Thirteen-Week Oral Toxicity Study in Cynomolgus Monkeys
([redacted]615-004)

Summary Review
(Complete review attached in Appendix)

PURPOSE:

To assess the toxicity of Hectorol in monkeys when administered orally for 13 weeks.

EXPERIMENTAL DESIGN:

Testing Facility: [redacted]
Study #: 615-004
Study Initiated: 10/30/89
Study Completed: 10/1/90
Dose & Formulation: 0, 0.06, 0.39, & 2.5 ug/kg oral single dose, in 1 ml/kg of coconut oil.
Batch of drug: lot K004.
Food: [redacted] certified primate chow 5048.
Monkeys were allowed free access to food and tap water.
GLP statement: GLP and QA signed and dated 10/1/90.
Animals: 32 Cynomolgus monkeys were obtained from [redacted] Primate.

Group:	Dose (ug/kg):	# of Animals
0	0	4 male + 4 Female
1	0.06	4 male + 4 Female
2	0.39	4 male + 4 Female
3	2.5	4 male + 4 Female

Dose Selection: not described.

RESULTS

SUMMARY TABLE

(Table lists all statistically significant drug related findings. See attached review for details.)

EFFECT/DOSE	0	0.06 ug/kg/d	0.39 ug/kg/d	2.5 ug/kg/d
Number/sex:	4	4	4	4
MORTALITY (M/F):	0/0	0/0	0/0	0/0
CLINICAL SIGNS:		No treatment related effects.		
BODY WEIGHT:		No treatment related effects.		Reduced wt. gain in Females (not significant).
FOOD CONSUMPTION:		No treatment related effects.		
HEMATOLOGY:		No treatment related effects.		
BLOOD CHEMISTRY:		No treatment related effects.		
URINALYSIS:		No treatment related effects.		
ORGAN WEIGHTS:		No treatment related effects.		
GROSS PATHOLOGY:		No treatment related effects.		
HISTO-PATHOLOGY:		No treatment related effects.		

SUMMARY and CONCLUSIONS:

Drug:	NOAEL (ug/kg/day)	HED* (ug/kg/day)	Human dose multiple**	LOAEL (ug/kg/day)	HED* (ug/kg/day)	Human dose multiple**
TSA-870:	>2.5	>0.8	>11.5	>>2.5	>>0.8	>>11.5

* HED on a mg/m^2 basis.

** Assume human dose of 3x10 ug/week = 0.07 µg/kg/day

The doses selected for this study were too low to see expected vitamin D mediated toxicities. NOAEL and LOAEL could not really be determined.

APPEARS THIS WAY ON ORIGINAL

One year Oral Toxicity Study in Cynomolgus Monkeys ([redacted] 295-135)

Summary Review
(Complete review attached in Appendix)

PURPOSE:

To assess the toxicity of Hectorol in monkeys when administered orally for 1 year.

EXPERIMENTAL DESIGN:

Testing Facility: [redacted]
Study #: 295-135
Study Initiated: 8/91
Study Completed: 6/30/93
Dose & Formulation: 0, 0.06, 0.6, 6 & 20 ug/kg oral single dose, in 1 ml/kg of coconut oil.
Batch of drug: lot K015.
Food: [redacted] certified primate chow 5048.
Monkeys were allowed free access to food and tap water.
GLP statement: GLP and QA signed and dated 6/30/93.
Animals: 50 Cynomolgus Monkeys, [redacted]

Group:	Dose (ug/kg):	# of Animals:
0	0	5 male + 5 Female
1	0.06	5 male + 5 Female
2	0.6	5 male + 5 Female
3	6	5 male + 5 Female
3	20	5 male + 5 Female

Dose Selection: not described.

RESULTS

SUMMARY TABLE

(Table lists all statistically significant drug related findings. See attached review for details.)

DOSE (ug/kg/d):	0	0.06	0.6	6.0	20
Number/sex:	5	5	5	5	5
MORTALITY (M/F):	0/0	0/0	0/0	2/0	1/4
CLINICAL SIGNS:		No TRE	No TRE	Decreased activity defecation (F) Ataxia, emesis, thin, decreased defecation, hunched posture, & inappetence in animals that died. 6/7deaths probably caused by chronic nephritis.	
BODY WEIGHT:		No TRE	No TRE	Wt loss (F)	Variable weights.
FOOD CONSUMPTION:		No TRE	No TRE	No TRE	No TRE
HEMATOLOGY:		No TRE	No TRE	No TRE	No TRE
BLOOD CHEMISTRY:		No TRE	No TRE	⇑Ca	⇑Ca ⇑P (F)
URINALYSIS:		No TRE	No TRE	⇑Ca ⇑P	⇑Ca ⇑P
ORGAN WEIGHTS:		No TRE	No TRE	⇑kidney weight (F)	⇑kidney weight ⇑relative lung wt. (M)

GROSS PATHOLOGY:		No TRE	No TRE	No TRE	No TRE

HISTOPATHOLOGY:

EFFECT/DOSE (ug/kg/d)*	Incidence of treatment related pathological findings (M/F):				
	0	0.06	0.6	6.0	20
Hyperostosis -thickening of cortical and trabecular with marrow reduction		No TRE	No TRE	0/1 mild,	3/4 mild,
Mineralization of lung		No TRE	No TRE	2/5 mild	4/5 mild
Mineralization of uterus		No TRE	No TRE	No TRE.	-/1 moderate
Mineralization of ovaries		No TRE	No TRE	-/1 mild	-/2 mild, -/1 moderate
Mineralization of trachea		No TRE	No TRE	3/3 mild,	3/4 mild,
Mineralization of stomach submucosa		No TRE	No TRE.	2/2 mild, 2/2 moderate	4/1 mild, 1/3 moderate
Mineralization of aorta		No TRE	No TRE	No TRE.	1/1 moderate
Renal interstitial nephritis		No TRE	No TRE.	1/0 mild	1/0 mild
Mineralization of kidney		No TRE	No TRE	3/3 mild, 0/1 moderate	2/4 mild, 1/0 moderate
Mineralization of heart		No TRE	No TRE.	1/0 mild	0/1 mild, 1/2 moderate
Myocardial degeneration		No TRE	No TRE.	2/4 mild	2/3 mild, 1/1 moderate

SUMMARY and CONCLUSIONS:

The NOEL for oral Hectorol can be considered 0.6 ug/kg/day in a one-year monkey study. The toxicities seen were consistent with excess vitamin D. Females were at least as sensitive as males.

Drug:	NOAEL (ug/kg/day)	HED* (ug/kg/day)	Human dose multiple**	LOAEL (ug/kg/day)	HED* (ug/kg/day)	Human dose multiple**
[redacted]870:	0.6	0.2	2.9 x	6	2	29 x

* HED on a mg/m^2 basis.

** Assume human dose of 3x10 ug/week = 0.07 µg/kg/day

APPEARS THIS WAY ON ORIGINAL

Overall Summary and Conclusions of Toxicity Studies:

Acute Toxicity

Acute effects of 1α-OH-D_2 were remarkably consistent in rats and mice, including the fact that in both species the sensitivity to 1α-OH-D_2 was 10-fold lower P.O. than IP. Common dose dependent findings were; reduced motility, dyspnea, hyporeactivity, ataxia, abdominal position, diarrhea (in rats) reduced defecation (in mice) and body weight, congestion of the stomach mucosa (P.O. studies) tan foci on the hearts and tan or red foci on the kidneys of surviving animals. Renal discoloration, congestion of the lungs and staining around the mouth and anus and small spleens were noted in deceased animals. These toxicities reflect agonal changes and the pharmacological effect of the drug to cause hypercalcemia as well as effects secondary to hypercalcemia.

MICE Route:	Minimum lethal dose (ug/kg)	Human equivalent dose, HED* (ug/kg)	LD_{50} (ug/kg)	Human equivalent dose, HED* (ug/kg)	Human dose multiple, HDM**
P. O. males	320	26	449	37	222 x
P. O. females	630	52	495	41	246 x
I. P. males	30	2.5	35	3	19 x
I. P. females	60	5	30-60	2.5-5	16 – 30 x

RATS Route:	Minimum lethal dose (ug/kg)	Human equivalent dose, HED* (ug/kg)	LD_{50} (ug/kg)	Human equivalent dose, HED* (ug/kg)	Human dose multiple, HDM**
P. O. males	1250	208	1700	283	1700 x
P. O. females	2500	416	1800	300	1800 x
I. P. males	33	6	25	4	25 x
I. P. females	65	10	70	12	75 x

* HED in units of ug/kg by comparison of doses on a mg/m^2 basis

** HDM on basis of single human 10 ug dose (0.167 ug/kg/day)

Males appear to be slightly more sensitive to the drug in all cases. In this regard, male rats have been shown to metabolize vitamin D rather differently from female rats and some other mammals (see Pharmacokinetic studies below). Another consistent finding was that the minimum lethal dose and the LD-50 are very close together indicating a very steep toxicity curve. However, this LD-50 > 20-times the proposed human oral dose (when doses are compared on a mg/m^2 basis).

Repeated dose (<6-month) toxicity:

Four repeated dose toxicity studies were conducted with 1α-OH-D_2 in rats (4- and 13-weeks) and monkeys (2- and 13 weeks) however, the longer term (13-week) monkey study was conducted at too low a dose and the shorter term rat study was conducted only in males. These studies taken together show the time and dose dependence of the effects of 1α-OH-D_2. All of these effects reflect the pharmacological actions of Vitamin D and are consistent with results from other toxicity testing: Diminished weight gain, pale kidneys with white foci, hypercalcemia with calciuria and phosphaturia, calcification of myocardium, selected blood vessels, gastric mucosa and muscle and kidney with accompanying tubular degeneration. Dose-dependent, increased proliferation of cancellous bone was a common finding in rats and (when severe) may affect bone marrow function, resulting in hematological changes.

Study:	NOAEL (ug/kg/day)	HED* (ug/kg/day)	HED* (ug)	Human dose multiple (HDM)*,**	Human exposure multiple (HEM)***
4-Wk Rat	0.1	0.017	1 ug	0.24 x	2 x
13-Wk Rat	0.06	0.01	0.6 ug	0.14 x	1.2 x
2-Wk Monkey	6	2	120 ug	29 x	9.4 x
13-wk Monkey	>2.5	>0.83	>50	>11.5 x	>3.8 x

* HED and HDM on a mg/m^2 basis

** Assume human dose of 3x10 ug/week = 0.07 ug/kg/day

*** Exposure multiple for 1,25-OH_2-D2 metabolite, based on 26- or 52-week toxicokinetic study results

The most sensitive indicator of toxicity to oral 1α-OH-D_2 was hypercalcemia. No significant sex related differences in sensitivity in rats or monkeys were observed. Monkeys appeared to be approximately 100-fold less sensitive than rats to the pharmacological/toxicological actions of the drug, i.e., when doses were compared on the basis of mg/m^2 body surface area. The NOAEL in rats is only a fraction of the proposed human dose, while the NOAEL in monkeys is a considerable multiple of that dose (dose comparison on a mg/m^2 basis). The low rat NOAELs observed in these toxicity studies do not necessarily cause concern about the proposed human dose because the toxicities all appear to result from the pharmacological action of the drug to cause hypercalcemia and related tissue pathology. Nevertheless, the toxic effects suggest a significant risk of relatively short-term exposure to Vitamin D in excess of the levels required to normalize calcium and PTH levels in patients.

One-Year Oral Toxicity Studies

One-year oral toxicity studies were conducted in rats and monkeys. Dose dependent toxic effects of 1α-OH-D_2 reflected the physiologic actions of vitamin D: diminished weight gain, renal calcification, hypercalcemia with calciuria and phosphaturia and calcification of blood vessels in numerous organs with accompanying tissue damage. All of these effects reflect the pharmacological actions of Vitamin D and are consistent with results from other toxicity testing. One effect, which was seen clearly for the first time in the rat study, was the hyperostosis in bone. This effect was dose dependent and was seen to some extent in all bones. This effect was not clearly observed in shorter-term studies probably because it takes a long time to develop. In moderate to severe cases the hyperostosis lead to diminished medullary space. This may be the cause of the extramedullary hematopoiesis and the changes in hematology.

Study:	NOAEL (ug/kg/day)	HED* (ug/kg/day)	Human dose multiple (HDM)*,**	Human exposure multiple (HEM)***
1-year Rat	0.02	0.003	0.043 x	0.42 x
1-year Monkey	0.6	0.2	2.9 x	1.6 x

* HED and HDM on a mg/m^2 basis

** Assume human dose of 3x10 ug/week = 0.07 ug/kg/day

*** Exposure multiple for 1,25-OH_2-D2 metabolite, based on 26- or 52-week toxicokinetic study results

The NOAELs, when expressed as human dose multiples calculated by comparing doses on the basis of mg/m^2 body surface area, are fairly low, particularly in the rat. However, in the case of the rat, the human dose multiple is a 10-fold underestimate of the human exposure multiple. Parenthetically, the exposure multiples relate not to the parent compound (1a-OH-D2) but to its main active metabolite 1a,25-OH_2-D2 (see ADME section, p.62). In contrast to the rat, the human exposure multiple for the 1-year monkey study NOAEL is lower than (0.5x) the human dose multiple.

The low NOAELs, either in terms of HDM or HEM, observed in these toxicity studies do not necessarily cause concern about the proposed human dose because the toxicities all appear to result from the pharmacological action of the drug to cause hypercalcemia and related tissue pathology. Nevertheless, the toxic effects suggest a significant clinical risk of exposure to vitamin D in excess of the levels required to normalize calcium and PTH levels in patients. Patients with renal failure will be less sensitive to these effects and will be monitored to avoid hypercalcemia and secondary complications.

SPECIAL TOXICITY STUDIES

Summary reviews of special toxicity studies

I	A 28-Day IV Study in Rats (Summary, see attached complete review)
II	Effect on Serum Biochemistry in Rats
III	Biological Response to 1-OH-D2 in *Macaca fascicularis*
IV	Control Urinalysis Study in Cynomolgous Monkeys
V	Overall Summary and Conclusions of Special Toxicity Studies.

A 28-Day IV Study in Rats (Summary; see attached complete review)

METHODS

SD Crl:CD®BR VAF/Plus® Rats, 10 /sex/group were dosed 0, 0.025, 0.25 and 2.5 µg/kg/day for 28-29 days. Organs examined microscopically were eyes, heart, injection site, kidneys and gross lesions from all animals.

RESULTS

Drug related toxicities reported were primarily limited to the high dose group and was largely related to the physiological activities of the test agent. The primary toxicity findings included the following:

- Loss of body weight, primarily in high dose males (-27%). Weight loss in the mid dose males and high dose females ranged from 3-7%.
- Decreased food consumption in high dose males.
- Slight increase in RBC and Hb in high dose males.
- Increases in serum calcium and phosphorus in mid and high dose females; in males, increases in calcium were found in the low and high dose, but not at the mid dose.
- Increase in total urine volume in treated males, reaching statistical significance in high dose males. There was a slight decrease in specific gravity with the increase in volume.
- Increase in abundance of amorphous crystals in the urine of high dose groups.
- Two high dose females had dilated kidney pelvis. One of these also had a distended ureter.
- High dose males: minimal to mild mineralization of large vessels at the base of the heart and in the tunica media of the ciliary artery of 4/10 males and in the lumen of the collecting ducts in the renal medulla in 9/10 males. There were also more calculi observed in the renal pelvis of high dose males compared to controls.
- Increase in number and severity of corneal crystals in both sexes, particularly in the high dose groups (100% of high dose males, 40% of high dose females). noted in the opthalmic exam but not in the histopathology report. Corneal crystals are not uncommon in rats and rats seem to develop them in response to several analogs of vitamin D. It is unknown whether this effect is a primary effect or secondary to hypercalcemia.

CONCLUSIONS

Significant toxicity (weight loss, hypercalcemia, mineralization of heart and kidney, and dilated renal pelvis) were observed at the 2.5 ug/kg/d dose. The males appeared to be more sensitive to these effects. The NOAEL is 0.25 µg/kg/day for both male and female rats are administered a bolus intravenous injection of BCI-101 for a minimum of 28 consecutive days.

Additional Special Toxicity Studies:

Study#	Study Title:	Species	Route	Dose	Purpose:	NDA Volume
001-91	Effect on Serum Biochemistry in Rats	Rat	Oral gavage	0,6,20,100 ug/kg/d 6M/6F 14 days	The study was conducted at the request of the FDA to demonstrate frank hypercalcemia. The study also evaluated metabolites and endogenous calcitriol.	16
[redacted] 053	Biological Response to 1-OH-D2 in *Macaca fascicularis*	Macaca Monkey	Oral	2.5, 25 ug/kg/d 12 Fem. 8-9 days	Study conducted to determine the range and variability of urinary calcium values after oral administration of 1α-OH-D_2.	16
[redacted] 615-008	Control Urinalysis Study in Cynomolgous Monkeys	Cynomolgus Monkey	N/A	N/A 4 Males	Study conducted to determine the range and variability of urinary calcium values in control monkeys.	16

Findings:

Study#	Study Title:	Results:
[redacted] 001-91	Effect on Serum Biochemistry in Rats	**Mortality:** 1 HD male was sacrificed on day 11. **Clin signs:** MD females; flacid body tone. HD males & females; flaccid body tone, decreased activity, abdominal stance, diarrhea, and chromodacryorrhea (bloody discharge from eyes) **Body Weigts, Food Consumption:** HD M&F decreased. **Blood Chemistry:** Dose related significant increases in calcium (higher in M), HD M&F significantly decreased Phosphate. **Necropsy:** LD & HD enlarged adrenals. HD, white striations on heart, stomach, colon and pale kidneys. **Metabolism:** Dose dependent increases in 1α,25$(OH)_2D_2$ and 1α,24$(OH)_2D_2$ and decreased 1α ,25$(OH)_2D_3$ with increasing doses of 1α-OH-D_2 as reported in the comparative study of 1α-OH-D_2 and 1 α-OH-D_3 (see above, page 18).
[redacted]	Biological Response to 1-OH-D2 in *Macaca fascicularis*	The same monkeys were used for two cycles of dosing; 9 days at 2.5 ug/kg/d, 6 days off and 9 more days at 25 ug/kg/d. Urine chemistry and plasma metabolites were the primary results reported. Both cycles produced significant increases in calcium and phosphorous / creatinine in the urine. There were dose dependent increases in 1α,25$(OH)_2D_2$ (active metabolite) and decreased 1α,25$(OH)_2D_3$ (endogenous and dietary sources) with increasing doses of 1α-OH-D_2.
[redacted] 615-008	Control Urinalysis Study in Cynomolgous Monkeys	In untreated Cynomolgous Monkeys the baseline urine values for pH, creatinine, calcium and phosphorous were highly variable and suggest that accurate determination of effects on these parameters will be difficult to determine in this species.

Overall Summary and Conclusions of Special Toxicity Studies:

- Toxic effects in these studies were consistent with toxicities noted previously in standard toxicity studies.
- Metabolism of 1α-OH-D_2 was also consistent with other more thorough studies, but confirmed that results could be extrapolated to higher doses (100 ug/kg/d) in rats.
- The urinalysis study in Cynomolgous Monkeys confirmed the variability in measurements of urinary parameters.

APPEARS THIS WAY ON ORIGINAL

GENETIC TOXICOLOGY STUDIES

Bacterial Mutagenicity Assay of [redacted]-870. ([redacted]-14-44)

PURPOSE:

To examine the mutagenic potential of [redacted]-870 in the "Ames Assay"; to induce reverse mutations in, [redacted]1535, [redacted]1537, [redacted]100, [redacted]98, and WP2uvrA- with and without metabolic activation with phenobarbital and 5,6 benzoflavone induced rat liver microsomes (S9).

EXPERIMENTAL DESIGN:

Testing Facility:	[redacted]
Date of experiment:	Spring 1991
Study Report Written:	6/14/91
GLP statement, Q/A:	GLP and QA statements included and signed 6/14/91.
Protocol:	Ames[1,2] plate incorporation assay with 20 minute preincubation with 10% S9.
Criteria for positive result:	2-fold (for [redacted]-98, [redacted]-100 and E. coli) or 3-fold (for [redacted]-1535 and [redacted]-1537) increase over controls in a dose dependent fashion.
Bacterial Strains:	Salmonella; [redacted]1535, [redacted]1537, [redacted]100, [redacted]98, and E. coli.: WP2uvrA-
Activation System:	10 % S9 induced with phenobarbital and 5,6 benzoflavone.
Quality of S9:	The quality of the S9 was ascertained only by positive controls, not by measurement of P450-1A (7-ethoxyresorufin O-deethylase) P450-1B (benzphetamine N-demethylase) or P450-3A (Erythromycin N-demethylase).
Dose & Formulation:	31, 62, 125, 250, 500, 1000, and 2000 ug/plate. Lot K009 Dissolved in DMSO.
Dose Selection:	Top dose is beyond limit of solubility. No growth inhibition was observed.
Positive Control:	Nine appropriate positive and negative controls were tested: **(-)S9:** [redacted]-1537, 9-amino acridine (40 ug); TA-1535, sodium azide (0.5 ug) and N-ethyl-N-nitro-N-nitrosoguanidine (5 ug); [redacted]-98, TA-100 and WP2uvrA, 2-(2-furyl)-3-(5-nitro-2-furyl)-acrylamide (40 ug) and p-dimethylaminobenzenediazo-sulfonate (25 ug). **(+)S9:** All, 2-amino anthracene (1-4 ug); [redacted]98, [redacted]-100 & [redacted]1537, 3,4-benz(a)pyrene (5 ug); WP2uvrA, diethylnitrosamine (5 ug); [redacted]1535, cyclophosphamide (200 ug).

RESULTS:

There was some precipitation of the drug at the highest concentration tested +/- S9. The 1000 ug/plate samples without S9 also precipitated. [redacted]-870 did not induce any increase in the number of

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[1] Ames,B.N.; McCann,J.and Yamasaki,E. Methods for detecting carcinogens and mutagens with the Salmonella Mammalian Microsome Mutagenicity Test. Mutation Research 31(6):347-364, 1975.
[2] Maron, D.M. and Ames,B.N. Revised methods for the Salmonella mutagenicity test. Mutation Research 113(3-4):173-215. 1983.

revertant colony counts in the presence or absence of S9 at any concentration (vs. controls and vs. historical values). Negative and positive controls assured that the test was valid. The results constitute a negative result for the in vitro mutagenicity test in the bacterial/microsomal activation assay.

CONCLUSIONS:

The Ames assay does not indicate a mutagenic potential for [redacted]-870.

L5178Y^{-} Mouse Lymphoma Cell TK $^{+/-}$ Forward Gene Mutation Assay of BCI-101. ([redacted] 0313FB14.001)

PURPOSE:

To examine the mutagenic potential of [redacted]-870 at the TK locus by using the Mouse Lymphoma mutagenesis assay with and without activation with Aroclor induced S9.

EXPERIMENTAL DESIGN:

Testing Facility:	[redacted]
Date of experiment:	8/27/96
Study Report Written:	3/19/97
GLP statement, Q/A:	GLP and QA statements included.
Protocol:	L5178Y Mouse Lymphoma Cell TK $^{+/-}$ Forward Gene Mutation Assay[3,4,5]
Criteria for positive result:	2-fold increase over controls in a dose dependent fashion.
Activation System:	10 % S9; Aroclor 1254-induced male Sprague Dawley rat liver homogenate.
Dose & Formulation:	0, 0.7, 2, 7, 20, 33, 67, and 100 ug/ml activated. (13% survival at 100 ug/ml). 0, 0.7, 2, 7, 13, 20, 33, and 67 ug/ml unactivated. (13% survival at 33 ug/ml). Lot K038 Dissolved in DMSO.
Dose Selection:	After an initial cytotoxicity test (up to the limit of solubility), the maximum dose was set at 67 ug without S9 and 100 ug with activation Survival at these concentrations was 16% and 13% respectively.
Quality of S9:	The quality of the S9 was ascertained only by positive controls, not by measurement of P450-1A (7-ethoxyresorufin O-deethylase) P450-1B (benzphetamine N-demethylase) or P450-3A (Erythromycin N-demethylase).
Positive Control:	Appropriate positive and negative controls were tested: **(-)S9:** ethyl methanesulfonate (250 ug/ml) **(+)S9:** 3-methylcholanthrene (2.5 ug/ml)

RESULTS:

There was no significant increase in the frequency of mutant colonies in the absence of S9. There was a slight (3-fold) increase in the frequency of mutant colonies in the presence of S9 in the 33 ug/ml sample however, the colony size distribution in this group was similar to the distribution in the control samples, and there was no increase in the frequency of mutant colonies in the two higher concentrations. In a confirmatory assay there was no increase in the mutation frequency in any groups in the presence of S9. In this experiment there was a slight 2- to 3-fold increase in the absence of S9 in the 0.6, 6 and 13 ug/ml samples. These increases apparently are the result of random fluctuations of the spontaneous mutation frequency. Negative and positive controls assured that the test was valid. The results constitute a negative result for the Mouse Lymphoma Mutagenesis assay.

CONCLUSION:

The Mouse Lymphoma assay does not indicate a mutagenic potential for [redacted]-870.

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[3] D. Clive and J. F. Spector. Laboratory procedure for assessing specific locus mutations at the TK locus in cultured L5178Y mouse lymphoma cells. *Mutat.Res.* 31 (1):17-29, 1975.

[4] D. Clive, K. O. Johnson, J. F. Spector, A. G. Batson, and M. M. Brown. Validation and characterization of the L5178Y/TK+/- mouse lymphoma mutagen assay system. *Mutat.Res.* 59 (1):61-108, 1979.

[5] C. S. Aaron, G. Bolcsfoldi, H. R. Glatt, M. Moore, Y. Nishi, L. Stankowski, J. Theiss, and E. Thompson. Mammalian cell gene mutation assays working group report. *Mutat.Res.* 312 (3):235-239, 1994.

Chemical Induction of Chromosome Aberration in Cultured Human Lymphocytes using BCI-101. ([redacted] 0324FB14.001)

PURPOSE:

To assess the potential for [redacted]-870 to induce chromosome aberrations in cultured human peripheral blood lymphocytes, with and without exogenous activation by 2% Aroclor-induced rat liver post mitochondrial supernatant (S9 fraction).

EXPERIMENTAL DESIGN:

Testing Facility:	[redacted]
Date of experiment:	9/5/96
Study Report Written:	9/4/97
GLP statement, Q/A:	GLP and QA statements included.
Protocol:	Human Lymphocyte Chromosomal Aberration Test[6,7]. Lymphocytes were grown for 48 h in culture medium. Medium was changed to + or - activation medium containing TSA-870 for 5, 23 or 46 h. Samples exposed for 5 h were washed and allowed to grow for 18 or 41 more hours. All samples were then incubated for 2h with Colcemid to arrest cells in metaphase before harvesting.
Dose Selection:	After an initial cytotoxicity test at up to the limit of solubility: 0, 0.007, 0.02, 0.07, 0.2, 0.7, 2, 7, 20, 70, and 200 ug/ml (+/- S9), in which 200 ug/ml suppressed mitosis >90%, an in which lower concentrations were unaffected, the maximum dose was set at 100 ug.
Dose & Formulation:	Chromosomal aberration tests were at: 1(-S9), or 7, 33, 67, and 100 ug/ml (+/- S9) (duplicate cultures) Lot K038 Compound dissolved in DMSO.
Criteria for positive result:	Reproducible, significant increase over controls in a dose dependent fashion.
Activation System:	2% S9; Aroclor 1254-induced male Sprague Dawley rat liver homogenate.
Quality of S9:	The quality of the S9 was ascertained only by positive controls, not by measurement of P450-1A (7-ethoxyresorufin O-deethylase) P450-1B (benzphetamine N-demethylase) or P450-3A (Erythromycin N-demethylase).
Positive Control:	Appropriate positive and negative controls were tested: **(-)S9:** Mitomycin C (0.250 ug/ml) **(+)S9:** Cyclophosphamide (40 ug/ml)

RESULTS AND DISCUSSION:

In Assay 1, Schedule II, an increase as compared to vehicle controls in **% aberrant cells** (2.8x) and in # of aberrations per cell (14x) was observed at 100 ug/ml (see table below). The effect was

[6] H. J. Evans and M. L. O'Riordan. Human peripheral blood lymphocytes for the analysis of chromosome aberrations in mutagen tests. *Mutat.Res.* 31 (3):135-148, 1975.D.

[7] S. M. Galloway, M. J. Aardema, M. Jr Ishidate, J. L. Ivett, D. J. Kirkland, T. Morita, P. Mosesso, and T. Sofuni. Report from working group on in vitro tests for chromosomal aberrations. *Mutat.Res.* 312 (3):241-261, 1994.

statistically significant ($p < 0.05$ and $p < 0.01$), dose dependent ($p<0.05$, and $p< 0.01$) and was repeated in a second assay. A dose-dependent increase in the % aberrant cells and # aberrations/cell was also observed in cultures treated in Schedule I (+S-9). The individual responses at 67 or 100 ug/ml in Schedule I however were not significantly increased.

The aberrations were broken down into 9 types of aberrations. In both experiments the increase in aberrations is accounted for by increases in two subtypes of aberrations; chromatid and chromosome deletions. This meets the stated criteria for a positive signal.

The Sponsor argues that this result was within "historical" control values in one of the two assays and is therefore only a statistical aberration. Historical control values were, however, not provided. Values given were pooled untreated and vehicle control values obtained in the current study (I).

ASSAY 1

Schedule	+/- S9	Exposure (h)	Growth (h)	MI depression	Effect (% Aberrant cells)	Effect (Aberrations/cell)
I	+	5	18	MI increased (I)	67 ug/ml, slight NS increase (from 2% to 5.5%) 100 ug/ml, slight NS increase (from 2% to 6%)	67 ug/ml, slight NS increase (from 0.02 to 0.1) 100 ug/ml, NS increase (from 0.02 to 0.08)
				32%	CP 40 ug/ml (positive control), significant increase (from 2% to 37%)	CP 40 ug/ml (positive control), significant increase (from 0.02 to 0.78)
II	+	5	41	40% 79%	67 ug/ml, slight NS increase (from 1.5% to 2.5%) 100 ug/ml, significant increase (from 1.5% to 7.8%)	67 ug/ml, NS increase (from 0.015 to 0.065) 100 ug/ml, significant increase (from 0.015 to 0.22)
III	-	5	18	<45%	No significant effects, 100 ug was not tested.	
IV	-	23	-	<46%	No significant effects, 100 ug was not tested.	
V	-	46	-	<66%	No significant effects, 100 ug was not tested.	

NS = non-significant

The results obtained with Schedule II were confirmed in a repeat assay:

ASSAY 2

Schedule	+/- S9	Exposure (h)	Growth (h)		Effect (% Aberrant cells)	Effect (Aberrations/cell)
I	+	5	18	<84%	No significant or NS effects, no increases	No significant or NS effects
				30%	CP 40 ug/ml (positive control), significant increase (from 2.5% to 23%)	CP 40 ug/ml (positive control), significant increase (from 0.04 to 0.40)
II	+	5	41	20% 51%	67 ug/ml, slight NS increase (from 0.5% to 2.0%) 100 ug/ml, significant increase (from 0.5% to 5.0%)	67 ug/ml, NS increase (from 0.005 to 0.040) 100 ug/ml, NS increase (from 0.005 to 0.075)
III	-	5	18	<73%	No significant effects, 100 ug was not tested.	
IV	-	23	-	<82%	No significant effects, 100 ug was not tested.	
V	-	46	-	<92%	No significant effects, 100 ug was not tested.	

CONCLUSIONS:

The Human Lymphocyte assay indicates that [redacted]-870 can induce structural chromosomal aberrations in the presence of metabolic activation with S9. The effect is statistically significant, dose dependent and reproducible.

This Reviewer considers Hectorol to be positive in the human lymphocyte chromosomal aberration test under the conditions of the test protocol.

Mouse Micronucleus Assay of [redacted]-870 ([redacted]-14-37)

PURPOSE:

To assess the potential for [redacted]-870 to induce chromosomal damage *in vivo* as indicated by the appearance of micronuclei in polychromatic erythrocytes in mice exposed to the drug. This assay detects clastogens that break chromosomes and can detect aneuploidy.

EXPERIMENTAL DESIGN:

Testing Facility:	[redacted]
Date of experiment:	Spring 1991
Study Report Written:	4/22/91
GLP statement, Q/A:	GLP and QA statements included and signed 4/12 and 4/15/91.
Protocol:	Mouse Micronucleus assay[8]. 24 h after the last dose, mice were killed and slides were prepared of the marrow cells from their femurs and stained with Giemisa- and new methylene blue.
Criteria for positive result:	2-fold increase over controls in a dose dependent fashion.
Mouse Strains:	Adult male C3HxSWV F1 mice from [redacted] 5 Mice per group.
Dose & Formulation:	Single oral doses of 0.25, 0.5, and 1 mg/kg or 4 daily oral doses of 0.25 mg/kg. Lot EC1aOH927903, dissolved in coconut oil.
Dose Selection:	2 mg/kg was the minimum lethal dose seen in a prior dose ranging study. The highest dose in this study (1 mg/kg) is a reasonably high dose. All animals experienced significant weight loss.
Controls:	Appropriate positive and negative controls were tested: Positive, Mitomycin C (2 mg/kg, IP); Negative, Coconut Oil, (10 ml/kg).

RESULTS:

None of the groups treated with [redacted]-870 had significantly ($P < 0.05$, Student's t-test) more reticulocytes, indicating that there was no bone marrow suppression.

None of the groups treated with [redacted]-870 had significantly ($P < 0.05$, Student's t-test) more micronucleated polychromatic erythrocytes (PCEs) than the control group.

There was a highly significant increase in the number of micronucleated PCEs in the positive control samples.

CONCLUSIONS:

[redacted]-870 was not clastogenic *in vivo* in the mouse micronucleus assay under the conditions described.

APPEARS THIS WAY ON ORIGINAL

[redacted]

Overall Summary and Conclusions of Genetic Toxicology Studies:

- The Ames and Mouse Lymphoma assays do not indicate a mutagenic potential for [redacted]870.
- The Mouse Micronucleus assay does not indicate a clastogenic potential for [redacted]870.
- The Human Lymphocyte assay indicates that [redacted]870 can induce chromosomal damage in the presence of metabolic activation with S9. The effect observed was statistically significant in samples treated with 100 ug/ml BCI-101. The sponsor claims that the result is merely a statistical aberration and is within the range of historical control values. This Reviewer feels that the results is positive and needs to be mentioned in the label.

REPRODUCTIVE TOXICITY STUDIES

I	Fertility and Reproductive Performance in Rats. ([redacted]-14-94)
II	Developmental Toxicity in Rats. ([redacted] 3422.1)
III	Developmental Toxicity in Rabbits. ([redacted] 3422.3)
IV	A perinatal and Postnatal Study in Rats.
V	Overall Summary and Conclusions of Reproductive Toxicity Studies.

Fertility and General Reproductive Performance in Rats (Formerly Segment I): Study No. [redacted]-14-94

Purpose:

To evaluate the effects of [redacted]-870 on fertility, mating behavior, gonadal function and early embryonic development in rats dosed prior to mating and through early gestation. Males, females and embryos were examined after Day 13 of Gestation.

Experimental design:

Testing Facility: [redacted]

Study #: [redacted]-14-94
Study Initiated: 5/18/91
Study Completed: 9/25/92
Dose & Formulation: 0, 0.06, 0.39, 2.5 ug/kg/day, orally by gavage in coconut oil. Males: 9 weeks prior to mating and until the pups were weaned (6 more weeks). Females: 2 weeks prior to mating, while mating, and until weaning (gestation day 19).
Batch of drug: lot EC1aOH927903
GLP statement: Included and signed 9/18/92.
Animals: 54 Jcl:Wistar Rats from [redacted] Age: male, 4 weeks, female 9 weeks were quarantined and acclimated for 2 weeks before initiating the experiment. Males weighed 136-154 g, females weighed 191-213 g.

Group:	Dose (ug/kg):	# of Animals:
1	0	27 Males and 27 Females, 15 necropsied on gest. day 20, 12 allowed to deliver.
2	0.06	27 Males and 27 Females, 15 necropsied on gest. day 20, 12 allowed to deliver.
3	0.039	27 Males and 27 Females, 15 necropsied on gest. day 20, 12 allowed to deliver.
4	2.5	27 Males and 27 Females, 15 necropsied on gest. day 20, 12 allowed to deliver.

All males were killed after about 15 weeks. Females were killed on gestation day 20, or after weaning (day 22) and mothers and fetuses examined. 5 male rats (5) from each group were used for a 14 day recovery arm. Pharmacokinetic measurements were not made.

Dose Selection:

The doses are based on the 13-week rat toxicity study in which the low dose of 0.06 ug/kg/day was the NOAEL and the high dose yielded kidney and soft tissue mineralization and diminished weight gain after one month. The high dose (2.5 ug/kg/day) is 35 times (on a mg/kg basis) the expected human dose (30 ug/week~0.07 ug/kg/day). On a mg/m^2 basis, the high dose is 5.9 times the recommended initial human dose.

Results and discussion:

F0 Observed effects:

There was no significant drug related effect except corneal opacities in males after 10 weeks.

F0 Mortality:

No deaths reported. No animals were sacrificed prematurely.

F0 Body Weight:

The high dose M & F did not gain as much as controls and the HD males lost weight after 14 weeks.

F0 Food Consumption:

HD dams had decreased consumption during gestation. In all other groups there was no effect on food consumption.

F0 Blood Chemistry:

Mid and high dose animals had statistically significantly elevated serum calcium and phosphorous. These are expected pharmacological actions of the drug.

F0 Estrous Cycles:

There were no effects on estrogen levels or estrus.

F0/F1 Reproductive Evaluation (mating performance):

No drug related changes were noted in any reproductive indices: Copulation, Fertility, Fecundity and Copulatory Interval.

F0/F1 Uterine observations (Necropsy day 20):

Number of corpora lutea, implantation sites, viable fetuses, dead fetuses, resorptions and pre and post implantation losses were unaffected by exposure to drug.

Examination of Fetuses and Placentae:

No treatment related abnormalities. 2/14 HD females had pre-implantation losses that accounted for the decreased live fetuses in this group.

Postnatal observation:

No adverse effects on pup mortality. Development of pups, eye opening, incisor eruption tended to be slightly accelerated in all treated groups. Grip strength and geotaxis were normal.

F1 Necropsy:

No treatment related differences.

Summary and Conclusions:

The range of doses tested covered previously determined toxic dose levels in the rat (2.5 ug/kg/day). Expected changes in serum calcium and phosphorous and weight were observed as was an increase in corneal opacities which was determined to be due to calcification. No toxic or adverse effects on reproduction or fertility were observed in this study at dose up to 2.5 ug/kg/day (6x expected human dose, ug/m2 comparison).

Developmental Toxicity of BCI-101 in Rats (Formerly Segment II). [redacted] Study #3422.1

Purpose:

To evaluate the effects of BCI-101 exposure during Gestation Days 6-17 on fetal development in rats. Dams and fetuses were examined on Day 20 of Gestation.

Experimental design:

Testing Facility: [redacted]
Study #: 3422.1
Study Initiated: 8/27/96.
Study Completed: 4/18/97.
Dose & Formulation: 0, 1, 10, 100, 0, 20 ug/kg/day, oral, in coconut oil. Gestation Days 6-17.
Batch of drug: lot K-038
GLP statement: **Included.**
Animals: 125 + 80 additional Crl:CD-BR VAF plus female rats from [redacted] and mated with male rats of the same strain from a colony maintained at [redacted] Animals were housed individually and fed [redacted] certified rodent chow 5002.

Group:		Dose (ug/kg):		# of Animals:	
	1		0		25 Females
	2		1		25 Females
	3		10		25 Females
	4		100		25 Females
	5		0		25 Females
	6		20		25 Females

All dams were killed on day 20 and mothers and fetuses examined. Pharmacokinetic measurements were not made.

Dose Selection:

0, 1, 10 and 100 ug/kg/d was initially selected in order to cover a wide range with previous experience only from acute studies. When all the females in the 100 ug/kg/d group died another segment was conducted with 0 and 20 ug/kg/d.

Results

Observed effects:

There was no significant drug related effect below 20 ug/kg/d. 20 and 100 ug groups showed emaciation, soft or no feces and stained fur. Agonal changes such as hunched posture were noted in the 100 ug group prior to death.

Mortality:

24/25 rats in the 100 ug/kg/d group died or were killed early.

Body Weight:

Weight gain was reduced in the 10, 20 and 100 ug/kg/d groups and body weights were reduced by 6%, 5-11%, and 14-30% respectively.

Food Consumption:

There was a significant reduction in food consumption in the 20 and 100 ug/kg/d groups during the interval of drug exposure.

Blood Chemistry:

All drug exposed groups had slightly elevated serum calcium but this was not statistically significant, serum phosphorous levels were significantly elevated in the 10 ug group. The effect on calcium is an expected pharmacological action of the drug and not a toxic effect, however it could affect the development of the fetus. Samples were only collected at one time point, probably at necropsy (3 days after the last dose) which would explain the minimal effect on blood chemistry.

Gross Pathology (maternal):

No drug related changes were noted except in the HD group, where mottled heart, lungs, stomach and kidneys were noted. This is consistent with previously noted effects of hypercalcemia at this dose. At the 10-20 ug/kg/d level hypercalcemia would be expected but it would take 2-weeks to a month to demonstrate these effects.

Litter observations:

No treatment related findings in the 1, 10 or 20 ug/kg/d groups. Number of corpora lutea, viable fetuses, early and late resorptions, pre- and post-implantation losses, fetal sex ratios and fetal weights were comparable to controls. In the one surviving HD rat a high number of early resorptions and 35% fetal weight reduction may be due to the maternal toxicity in this dam.

Fetal Alterations:

There were no increased incidence in any external, visceral or skeletal abnormalaties in the fetuses. Individual incidence of several additional malformations were reported. One 10 ug/kg/day fetus had anal atrisia, one 20 ug/kg/d fetus and 2 control fetuses had micropthalmia. The distribution and incidence did not suggest a relationship to treatment. There was also no effect on the total incidence of all malformations.

Summary and conclusions:

When 1α-OH-D_2 was administered to gravid female rats on Gestation days 6 through 17 there were no adverse effects on fetal growth or survival at up to 20 ug/kg/d. At the maximum dose tested (100 ug/kg/day) toxic maternal effects (decreased weight gain and calcification of heart, kidneys, lungs and stomach) were noted. The high dose fetuses appeared subject to a possible increased rate of resorption but there was inadequate maternal survival (1/25) to determine any consistent effect.

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Developmental Toxicity of BCI-101 in Rabbits (Formerly Segment II). [redacted] Study #3422.3

Purpose:

To evaluate the effects of BCI-101 exposure during Gestation Days 6-18 on fetal development in rabbits. Dams and fetuses were examined on Day 29 of Gestation.

Experimental design:

Testing Facility: [redacted]
Study #: 3422.3
Study Initiated: 11/8/96.
Study Completed: 4/18/97.
Dose & Formulation: 0, 0.03, 0.1, & 0.3 ug/kg/day, oral gavage in coconut oil. Gestation Days 6-18.
Batch of drug: lot K038
GLP statement: **Included.**
Animals: 88 mated New Zealand White does from [redacted]

Group:		Dose (ug/kg):		# of Animals:	
	1		0		20 Females
	2		0.03		20 Females
	3		0.1		20 Females
	4		0.3		20 Females

All does were killed on day 29 and mothers and fetuses examined. Pharmacokinetic measurements were not reported.

Dose Selection:
A dose range finding study was conducted [redacted] 3422.5) (not reviewed) in pregnant rabbits exposed to 0, 0.003, 0.01, 0.03, 0.1, and 0.3 ug/kg/day on Gestation Days 6-18

Results:

Observed effects:
There was some dose related evidence of constipation (a consequence of hypercalcemia) in all treated dose groups, namely few, small or no feces.

Mortality:
No deaths reported. No animals were sacrificed prematurely.

Body Weight and Food Consumption:
There was dose related decreased body weight, body weight gain and food consumption in the MD and HD dams.

Blood Chemistry (maternal):
HD rabbits had increases in serum calcium determined at necropsy. These effects are an expected pharmacological action of the drug and not a toxic effect, however it could affect the development of the fetus. It is surprising that there is any effect on serum chemistry 11 days after the last dose however, this result is consistent with the high dose rats in the rat segment 2 study.

Gross Pathology (maternal):

The only potential drug related changes noted were multiple kidney foci in 2, 12 and 14 dams at necropsy. These lesions are consistent with mineralization often encountered with this class of compounds.

Litter observations:

No significant treatment related findings in any groups. Number of corpora lutea, viable fetuses, early and late resorptions, pre- and post-implantation losses, and fetal sex ratios were comparable to controls. Mean fetal weights were reduced ~9% in the MD and HD groups and there was a slight increase in the number of late resorptions in the HD group but these differences were not statistically significant.

Fetal Alterations:

There were no significant increases in observed alterations however, in the HD group there was a slight increase in the number of litters with unossified sternebrae and costal cartilage variations.

Summary and conclusions:

When BCI-101 was administered to gravid female rabbits on Gestation days 6 through 18 there were no adverse effects on fetal or maternal health at the LD of 0.03 ug/kg/d. At the MD and HD there was a slightly increased rate of resorption (not significant). This is consistent with the rat Segment II study, where there was an increase in resorptions in the one HD dam that survived. Despite evidence of maternal toxicity (weight loss and renal foci) at the MD and HD, there was no statistically significant evidence of fetal toxicity, although there was some incidence of delayed ossification in the HD litters.

A Perinatal and Postnatal Study in Rats with BCI-101, (Segment 3). [redacted] Study #3422.4

Purpose:

To evaluate the effects of BCI-101 exposure from Gestation Day 6 - Lactation Day 20 on maternal, fetal and neonatal development as well as the reproductive capacity of the F1 generation in rats. Dams and pups were sacrificed on Day 21 of Lactation except 20 F1 pups/group that were allowed to continue as parental animals . These F1 pups were mated and subsequently examined with the F2 pups on Day 21 of their subsequent Lactation.

Experimental design:

Testing Facility: [redacted]
Study #: 3422.4
Study Initiated: 8/28/96.
Study Completed: 10/8/97.
Dose & Formulation: 0, 0.25, 2.5, 15 & 25 ug/kg/day, oral gavage in coconut oil, Gestation Day 6 - Lactation Day 20.
Batch of drug: K038
GLP statement: **Included.**
Animals: 125 of 160 Sprague Dawley Crl:CD-BR VAF/Plus Females cohabited individually in nesting boxes with 1 of 125 males (5 Mo. old) [redacted] Rodent Chow #5002.

Group:	1	Dose (ug/kg):	0	# of Animals:	25 Females
	2		0.25		25 Females
	3		2.5		25 Females
	4		15		25 Females
Discontinued	5	Discontinued	25	Discontinued	25 Females

All females were allowed to deliver in individual nesting cages.
Nursing and nesting behavior and retrieval were monitored.
On Lactation Day 4 the litters were culled to a maximum size of 8 (4 of each sex when possible).
Pups development was carefully examined.
Dams were killed on Lactation Day 21 and examined.
1 male and 1 female from each litter were selected for breeding.
Nonselected pups were necropsied.
At 12 weeks of age F1 pairs were mated (avoiding siblings).
Weight and behavior was closely monitored.
F1 females allowed to litter, F1 males necropsied, and F2 pups culled on lactation day 4.
F2 pups examined on lactation day 21.
All F1 animals were necropsied on lactation Day 21.

Pharmacokinetic measurements were not made and there were no satellite groups.

Dose Selection:

No rationale was provided. However, a wide range of doses was selected and during the course of the experiment the Segment 3 study showed that the HD group might show excessive toxicity so this group was terminated early. The remaining high dose (15 ug/kg/d, 90 ug/m^2) is 35 times the recommended initial human dose (30 ug/week, 0.07 ug/kg/day), on a mg/m^2 basis.

Results

F0 Generation

Observed effects:

There was no observed significant drug related toxicity. All findings were randomly distributed among all groups.

Mortality:

No deaths reported. One 0.25 and one 2.5 ug/kg/d females were killed on day 25 and found to be nongravid despite evidence of mating.

Body Weight and Food Consumption:

Food consumption and subsequently body weights were significantly decreased in the HD 15 ug/kg/d females during dosing (8-10%). Although food consumption increased mean body weights remained significantly lower in the HD females during lactation

Blood Chemistry:

Only calcium and phosphorous were monitored. Both were increased in a dose dependent manner with calcium in the HD dams reaching statistically significantly increased level.

	0	0.25 ug/kg/d	2.5 ug/kg/d	15 ug/kg/d
Phosphorous mg/dl	8.04	8.8	9.19	9.21
Calcium mg/dl	11.73	12.45	12.75	13.71 * p<0.001

In this study measurements were made at necropsy the day after the last dose was given. These effects are an expected pharmacological action of the drug and not a toxic effect, however it could affect the development of the fetus.

Gestation, Parturition and Lactation:

No effect on gestation lengths, delivery duration, nesting behavior or lactation.

Litter Retrieval:

Slightly lower in controls. This is not considered meaningful.

Gross Pathology (maternal):

There were no remarkable findings in any group. A similar number of implantation scars were noted in each group.

F0 Summary:

Maternal toxicity was observed in the HD group in the form of hypercalcemia and hyperphosphatemia and decreased weight, weight gain and food consumption. No effects were noted at lower doses.

F1 Generation:

Litter Data:

There were no treatment related differences in pup viability, live/dead pups, litters, litter size or male/female ratios.

Pup Necropsy Observations:

No treatment related findings were reported.

Pup Development and Functional Tests:

Pinnae detachment, surface righting response, cliff aversion, eye and vaginal opening, startle and auditory responses were all comparable among all groups.

Pup Behavioral Tests:

No differences in; open field parameters, swimming trials and T-maze tests.

Survival and Body Weight Changes:
Growth and survival of all groups was similar.

Copulation, Fertility and Precoital Intervals:
Copulation and fertility indices were comparable among groups, precoital interval, and pregnancy rates.

Biochemistry:
No differences noted in serum calcium or phosphorous.

Necropsy (F1 adult):
There were no remarkable observations and no differences between groups.

F2 Generation:

Litter Data:
There were no treatment related differences in pup viability, live/dead pups, litters, litter size or male/female ratios.

Pup clinical and Necropsy Observations:
No treatment related findings were reported.

Survival and Body Weight Changes:
Body weight and weight change and survival of all groups was similar.

Summary and conclusions:

Although maternal toxicity was observed in the HD group (15 ug/kg/day) in the form of hypercalcemia and decreased body weight, body weight gain and food consumption, there were no developmental or reproductive effects at any dose.

Overall Summary and Conclusions of Reproductive Toxicity Studies:

Segment 1:

The Segment I study revealed no significant toxicity in rats reproduction or fertility when given up to 2.5 ug/kg/day. Expected changes in serum calcium and phosphorous and weight were observed as was an increase in corneal opacities which was determined to be due to calcification. No toxic or adverse effect on reproduction or fertility was observed in this study.

Segment 2:

When 1α-OH-D_2 was administered to gravid female rats on Gestation days 6 through 17 there were no adverse effects on fetal growth or survival at up to 20 ug/kg/d. At the maximum dose tested (100 ug/kg/day) toxic maternal effects (decreased weight gain and calcification of heart, kidneys, lungs and stomach) were noted. The high dose fetuses appeared subject to a possible increased rate of resorption but there was inadequate maternal survival (1/25) to determine any consistent effect.

When BCI-101 was administered to gravid female rabbits on Gestation days 6 through 18 there were no adverse effects on fetal or maternal health at the LD of 0.03 ug/kg/d. At the MD (0.1 ug/kg/d) and HD (0.3 ug/kg/d) there was slightly increased rate of resorption (not significant). This is consistent with the rat Segment II study, where there was an increase in resorptions in the one HD dam that survived. Despite evidence of maternal toxicity (weight loss and renal foci) at the MD and HD, there was no statistically significant evidence of fetal toxicity although there were some incidence of delayed ossification in the HD litters.

Segment 3:

Although maternal toxicity was observed in the HD group (15 ug/kg/d) in the form of hypercalcemia and decreased weight, weight gain and food consumption, no maternal effects were noted at lower doses and there were no developmental or reproductive effects at any dose

In conclusion, the lowest threshold of maternal and fetal effects was seen in the rabbit Segment II study which showed maternal effects at the 0.1 and 0.3 ug/kg MD and slightly increased resorptions with delayed fetal ossification at the 0.3 ug/kg/day HD.

ADME STUDIES

I	ADME of Radiolabeled 1-OH-D2 in Rats
II	Single and multiple dose PK Studies in Rats
III	ADME of Radiolabeled 1-OH-D2 in Cynomolgus Monkeys
IV	Single and multiple Dose PK Studies in Cynomolgus Monkeys
V	Overall Summary and Conclusions of ADME Studies.
VI	Comparison Animal-Human PK

BACKGROUND

The test compound is a synthetic vitamin D2 analog (1a-OH-VitD2). It is converted to 1a-OH, 25-OH-D2 in the liver, and does not need 1-OH-lation in the kidney. Normally, Vit D from skin (D3) or food (D2) is absorbed into the circulation, and hydroxylated by microsomal enzymes (P450c25), mainly in liver, to 25-OH-VitD. The 25-OH-D2 is then hydroxylated by 1-a-hydroxylase in mitochondria of renal tubules to the main active metabolite, 1a,25(OH)2-VitD. The liver conversion is not tightly regulated, but the kidney conversion is (rate-limiting). The renal 1-a-hydroxylase enzyme is stimulated by PTH, and by (PTH-induced) hypophospatemia. Vitamin D metabolites are bound to a specific vitamin D binding serum globulin.

In humans the plasma level of 25OH-VitD3 is ca. 30 ng/ml. By contrast, the level of 1,25OH-VitD3 can be as low as 30 pg/ml (1/1000 x the 25-OH form). 1,25(OH)2-vitD (D2 or D3) stimulates intestinal Ca uptake by inducing synthesis of a calcium binding protein in the intestinal cell, and is ca. 100 times more potent than 25OH-VitD in doing this. 1,25(OH)2-VitD also reduces Ca excretion by the kidney, and preserves bone by its effect on the osteoblast.

Other kidney metabolites of 25OH-VitD are 25,26-(OH)2-VitD, 1,24,25-OH3-VitD, 1,25,26-(OH)VitD, and 25OH-26,23-lactone. 1a-Vit D2, but not 1a-Vit D3, can be activated in the liver by hydroxylation at the 24-site, resulting in 1a,24-(OH)2VitD2. Hydroxylation of vitamin D is also the initial step in metabolic deactivation. Excretion of VitD metabolites occurs in the bile, and there may be enterohepatic circulation of OH-metabolites.

Vit D acts as a classic steroid hormone. Receptors are found in intestine, bone, parathyroid glands, pancreas, pituitary, placenta etc.

There are 2 kinds of vitamin D assays: competitive protein-binding assay (using radiolabeled ligand), and radioimmunoassay: both involve competition in vitro between a radioligand and the vitamin D sterol of interest, for available sites on a binding protein. Vitamin D and metabolites can also be separated and assayed chromatographically.

ADME Studies Reported in this NDA

Reviewers Study Nr.	NDA Volume	Species	Title	Study Id	Dose (ug/kg)	Dose (route)	Measured	Samples
R1	1.24, p.062	Rat	Pharmacokinetics of TSA-870 in Rats and Monkeys	[redacted]-14-107	2.5	single (iv), single (po) 7 days (po)	Radiolabeled compound, 1aOHD2, 1a,25OH2D2	Blood, urine, feces, tissues, bile
R2	1.24, p.118	Rat	Pharmacokinetics of BC-101 in Rats	[redacted]849-BCI-001-91	0, 0.39	single (po)	1a,25OH2D2 1a,24(OH)2D2	Plasma
R3	1.24, p.163	Rat	Pharmacokinetics of BC-101 in Rats	[redacted]849-BCI-002-91	0, 0.39, 2.5	7 days	1a,25OH2D2 1a,24(OH)2D2 1a,25OH2D3 (calcitriol)	Plasma
R4	1.16, p.016	Rat	Effect of BC-101 on Serum Biochemistry in Rats	[redacted]464-BCI-001-91	0, 6, 20, 100	14 days (po)	1a,25OH2D2, 1a,24(OH)2D2 1a,25OH2D3 (calcitriol)	Plasma
R5	1.24, p.211	Rat	Analysis of 1a,25-dihydroxyvitamin D2 in serum samples from IRDC Study No. 295-136 (One-year oral toxicity study in rats)	Cont Assays Corp BCI Report No. T-105	0, 0.02, 0.06, 0.55, 5	52 weeks (po)	1a,25OH2D2	Serum
R6	1.6, p.112	Rat	Four Week Oral Comparative Toxicity Study of 1-OH-D2 and 1-OH-D3 in rats	[redacted]-14-90	0.1, 0.5, 2.5, 12.5	4 weeks	1a,25OH2D2 1a,25OH2D3 (calcitriol)	Plasma
R7	1.25, p.007	Rat	Pharmacokinetics of BCI-2-125, BCI-3-125, and LR-103 following Single Oral Dose Administration to Rats	[redacted]849-BCI-001-92	0, 0.15, 0.39	single (po)	1a,25(OH)2D2 1a,25(OH)2D3 1a,24(OH)2D2	Plasma
M1	1.24, p.062	Monkey	Pharmacokinetics of TSA-870 in Rats and Monkeys	[redacted]-14-107	2.5	single (iv), single (po)	Radiolabeled compound, 1aOHD2, 1a,25OH2D2	Blood, urine, feces
M2	1.24, p.243	Monkey	Single-Dose Pharmacokinetic Study in Cynomolgus Monkeys	[redacted] Report 053	0.39	single (iv), 8 days (po)	1a,25OH2D2	Serum
M3	1.24, p.292	Monkey	Analysis of 1a,25-dihydroxvitamin D2 in serum samples from IRDC No.295-135 (One Year Oral Toxicity Study In Cynomolgus Monkeys)	Cont Assays Corp BCI Report No. T-106	0.06, 0.6, 6, 20	52 weeks (po)	1a,25OH2D2	Serum

[redacted]-870 = 1a-OH-D2
BCI-101= 1a-OH-D2
BCI-2-125 = 1a,25(OH)2D2
BCI-3-125 = 1a,25(OH)2D3
[redacted]-103 = 1a,24(OH)2D2

RAT STUDIES

(A) Pharmacokinetic data from radiolabeled single and 7-day dose studies in rats

R1. Pharmacokinetics of [redacted]-870 in Rats and Monkeys ([redacted]-14-107)

Methods

Rats: Male Jcl:SD rats (260-330g)
Dose: ^{3}H-1a-OH-D2 (single oral or i.v., or 7 days oral) (2.5 µg/kg)
Plasma collected : 12 time points between 1-168h post oral dose
11 time points between 0.1-48h post iv dose
Excreta collected: For 168h post single dose, for 120h after last multiple dose