	28	21	14
Number of late embryo/foetal deaths	1	0	1	2
Number of dead foetuses	0	0	0	0
Mean % post-implantation loss	6.1	8.7	5.4	4.1
Number of live foetuses	341	352	371	336
Mean number per female	14.8	14.7	14.8	14.6
Standard deviation	2.1	3.1	2.4	3.5
Mean % of implantations	93.9	91.3	94.6	95.9

Table-17.

## Group mean litter weights (g) / foetal data

Group	:	1	2	3	4
Test article	:	Control	Lanthanum Carbonate		
Dose level (mg/kg/day)	:	0	200	600	2000

	Group 1	Group 2	Group 3	Group 4
Number of females with live foetuses at scheduled kill	23	24	25	23
Number of live foetuses	341	352	371	336
Mean number per female	14.8	14.7	14.8	14.6
Standard deviation	2.1	3.1	2.4	3.5
Number of male foetuses	154	174	181	187
Number of female foetuses	187	178	190	149
Mean % male foetuses	45.1	49.5	48.3	53.3*
Mean litter weight	60.4	59.5	61.6	60.8
Standard deviation	8.9	12.8	10.2	15.1
Mean foetal weight	4.07	4.05	4.15	4.15
Standard deviation	0.16	0.24	0.24	0.31
Mean foetal weight - males only	4.20	4.14	4.25	4.25
Standard deviation	0.18	0.28	0.30	0.32
Mean foetal weight - females only	3.98	3.96	4.06	4.05
Standard deviation	0.19	0.24	0.23	0.36
Mean placental weight	0.61	0.69	0.62	0.67
Standard deviation	0.05	0.31	0.06	0.26

\* $p < 0.05$ , \*\* $p < 0.01$ , \*\*\* $p < 0.001$ 

Table 18.

## Foetal examination : summary of group mean data

Group	:	1	2	3	4
Test article	:	Control	Lanthanum Carbonate		
Dose level (mg/kg/day)	:	0	200	600	2000

	Group 1	Group 2	Group 3	Group 4
Combined examination (external/visceral/skeletal)				
Total number of litters examined	23	24	25	23
Total number of fetuses examined	341	352	371	336
Number with major abnormalities	1	1	7	3
Mean % of fetuses examined	0.3	0.3	2.0	0.9
Number of litters affected	1	1	2	2

Table 19.

## Foetal examination : summary of group mean data

Group	:	1	2	3	4
Test article	:	Control	Lanthanum Carbonate		
Dose level (mg/kg/day)	:	0	200	600	2000

	Group 1	Group 2	Group 3	Group 4
External and visceral examination				
Total number of litters examined	25	24	25	23
Total number of fetuses examined	341	352	371	336
Number with major abnormalities	1	1	7	3
Mean % of fetuses examined	0.3	0.3	2.0	0.9
Number of litters affected	1	1	2	2
Number with minor abnormalities	3	1	4	3
Mean % of fetuses affected	0.9	0.3	1.1	0.8
Number of litters affected	3	1	3	3
Number with variations	46	68	85	72
Mean % of fetuses affected	13.6	22.0	23.7	20.6
Number of litters affected	16	19	21	18

Table 19 (continued)

Foetal examination : summary of group mean data					
Group	:	1	2	3	4
Test article	:	Control	Lanthanum Carbonate		
Dose level (mg/kg/day)	:	0	200	600	2000
		Group 1	Group 2	Group 3	Group 4
Skeletal examination					
Total number of litters examined		23	24	25	23
Total number of foetuses examined		175	182	192	172
Number with major abnormalities		0	0	3	1
Mean % of foetuses examined		0.0	0.0	1.7	0.6
Number of litters affected		0	0	1	1
Number with minor abnormalities		9	19	17	21
Mean % of foetuses affected		5.4	13.5	9.2	11.7
Number of litters affected		9	13	12	8
Number with variations		169	178	187	167
Mean % of foetuses affected		96.4	97.9	97.2	97.1
Number of litters affected		23	24	25	23

Table 19 (continued)

## 2. Developmental Toxicity Study in Rabbits

**Key findings:** Oral administration of lanthanum carbonate at 1500 mg/kg/day to pregnant rabbits during organogenesis was associated with increased incidences of pre- and post-natal losses, reduced litter and fetal weights, delayed skeletal ossification and maternal toxicity. Lower dose levels (750 and 250 mg/kg/day) did not produce any adverse effects on either dam or fetus.

**Testing facility:**

[ ]

**Study number:** SPD/57/R

**Study dates:** Initiation Date – July 10, 1996

Completion Date -- August 8, 1996

**GLP compliance:** The study was conducted in accordance with UK GLP Compliance Program

**QA report:** Yes

**Animals:** Timed-mated female New Zealand White rabbits, about 4 months of age and weighing between 3 and 4 kg, were obtained from C

by day 2 of pregnancy (day 0 of pregnancy was the day of mating). They were acclimatized for 4 to 5 days before the initiation of dosing. Animals were housed individually in grid-bottom metal cages suspended over paper-lined trays. A pelleted standard rabbit diet (manufactured by C and tap water were available *ad libitum*.

Eighty females were randomly assigned to four groups of 20 animals each.

**Dose levels and mode of administration:** The doses employed were 0 (vehicle control), 250, 750 and 1500 mg/kg/day.

Lanthanum carbonate (batch numbers 6268/960303 and 6268/960304), suspended daily in 0.5% aqueous carboxymethylcellulose, was administered by oral gavage from day 6 to day 18 of pregnancy, at a dose volume of 5 ml/kg.

Analyses of the samples of test article formulations (prepared on the first day of dosing and on a day toward the end of the dosing period) showed that the lanthanum content (specification - [            ]) and the total metal impurities (specification - not more than [            ] ppm) were within the specification limits.

[It is stated that the doses for the present study were selected based on the results from an oral administration rangefinding study conducted in pregnant rabbits (dosed from days 6 to 18 of pregnancy) at dose levels of 0 (vehicle control), 250, 500, 1000 and 1500 mg/kg/day. In that study, the 1500 mg/kg/day dose produced maternal toxicity (reduction in body weight gain and food consumption, and reduced fecal production) and reduction in fetal body weight. There were no significant treatment-related effects in lower dose groups. Hence, 1500 mg/kg/day, a dose that produced slight maternal toxicity, was selected as the high dose for the rabbit developmental toxicity study. A no-effect level of 250 mg/kg/day was chosen as the low dose and 750 mg/kg/day was selected as the mid dose for the study.]

**Observations and measurements:** All animals were checked daily for mortality and clinical signs of toxicity. Body weights were recorded on days 0, 3, 6 to 18, 22, 25 and 28 of pregnancy. Food consumption was recorded daily from days 3 to 6 of pregnancy and every two days thereafter.

All surviving animals were sacrificed on day 28 of pregnancy by an intravenous administration of sodium pentobarbitone solution. The thoracic and abdominal cavities were opened and all major organs examined. Organs or tissues showing macroscopic abnormalities were stored in neutral buffered formaldehyde.

The pregnancy status was confirmed, and the numbers of corpora lutea, implantation sites, early and late resorptions, and dead and live fetuses were recorded. The live fetuses were removed, and fetal and placental weights, and external fetal abnormalities were recorded. Pre- and post- implantation losses were determined.

All live fetuses were euthanized, weighed, and briefly fixed in alcohol. Then they were skinned, dissected and examined for visceral malformations, and their sex was determined. The fetuses were eviscerated and placed back in alcohol. After at least 12 hours fixation, a razor blade cut was made through the head and the brain examined. The fetuses were then cleared in potassium hydroxide, stained with Alizarin red S and examined for skeletal malformations and variations.

Structural congenital abnormalities that impair or potentially impair the survival or constitution of the fetus were classified as major abnormalities. Other defects were classified as minor abnormalities. Commonly observed variations in the degree of ossification from that expected of a day 28 gestation fetus, together with deviations in the numbers of thoracic vertebrae and ribs, lumbar vertebrae and caudal vertebral centra and neural arches were classified as variants.

All skeletal specimens were stored in aqueous glycerol with thymol.

Analysis of variance was performed on all parameters. Residuals from this preliminary analysis were examined for heterogeneity of variance using Levene's test. If the Levene's test was significant at the 1% level, then the particular variable was subjected to non-parametric analyses using Kruskal-Wallis ANOVA test followed by Shirley's non-parametric version of Williams test. If the Levene's test was not significant at the 1% level, then William's test was done to compare treated and control groups.

## Results

### *Maternal Toxicity*

One high dose female (animal # 72) aborted (7 fetuses) on day 25 of pregnancy. Previous clinical signs observed in this animal included reduced fecal output (liquid/loose feces), reduction in body weight and presence of mucus on the tray liner. Necropsy findings included staining of the fur (red), distended stomach with dark fluid and an empty colon.

There were no mortalities or other premature sacrifices during the study.

A higher incidence of reduced fecal output, with liquid/loose feces, was noted in high dose animals compared to controls.

A reduction in body weight gain was noted in the high dose group during the first few days of dosing (days 6 to 10 of pregnancy). During the remainder of the dosing period and for the post-dosing period, the body weight gains in this group were generally higher than control. However, the overall body weight gain for the high dose group during the dosing period (days 6 to 18 of pregnancy) was significantly lower than that of control. The body weight gains in other treatment groups were generally similar to that of control.

The food consumption values for the high dose animals were lower than control values throughout the dosing period, the differences attaining statistical significance for the period between days 6 and 10 of pregnancy. The food consumption during the post-dosing period was similar to that of the pre-dosing period. There were no treatment-related effects on food consumption in other groups.

There were no remarkable macroscopic findings observed at the scheduled necropsy.

The pregnancy rates were 90, 90, 95 and 95% at 0, 250, 750 and 1500 mg/kg/day, respectively.

### *Developmental Toxicity*

The numbers of corpora lutea, implantations and early and late embryo/fetal deaths, and the mean percent pre- and post-implantation losses are presented in Table 20.

At 1500 mg/kg/day, the mean pre-implantation loss (16.7%) and the mean post-implantation loss (10%) values were higher than their respective control values (8.8 and

4.7%). [Historical control values: mean pre-implantation loss = 12.8% (range = 2.5-26.7%) and mean post-implantation loss = 9.6% (range = 3.8-15.9%)]. There was no effect of treatment on pregnancy parameters in other treated groups.

Total numbers of live fetuses per group, mean number of fetuses per dam, numbers of male and female fetuses, mean litter weight and mean fetal and placental weights are given in Table 21.

There was no treatment-related effect on fetal sex ratio. The mean litter weight and mean fetal weights (male, female and total) were lower in the high dose group than in the control group (not statistically significant). Fetal weights in the other treated groups were similar to those of controls. The placental weights were lower than control in all treated groups (not dose-dependent); the difference achieved statistical significance at mid and high dose levels.

The fetal and litter incidences of external, visceral and skeletal malformations and variations are summarized in Table 22. There were no significant treatment-related effects on the overall incidences of external, visceral or skeletal malformations or variations.

Increased trends in the incidences of minor skeletal malformations [incomplete or absence of ossification of the parietal bone, one or more metacarpel (forelimb) or astragalus (hindlimb)] or variations (incomplete ossification of one or more phalanges of the hind limbs) were observed in treated groups (Table 23). It is noted that the incidences of the above abnormalities were within the sponsor's respective historical control ranges.

**Evaluation:** Oral administration of lanthanum carbonate at 1500 mg/kg/day (about 5 times the maximum recommended human dose of 3000 mg lanthanum/day, on a mg/m<sup>2</sup> basis) to pregnant rabbits during organogenesis was associated with increased incidences of pre- and post-natal losses, reduced litter and fetal weights, delayed skeletal ossification, and maternal toxicity (reduced weight gain and food consumption and decreased fecal output). However, the incidences of the above abnormalities were within the sponsor's historical control ranges. Lower dose levels did not produce any adverse effects on either dams or fetuses.

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Group mean uterine / implantation data					
Group	:	1	2	3	4
Test article	:	Control	Lanthanum Carbonate		
Dose level (mg/kg/day)	:	0	250	750	1500
		Group 1	Group 2	Group 3	Group 4
Number of females with implantations at scheduled kill		18	18	19	18
Number of corpora lutea		181	201	199	190
Mean number per female		10.1	11.2	10.5	10.6
Standard deviation		1.7	4.0	1.7	1.9
Number of implantations		165	172	184	160
Mean number per female		9.2	9.6	9.7	8.9
Standard deviation		1.8	2.9	1.9	2.8
Mean % pre-implantation loss		8.1	13.1	7.0	16.7
Number of early embryo/foetal deaths		3	9	2	10
Number of late embryo/foetal deaths		3	7	3	5
Number of dead fetuses		1	0	0	0
Mean % post-implantation loss		4.7	7.3	2.7	10.0
Number of live fetuses		158	156	179	145
Mean number per female		8.8	8.7	9.4	8.1
Standard deviation		2.0	2.2	1.8	2.9
Mean % of implantations		95.3	92.7	97.3	90.0

Table 20.

Group mean litter weights (g) / foetal data				
Group	1	2	3	4
Test article	Control	Lanthanum Carbonate		
Dose level (mg/kg/day)	0	250	750	1500
	Group 1	Group 2	Group 3	Group 4
Number of females with live foetuses at scheduled kill	18	18	19	18
Number of live foetuses	158	156	179	145
Mean number per female	8.8	8.7	9.4	8.1
Standard deviation	2.0	2.2	1.8	2.9
Number of male foetuses	74	66	87	75
Number of female foetuses	84	90	92	70
Mean $\bar{x}$ male foetuses	46.3	41.1	49.2	50.4
Mean litter weight	320.1	311.5	340.6	276.1
Standard deviation	62.2	72.5	48.9	90.6
Mean foetal weight	36.9	36.3	36.7	34.9
Standard deviation	2.7	3.3	4.7	3.3
Mean foetal weight - males only	37.1	36.6	36.3	35.0
Standard deviation	3.2	2.9	4.7	4.4
Mean foetal weight - females only	36.8	35.9	37.0	34.0
Standard deviation	3.0	4.3	4.8	3.4
Mean placental weight	4.80	4.44	4.26*	4.34*
Standard deviation	0.77	0.85	0.59	0.58

\* - p<0.05    \*\* - p<0.01    \*\*\* - p<0.001

Table 21.

Foetal examination : summary of group mean data				
Group	1	2	3	4
Test article	Control		Lanthanum Carbonate	
Dose level (mg/kg/day)	0	250	750	1500
	Group 1	Group 2	Group 3	Group 4
External and visceral examination				
Total number of litters examined	18	18	19	18
Total number of fetuses examined	158	156	179	145
Number with major abnormalities	3	2	2	1
Mean % of fetuses examined	2.4	2.2	1.4	0.8
Number of litters affected	2	2	2	1
Number with minor abnormalities	50	52	69	43
Mean % of fetuses affected	30.2	35.1	37.9	27.7
Number of litters affected	16	16	16	12
Number with variations	0	0	0	0
Mean % of fetuses affected	0.0	0.0	0.0	0.0
Number of litters affected	0	0	0	0

Table 22.

Foetal examination : summary of group mean data

	Group 1	Group 2	Group 3	Group 4
Skeletal examination				
Total number of litters examined	18	18	19	18
Total number of foetuses examined	158	156	179	145
Number with major abnormalities	3	3	0	2
Mean % of foetuses examined	1.9	1.9	0.0	1.3
Number of litters affected	3	2	0	2
Number with minor abnormalities	26	37	47	40
Mean % of foetuses affected	17.4	23.2	24.5	28.1
Number of litters affected	13	15	15	15
Number with variations	157	153	176	145
Mean % of foetuses affected	99.4	96.5	98.3	100.0
Number of litters affected	18	18	19	18

Table 22 (continued)

Foetal examination : summary of group mean data

	Group 1	Group 2	Group 3	Group 4
Combined examination (external/visceral/skeletal)				
Total number of litters examined	18	18	19	18
Total number of foetuses examined	158	156	179	145
Number with major abnormalities	4	4	2	3
Mean % of foetuses examined	2.9	3.3	1.4	2.1
Number of litters affected	3	3	2	2

Table 22 (continued)

Skeletal examination of fetuses : group mean data  
 Number of fetuses affected (group mean percent)

Group	1	2	3	4
Test article	Control	Lanthanum Carbonate		
Dose level (mg/kg/day)	0	250	750	1500

Key Finding	Type	Group 1	Group 2	Group 3	Group 4
Total number of fetuses examined		158	156	179	145
Total number of litters examined		18	18	19	18
Skull					
L Acephaly	Major	1 (0.5)	0 (0.0)	0 (0.0)	0 (0.0)
15 Fontanelle- Anterior: Increased in size	Minor	2 (1.4)	4 (2.0)	1 (0.6)	11 (5.6)
16 Fontanelle- Posterior: Increased in size	Minor	1 (0.9)	0 (0.0)	0 (0.0)	2 (0.9)
a One or more: Fissure/plaque of bone integral to normal structure of bone	Variant	7 (4.6)	4 (2.4)	10 (5.7)	8 (6.9)
M Parietal: Absent	Major	0 (0.0)	1 (0.8)	0 (0.0)	0 (0.0)
17 Parietal: Incomplete ossification	Minor	0 (0.0)	2 (1.3)	8* (4.2)	5 (4.3) T*
18 Parietal: Fused	Minor	0 (0.0)	1 (0.8)	3 (1.5)	0 (0.0)
N Parietal: Fused	Major	1 (0.5)	0 (0.0)	0 (0.0)	0 (0.0)
O Interparietal: Absent	Major	0 (0.0)	1 (0.8)	0 (0.0)	0 (0.0)
19 Interparietal: Incomplete ossification	Minor	1 (0.9)	1 (0.8)	0 (0.0)	3 (1.5)
P Occipital: Absent	Major	0 (0.0)	1 (0.8)	0 (0.0)	0 (0.0)
20 Maxilla- Uni- or bilateral: Not ossified	Minor	0 (0.0)	2 (1.0)	0 (0.0)	0 (0.0)
b Maxilla- Uni- or bilateral: Incomplete ossification	Variant	39 (23.6)	43 (29.1)	36 (20.7)	53 (32.1)
21 Hyoid: Cornua bent	Minor	4 (3.5)	4 (2.7)	1 (0.5)	1 (1.9)
22 Zygomatic arch and maxilla- Uni- or bilateral: Premature partial fusion	Minor	7 (4.2)	7 (5.8)	11 (5.0)	2 (1.9)

T - trend test \* - p<0.05 \*\* - p<0.01 \*\*\* - p<0.001

Table 23.

Skeletal examination of fetuses : group mean data  
 Number of fetuses affected (group mean percent)

Key Finding	Type	Group 1	Group 2	Group 3	Group 4
Total number of fetuses examined		158	156	179	145
Total number of litters examined		18	18	19	18
Cervical vertebra					
23 One or more centra: Not ossified	Minor	0 (0.0)	1 (0.6)	0 (0.0)	1 (0.5)
24 One or more centra: Offset	Minor	0 (0.0)	1 (0.6)	0 (0.0)	0 (0.0)
25 One or more centra: Bilobed	Minor	0 (0.0)	1 (0.6)	0 (0.0)	0 (0.0)
26 One or more centra: Hemiacentric	Minor	0 (0.0)	1 (0.6)	0 (0.0)	0 (0.0)
27 One or more centra: Asymmetrically ossified	Minor	0 (0.0)	1 (0.6)	2 (1.0)	0 (0.0)
Cervical, thoracic, lumbar, sacral or caudal vertebra					
Q One or more: Scoliosis	Major	0 (0.0)	1 (0.6)	0 (0.0)	1 (0.8)
Thoracic vertebra					
28 Number of vertebra: 11	Minor	0 (0.0)	1 (0.6)	0 (0.0)	0 (0.0)
c Number of vertebra: 13	Variant	73 (49.6)	75 (44.5)	77 (42.2)	85 (60.9)
29 One or more centra: Hemiacentric	Minor	0 (0.0)	1 (0.6)	0 (0.0)	0 (0.0)
30 One or more centra: Asymmetrically ossified	Minor	0 (0.0)	1 (0.6)	0 (0.0)	0 (0.0)
31 One or more centra: Bilobed	Minor	0 (0.0)	0 (0.0)	0 (0.0)	1 (0.5)

Table 23 (continued)

Skeletal examination of fetuses : group mean data  
 Number of fetuses affected (group mean percent)

Key Finding	Type	Group 1	Group 2	Group 3	Group 4
Total number of fetuses examined		158	156	179	145
Total number of litters examined		18	18	19	18
Lumbar vertebra					
32 Number of vertebra: 5	Minor	0 (0.0)	0 (0.0)	1 (0.4)	0 (0.0)
d Number of vertebra: 6	Variant	43 (29.0)	48 (28.4)	28 (14.3)	42 (33.8)
e Number of vertebra: 8	Variant	1 (0.7)	2 (1.3)	7 (3.9)	6 (3.6)
33 One or more neural arch: Additional ossification centre	Minor	1 (0.6)	0 (0.0)	0 (0.0)	0 (0.0)
Sacral vertebra					
R One or more centra: Absent	Major	0 (0.0)	1 (0.6)	0 (0.0)	0 (0.0)
S One or more neural arch: Absent	Major	0 (0.0)	1 (0.6)	0 (0.0)	0 (0.0)
Caudal vertebra					
T One or more: Tail agenesis	Major	0 (0.0)	1 (0.6)	0 (0.0)	0 (0.0)
f Number of centra: <=14	Variant	17 (13.6)	11 (6.2)	18 (9.0)	29 (17.0)
g Number of neural arches: <=6	Variant	10 (7.9)	4 (2.3)	6 (3.2)	13 (6.8)
34 One or more centra: Misplaced	Minor	0 (0.0)	0 (0.0)	0 (0.0)	1 (0.5)
35 One or more centra: Offset	Minor	0 (0.0)	2 (1.1)	0 (0.0)	1 (0.8)
Rib					
36 Rib- Bilateral: 11 pairs	Minor	0 (0.0)	1 (0.6)	0 (0.0)	0 (0.0)

Table 23 (continued)

Skeletal examination of fetuses : group mean data  
 Number of fetuses affected (group mean percent)

Key Finding	Type	Group 1	Group 2	Group 3	Group 4
Total number of fetuses examined		158	156	179	145
Total number of litters examined		18	18	19	18
Rib (continued)					
37 Rib- Uni- or bilateral: Cervical	Minor	0 (0.0)	1 (0.6)	3 (1.6)	1 (1.4)
38 One or more: Ossified outgrowth	Minor	0 (0.0)	1 (0.6)	0 (0.0)	0 (0.0)
U One or more: Fused	Major	0 (0.0)	1 (0.6)	0 (0.0)	0 (0.0)
V One or more: Absent	Major	0 (0.0)	0 (0.0)	0 (0.0)	1 (0.8)
h 13th- Uni- or bilateral: Extra	Variant	73 (49.6)	76 (45.1)	78 (42.8)	86 (61.6)
i 13th- Uni- or bilateral: Vestigial	Variant	30 (20.2)	24 (15.1)	22 (12.3)	21 (14.8)
j 13th- Uni- or bilateral: Floating	Variant	15 (10.3)	13 (7.6)	15 (8.6)	10 (6.7)
39 One or more: Floating	Minor	0 (0.0)	1 (0.6)	0 (0.0)	0 (0.0)
Sternum					
40 Additional centra- One of more: Ossified	Minor	1 (0.6)	0 (0.0)	1 (0.5)	0 (0.0)
41 2nd sternebra: Incomplete ossification	Minor	0 (0.0)	0 (0.0)	0 (0.0)	2 (1.0)
42 One or more: Fused	Minor	1 (0.9)	0 (0.0)	0 (0.0)	1 (0.6)
43 One or more: Mis-shapen or misaligned	Minor	3 (2.0)	2 (1.2)	1 (0.5)	7 (4.0)
W One or more: Fused	Major	1 (0.9)	0 (0.0)	0 (0.0)	1 (0.5)

Table 23 (continued)

Skeletal examination of fetuses : group mean data  
 Number of fetuses affected (group mean percent)

Key Finding	Type	Group 1	Group 2	Group 3	Group 4
Total number of fetuses examined		158	156	179	145
Total number of litters examined		18	18	19	18
Sternum (continued)					
44 4th sternebra: Incomplete ossification	Minor	0 (0.0)	0 (0.0)	0 (0.0)	1 (0.6)
k 5th sternebra: Not ossified	Variant	17 (10.0)	31 (21.4)	25 (13.6)	28 (18.9)
m 5th sternebra: Incomplete ossification	Variant	53 (33.0)	44 (28.4)	38 (20.9)	39 (26.0)
n 6th sternebra: Not ossified	Variant	3 (2.6)	6 (3.4)	0 (0.0)	11 (10.2)
o 6th sternebra: Incomplete ossification	Variant	18 (13.4)	22 (13.6)	30 (15.5)	25 (14.8)
Pelvic girdle					
45 Entire: Asymmetric insertion	Minor	5 (3.2)	1 (0.6)	9 (4.9)	4 (2.4)
46 Pubis- Uni- or bilateral: Not ossified	Minor	0 (0.0)	0 (0.0)	0 (0.0)	2 (1.2)
47 Pubis- Uni- or bilateral: Incomplete ossification	Minor	6 (3.9)	11 (6.1)	6 (3.4)	12 (7.3)
Forelimb					
p Epiphyses: Not ossified	Variant	25 (16.1)	48 (29.6)	41 (21.0)	45 (30.8)
q Proximal or distal epiphyses of humerus only: Not ossified	Variant	39 (26.5)	32 (19.7)	36 (20.1)	33 (21.3)
48 One or more metacarpal: Not ossified	Minor	1 (0.6)	3 (2.8)	9 (4.0)	7 (3.6) T*

T - trend test \* - p<0.05 \*\* - p<0.01 \*\*\* - p<0.001

Table 23 (continued)

Skeletal examination of fetuses : group mean data  
 Number of fetuses affected (group mean percent)

Key Finding	Type	Group 1	Group 2	Group 3	Group 4
Total number of fetuses examined		158	156	179	145
Total number of litters examined		18	18	19	18
Forelimb (continued)					
49 One or more phalange: Not ossified	Minor	1 (0.9)	2 (1.2)	1 (0.7)	3 (4.3)
r One or more phalange: Incomplete ossification	Variant	29 (19.4)	26 (17.0)	42 (21.3)	32 (24.7)
50 One or more claw: Absent	Minor	0 (0.0)	0 (0.0)	0 (0.0)	1 (0.6)
Hindlimb					
s Epiphyses: Not ossified	Variant	75 (46.5)	88 (55.4)	100 (53.3)	82 (53.2)
t Proximal epiphyses of tibia or distal epiphyses of femur only: Not ossified	Variant	59 (37.9)	49 (30.3)	54 (31.5)	50 (36.5)
51 Femur- Uni- or bilateral: Incomplete ossification	Minor	0 (0.0)	0 (0.0)	1 (0.4)	0 (0.0)
52 Tibia- Uni- or bilateral: Incomplete ossification	Minor	0 (0.0)	0 (0.0)	1 (0.4)	0 (0.0)
53 Fibula- Uni- or bilateral: Incomplete ossification	Minor	0 (0.0)	0 (0.0)	1 (0.4)	0 (0.0)
54 Astragalus- Uni- or bilateral: Not ossified	Minor	0 (0.0)	0 (0.0)	2 (0.8)	3 (3.0) T*
u One or more phalange: Incomplete ossification	Variant	4 (3.0)	5 (3.0)	15 (7.3)	15 (10.5) T*

T - trend test \* - p<0.05 \*\* - p<0.01 \*\*\* - p<0.001

Table 23 (continued)

### 3. Pre- and Post-Natal Developmental Toxicity Study in Rats

**Key findings:** Oral administration of lanthanum carbonate in rats at 2000 mg/kg/ day from implantation to the end of lactation caused delayed eye opening, reduction in weight gain, and delayed sexual development (preputial separation and vaginal opening) of the offspring. Mating performance and fertility of offspring were unaffected by the maternal treatment.

**Testing facility:** [ ]

**Study number:** SPD/58/R

**Study dates:** Initiation Date – January 1997

Completion Date – June 1997

**GLP compliance:** The study was conducted in accordance with UK GLP Regulations

**QA report:** Yes

**Animals:** Timed-mated female Sprague-Dawley derived rats of the — (SD) IOPS-Caw strain (VAF plus), about 10 weeks of age and weighing between 265 and 364 g, were obtained from [ ] by day 4 of pregnancy (day 0 of pregnancy was the day of observation of a sperm positive smear or vaginal plug). The animals were housed in solid-bottomed polypropylene cages, with sawdust as bedding. A pelleted — Rat and Mouse — expanded diet (manufactured by [ ] and tap water were provided *ad libitum*.

One hundred females were assigned to four groups of 25 animals each on day 5 of pregnancy using a bodyweight stratification technique.

**Dose levels and mode of administration:** The doses employed were 0 (vehicle control), 200, 600 and 2000 mg/kg/day.

Lanthanum carbonate (batch number 6268/960105), suspended on the day of use in 0.5% aqueous carboxymethylcellulose, was administered once daily by oral gavage, from day 6 of pregnancy to day 20 post-partum, at a dose volume of 10 ml/kg.

Analyses of the samples of formulations (prepared on the first day of dosing and in weeks 3 and 5) showed that the concentrations were within 87 to 97% of the nominal values.

The doses were selected based on a preliminary study (0, 200, 600, 1000 or 2000 mg/kg/day) in rats (same strain) that was used for the dose selection for the fertility and

embryonic development study in rats. (The results of the preliminary study are presented under the fertility and development study in rats.)

### **Observations and measurements**

#### *F0 Maternal Animals*

All females were checked twice daily for mortality and daily for clinical signs. Body weights were recorded daily from day 5 of pregnancy until necropsy on postnatal day (pnd) 21. (Body weights recorded on days 5, 6, 7, 8, 9, 12, 15 and 20 of pregnancy and on post-natal days 0, 4, 7, 14 and 21 were the only body weights reported.) Food consumption was recorded during pregnancy and on pnd 1 to 14. (Food consumptions over days 5 to 6, 6 to 9, 9 to 12, 12 to 15, and 15 to 20 of pregnancy and during pnd 1 to 7 and 7 to 14 were reported.)

Beginning on day 21 of pregnancy, all F0 females were observed at about 30-minute intervals between the hours of 0700 and 2400 for evidence of parturition; the times of onset and completion of parturition were recorded.

F0 dams were allowed to rear their pups to pnd 21.

All F0 dams were killed after weaning their offspring on pnd 21. All major organs were examined, and ovaries, pituitary and adrenals were weighed. Ovaries, uterus, cervix, vagina, pituitary, adrenals and any gross lesions were fixed in neutral buffered formalin.

#### *F1 Progeny*

The litter size and sex of the pups were recorded soon after birth and daily thereafter until pnd 21. The pups were examined for gross malformations. Clinical signs were recorded daily. A necropsy was conducted on any pup that died or was killed prematurely.

On pnd 4, the size of each litter was adjusted to eight (four per sex, if possible). Litters of less than eight were not adjusted. All culled pups were necropsied.

The pups were weighed individually soon after birth (day 0), and on pnd 4, 7, 14 and 21.

During the lactation period (up to pnd 21), the pups were observed for ear opening (daily from day 0 to day 4), eye opening (daily up to day 16), static righting reflex (on day 5), startle response (on day 15) and pupillary light reflex (on day 21).

After weaning, 20 F1 pups/sex/group were randomly selected for detailed post-weaning examinations and rearing to sexual maturity. The remaining pups were subjected to necropsy.

The selected pups were examined daily for clinical signs, and weighed weekly. Ophthalmoscopic examination (pnd 28-35) and assessments of auditory function (pnd 28-35) and

E-maze learning potential (pnd 28) were performed. All males were examined daily for balanopreputial separation from pnd 35, and females were examined daily for vaginal opening from pnd 28.

When the selected F1 animals reached 13 weeks of age, each female was mated with a male from the same group for up to 7 days. Vaginal smears were taken daily until sperm was found in the smear. The male was removed from the cage on the day of mating. If a female had not mated within seven days, the male was removed and another male that had previously mated was substituted.

The F1 pregnant females were weighed on days 0, 7, and 13 of pregnancy, and were necropsied on day 13. All major organs were examined grossly; ovaries, pituitary and adrenals were weighed. Organs or tissues showing gross abnormalities were fixed in neutral buffered formaldehyde. Pregnancy status and the numbers of corpora lutea, implantation sites, early and late resorptions, and live embryos were recorded.

The F1 females that showed no evidence of mating after the second mating were necropsied 13 days after the end of the mating period.

The F1 males were killed about 2 weeks after the end of the mating period, major organs examined, and the testes, epididymides and adrenals were weighed. The testes were preserved in Bouin's solution and the epididymides and any other organs or tissues showing gross lesions were preserved in neutral buffered formaldehyde.

Gestation, live birth and viability indices were calculated.

Analysis of variance (ANOVA) was performed on all parameters. Residuals from this preliminary analysis were examined for heterogeneity of variance using Levene's test. If the Levene's test was significant at the 1% level, then the particular variable was subjected to non-parametric analyses using Kruskal-Wallis ANOVA test followed by Shirley's non-parametric version of Williams test. If the Levene's test was not significant at the 1% level, then William's test was done to compare treated and control groups.

The nominal data were analyzed using the Fisher's exact test.

## **Results**

### *F0 Maternal Animals*

Pale and reduced quantities of feces were observed in treated groups during the late pregnancy and early lactation periods. There were no other clinical signs observed in the study.

There was no effect of treatment on maternal body weight, body weight gain or food consumption during pregnancy or lactation except that the food consumption of the high dose group was significantly lower than control during days 7 to 14 of lactation.

The pregnancy and litter data are summarized in Table 24. One high dose female littered only one pup, which was dead. All remaining pregnant females produced live litters; the gestation indices were 100, 100, 100 and 95.5% for 0, 200, 600 and 2000 mg/kg/day, respectively. The mean durations of gestation and parturition were similar across control and treated groups.

Six females failed to rear their offspring: two from the control group (#s 8 and 17), three at 600 mg/kg/day (#s 52, 63 and 65) and one at 2000 mg/kg/day. Since there was no dose relationship or any effect on pup survival in the remaining litters of these groups, these losses were considered to be unrelated to treatment.

The necropsy of F0 females showed no remarkable findings. There were no treatment related effects on the absolute or relative body weights of pituitary, adrenals and ovaries.

#### *F1 Progeny*

There was no effect of maternal treatment with lanthanum carbonate at any dose level on the mean number of pups born, mean live birth index, sex ratio of pups or viability indices (Table 24).

The pup body weights for both sexes were generally similar across control and treated groups on pnd 0. Beginning from pnd 7 (pnd 7, 14 and 21), the pup body weights at 2000 mg/kg/day were significantly lower than control in both boxes (Table 25). In females, reduction in body weight was noted in all treated groups on pnd 4.

The overall body weight gain for the high dose group was significantly reduced for both sexes for pnd 0 to 21. Significantly reduced body weight gain was also noted in mid dose females (Table 25).

The percentage of pups with eyes open on pnd 16 was significantly reduced at the high dose compared to control. Seven of the 21 litters of the high dose group had 50% or more of pups with delayed eye opening on day 16. The times of eye opening in other dose groups were similar to control. There were no effects of maternal treatment on the time of ear opening, or on the presence of righting, startle or pupillary light reflexes.

There were no clinical signs observed in F1 generation animals, except that at the high dose, pale body and piloerection were noted immediately after weaning.

The body weights of high dose F1 males were lower than control throughout the study, the differences being statistically significant from week 5 to 14 (except for week 12). In high dose females, body weights were lower than control, the differences attaining statistical significance from week 5 to 8. There were no treatment-related effects on body weights in lower dose groups.

The body weights during pregnancy (F1) in all treatment groups were similar to control.

Group mean pregnancy and litter data - F0 generation females and F1 generation litters

Group	1	2	3	4								
Treatment	Control		Lanthanum carbonate									
Dosage (mg/kg/day)	0	200	600	2000								
Group	Number littered (N)	Gestation index	Mean duration of gestation (days) ± S.D.	Mean duration of parturition (hours) ± S.D.	Mean number of pups born ± S.D.	% males	Mean live birth index	Mean viability index 1	Mean viability index 2	Mean viability index 3	Mean viability index 4	Mean cumulative survival index
1	21	100.0	21.7 ± 0.2	2.2 ± 0.6(18)	14.0 ± 3.7	44.4(20)	93.6	87.6	100.0(19)	100.0(19)	100.0(19)	82.7
2	22	100.0	21.8 ± 0.3	2.5 ± 1.6(20)	14.4 ± 2.4	51.0	98.0	100.0	100.0	99.4	99.4	96.9
3	20	100.0	21.9 ± 0.4	2.3 ± 0.7(17)	14.6 ± 2.4	50.6(17)	88.6	76.8	100.0(17)	100.0(17)	100.0(17)	75.5
4	22	95.5	21.8 ± 0.5	2.2 ± 0.7(18)	12.9 ± 4.4	52.1	95.2	97.5(21)	98.8(21)	100.0(21)	99.4(21)	91.1(21)
Analysis of variance		NS	NS	NS	NS	NS	NS			NS	NS	
Kruskal- Wallis test								p<0.01		NS		NS

N = number of animals in mean  
( ) = N, where N differs from original

Table 24.

Table 25.

Group mean pup bodyweights and bodyweight gains (g) ± S.D. : F1 generation litters

Group	1	2	3	4	
F0 Treatment	Control		Lanthanum carbonate		
F0 Dosage (mg/kg/day)	0	200	600	2000	
<b>Males</b>					
Bodyweight (day post partum)	Group				Analysis of variance
	1	2	3	4	
N	21	22	19	21	
0	6.3 ± 0.6	6.2 ± 0.3	6.4 ± 0.5	6.4 ± 0.5(20)	NS
4	9.2 ± 1.1(19)	8.5 ± 0.8	8.8 ± 1.0(16)	8.6 ± 1.1	NS
4pc	9.3 ± 1.0(19)	8.5 ± 0.8	8.9 ± 1.0(16)	8.7 ± 1.1	NS
7	14.9 ± 1.6(19)	14.3 ± 1.7	14.8 ± 1.8(16)	13.3 ± 1.6**	p<0.05
14	32.6 ± 2.8(19)	31.9 ± 2.7	31.9 ± 2.8(16)	27.5 ± 3.4***	p<0.001
21	52.1 ± 3.6(19)	51.5 ± 4.5	51.9 ± 4.3(16)	43.2 ± 6.3***	p<0.001
Bodyweight gain (days 0 - 21)	45.8 ± 3.6(19)	45.3 ± 4.4	46.3 ± 5.0(16)	36.8 ± 6.0(20)***	p<0.001
<b>Females</b>					
Bodyweight (day post partum)	Group				Analysis of variance
	1	2	3	4	
N	21	22	20	21	
0	6.0 ± 0.5	5.9 ± 0.3	6.0 ± 0.5	6.1 ± 0.6(20)	NS
4	9.0 ± 0.9(19)	8.2 ± 1.0*	8.3 ± 0.8(17)*	8.3 ± 1.1*	NS
4pc	9.1 ± 0.9(19)	8.2 ± 0.9(21)**	8.3 ± 0.9(17)**	8.3 ± 1.0*	p<0.05
7	14.6 ± 1.4(19)	13.9 ± 2.0	13.8 ± 1.5(17)	12.7 ± 1.6***	p<0.01
14	32.0 ± 2.5(19)	31.3 ± 3.0	30.2 ± 3.0(17)	26.7 ± 3.6***	p<0.001
21	52.0 ± 3.6(19)	50.7 ± 4.6	48.4 ± 5.8(17)*	42.2 ± 6.3***	p<0.001
Bodyweight gain (days 0 - 21)	45.9 ± 3.7(19)	44.8 ± 4.5	42.4 ± 5.6(17)*	36.1 ± 5.9(20)***	p<0.001

N = number of litters in mean

() = N, where differs from original

pc = post culling

\* = significantly different from the control, p&lt;0.05, Williams' test

\*\* = significantly different from the control, p&lt;0.01, Williams' test

\*\*\* = significantly different from the control, p&lt;0.001, Williams' test

There were no treatment-related effects on E-maze learning, auditory function or ophthalmoscopy.

Preputial separation, a criterion for sexual development, was significantly delayed in high dose males compared to control. A dose-related delay in vaginal opening was noted in females from all treated groups (Table 26).

The mean number of days taken for mating, and the copulation and fertility indices were similar across control and treated groups.

The pregnancy rates for F1 females were 84.2, 94.7, 85.0 and 100% at 0, 200, 600 and 2000 mg/kg/day, respectively. There were no differences in the numbers of corpora lutea, implantations and live embryos, or on the extent of pre- and post-implantation losses that were considered to be related to F0 maternal treatment with lanthanum chloride (Table 27).

Necropsy of F1 offspring (pups that died, or were killed at culling on pnd 4, at weaning on pnd 21 or terminally) showed no treatment related findings.

There was no effect of F0 maternal treatment on F1 organ weights except that the mean absolute pituitary weight was lower in the high dose male group than in control, with no significant effect on relative pituitary weight.

**Evaluation:** In rats, oral administration of lanthanum carbonate at 2000 mg/kg/day from implantation to the end of lactation caused delayed eye opening, reduction in body weight gain and delayed sexual development (preputial separation and vaginal opening) of the offspring. Mating performance and fertility of offspring were unaffected by the maternal treatment with lanthanum carbonate.

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Table 26.

Group mean sexual development observations  $\pm$  S.D. : F1 generation

Group	1	2	3	4
F0 Treatment	Control		Lanthanum carbonate	
F0 Dosage (mg/kg/day)	0	200	600	2000
Group	N	Day preputial separation observed	Day vaginal perforation observed	
1	20	44.3 $\pm$ 1.5	33.6 $\pm$ 1.5	
2	20	44.6 $\pm$ 1.3	34.0 $\pm$ 2.2*	
3	20	44.8 $\pm$ 2.2	34.5 $\pm$ 1.5**	
4	20	46.7 $\pm$ 3.4**	35.4 $\pm$ 2.3***	
Analysis of variance		p<0.01	p<0.001	

N = number of animals in mean

\* = significantly different from the control, p<0.05, Williams' test

\*\* = significantly different from the control, p<0.01, Williams' test

\*\*\* = significantly different from the control, p<0.001, Williams' test

Group mean uterine / implantation data					
Group	:	1	2	3	4
FO Test article	:	Control	Lanthanum Carbonate		
FO Dose level (mg/kg/day):		0	200	600	2000
		Group 1	Group 2	Group 3	Group 4
Number of females with implantations at scheduled kill		16	18	17	20
Number of corpora lutea		283	300	288	324
Mean number per female		17.7	16.7	16.9	16.2
Standard deviation		1.4	2.3	1.6	1.8
Number of implantations		261	281	275	299
Mean number per female		16.3	15.6	16.2	15.0
Standard deviation		2.4	2.5	1.6	3.8
Mean % pre-implantation loss		7.8	6.1	4.3	8.6
Number of early embryo deaths		15	14	10	17
Number of late embryo deaths		0	0	0	0
Number of dead embryos		2	1	1	1
Mean % post-implantation loss		6.3	5.5	3.9	5.5
Number of live embryos		244	266	264	281
Mean number per female		15.3	14.8	15.5	14.1
Standard deviation					
Mean % of implantations		93.7	94.5	96.1	94.5

Table 27.

## VIII. SPECIAL TOXICOLOGY STUDIES

*Sponsor's summaries of special toxicity studies are provided below.*

### **An Assessment of the Effects of Lanthanum on Bone and Other Tissues in a Chronic Renal Failure Rat Model (Report No. R00081-LAM-IIIQ; LAN-02)**

The study objective was to further investigate the distribution of lanthanum after oral dosing in several tissues, and more specifically the effects on bone turnover in a rat model of chronic renal failure using various doses of lanthanum to investigate dose-response relationships.

Male Wistar rats were separated into 10 groups. The first 5 groups of 8 animals were sham operated, and the remaining 5 groups underwent 5/6 nephrectomy by ligation of two of the three branches of the renal artery of the left kidney, followed by removal of the right kidney one week later. A group of 25 animals underwent the same procedures with a delay of 14-weeks to supplement any losses with the first groups. Animals were dosed at 100, 500, 1000, or 2000 mg/kg/day for 12 weeks. Blood collection occurred for serum and plasma prior to CRF, after the 2-week stabilization period, and at 3-month intervals during dosing at 4, 8, and 12 weeks. Urine samples were collected 24-hours after prior to blood collection.

The results suggest that animals with CRF developed hyperthyroidism. At the higher lanthanum carbonate dose (1000 and 2000 mg/kg/day) there were effects superimposed on this, suggesting the presence of reduced bone formation in a few animals. There was no consistent relationship between bone lanthanum concentrations and osteomalacia. The results suggest that the osteomalacia in the renal impaired rats was unlikely to have been a direct concentration-related toxic response to the lanthanum present in the bone.

In conclusion, osteomalacia occurred in a small number of 5/6<sup>th</sup> nephrectomized rats given lanthanum carbonate at 1000 and 2000 mg/kg/day. The mechanism by which lanthanum induced osteomalacia remains unclear, however, lanthanum accumulates in bone after repeated administration. A direct concentration-related toxic response to the presence of drug in this tissue seems unlikely, because considerably higher bone concentrations measured in longer-term animal studies have resulted in no evidence of bone toxicity.

**Table 5-54 An Assessment of the Effects on Bone and Other Tissues in a Chronic Renal Failure Rat Model**

<b>Name of Company:</b> Shire Pharmaceuticals Group		<b>Report No:</b> R00081-LAM-III(Q) (LAN-02)									
<b>Species/Strain:</b> Rat/Wistar, males only		<b>Route:</b> Oral (gavage)								<b>Duration of Treatment:</b> 12 weeks	
<b>Weight Range on Day 1:</b> Males 384 - 562 g		<b>Test Material:</b> Lanthanum Carbonate Batch No: B1066-980601								<b>Dosing Frequency:</b> Once daily	
<b>Age on Day 1:</b> 14 weeks old		<b>Vehicle:</b> 2% w/v carboxymethyl-cellulose								<b>Necropsy Dates:</b> 3 May 2000	
<b>Dose Volume:</b> 10 ml/kg		<b>Treatment of Controls:</b> Vehicle at 10 ml/kg									
<b>No Observable Adverse Effect Level (NOAEL):</b> 500 mg (salt)/kg/day		<b>Study in Compliance with GLP:</b> No								<b>Main Test Facility:</b> 	
<b>Model of Chronic Renal Failure:</b> A 5/6 nephrectomy was carried out in 2 stages. Ligation of 2 of the 3 branches of the left renal artery was followed one week later by right nephrectomy. Dosing started 2 weeks later when stable chronic renal insufficiency had been achieved. Control animals with normal renal function were sham-operated.											
<b>Measurements and Observations:</b> Mortality: daily, clinical observations: daily; body weight: once weekly-Weeks 1-12; food and water consumption: daily Weeks 1-12; serum and plasma lanthanum concentration measurements: Weeks -4, 0, 4, 8, 12; clinical chemistry and urinalysis: Weeks -4, 0, 4, 8, 12; macroscopic and microscopic pathology, bone histomorphometry and tissue lanthanum concentration measurements: Week 12.											
<b>Study Design:</b>		<b>Normal Renal Function (Sham Operated)</b>					<b>Chronic Renal Failure</b>				
<b>Dose (mg (salt)/kg/day)</b>		<b>Vehicle</b>	<b>100</b>	<b>500</b>	<b>1000</b>	<b>2000</b>	<b>Vehicle</b>	<b>100</b>	<b>500</b>	<b>1000</b>	<b>2000</b>
<b>No. animals</b>		10	10	10	11	8	14	14	13	14	10
<b>Important Findings:</b> Treatment (lanthanum carbonate) related findings are shown in bold											
<b>No. deaths<sup>1</sup></b>		1	2	1	1	4	5	7	5	7	5
<b>Clinical signs</b>		No treatment-related findings									
<b>Body weight</b>		No treatment-related findings									
<b>Food intake</b>		No treatment-related findings									
<b>Water intake</b>		No treatment-related findings									
<b>Plasma Toxicokinetics<sup>2</sup>:</b> Mean lanthanum concentration (ng/ml)											
<b>Week 4</b>		2.50	1.22	5.40	1.91	1.50	1.56	1.55	2.91	2.15	6.71
<b>Week 8</b>		2.28	0.88	2.95	1.58	2.95	0.93	0.94	1.57	2.47	1.85
<b>Week 12</b>		2.04	1.11	1.85	1.83	3.02	0.92	0.70	1.62	1.73	9.39
<b>Clinical Chemistry:</b>											
<b>Serum Creatinine (mg/dL)<sup>2</sup>:</b>											
<b>Week 0</b>		0.47	0.45	0.47	0.46	0.53	1.37	1.13	1.26	1.29	1.56
<b>Week 4</b>		0.46	0.40	0.46	0.45	0.53	1.40	1.24	1.31	1.33	1.60
<b>Week 8</b>		0.46	0.47	0.50	0.44	0.60	1.83	1.54	1.38	1.54	2.08
<b>Week 12</b>		0.47	0.46	0.50	0.43	0.55	3.20	1.57	1.85	1.80	2.18

**Table 5-54 An Assessment of the Effects on Bone and Other Tissues in a Chronic Renal Failure Rat Model (continued)**

Name of Company: Shire Pharmaceuticals Group		Report No: R00081-LAM-IIIQ (LAN-02) Report Date: August 2000								
Dose (mg (salt)/kg/day)	Normal Renal Function (Sham Operated)					Chronic Renal Failure				
	0	100	500	1000	2000	0	100	500	1000	2000
<b>Serum PTH (pg/mL) <sup>2</sup>:</b>										
Week 0	64.1	73.25	75.0	66.6	79.8	261.4	198.1	220.6	219.9	227.6
Week 4	73.8	90.8	97.3	138.3	109.0	289.2	266.4	242.3	333.9	193.4
Week 8	81.6	110.1	118.4	124.9	148.3	719.4	451.3	292.4	475.6	286.8
Week 12	92.2	89.0	191.6	77.1	85.8	979.8	580.4	402.0	427.1	124.8
<b>Serum Calcium (mg/dL):</b>										
Week 12	10.04	10.03	9.97	9.68	9.80	10.70	10.31	10.49	10.17	10.92
<b>Serum Phosphate (mg/dL):</b>										
Week 0	7.72	7.37	7.42	7.29	7.88	6.51	7.26	7.28	6.97	8.60
Week 4	7.16	6.37	7.70	6.36	6.73	7.00	6.99	6.93	6.96	5.40
Week 8	6.67	6.58	6.32	6.18	5.75	6.96	6.99	7.26	6.21	6.04
Week 12	6.77	6.59	7.01	6.32	6.15	10.08	8.09	7.71	6.03	5.56
<b>Serum Osteocalcin (ng/ml) <sup>2</sup>:</b>										
Week 0	37.8	47.0	27.9	31.9	20.4	112.4	109.6	83.4	82.8	148.5
Week 4	40.2	49.3	34.6	32.4	29.7	208.2	220.2	178.5	197.7	154.4
Week 8	15.9	18.7	18.2	18.3	25.8	95.9	88.3	74.4	119.7	98.9
Week 12	44.8	35.9	26.3	25.2	31.1	206.2	109.8	128.4	103.9	95.1
<b>Serum Alkaline Phosphatase (IU/L):</b>										
Week 12	53.4	64.3	65.3	64.7	46.5	51.1	74.3	54.3	58.9	48.5
<b>Urinalysis:</b>										
<b>Urine Volume (mL):</b>										
Week 12	53.6	31.3	43.7	41.6	39.3	37.8	39.9	49.3	40.1	51.4
<b>Urine Creatinine (mg/24h):</b>										
Week 12	19.3	19.0	23.6	20.9	22.1	18.6	19.0	24.2	17.8	19.0
<b>Urine Protein (mg/24h) <sup>2</sup>:</b>										
Week 0	0.135	0.122	0.124	0.140	0.126	0.205	0.448	0.185	0.323	0.216
Week 4	0.131	0.125	0.122	0.125	0.149	1.228	1.653	0.596	1.135	0.561
Week 8	0.150	0.163	0.153	0.182	0.130	1.708	1.585	1.266	1.756	0.974
Week 12	0.185	0.105	0.100	0.16	0.111	2.060	1.661	1.508	1.749	1.748
<b>Urine Calcium (corrected) (mg/24h) <sup>2</sup>:</b>										
Week 0	0.58	0.74	0.54	0.46	0.58	5.79	4.24	3.93	5.23	6.19
Week 4	0.43	0.37	0.44	0.31	1.21	2.61	3.42	0.92	2.14	2.76
Week 8	0.32	0.53	0.44	0.29	0.45	1.68	0.71	1.13	1.54	1.51
Week 12	0.52	0.35	0.29	0.28	0.25	0.90	0.66	1.56	1.01	3.63
<b>Urine Phosphorus (mg/24h):</b>										
Week 0	20.91	19.56	17.25	16.96	18.29	18.31	18.26	17.41	21.56	15.03
Week 4	18.65	16.82	11.57	9.44	16.51	19.15	17.34	10.34	6.56	5.13
Week 8	16.40	12.93	7.30	6.76	13.84	17.52	16.88	10.12	3.79	2.36
Week 12	13.53	12.35	7.30	14.19	7.15	8.54	8.89	7.65	3.68	1.13

**Table 5-54 An Assessment of the Effects on Bone and Other Tissues in a Chronic Renal Failure Rat Model (Continued)**

Name of Company: Shire Pharmaceuticals Group		Report No: R00081-LAM-IIIQ (LAN-02) Report Date: August 2000								
Study Design:	Normal Renal Function (Sham Operated)					Chronic Renal Failure				
Dose (mg (salt)/kg/day)	0	100	500	1000	2000	Vehicle	100	500	1000	2000
<b>Urine Phosphorus (% Animals with Concentrations Below LLoQ):</b>										
Week 0	0	0	0	0	0	0	0	0	0	0
Week 4	0	0	0	0	0	0	0	0	14	40
Week 8	0	0	0	30	0	0	0	13	43	60
Week 12	0	0	11	0	0	0	14	38	57	100
<b>Urine Leucocytes (Mean Dipstick Score) <sup>1</sup>:</b>										
Week 0	0.3	0.3	0.1	0.3	0.0	1.3	1.6	0.8	2.0	1.4
Week 4	0.3	0.5	0.0	0.5	0.3	3.1	3.0	2.6	2.7	2.8
Week 8	0.7	0.5	0.1	0.7	0.8	3.0	3.0	3.0	2.7	3.5
Week 12	0.4	0.4	0.0	1.0	0.8	3.0	3.4	3.6	3.4	4.0
<b>Urine Erythrocytes (Mean Dipstick Score) <sup>1</sup>:</b>										
Week 0	0.4	0.6	0.4	0.2	1.3	1.0	0.9	0.8	0.7	0.8
Week 4	0.2	0.3	0.4	0.9	0.8	1.4	1.3	1.1	0.9	1.6
Week 8	0.2	0.0	0.1	0.3	1.0	1.3	0.9	0.9	0.3	1.0
Week 12	0.3	0.3	0.1	0.4	0.8	0.4	0.3	0.9	1.0	0.4
<b>Bone Histomorphometry: Mean Values</b>										
Bone Area (%)	20.4	14.6	18.1	11.0	16.6	25.7	20.0	25.6	25.5	32.4
Osteoid Area (%)	0.70	0.50	0.30	0.95	0.50	3.80	4.30	4.30	7.30	3.10
Osteoid Perimeter (%)	4.50	5.45	3.50	8.25	5.80	27.3	31.4	27.8	45.5	31.3
Osteoid Width (µm)	3.5	3.1	3.1	3.5	4.0	5.3	5.3	5.0	6.4	5.5
Double Labelled Perimeter (%)	13.8	8.00	14.5	14.3	19.0	12.3	14.1	15.9	2.50	3.45
Mineral Apposition Rate (µm/day)	1.97	1.70	1.69	1.98	1.59	3.03	2.75	2.83	1.61	0.90
Bone Formation Rate (µm <sup>2</sup> /mm <sup>2</sup> /day)	1547	759	1512	1476	1255	3221	2944	3906	926	1443
Mineralization Lag Time (days)	0.60	0.65	0.50	1.40	0.75	2.10	2.10	1.90	19.4	4.65
Eroded Perimeter (%)	0.70	1.40	1.00	2.35	2.15	4.50	1.20	2.70	2.10	1.40

**Table 5-54 An Assessment of the Effects on Bone and Other Tissues in a Chronic Renal Failure Rat Model (Continued)**

Name of Company: Shire Pharmaceuticals Group		Report No: R00081-LAM-IIIQ (LAN-02) Report Date: August 2000								
Classification of Renal Osteodystrophy: Number of affected animals										
Treatment:	Normal Renal Function (Sham Operated)					Chronic Renal Failure				
Dose (mg salt/kg/day)	0	100	500	1000	2000	0	100	500	1000	2000
No. Rats Examined	8	8	9	10	4	7	7	8	7	4
Normal bone histology	5	7	6	6	3	2		3	-	
Adynamic bone	1	-	2	2	-	-	1	-	-	1
Mixed lesions	1	-	-	1		1	4	1	3	1
Mild hyperparathyroid.	1	-	1	1	1	4	2	4	1	1
Osteomalacia	-	1	-	-	-	-	-	-	3	1
Microscopic Pathology (Other Tissues): Number of affected animals										
No. Rats Examined	9	8	9	10	4	9	7	8	7	5
Kidney:										
Chronic progressive nephropathy	1	1	0	3	0	8	7	8	7	5
Tissue Lanthanum Concentrations (ng/g wet weight): Medians										
Femur	63	171	482	605	1154	75	198	1221	1411	1582
Brain	4	4	14	18	11	5	5	12	23	53
Kidney	21	60	593	607	1007	73	131	724	1140	1222
Liver	6	62	499	468	982	15	304	1778	2780	3540
Pancreas	47	58	92	84	189	89	192	237	62	213
Testes	14	10	18	24	83	6	6	22	25	25
Epididymides	28	17	29	39	211	17	17	39	51	170
Spleen	10	21	68	50	102	29	61	95	94	137
Heart	4	11	48	41	118	21	19	32	53	58
Lung	20	35	275	77	236	23	32	93	127	277
Sciatic Nerve	108	155	68	84	53	106	77	61	135	317
Spinal Cord	16	24	34	112	61	26	34	82	45	91
Eyes	20	25	126	123	739	12	27	44	109	274

**Table 5-54 An Assessment of the Effects on Bone and Other Tissues in a Chronic Renal Failure Rat Model (Continued)**

<b>Name of Company:</b> Shire Pharmaceuticals Group	<b>Report No:</b> R00081-LAM-IIIQ (LAN-02) <b>Report Date:</b> August 2000
<b>Noteworthy Findings:</b> There were no drug related effects on mortality, food consumption, body weight or body weight gain. Rats with CRF in the control group developed hyperparathyroidism (increased osteoid area, bone formation rate, and eroded perimeter). At the 1000 and 2000 mg/kg/day doses, additional effects were superimposed on this, suggesting the presence of reduced bone formation (osteomalacia) in a few animals. There was no consistent relationship between bone lanthanum concentration and osteomalacia in the rats with CRF. Thus osteomalacia was unlikely to have been a result of a toxic response to lanthanum.	
<b>Conclusion:</b> Osteomalacia occurred in renally impaired rats dosed for 12 weeks with lanthanum carbonate at 1000 mg salt/kg/day (3 of 7 rats) and 2000 mg salt/kg/day (1 of 4 rats), but not in rats with normal renal function given the same doses. The marked reduction in urine phosphate output suggested that the osteomalacia was secondary to phosphate depletion, a mechanism widely reported in the literature, and consistent with lanthanum carbonate's potent phosphate binding activity. A direct toxic mechanism was considered unlikely in view of the relatively low bone lanthanum concentrations in the affected groups compared with those measured in a variety of animal species during chronic toxicity and carcinogenicity studies, in which no bone toxicity was observed.	
<b>Notes:</b> NRF - Normal renal function CRF - Chronic renal failure LLoQ - Lower limit of quantification <sup>1</sup> Mortality in CRF groups was generally higher than in the corresponding NRF groups, suggesting that severe impairment of renal function was responsible for many of the deaths. Others were attributed to the blood collection technique. None was considered directly related to lanthanum administration. <sup>2</sup> CRF resulted in significant and expected increases in serum creatinine, osteocalcin (a marker for bone formation), and PTH; and in urine protein, calcium, leucocytes and erythrocytes compared to the equivalent NRF groups. <sup>3</sup> Plasma and tissue lanthanum levels were variable, the ranges often being disproportionately affected by occasional very high results. In the lungs exceptionally high lanthanum concentrations were found in occasional animals suggesting that partial misplacement of the dose may have occurred. Other very high values may have resulted from cross contamination from gut luminal contents during necropsy.	

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**An Assessment of the Effects of Lanthanum on Bone and  
Other Tissues in a Chronic Renal Failure Rat Model –  
Report Addendum Report No. R00081-LAM-IIIQ; LAN-02)**

The purpose of this addendum was to further investigate whether in the rats with chronic renal failure, a disturbed vitamin D metabolism in combination with lanthanum-induced phosphate depletion might have been responsible for the observed bone lesions. Serum 25(OH)-vitamin D, and 1,25(OH)<sub>2</sub>-vitamin D<sub>3</sub> levels were measured in a limited number of animals from the R00081-LAM-IIIQ study.

The results indicated that serum 1,25(OH)<sub>2</sub>-vitamin D<sub>3</sub> levels in CRF rats did not differ from those in rats with normal renal function nor did it differ between lanthanum-treated and vehicle-treated animals. 25(OH)<sub>2</sub>-vitamin D<sub>3</sub> levels in CRF rats were significantly lower than those measured in rats with normal renal function and remained low in the lanthanum treated animals in a comparable way. These results coincide with those from the previous report.

The findings present evidence that lanthanum carbonate treatment in chronic renal failure rats does not alter the serum concentrations of hydroxylated vitamin D metabolites.

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**Table 5-55 An Assessment of the Effects of Lanthanum on Bone and Other Tissues in a Chronic Renal Failure Rat Model – Report Addendum**

<b>Name of Company:</b> Shire Pharmaceuticals Group		<b>Shire Report No:</b> R00081-LAM-IIIQ (LAN-02) Addendum <b>Report Date:</b> June 2001	
<b>Species/Strain:</b> Rat/Wistar, males only		<b>Route:</b> Oral (gavage)	<b>Duration of Treatment:</b> 12 weeks
<b>Study in Compliance with GLP:</b> No		<b>Test Material:</b> Lanthanum Carbonate <b>Batch No:</b> B1066-980601	<b>Testing Facility:</b> 
<b>Study Objectives:</b> To further investigate whether in the rats with chronic renal failure, a disturbed vitamin D metabolism in combination with lanthanum –induced phosphate depletion might have been responsible for the observed bone lesions Serum 25(OH)-vitamin D, and 1,25(OH) <sub>2</sub> -vitamin D <sub>3</sub> levels were measured in a limited number of animals from the R00081-LAM-IIIQ study.			
<b>Important Findings:</b>			
<b>Study Group</b>		<b>Mean</b>	
<b>Animals with Normal Renal Function</b>		<b>1,25-(OH)<sub>2</sub>-D3 (pg/ml)</b>	<b>25-(OH)-D3 (ng/ml)</b>
Vehicle		100	28
100 mg/kg		95	31
1000 mg/kg		86	25
2000 mg/kg		136	30
<b>Animals with Chronic Renal Failure</b>		<b>1,25-(OH)<sub>2</sub>-D3 (pg/ml)</b>	<b>25-(OH)-D3 (ng/ml)</b>
Vehicle			
100 mg/kg		121	10.4
1000 mg/kg		111	8.2
2000 mg/kg		98	13.7
<b>Conclusion:</b> The findings present evidence that lanthanum carbonate treatment in chronic renal failure rats does not alter the serum concentrations of hydroxylated vitamin D metabolites.			

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**An Assessment of the Effects of Renagel® (Sevelamer) on Bone and Comparison with Lanthanum in a Chronic Renal Failure Rat Model (Report No. R00363-LAM-IIIQ; LAN-03)**

This study was conducted to investigate the hypothesis that osteomalacia can be induced in chronic renal failure (CRF) rats as a result of phosphate depletion associated with excessive doses of a phosphate binder. The effects of lanthanum carbonate on bone were compared with those of sevelamer, a non-absorbed, non-metallic phosphate binder.

Thirty male Wistar rats underwent 5/6 nephrectomy to induce chronic renal failure (CRF). Dosing, started two weeks after the second operation, was by oral gavage with either sevelamer (500 mg/kg/day or 1000 mg/kg/day), lanthanum carbonate (1000 mg/kg/day), or vehicle (2% carboxymethylcellulose) for 12 weeks.

Blood and urine samples were obtained at baseline (prior to induction of CRF); prior to treatment with vehicle, sevelamer or lanthanum carbonate; every 4 weeks (blood) and every 2 weeks (urine) during the treatment period.

At the end of the study animals were euthanized, and tibias were examined for bone histology and histophometry. The following parameters were studied: Total bone area, osteoid area, osteoid width, osteoid perimeter, double labeled perimeter, mineral apposition rate, mineralization lag time, and bone formation rate.

There were no significant differences in animal mortalities, food consumption or weights between groups.

There were no significant differences in serum creatinine, phosphate, calcium, or alkaline phosphatase levels between groups. There was a slight tendency towards higher serum iPTH levels after installation of CRF, indicative of the development of hyperparathyroidism.

There were no significant differences in proteinuria, between groups, which increased as a function of time due to induction of renal failure. Calciuria did not differ between groups, but was significantly increased over baseline levels in all groups.

Phosphaturia decreased over time in all treatment groups, reaching significance in the lanthanum carbonate-treated group vs. the vehicle group by Week 2. Initially (Week 2 to Week 6) this decrease was more pronounced in the lanthanum treated group, but was significant after 6 and 8 weeks in the high and low-dose sevelamer groups. By the end of the study, urine phosphate output was markedly reduced suggesting the presence of phosphate depletion.

Bone histology revealed a tendency towards an increase in osteoid area, and decrease in bone formation rate relative to the control, group in the sevelamer 1000 mg/kg/day group, and to a lesser extent in the lanthanum 1000 mg/kg/day group. Four of 7 rats in the

sevelamer group and 1 of 4 rats in the lanthanum carbonate group were affected with impaired mineralization (referred to as osteomalacia in previous studies).

Serum 25-(OH) vitamin D<sub>3</sub> concentration did not differ significantly between groups, although there was a tendency towards lower levels in the sevelamer treated animals. In contrast, the serum 1,25-(OH)<sub>2</sub> vitamin D<sub>3</sub> levels were significantly lower ( $p < 0.05$ ) in the sevelamer treated animals than either the control or lanthanum-treated animals, with an apparent dose-dependent effect. Due to the impaired vitamin D absorption from the gastrointestinal tract because of the sevelamer and higher turnover of 25-(OH) vitamin D<sub>3</sub> due to renal failure, these animals are less able to absorb the remaining non-bound phosphate in the gastrointestinal tract, leading to an increased need for mobilization of phosphate out of bone.

In conclusion, the administration of relatively high doses of sevelamer (1000 mg/kg/day) to rats with CRF induced osteomalacic lesions most probably resulting from increased bone demineralization, secondary to phosphate depletion. These effects on bone were similar to those observed in previous studies with lanthanum carbonate.

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## IX. OVERALL SUMMARY AND EVALUTION

Lanthanum carbonate hydrate is being developed as a phosphate binding agent for the treatment of hyperphosphatemia in patients with end stage renal disease. Chronic renal failure has been shown to cause a progressive decline in the kidneys' ability to excrete phosphate, produce active vitamin D and maintain calcium and phosphorus homeostasis. Hyperphosphatemia and decreased vitamin D levels can cause increased parathyroid hormone levels or secondary hyperparathyroidism, which may result in renal osteodystrophy including osteomalacia. Moreover, higher serum phosphorus can precipitate calcium, leading to higher incidence of visceral and vascular calcification. Phosphate binding agents decrease the gastrointestinal absorption of phosphates.

Lanthanum ions bind with dietary phosphate in the stomach, forming highly insoluble lanthanum phosphate that is excreted in the feces, thereby significantly reducing the absorption of dietary phosphate. It is noted that the low intrinsic systemic toxicity, due to poor GI absorption of lanthanum, together with its high phosphate binding capacity, make lanthanum carbonate a good candidate for the clinical management of hyperphosphatemia.

It is proposed that lanthanum carbonate be started at a dose of 750 mg lanthanum daily, and the dose be titrated weekly to a level (up to 3000 mg lanthanum per day) that achieves maintenance of acceptable serum phosphate levels.

The nonclinical studies conducted with lanthanum carbonate are summarized below.

In *in vitro* studies, lanthanum carbonate showed high affinity for phosphorus binding [with low levels of released metal ions (considered to be an index for absorption) detected in solution] at a pH range of 3 to 7, maximum binding occurring within 15 to 30 minutes. These studies also showed that lanthanum carbonate, when present in a two-fold molar excess, removed >97% of the available phosphorus at pH 3 and 5 and >66% at pH 7, with a maximum metal ion concentration of 302 ppm at pH 3, declining to 1 ppm at pH 7. The effects of lanthanum were comparable to aluminum hydroxide but superior to calcium salts.

The effect of lanthanum carbonate on the transport of labeled ( $^{32}\text{P}$ ) phosphate across gut lumen was studied in isolated perfused rat gut preparations, and compared with the effects of equimolar doses of aluminum hydroxide. At low dose levels (9.2 mg lanthanum carbonate/kg or 1.35 mg aluminum hydroxide/kg), there was little difference between lanthanum carbonate (no inhibition) and aluminum hydroxide (4.8% inhibition), compared to control values, in their ability to inhibit phosphate transport. At higher doses, lanthanum carbonate (30.8 mg/kg; 34.4% inhibition) was shown to be more efficient at binding phosphate than the equimolar dose of aluminum hydroxide (4.56 mg/kg; 17.5% inhibition).

An *in vivo* study in rats showed that single dose oral administration of lanthanum carbonate (1.5 or 2000 mg/kg) produced dose-dependent reductions (23 to 24% at 1.5

mg/kg and 33 to 41% at 2000 mg/kg) in plasma or serum phosphate levels at 8 and 24 hr post-dose, with no effects on plasma or serum calcium levels. The high dose caused reductions in urinary volume and urinary calcium levels over 0 to 6 hr post-dose, with no change in urinary phosphate levels.

Daily administration of lanthanum carbonate (1000 mg/kg, po) to rats with normal renal function for 7 days prior to oral administration of labeled phosphate (60 mg/rat) resulted in the excretion of about 56% of the administered phosphate dose in the feces and 2% in urine during the 0 to 144 hr post-dose period, with most of the radioactivity being excreted within the first 48 hours. (In control rats, 31% of the administered phosphate dose was excreted in the feces and 12% in the urine.) The effect of lanthanum carbonate on fecal phosphate excretion was comparable to the effects seen with equimolar doses of aluminum hydroxide and calcium carbonate, but greater than that seen with sevelamer, the polymeric resin. Reduction in urinary phosphate excretion was similar for all drugs. The increased fecal excretion of phosphate is consistent with a reduction in the absorption of phosphorus due to the binding and precipitation of insoluble phosphate salts in the gastrointestinal tract.

Lanthanum carbonate at single oral doses of 200, 500 or 1000 mg/kg did not produce any effects on Irwin Screen parameters (visual placing, grip strength, twitches, tremors, respiration and behavioral observations) or body temperature up to 4 hours post-dose in male CD-1 mice.

Single oral doses of lanthanum carbonate (200, 500 or 1000 mg/kg) had no effect on spontaneous motor activity in male CD-1 mice.

Lanthanum carbonate at single oral doses of 500, 1000 or 2000 mg/kg produced no significant proconvulsant or anticonvulsant effects in metrazol- or electroshock-induced convulsions in male CD-1 mice.

Lanthanum carbonate (500, 1000 or 2000 mg/kg, po) did not produce any statistically significant effects on hexobarbital-induced sleeping time in male or females mice. The reference compound, chlorpromazine hydrochloride (15 mg/kg), significantly prolonged hexobarbital-induced sleeping time in mice (both sexes).

Intraduodenal administration of lanthanum carbonate at 0, 200, 600 or 2000 mg/kg had no effect on blood pressure, heart rate, left ventricular pressure, femoral flow rate, peripheral resistance, and on EKG (QRS amplitude, PR, ST and QT intervals, and QTc value) and respiratory (rate, tidal volume and minute volume) parameters in anesthetized dogs.

Oral administration of lanthanum carbonate at 200, 500 and 1000 mg/kg had no significant effect on intestinal motility of rats. At 1000 mg/kg, charcoal meal content in the small intestine was reduced, indicating delayed gastric emptying at this dose.

In a pylorus ligated rat model, a single oral administration of lanthanum carbonate at 200, 500 or 1000 mg/kg had no effect on the volume of gastric fluid secretion or pepsin activity, but the hydrogen ion concentration was reduced in a dose-dependent manner, with the reduction being significant ( $p < 0.01$ ) at 1000 mg/kg. This reduction in pH is attributed to the neutralization of the gastric content by lanthanum carbonate.

Oral administration of lanthanum carbonate (200, 500 or 1000 mg/kg) had no effect on urinary volume or electrolyte concentrations at 3 or 6 hr post dose. At 24 hr post dose, a significant reduction in urinary phosphate excretion at 500 or more mg/kg, and an increase in urine volume and urinary sodium output at 1000 mg/kg were noted. There was no effect on fecal output.

Single oral doses of lanthanum carbonate (200, 500 or 1000 mg/kg) did not cause any gastric lesions in rats, when examined macroscopically 3-hr post dose, while aspirin (reference compound; 150 mg/kg) caused significant stomach lesions ( $p < 0.01$ ). In a separate study, it was shown that pretreatment with a single dose of lanthanum carbonate (200, 500 or 1000 mg/kg, po), 2 hr before the administration of aspirin (150 mg/kg), reduced the incidence of aspirin-induced gastric lesions in rats. A similar reduction in aspirin-induced gastric lesions was also observed after pretreatment with 6% sodium chloride, the protective effect being attributed to its hypertonicity. The gastroprotection effect of lanthanum carbonate is attributed to its hypertonicity and neutralizing capacity.

In *in vitro* studies, the effects of lanthanum carbonate (100, 500, 1000, 5000 and 15000 ng/ml) on bone resorption, differentiation of osteoclasts and osteoblasts, and bone formation were investigated. In these studies, lanthanum had no effects on bone resorption by mature osteoclasts at any concentration tested. At concentrations of 500 to 15000 ng/ml, lanthanum produced a concentration-dependent inhibition of osteoclast differentiation. The effects of lanthanum on osteoblast differentiation were mixed, with stimulation observed at the low concentration (100 ng/ml) and inhibitory effects at high concentrations (5000 and 15000 ng/ml). In contrast, substantial activation of the bone formation activity of mature osteoblasts occurred at all concentrations of lanthanum tested.

Lanthanum is minimally absorbed by rats and dogs after oral administration as the carbonate salt. Oral bioavailability was estimated to be 0.0007% in the rat and 0.00005% in the dog. In general, oral administration of increasing doses of lanthanum carbonate (200 to 2000 mg/kg/day) resulted in dose-dependent, but less than proportional [especially at higher dose levels (1500 and 2000 mg/kg/day in rat and dog, respectively)], increased levels of systemic exposure. At the highest dose level tested in mice, rats, rabbits and dogs, exposure was 2 to 10 fold higher than that observed in placebo-treated animals. (Low endogenous systemic levels of lanthanum, close to the limit of quantification of the assay, were generally seen in placebo-treated animals.) Generally, no significant gender differences were noted in systemic exposure.

The time to peak plasma concentration ( $T_{max}$ ) after drug administration varied in different species. The mean  $T_{max}$  values in healthy human subjects ranged from 3 to 6 hours

compared to 1 to 6 hours in mice, 2 to 8 hours in rats, 8 hours in rabbits and 1 to 9 hours in dogs.

Comparison of mean plasma levels and  $AUC_{(0-24h)}$  values after single and repeat dose administration of lanthanum carbonate showed no evidence of accumulation following repeated dosing in rats, mice and rabbits. However in dogs, comparison of AUC values at doses of 200, 600 and 2000 mg/kg/day across studies at weeks 4, 13 and 25 showed that the exposure at week 13 was increased relative to values at week 4, but were comparable or higher than respective values at week 25, suggesting that exposure had reached steady state levels by week 13 in dogs. It is also noted that the plasma lanthanum concentrations were comparable during weeks 26 and 51 of the 52-week oral toxicity study in dogs.

It is noted that at steady state, plasma lanthanum exposures ( $AUC_{(0-24h)}$ ) at maximum tolerated doses in animal oral toxicity studies exceeded the exposure in humans (at the maximum recommended dose of 3 g lanthanum per day) by up to 7 fold (Table on page 27).

The plasma elimination half-life ( $t_{1/2}$ ) of lanthanum after oral administration was estimated to be about 36 hours in man, 9 hours in mouse, 13 to 20 hours in rat, 20 hours in rabbit and 20 to 26 hours in dog. Because of the retention of lanthanum in tissue compartments, the  $t_{1/2}$  values appears to relate to an initial plasma clearance phase, and not the true terminal elimination half-life of the drug.

*In vitro* studies using blood samples from mouse, rat, rabbit, dog and human showed that lanthanum binds extensively to plasma proteins, with mean protein binding exceeding 99.5% in all species.

The distribution of lanthanum in tissues was determined following single and/or repeated oral or iv dosings in different species. The absorbed portion of an oral dose of lanthanum is widely distributed to tissues, and the concentrations vary considerably depending on the tissue type. Many tissues retain concentrations significantly above those in plasma.

Lanthanum concentrations were found to be low in the majority of tissues after chronic oral dosing for 80 weeks in the mouse, 78 weeks in the rat and 52 weeks in the dog at maximum tolerated doses (1500 mg/kg/day in mice and rats, and 2000 mg/kg/day in dogs). Tissues that generally had low median concentrations (1  $\mu\text{g/g}$  wet tissue or less) include aorta, adrenals, brain, heart, kidney, ovary, pituitary, prostate, sciatic nerve, skeletal muscle, spleen, testes, urinary bladder, uterus and vagina. Tissue lanthanum concentrations were found to be higher after iv administration than after oral dosing. It was shown that even at the maximum tolerated iv dose in dogs (1 mg/kg/day), the range of median concentrations of lanthanum in brain and cerebrospinal fluid (CSF) was 0.035 to 0.162  $\mu\text{g/g}$  wet tissue and 0.22 to 0.85 ng/ml, respectively. The concentrations in the CSF were more than 3 orders of magnitude lower than  $C_{\text{min}}$  plasma concentrations. These results are consistent with published studies showing that lanthanum does not cross the blood:brain barrier due to its inability to pass through intercellular tight junctions and its low potential for trans-cellular transport across endothelial cells. This is in contrast to

aluminum (used as a phosphate binding agent for the treatment of renal dialysis patients) which accumulates in the brain causing CNS toxicity (encephalopathy).

Intermediate levels of lanthanum (1.0 to 10 µg/g wet tissue) were seen in femur (shaft and plate), liver and sternum after chronic oral administration at the above maximal dose levels in mice, rats and dogs.

Femur lanthanum concentrations observed in animal studies (at MTD) are summarized below, along with the bone lanthanum levels seen in dialysis patients (bone biopsy) after one year of lanthanum treatment (500 to 3750 mg lanthanum/day).

**Femur Lanthanum Concentrations at Maximum Tolerated Doses in Animals**

Species (Report)	Route	Duration of Dosing	Dose (mg (salt)/kg/day)	Medians (µg/g wet tissue)	Overall Range (µg/g wet tissue)
Man (dialysis patient) (LAM-IV- 303)	po	Up to 52 weeks	Up to 143 <sup>a</sup>	1.8	
Rat (SPD0099)	po	4 weeks	1500	0.35 - 0.58	
Rat (SPD/87/C)	po	78 weeks	1500	2.2 - 4.4	
Rat (SPD0102)	iv	4 weeks	0.3	6.9 - 8.2	
Rat - Uremic (LAN-02)	po	13 weeks	2000 (NRF) 2000 (CRF)	1.2 1.6 <sup>Ost</sup>	
Dog (SPD0100)	po	4 weeks	2000	0.33 - 0.77	
Dog (SPD/66/TK)	po	26 weeks	2000	2.0 - 3.3	
Dog (SPD/66/TK)	po	52 weeks	2000	1.8 - 3.9	
Dog (SPD0104)	iv	4 weeks	1.0	26.0 - 54.5	
Mouse (SPD/88/C)	po	80 weeks	1500	3.6 - 8.1	

CRF - chronic renal failure

NRF - normal renal function

Ost - associated with osteomalacia

a - range of patient doses 500 to 3750mg/day, ie 19 to 143 mg (salt)/kg/day for a 50kg patient.

The above data indicate that chronic oral administration of lanthanum carbonate for 78 weeks in rats at 1500 mg/kg/day and 52 weeks in dogs at 2000 mg/kg/day, was associated with an increased (5-8 fold) median concentrations of lanthanum in femur compared to respective values after 4-weeks of dosing. In dogs, bone lanthanum concentrations were comparable between 26 and 52 weeks, indicating that a steady state level (with no further accumulation of lanthanum in this tissue) was achieved after 26 weeks of treatment.

The median bone lanthanum concentration in dialysis patients (1.8 µg/g wet tissue) was lower than the levels seen in bones in chronic animal studies.

In renally impaired rats dosed for 12 weeks at 2000 mg/kg/day (some of which developed osteomalacia), the median femur lanthanum concentration (1.6 µg/g wet tissue) was comparable to or lower than the bone lanthanum levels seen in chronic studies in normal animals (rats, dogs and mice). Although the bone lanthanum levels were higher in long-term studies, no bone pathology was seen in these studies. Moreover, the median bone lanthanum levels were mostly the same in renally impaired and renally competent rats.

Median liver lanthanum concentration generally remained below 10 µg/g in all species after chronic oral administration. In dogs, the liver lanthanum levels were higher after 26 weeks of treatment than after 4 weeks, but there was little further increase between 26 and 52 weeks, indicating achievement of steady state levels after about 26 weeks of treatment.

High concentrations of lanthanum were seen in the GI tract, especially in the stomach, after chronic oral administration. The highest median stomach concentrations achieved in long term studies at MTD were 2189 µg/g in mice (80 week dosing), 827 µg/g in rats (78 weeks dosing) and 349 µg/g in dogs. In rodents, glandular stomach had higher concentrations than non-glandular stomach. After 4 weeks of oral dosing in rats, the median concentrations in the glandular stomach were 46 to 90 µg/g compared to 4 to 20 µg/g in the non-glandular stomach.

The clearance of lanthanum from tissues was studied in rats and dogs that received drug treatment for 4 weeks followed by a recovery period of 4 or 26 weeks.

In rats, after 4 weeks of oral administration of lanthanum carbonate at 1500 mg/kg/day, the median concentration of lanthanum was less than 1 µg/g in the majority of tissues including femur, liver, kidneys, heart and teeth. The median concentration in the brain was at the lower limit of quantification for the assay. The highest concentrations (up to 90 µg/g) were found in the GI tract and associated lymph nodes. The patterns of distribution and clearance of lanthanum in male and female rats were generally similar.

After a 4 week drug-free period, more than 99% of drug was cleared from the lower GI tract (cecum, colon and rectum), more than 90% from the non-glandular stomach and ileum, and more than 75% from the liver. In contrast, there was no appreciable clearance from the glandular stomach and upper small intestine (duodenum and jejunum) over this period. Clearance from bone, cartilage and teeth was slow. After a 26 week drug-free period, more than 50% of the levels observed at the end of the dosing period were present in the glandular stomach, duodenum, mesenteric lymph nodes, jejunum, spleen, femur, sternum and trachea.

In the dog, after 4 weeks of oral drug administration (2000 mg/kg/day), the pattern of tissue clearance was similar to that seen in the rat, except that drug was cleared more rapidly from upper small intestine (duodenum) and teeth, and more slowly from liver after a 4 week drug-free recovery period. At the end of the 26 week recovery period, tissues that retained more than 50% of the level observed at the end of the dosing period included stomach (fundus), liver, femur (growth plate and shaft), spleen and sternum.

In summary, the distribution and clearance studies in rats and dogs showed that lanthanum is cleared steadily from most tissues, but very slowly from stomach, spleen, sternum and bone (rat and dog), small intestine (rat) and liver (dog).

Drug metabolism studies were not necessary for lanthanum carbonate because of its elemental and inorganic form. It has been shown that biochemical interactions with lanthanides are ionic and, therefore, the formation of covalently bound organic metabolites *in vivo* is extremely unlikely. Within tissues, lanthanum is predominantly found in the extracellular compartment (outer surface of cell membranes) because of its inability to pass through the plasma membrane of healthy cells. This is consistent with the high affinity of lanthanides for ionic binding to surface ligands, including membrane proteins and phospholipid bilayers.

In an *in vitro* study, it was shown that lanthanum carbonate was not a significant inhibitor of any of the human liver cytochrome P450 (CYP 1A2, 2C9/10, 2C19, 2D6 and 3A4/5) examined, indicating a low potential for drug-drug interactions *in vivo*.

Since the absorption was minimal, most of an oral dose of lanthanum carbonate was excreted in the feces. In the rat, 99.3% of the administered dose was recovered in feces, and in the dog, 93.4% was recovered either in feces or vomit within 7 days. Recovery of the dose from urine was very low, about 0.004% in rats and 1.14% in dogs over the same period. In man, urinary excretion in healthy subjects represented 0.000031% of the administered dose.

In bile-duct cannulated rats, following administration of a single iv dose of lanthanum chloride, about 79% of the total dose was recovered in the bile (over the first 120 hr post-dose), indicating that biliary excretion is the predominate route of elimination for circulating lanthanum in rats.

Single dose oral administration toxicity studies in rats and mice indicated that the maximum tolerated dose was greater than 2000 mg lanthanum carbonate/kg in these species.

In a twenty-six week oral toxicity study in rats (0, 100, 600 and 2000 mg/kg/day), dose-related increased incidences of stomach lesions (hyperplasia of the fundus epithelium and mucus cells, sub-mucosal inflammation and hyperplasia at the limiting ridge) were seen in treated males and females, especially at the 600 and 2000 mg/kg/day dose levels, the incidence and severity of lesions being higher at the high dose. (Based on these results, and also considering the potential for progression to excessive inflammation and gastric ulceration over the course of the lifetime study, a dose of 1500 mg/kg/day was selected as the top dose for the 2-year rat bioassay.) After a 4-week recovery period, sub-mucosal inflammation and hyperplasia at the limiting ridge appeared to persist, however, the hyperplasia of the fundus epithelium was less prominent. Plasma lanthanum levels in weeks 13 and 26 were generally similar to that seen on day 1 of the study, indicating no significant accumulation with time. A dose-related increase in lanthanum levels was seen

in all four tissues (brain, femur, liver and kidney) analyzed; however, the sample size was small and there were wide variations between individual values.

Increased incidences of stomach lesions (epithelial hyperplasia of the limiting ridge and non-glandular region, and mucosal inflammatory cell infiltration in the glandular region) were also seen in a 13-week oral toxicity study in the mouse (0, 500, 1500 and 2000 mg/kg/day) at the top two dose levels. Inflammation of the glandular epithelium was more prevalent and severe at 2000 than at 1500 mg/kg/day. (Based on these results, 1500 mg/kg/day was selected as the top dose for the 99-week mouse bioassay.) The plasma levels at week 13 were generally similar to those on day 1. Higher levels of lanthanum, compared to controls, were seen in all four tissues (brain, femur, liver and kidney) analyzed from treated animals, especially in the femur.

In the dog, oral administration of 0, 200, 600 and 2000 mg lanthanum carbonate/kg/day for 52 weeks did not reveal any significant toxicity. Unlike in rodents, no stomach lesions were seen in the dog. The better gastric tolerance in dogs may be related to the ability to vomit to expel an irritant material, unlike in rodents. It was shown that lanthanum carbonate is better tolerated if administered with food in man and dogs. Nocturnal feeding habits result in rodents receiving the drug (during the day) on an empty or partially-empty stomach. It is believed that the longer duration of direct contact of lanthanum with the stomach wall, together with the inability to vomit an irritant material is likely to make rodents more susceptible than dogs to stomach lesions.

Two years of oral administration of lanthanum carbonate at 0, 0, 100, 500 and 1500 mg/kg/day did not produce any treatment-related increased incidence of tumors in the rat. The highest dose employed in the study (1500 mg lanthanum carbonate or 786 mg lanthanum/kg/day) is about 3 times the maximum recommended human dose (MRHD) of 3000 mg lanthanum/day (50 mg lanthanum/kg/day for a 60 kg subject) on a mg/m<sup>2</sup> basis.

In the mouse, oral administration of lanthanum carbonate at 0, 100, 500 and 1500 mg/kg/day for 99 weeks produced a significant increasing trend for stomach adenoma in male mice [0/50 (C), 0/50 (LD), 0/50 (MD) and 4/50 (HD) p = 0.0041]. The highest dose employed in the study is about 1.3 times the MRHD of 3000 mg lanthanum/day, on a mg/m<sup>2</sup> basis.

The Executive Carcinogenicity Assessment Committee, at their November 5, 2002 meeting, concluded that both rat and mouse carcinogenicity studies were adequately performed, and that the stomach adenomas observed in male mice were drug related.

In a fertility and embryonic development study, oral administration of lanthanum carbonate to male and female rats during pre-mating, mating and up to gestational day 17, at dose levels up to 2000 mg/kg/day (about 3.4 times the MRHD on a mg/m<sup>2</sup> basis) did not affect fertility or mating performance, or produce any harm to the fetus.

In a rabbit developmental toxicity study (0, 250, 750 and 1500 mg/kg/day), oral administration of lanthanum carbonate to pregnant rabbits at 1500 mg/kg/day (about 5 times the MRHD on a mg/m<sup>2</sup> basis) during organogenesis was associated with increased pre- and post-natal losses, reduced litter and fetal weights, delayed skeletal ossification and maternal toxicity (reduced weight gain and food consumption, and decreased fecal output). However, all values were within the sponsor's historical control range. Lower dose levels (750 and 250 mg/kg/day) did not produce any adverse effects on either dam or fetus.

In a pre- and post-natal study in rats (0, 200, 600 and 2000 mg/kg/day), oral administration of lanthanum carbonate at 2000 mg/kg/day (about 3.4 times the MRHD on a mg/m<sup>2</sup> basis) from implantation to the end of lactation caused delayed eye opening, reduction in body weight gain, and delayed sexual development (preputial separation and vaginal opening) of the offspring. Mating performance and fertility of offspring were unaffected by the maternal treatment with lanthanum.

Lanthanum carbonate tested negative for genotoxicity in *in vitro* (bacterial reverse mutation assay, and mammalian cell gene mutation and cytogenetic assays in Chinese hamster ovary cells) and *in vivo* (mouse micronucleus assay) test systems.

The following special toxicity studies were conducted with lanthanum carbonate.

In chronic renal failure (CRF, produced by 5/6<sup>th</sup> nephrectomy) rats, oral administration of lanthanum carbonate (0, 100, 500, 1000 and 2000 mg/kg/day) for 12 weeks produced osteomalacia (reduced bone formation and mineral apposition rates, and increased mineralization lag time) at 1000 (3 of 7 rats) and 2000 (1 of 4 rats) mg/kg/day dose levels. These bone effects were not seen in rats with normal renal function (NRF) given the same doses of lanthanum carbonate. Although there were dose-dependent accumulations of lanthanum in the bone in both CRF and NRF rats (table below), there was no significant difference in the lanthanum concentrations between NRF rats (in which no osteomalacia was seen at 2000 mg/kg/day; median bone lanthanum concentration = 1.2 µg/g wet tissue) and CRF rats (in which osteomalacia was seen at 2000 mg/kg/day; median bone lanthanum concentration = 1.6 µg/g wet tissue), indicating a lack of consistent direct relationship between bone lanthanum concentration and osteomalacia.

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**Femur lanthanum concentrations in normal and uraemic rats in Study LAN-02**

Species (Report No.)	Route	Duration of Dosing	Dose (mg (salt)/ kg/day)	Medians ( $\mu\text{g/g}$ wet tissue)	Overall Range ( $\mu\text{g/g}$ wet tissue)
Rat NRF (LAN-02)	po	13 weeks	100	0.17	
			500	0.48	
			1000	0.61	
			2000	1.2	
Rat CRF (LAN-02)	po	13 weeks	100	0.20	
			500	1.2	
			1000	1.4	
			2000	1.6	

CRF – chronic renal failure  
NRF – normal renal function

Furthermore, higher bone lanthanum levels than observed in CRF rats were seen in chronic toxicity study animals (Table on page 172) in which no bone toxicity was observed, again indicating that a direct concentration-related toxic effect of lanthanum on bone is unlikely. It is noted that the doses of lanthanum that produced osteomalacia in the renally impaired rats produced marked reductions (78 and 86% reduction at 1000 and 2000 mg/kg/day, respectively, at week 8) in urinary phosphate excretion, compared to NRF rats given the same doses of lanthanum carbonate, indicating a state of phosphate depletion in renally impaired animals. Furthermore, it was shown that in CRF rats (both vehicle and lanthanum treated), there was a significant reduction (about 50%) in serum 25-(OH) vitamin D<sub>3</sub> levels (with no change in serum 1,25 (OH)<sub>2</sub> vitamin D<sub>3</sub> levels), compared to vehicle and lanthanum treated NRF rats. (Vitamin D<sub>3</sub> is essential for the absorption of phosphate from the gut.)

Rats with normal renal function are able to maintain normal vitamin D activity to enable absorption of phosphate from the gut, and to reabsorb phosphate from the kidney to avoid the need to mobilize phosphorus from bone. It is hypothesized that in CRF rats, the compensatory mechanisms are compromised; reduction in 25-(OH) vitamin D<sub>3</sub> level leads to reduced absorption of phosphate from the gut, and even maximal renal phosphate reabsorption (resulting in hypophosphaturia) is inadequate to maintain serum levels, thus requiring phosphorus mobilization from the bone, resulting in the observed mineralization defect.

It is well known from the published literature that hypophosphatemia, by any cause, can result in impaired bone mineralization (osteomalacia). The hypothesis that osteomalacia can be induced in CRF rats as a result of phosphate depletion associated with high doses of a phosphate binder, and not by excessive accumulation or direct toxic effect of the compound on the bone, was tested using a non-absorbed, non-metallic phosphate binder, sevelamer, comparing its effects with that of lanthanum carbonate. It was shown that oral administration of sevelamer (1000 mg/kg/day) to CRF rats for 12 weeks produced impaired mineralization in 4 of 7 rats, while lanthanum carbonate produced bone

mineralization defects in 1 of 4 rats, indicating that the bone effects were secondary to phosphate depletion.

Dietary phosphorus restriction (using a phosphate deficient diet) has been reported to result in the rapid onset of osteomalacia within one week in CRF rats, whereas uremia or phosphate deficient diet alone did not produce the bone effects (Lieuallen, WG et al. 1990. The effects of uremia and dietary phosphorus on the bone of rats. Bone 11: 41-46.).

In summary, the osteomalacia observed in uremic rats is not considered to be due to a direct toxic effect of lanthanum on bone, but is associated with uremia and phosphate depletion in these animals. As hyperphosphatemia, rather than phosphate depletion, is a prerequisite for treatment of end stage renal disease patient with a phosphate binder, such bone changes are unlikely to occur during treatment with lanthanum carbonate.

All toxicity studies appeared to be adequately performed using maximum tolerated doses.

The glandular stomach adenomas observed in male mice were associated with proliferative changes due to gastric irritation induced by the very high stomach lanthanum concentration at the dose at which the tumors occurred in this species (2189  $\mu\text{g/g}$  in mice vs 827  $\mu\text{g/g}$  in rats and 349  $\mu\text{g/g}$  in dogs). No neoplastic lesions (benign or malignant) were associated with lanthanum carbonate administration in the rat. In view of the above and in the absence of evidence of a genotoxic potential for lanthanum carbonate, the mouse tumor finding is not considered to be an approvability issue.

The apparently drug-related effects on rabbit embryo/fetal survival and development occurred at a relatively high dose, about 5 times the MRHD (on a  $\text{mg/m}^2$ ) basis, and may have been secondary to maternal toxicity observed at this dose. Moreover, the values observed in the study were within the sponsor's historical control range. Although in a study in which rats were dosed from implantation through lactation, 2000  $\text{mg/kg/day}$  was associated with delayed eye opening and delayed sexual development of the offspring, mating performance and fertility of the offspring were unaffected by the maternal treatment. We do not consider the above reproductive toxicity findings to constitute an approvability issue.

In conclusion, there are no approvability issues for lanthanum carbonate based on the non-clinical toxicity-testing program.

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the approval package consisted of draft labeling

**XI. ATTACHMENT**

Dr. John Koerner's 'Review and Evaluation of Canine Toxicology and Pharmacokinetics Studies' is attached.

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