

CENTER FOR DRUG EVALUATION AND RESEARCH

APPLICATION NUMBER: NDA 21055

PHARMACOLOGY REVIEW(S)

Division of Oncology Drug Products, HFD-150
REVIEW AND EVALUATION OF PHARMACOLOGY AND TOXICOLOGY DATA
Review No. 1

Keywords: Targretin, bexarotene, RXR agonist, NDA

NDA #: 21-055

Serial #: 000 **Type:** NDA **Letter Dated:** 6/22/99 **Received by CDR:** 6/23/99

Information to be conveyed to the sponsor: Yes

Reviewer: Chang H. Ahn, Ph.D.

Review Completion Date: December 10, 1999

Sponsor: Ligand Pharmaceuticals Inc. San Diego, CA

Manufacturer: []

Drug:

Code Name: LG100069, LGD1069

Generic Name: bexarotene

Trade Name: Targretin® capsules

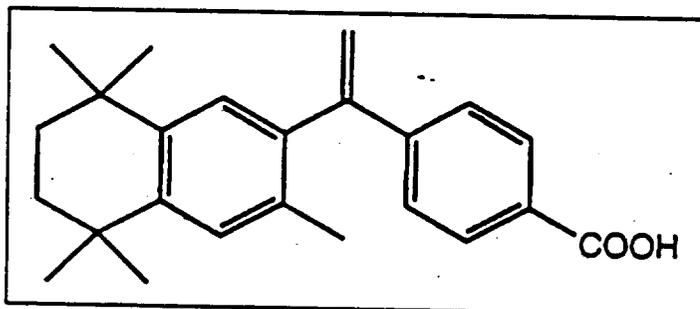
Secondary therapies: none

Chemical Name: 4-[1-(3,5,5,8,8-pentamethyl-5,6,7,8-tetrahydro-2-naphthalenyl)vinyl]benzene
carboxylic acid

CAS Registry Number: 153559-49-0

Molecular formula/weight: C₂₄H₂₈O₂ (348.48)

Structure:



Related INDs, NDAs, DMFs: IND []

Drug Class: Antineoplastic agent (retinoid analogue with RXR selectivity)

Indication: The treatment of patients with cutaneous manifestations of refractory or persistent early and refractory advanced stage CTCL.

Clinical Formulation:

<u>Ingredient</u>	<u>Amount (mg/capsule)</u>
Bexarotene	75.00
PEG 400, NF	}
Polysorbate 20, NF	
Povidone (K-90), USP	
Butylated hydroxyanisole, NF	
Gelatin, NF	
Sorbitol Special-glycerin blend	}
Titanium oxide, USP	

trace }

Dosage and administration: 300 mg/m²/day taken as a single oral daily dose with a meal for up to 97 weeks for CTCL patients.

Studies Reviewed within this submission:**Toxicology**

1. 28-Day oral toxicity study in rats (vol. 1.16, pp 2-409, RR-815-94-013)
2. 91-Day oral toxicity study in dogs (vol. 1.30, pp 105, to vol.1.32, RR-815-95-003,
3. 6-Month oral toxicity study in rats (vol. 1. 21, pp 2-, to vol. 1.27, RR-815-98-004)
4. 6-Month oral toxicity study in dogs (vol. 1.33,pp 2- to vol. 1.36, RR-815-98-003a)

Pharmacology**Mechanism of Action**

1. Transcriptional activation and receptor binding profile (vol. 1.10, pp276-341, RR-750-93-013/-004/011)
2. Repression of AP-1 activity (vol. 1.10, pp 390-397, RR-750-93-011)
3. Regulation of Fas-ligand expression and activation-induced T-cell apoptosis (vol. 1.11, pp 2-21, RR-750-98-002)

In vitro efficacy studies

1. Growth inhibitory effects on carcinoma cells of breast, head/neck and on primary tumor samples (vol. 1.10, pp398, to 1.11, pp38-80, RR-750-98-009/014, 93-013)
2. Induction of cell cycle arrest and differentiation in NB4 APL cells (vol. 1.11, pp 22-37, RR-750-98-003)

In vivo/Ex vivo studies

1. Effects on growth of human head and neck carcinoma xenografts in nude mice (vol. 1.11, pp 95-108, 132-145, 255-271, RR-740-94-007a, 93-014a, 750-98-005)
2. Effects on mammary carcinoma in the NMU-induced rat mammary tumor model (vol. 1.11, pp 160-223, RR-750-98-001/004/010)
3. Antikeratinizing activities in the rhino mouse (vol. 1.11, pp 146-159, RR-740-93-002)

Safety Pharmacology

1. Summary table of safety pharmacology (vol. 1.12, pp 1-261, RR-740-93-002/006/007/009/013/016/017)

Pharmacokinetics

1. Relative bioavailability of micronized LGD 1069 in sesame oil and in 10% PEG/Aqueous formulation in male rats (vol. 1.43, pp 207- , RR-845-96-003a)
2. Single oral pharmacokinetics study in rats (vol. 1.44, pp 2- , RR-815-98-013)
3. Single and repeat dose oral pharmacokinetics in rats (vol. 1.45, pp2-33, RR-845-98-007)
4. Three-way crossover oral bioequivalence study of two clinical formulations in dogs (vol. 1.46, pp83-163, RR-845-94-011)
5. Five-way crossover oral study in female beagle dogs (vol. 1.46, pp 205-315, RR-845-98-014)
6. Tissue distribution of [¹⁴C]LGD 1069 in rats (vol. 1.47, pp 188- , RR-845-97-002b)
7. Effects on hepatic microsomal cytochrome P450 and in vitro metabolism (vol. 1.48, pp 2-20, RR-845-98-009)
8. Metabolic fate in rats (vol. 1.48, pp 42-79, RR-845-98-001)
9. Binding to human plasma protein (vol. 1.57, pp 135-136, RR-845-99-002)

Reproductive Toxicology

1. Oral developmental toxicity study in rats (vol.1.38, pp 134- , RR-815-97-003b)
2. Oral dose-range development toxicity study in rats (vol. 1.39, pp 2-260, RR-915-97-004a)

Genetic Toxicology

1. Salmonella/E. coli mutation assays (vol. 1.39, pp262- /1.40, pp 2-65, RR-815-95-005/RR-815-95-004)

2. CHO chromosome aberration assay (vol. 1.40, pp 66-141, RR-815-97-008)
3. L5178Y mouse lymphoma cell tk+/tk- gene mutation assay (vol 1.40, pp 142- , RR-815-97-009)
4. In vivo bone marrow micronucleus assay in mice (vol. 1.41, pp 2-163, RR-815-97-010)

Phototoxicity

1. In vitro phototoxicity studies (summary; vol 1.41, pp165-243, RR-815-98-006)

Studies Not Reviewed within this submission:**Toxicology**

1. 5-Week repeated dose toxicity study in female rats (vol. 1.17, pp 2-97, RR-815-93-019a)
2. Effects in male rats via feed for 37 days (vol. 1.17, pp 98-125, RR-815-93-018a)
3. 6-Month oral toxicity study in rats: 3-Month interim report (vol. 1.18-1.20, RR-815-97-016)
4. 30-Day repeated dose oral range-finding study in dogs (vol. 1.30, pp 2-104, RR-815-93-017)
5. 28-Day dermal toxicity study in rats (vol. 1.37, pp 2- , RR-94-008)
6. 28-Day dermal toxicity study in rats (0.1% and 1% gel containing DEET) (vol. 1.38, pp 2-36, RR-815-98-005a)
7. Dermal sensitization study in guinea pigs (Buehler's technique modified) (vol. 1.38, pp 37-108, RR-815-94-009)
8. Guinea pig primary skin irritation for LGD 1069 and Retin-A 0.1% cream (vol. 1.38, pp 109-132, RR-815-94-010)

Pharmacology

1. Development of leiomyoma in the Eker rat uterine fibroid model (vol. 1.11, pp 224-234, RR-750-98-007)

Pharmacokinetics

1. Relative bioavailability study in dogs (vol. 1.46, pp 166-204, RR-845-98-013)
2. Pharmacokinetics of topical LGD1069 in male and female rats (vol. 1.48, pp 167-207, RR-845-94-008a)

Studies Previously Reviewed: The following studies were reviewed under IND []**Pharmacology**

1. Transcriptional activation (vol. 1.4, RR-750-93-004)
2. Retinoic acid receptor binding profile (vol 1.4, RR-750-93-013)
3. Effects on human leukemia cells, Kaposi's sarcoma, cervical carcinoma cells, head and neck squamous carcinoma and breast carcinoma cells (vol. 1.4, RR-750-93-008/-012; vol. 1.5, RR-750-93-007/-013)
4. Effects on human head and neck squamous cell carcinoma xenografts in nude mice (vol. 1.5, RR-740-93-014)
5. Effects on human cervical carcinoma xenografts in nude mice (vol. 1.5, RR-740-93-015)
6. Effects on cardiovascular and central nervous systems (vol. 1.5, RR-740-93-006/-007)

Pharmacokinetics

1. Toxicokinetics in rats (vol. 1.7, RR-845-93-021)
2. Toxicokinetics in dogs (vol. 1.6, RR-845-93-023)
3. Bioavailability in rats (vol. 1.6, RR-845-93-018)
4. Bioavailability and metabolism in dogs (vol. 1.6/1.7, RR-845-93-022/-024)
5. Tissue distribution and metabolism in mice (vol. 1.7, RR-845-93-017)
6. Metabolism in rats (vol. 1.7, RR-945-93-020)
7. Plasma protein binding (vol. 1.7, RR-845-93-025)

Toxicology

1. Acute oral toxicity study in rats (vol. 1.13, RR-815-93-020/-021)
2. Acute oral toxicity study in dogs (vol. 1.13, RR-815-93-015)

3. 28-Day repeat dose oral toxicity study in rats (vol. 1.14, RR-815-93-022)
4. 28-Day repeat dose oral toxicity study in rats (interim report, vol. 1.16, RR-815-94-013)
5. 28-Day repeat dose oral toxicity study in dogs (vol. 1.12, RR-815-93-016)

Note: Portions of this review were excerpted directly from the sponsor's submission.

INTRODUCTION AND DRUG HISTORY

Targretin is a synthetic retinoid analogue that is claimed to selectively activate retinoid X receptors. Targretin is the first among RXR-specific retinoids for which an NDA has been submitted. RAR-specific retinoids (e.g., all-trans retinoic acid, 13-cis-retinoic acid) and pan-RAR/RXR agonist (e.g., 9-cis-retinoic acid) are either NDA-approved or in clinical trials for oncologic indications. Targretin has demonstrated its antitumor activity against squamous cell carcinoma xenografts in nude mice and carcinogen-induced mammary tumors in rats. It has also shown to possess antikeratinizing activity in rhino mice. The sponsor initiated clinical trials and conducted two open-label, multicenter, multinational, historically-controlled, phase II-III studies in patients with CTCL. Based on the results of these phase II-III studies, the sponsor seeks an NDA approval for patients with refractory or persistent early and refractory advanced stage CTCL.

PHARMACOLOGY

Mechanism of Action

1. Transcriptional activation and receptor binding profile (vol. 1.10, pp276-341, RR-750-93-013/-004/011)

method- Transactivation- The recombinant DNA constructs were transiently transfected into sub-confluent (about 70%) CV-1 cells by calcium-phosphate coprecipitation. Following transfection, all subsequent steps were performed on a Biomek Automated Workstation (Beckman). Medium containing the DNA was removed from transfected cells after six h, cells were washed and LG1069 (10^{-12} – 10^{-5} M) was added. After 38 h the cells were washed and then lysed with 0.5% Triton-X 100 and assayed for luciferase and β -galactosidase activities using a luminometer and ELISA plate reader. The EC₅₀ was determined graphically. Receptor expression plasmids used in the cotransfection assay included pRS-hRAR α , pRS-hRAR β , pRS-hRAR γ , pRS-hRXR α , pRS-hRXR β , and pRS-hRXR γ .

Receptor binding- Sf21 cells (1.2×10^6 cells/ml) transfected with vectors containing receptor DNA encoding hRAR α , hRAR β , hRAR γ , mRXR α , mRXR β , or mRXR γ were grown in suspension culture, harvested and washed by centrifugation (1000 x g, 10 min, 4°C). Cell pellets were suspended in lysis buffer (10 mM Tris, pH7.5, 5 mM DTT, 2 mM EDTA, 1 mM PMSF, 1 ug/ml aprotinin, 1 ug/ml leupeptin) and homogenized. Lysates were obtained by centrifugation at 100,000 x g for 1 h at 4°C. The final volume for binding assays was 250 ul (10-40 ug extract protein, 5 nM [³H]ATRA or 10 nM [³H]9-cis-RA plus varying concentrations of competing LG1069 ($0 - 10^{-5}$ M) in 96-well minitube system. Incubations were carried out at 4°C until equilibrium was achieved. At the end of the incubation period, 50 ul of 6.25% hydroxyapatite was added in the wash buffer (100 mM KCl, 10 mM Tris HCl, either 5 mM CHAPS (for RXRs) or 0.5% Triton X-100 (for RARs)) which binds the receptor-ligand complex. The mixture was vortexed and incubated for 30 min at room temperature, centrifuged and the supernatant removed. The hydroxyapatite pellet was washed and the amount of receptor-ligand complex determined by liquid scintillation counting. After correcting for non-specific binding, IC₅₀ values (concentrations of competing ligand required to decrease specific binding by 50%) were determined graphically and K_d values were determined by application of the Cheng-Prusoff equation.

GLP statement- No

Results- LG1069 is a RXR-specific ligand with K_d values of 14-30 nM for RXRs (vs. 4804- >10,000 nM for RARs). Transactivation assays also confirmed RXR-mediated activity of LG1069 with EC₅₀ values of 19-27 nM for RXR (vs. >10,000 nM RARs).

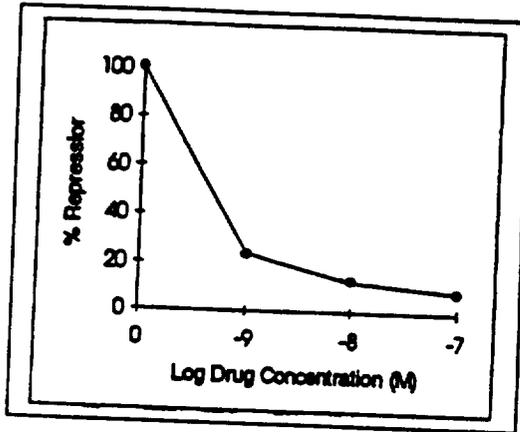
Receptor	EC ₅₀ (nM)	K _d (nM)
RAR α	>10,000	6298
RAR β	>10,000	>10,000
RAR γ	>10,000	4804
RXR α	25	30
RXR β	27	14
RXR γ	19	15

2. Repression of AP-1 activity (vol. 1.10, pp 390-397, RR-750-93-011)

method- Plasmid DNA constructs (a plasmid expressing RXR α (under the control of a constitutive promoter) and a plasmid which expresses the reporter enzyme luciferase under the control of a conditional promoter (collagenase) containing an AP-1 responsive element) were transiently transfected into HeLa cells by calcium-phosphate coprecipitation. After removing medium containing the DNA from transfected cells after 16 h, cells were washed and LG1069 (10⁻⁹ to 10⁻⁷M) was added. After another 16 h, AP-1 activity was induced by PMA, a phorbol ester, and cells were lysed 4-6 h later and luciferase activity was measured.

GLP statement- No

Results- LG1069 down-regulated transcriptional regulation by AP-1 in a dose-dependent manner in cells transfected with RXR α . The results suggest that LG1069 binding to the RXR α receptor can interfere with AP-1 activated gene expression.



3. Regulation of Fas-ligand expression and activation-induced T-cell apoptosis (vol. 1.11, pp 2-21, RR-750-98-002)

method- Apoptosis assay- (a) A T-cell hybridoma line 2B4, which rapidly expresses the Fas receptor and Fas-ligand (FasL)- their interaction transduces a death signal, was stimulated to undergo activation-induced apoptosis by culturing the cells in the presence of anti-T cell receptor monoclonal antibody in the presence or absence of 1 μ M LG1069. Apoptosis was determined by flow cytometry of cells exposed to propidium iodide as a vital dye. (b) The expression of functional FasL on 2B4 cells was assayed using Fas-expression L1210.fas target cells, which undergo apoptosis when exposed to FasL. 2B4 cells (1 x 10⁵/well-2.5 x 10⁴/well to give the ratio of effector:target cells were 4:1, 2:1 and 1:1) were activated by culturing the cells on anti-CD3 antibody-coated plates in the presence or absence of 1 μ M LG1069. After 4 h, 2.5x10⁴ [³H]TdR-labeled L1210.fas target cells were added per well. Controls for non-specific apoptosis included L1210 wild-type cells (Fas negative) and target cells cultured in the absence of 2B4 effector cells. The data is expressed as the percent DNA fragmentation per culture relative to controls.

GLP statement- No

Results- LG1069 (1 μ M) inhibited activation-induced apoptosis in 2B4 T-cell hybridoma cells. At 1 μ M, this apoptotic activity could be produced through interaction with RARs as well as RXR, suggesting its pan-retinoid activity rather than RXR-specific activity.

a. Inhibition of activation-induced apoptosis in 2B4 cells

Treatment	Apoptotic Cells (% total cells)	
	Un-activated 2B4 cells	Activated 2B4 cells
Vehicle	3.77 (0.58)	69.62 (8.40)
LG1069 1 μ M	3.6 (2.94)	50.2 (2.94)

b. Inhibition of functional FasL expression on anti-CD3 activated 2B4 cells detected as lysis of L1210.fas target cells

Treatment Effector:Target Ratio	L1210 wild-type and L1210.fas Target Cell Apoptosis (% Control)				
	Unactivated Control 2B4 Cells		Activated 2B4 Cells		
	L1210 Wild-type	L1210.fas	L1210 Wild-type	L1210.fas	
Vehicle	4:1	-0.99	0	11.05	56.05
	2:1	1.31	0.61	5.25	46.69
	1:1	1.19	0.42	9.6	35.1
	0:1	0	1.54	0	4.36
LG1069	4:1	1.03	08.25	8.06	36.82
	2:1	1.13	0	3.15	16.51
	1:1	0	0	0	8.04
	0:1	0.74	2.77	2.57	0.53

In vitro efficacy studies

1. Growth inhibitory effects on Kaposi's sarcoma derived cells, and carcinoma cells of breast, head/neck and on primary tumor samples (vol. 1.10, pp343, to 1.11, pp38-80, RR-750-98-009/014, 93-007/013)

method- Subconfluent monolayer cells (500 - 4 x 10⁴ cells/well) were cultured in 96-well plates and incubated for 24 h in the presence of various concentrations of LG1069. The effects of LG1069 on the growth of these cells were monitored in thymidine incorporation and/or cell number. For thymidine incorporation, 1 uCi[³H]Thymidine (43 Ci/mmol specific activity) per well was added for 18 h. The cells were released with trypsin/EDTA and precipitated with 10% trichloroacetic acid onto glass fiber filter mass using a multi-well cell harvester. Radioactivity incorporated into DNA as a direct measurement of cell growth, was measured by liquid scintillation counting. For cell counting, the viable cell number was determined with trypan blue and a hemocytometer or a Coulter cell counter after 1-3 days culture.

GLP statement- No

Results-

Cell Line	Study No.	Growth Inhibition (IC ₅₀ or % Inhibition at various LG1069 concentration)		
		IC ₅₀ (uM)	LG1069 (uM)	% Growth Inhibition
Breast - Primary tumor samples T47D (ER+) SK-BR-3 (ER-) MCF-7	RR-750-98-009	0.1	0.1	No inhibition
	RR-750-98-014		1	
	RR-750-93-013		1	
Cervix MF-180 Primary tumor samples	RR-750-93-012 RR-750-98-009	0.5	0.1	No inhibition
Colon Primary tumor samples	RR-750-98-009		0.1	No inhibition
Head and Neck 1483 SCC25 SqCC/Y1	RR-750-93-013		1	30%
			1	10%
			1	25%
Kaposi's sarcoma-derived cells	RR-750-93-007		1	40%
			10	75%
Leukemia Primary AML cells	RR-750-98-009		0.1	50%(3/5) to no inhibition (2/5)
Melanoma Primary tumor samples	RR-750-98-009		0.1	No inhibition
Ovary Primary tumor samples	RR-750-98-009		0.1	10% (3/4) -50% (1/4)

2. Induction of cell cycle arrest and differentiation in NB4 APL cells (vol. 1.11, pp 22-37, RR-750-98-003)

method- Cell cycle arrest- APL NB-4 cells ($1-2 \times 10^6$ cells) were treated in the presence or absence of LG1069 ($10^{-10} - 10^{-5}M$) for 4 days and then fixed by adding 3 ml 100% ethanol for 30 min and the fixed cells were washed in PBS, resuspended in 1 ml PBS containing 5 ul Rnase A/T1 cocktail and incubated 30 min on ice. 1 ml of the propidium iodide solution (25 ug/ml) was added, the cells incubated overnight at 4°C and then analyzed for DNA contents by flow cytometry.

Differentiation- Expression of the CD11b was used as a measure of differentiation. NB4 cells were treated with 1 uM LG1069 for 4 days and then were stained with a 10 ul phycoerythrin-conjugated mouse anti-human CD11b mAb for 45 min on ice. Cells were fixed in 1 ml paraformaldehyde (1% in PBS) and analyzed for CD11b by flow cytometry.

GLP statement- No

Results- Cell cycle G1 arrest- EC_{50} of LG1069 was approximately 1.5 uM (vs. RAR-specific TTNPB 650 pM and pan-agonist LG1057 30 nM). There was a modest increase in the number of cells (18.7%) expressing CD11b at 1 uM LG1069 compared to a vehicle control (vs. pan-agonist LG1057 10 nM with 33.1% CD11b positive cells and vehicle control with 1.1% CD11b positive cells). These results suggest that LG1069 is a weak inducer of G1 arrest and differentiation of NB4 cells and that the LG1069 may induce these effects through RAR-specific mechanism.

In vivo/Ex vivo studies

1. Effects on growth of human head and neck carcinoma xenografts in nude mice (vol. 1.11, pp 95-108, 132-145, 255-271, RR-740-94-007a, 93-014a, 750-98-005)

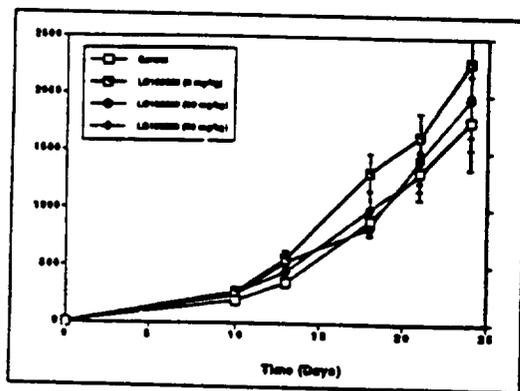
method- 1483 cell line- Xenografts were established by sc injection of 1×10^6 1483 cells at two sites dorsally on nude mice. LG1069 (3, 30 and 60 mg/kg in sesame oil; lot # LG10069-000Z004) was administered orally by gavage 48h later on a regimen of 5 days dosing/week. Xenograft growth was assessed by measurement of tumor volume.

HN9N and HN21P cell lines- nude mice were implanted sc with 1 mm^3 tumor pieces. Two days after transplantation, animals were treated with 60 mg/kg LG1069 (in sesame oil), po, daily. Tumor volumes were measured 2/week using Vernier calipers.

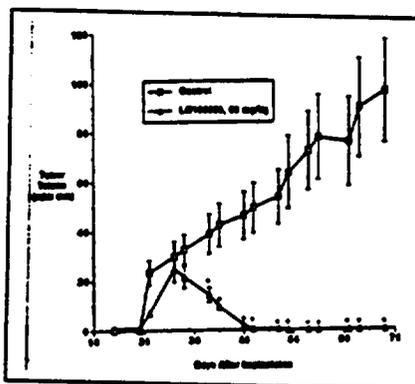
GLP statement- No

Results- LG1069 did not alter proliferation of rapidly-growing 1483 tumors, whereas tumors treated with LG1069 regressed to a non-measurable size by day 49 for HN9N and day 37 for HN21P, respectively. The regression was maintained up to 68 days after implantation.

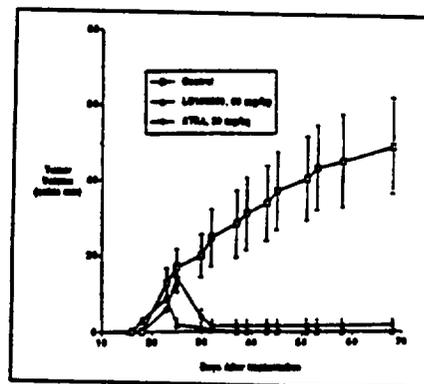
1484 Tumors



HN9N Tumors



HN21P Tumors



3. Effects on mammary carcinoma in the NMU-induced rat mammary tumor model (vol. 1.11, pp 160-223, RR-750-98-001/004010)

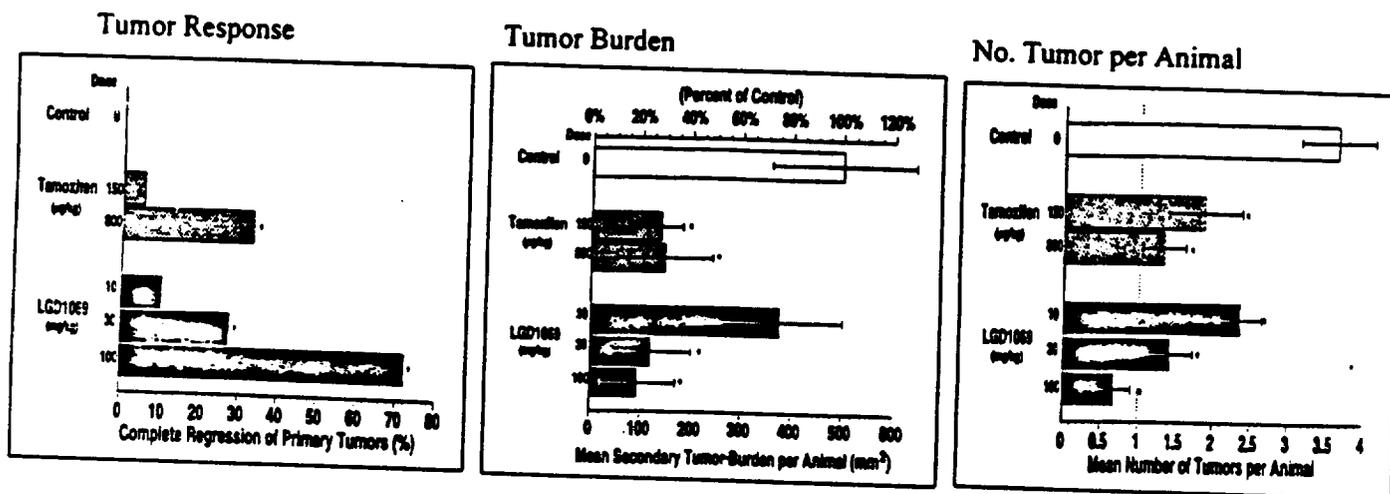
method- Female SD rats (6-13/group, 55 days old) received a single iv injection (via tail vein) of 50 mg/kg N-nitroso-N-methylurea (NMU) to induce mammary tumors. After tumors were established (75 mm^2), the tumor bearing rats were treated with daily oral doses of 0, 10, 30 or 100 mg/kg/day LG1069 (in 10% v/v PEG/Tween 80 and 90% of 1% w/v carboxymethylcellulose) by gavage (5 ml/kg) or 150 ug/kg or 800 ug/kg/day tamoxifen (s.c) for 6 weeks. At the end of the treatment period tumor response was measured.

GLP statement- No

Results- Tumor response- complete regression, partial regression and progression of primary tumors were 72.2%, 16.7% and 11.1% in 100 mg/kg/day LG1069 group and 33.3%, 33.3% and 33.3% in 800 ug/kg/day tamoxifen group, respectively. Complete regression of tumors in LG1069 groups was dose-dependent with 10.5%, 27.8% and 72.2% at 10, 30 and 100 mg/kg/day, respectively.

Tumor burden- Animals of LG1069-treated groups showed a dose-dependent decrease of up to 81% in tumor burden with 376, 121 and 95 mm² at 10, 30 and 100 mg/kg/day, respectively (vs. 498 mm² control animals).

Number of tumors per animal- Animals of LG1069-treated groups showed a dose-dependent decrease in the mean numbers of tumor per animal (0.69 in 100 mg/kg/day LG1069 vs 3.63 in control animals). Tamoxifen decreased the number to 1.33 at 800 ug/kg/day. These results suggest potential chemopreventive effects of LG1069.



4. Antikeratinizing activities in the rhino mouse (vol. 1.11, pp 146-159, RR-740-93-002)

method- Hairless female rhino mice (18 g, 6-8 weeks old) were topically applied by daily dose of 0, 0.05, 0.1 or 0.5% LG1069 (in ethanol:propylene glycol =70:30 v/v, 0.1 ml) (or 0.01, 0.05 and 0.1% ATRA) or received daily oral doses of 0, 10, 30 or 100 mg/kg/day LG1069 (in super refined sesame oil) by gavage (5 ml/kg) for 3 weeks. Mice were sacrificed 72h after the last dose and dorsal trunk skin was removed and placed into 0.5% acetic acid for 24-36h at 4°C. An area of skin (2x 5 cm) was partitioned and the epidermis was carefully peeled off. The epidermis was placed on a glass slide with the dermal side facing up, cleared in alcohol/xylene, and coverslipped with Permount for microscopic evaluation. Each mount the diameters of 10 utriculi in 5 random fields were measured with an Optomax Image Analysis System and mean utriculi diameter was calculated for each treatment group.

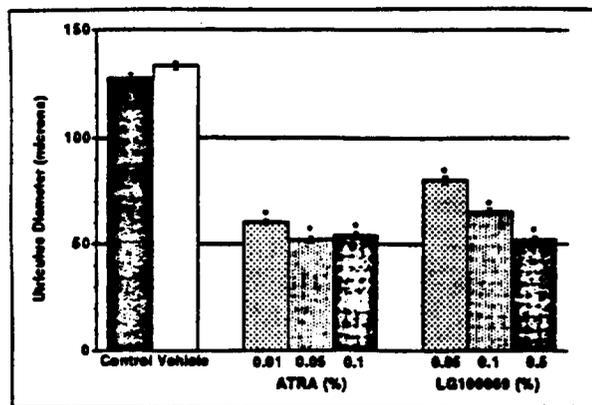
GLP statement-

Results- Topical administration- LG1069 produced dose-related decreases in utriculus diameter from 40% at 0.05% LG1069 to 61% at 0.5% LG1069 (vs. 0.01-0.1% ATRA were almost equally efficacious with approximately 60% decrease).

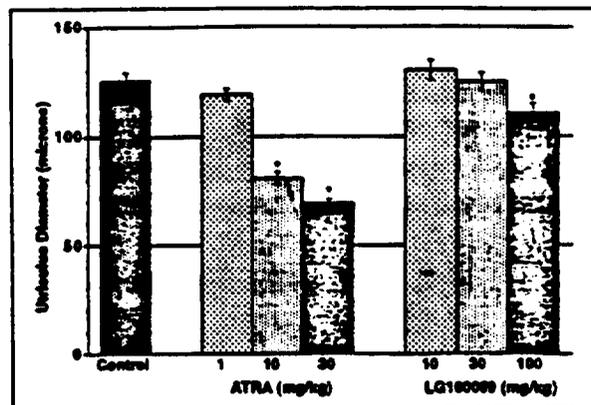
Oral administration- 100 mg/kg/day LG1069 produced only 13% decrease in utriculus diameter and 10 and 30 mg/kg doses were inactive. ATRA produced dose-dependent decreases with 6, 36 and 44% at 1, 10 and 30 mg/kg/day.

These results suggest that LG1069 may be an effective antikeratinizing agent when administered topically, but not an active agent following oral administration.

Topical Administration



Oral Administration



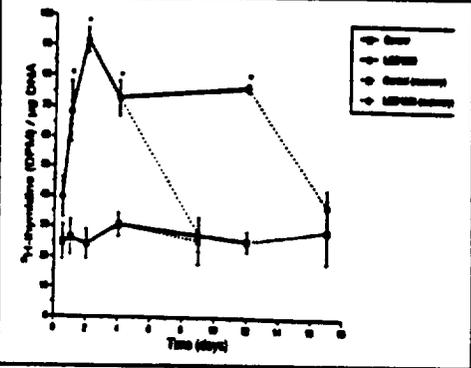
Overall Pharmacology Summary:

Study	Results/Effects
Cytotoxic Mechanisms	Receptor interaction- Kd of 14-30 nM for RXR and of >4800 nM for RAR Transactivation- EC ₅₀ of 19-27 nM for RXR and of >10,000 nM for RAR Apoptosis in 50% of activated 2B4 T-cell hybridoma cells by 1 uM LG1069 AP-1 inhibition- IC ₅₀ of approximately 0.3 nM in RXRα-transfected HeLa cells G1 cell cycle arrest- EC ₅₀ of 1.5 uM in NB4 APL cells
Growth Inhibition in vitro	LG1069 1 uM- inhibited growth of cancer cells from breast (SKBr-3 50%, MCF-7 80%), head and neck (10-30%) and Kaposi's sarcoma (40%). LG1069 0.1 uM- inhibited AML cells (up to 50%), breast cancer T47D (50%) and ovarian cancer cells (10-50%), but did not inhibit primary tumor-derived cells from breast, cervix, colon and melanoma.
In vivo Tumor Models	Head and Neck cancer xenograft in nude mice- LG1069 (60 mg/kg/day, po) induced complete regression of HN9N and HN21P-xenografted tumors by day 49 and day 37, respectively, but had no effect on 1483-xenografted tumors. The complete regressions were maintained up to 68 days post-implantation of tumor cells. NMU-induced rat mammary tumor- There observed dose-dependent effects of LG1069 on complete response, tumor burden and number tumors per animal. LG1069 dose at 100 mg/kg/day resulted in complete regression in 72% of primary tumors, 81% decrease in tumor burden, and significant decrease in number tumors/animal (0.69 vs 3.63 in control)
Epidermal Effect	Antikeratinizing effect in rhino mice was observed by LG1069 when administered topically (0.05 – 0.5%), but not when administered orally at dose up to 100 mg/kg/day.

SAFETY PHARMACOLOGY

1. Summary table of safety pharmacology (vol. 1.12, pp 1-261, RR-740-93-002/006/007/009/013/016/017)

Study	Method	Results/Effects	Study No.
Cardiovascular Assay	Normotensive male SD rats (10/group, b.w. 200-300 g) received daily oral doses of 0, 10, 30 and 60 mg/kg/day LG 1069 (lot # LG100069-000Z004; in sesame oil) by gavage (10 ml/kg) for 4 days. Mean arterial blood pressure and heart rate (plus clinical pathology) were measured directly from a chronic indwelling cannula in the abdominal aorta.	1. No effects on mean arterial blood pressure and heart rate. 2. 23% and 20% Increases in aPTT in 30 (p<0.01) and 60 mg/kg (p<0.01) groups, respectively. 3. No necropsy performed.	RR-740-93-006

Blood Coagulation	Male SD rats (8/group, b.w. 250 g, 8-9 wks of age) received daily oral doses of 0, 10 or 100 mg/kg/day LG1069 (lot # LG100069AD15) in sesame oil suspension admixed with powdered feed for 29 days. When PT and aPTT increased 2-fold, animals received 10 mg/kg vitamin K ₁ by ip (d17/18) or sc (d19/20).	<ol style="list-style-type: none"> 6/8 animals in 100 mg/kg group died due to bleeding disorders, while none died in 100 mg/kg LG1069 + vitamin K group. aPTT increased 3-fold in 10 mg/kg group and 4-fold in 100 mg/kg group over control at week 3. PT also increased 2-fold in 100 mg/kg group. Vitamin K restored PTs and aTPTTs to control levels after 4 days of treatment. 	RR-740-94-002
Hepatocyte Proliferation	Female SD rats received daily oral doses of 100 mg/kg/day LG1069 (lot # LG100069-000Z007, bw 180-200, 2 months of age) by gavage for 1, 2, 4, or 12 days. Two h prior to sacrifice, rats were injected with 50 uCi [³ H]thymidine by ip. At necropsy, livers were excised, homogenized and analyzed for [³ H]thymidine incorporation and DNA contents.	<ol style="list-style-type: none"> absolute liver weight increased significantly following 4 or 12 days dosing (8.4 g vs. 5.1 g control). Increased rate of [³H]thymidine incorporation into liver occurred during LG1069 administration and was reversible over a 5-day recovery period.  <ol style="list-style-type: none"> liver concentrations of protein, DNA and glycogen from LG1069-treated groups were not significantly different from control group. 	RR-740-94-009
Plasma Lipids	Male rabbits (5/group, bw 1.25-1.55 kg) received oral daily doses of 0, 1, 10 or 100 mg/kg/day LG1069 (in sesame oil; lot # LG100069-000Z004) by gavage (5 ml/kg) for 4 days. Blood for evaluation of lipids was collected at termination.	<ol style="list-style-type: none"> increases in plasma cholesterol (3-fold) and triglycerides (10-fold) in 100 mg/kg group. 1 and 10 mg/kg/day LG1069 dose levels were unremarkable. LDL and HDL were not affected. Dose-related increases in liver weight (control 40.1g, 1 mg/kg group 48.0g, 10 mg/kg group 50.2g, 100 mg/kg group 58.7g). Approximately 10% body weight loss by day 5 in 100 mg/kg group compared to controls. 	RR-740-93-016/017
Neuro-pharmacology	Male SD rats (10/group, bw 150-250g) received daily oral doses of 0, 10, 30 or 100 mg/kg/day LG1069 (in sesame oil; lot# LG100069-000Z004) by gavage (10 ml/kg) for 4 days. Ataxia, convulsions, alertness and spontaneous motor activity were measured.	<ol style="list-style-type: none"> No treatment-related effects on ataxia, convulsions, alertness or spontaneous motor activity. No change in body temperature observed. 	RR-740-93-007

Overall Safety Pharmacology Summary: Increases in aPTT (3-fold with 10 mg/kg/day, 4-fold with 100 mg/kg/day) in rats, increases in plasma cholesterol (3-fold) and triglycerides (10-fold) with 100 mg/kg/day LG1069 in rabbits and increases in absolute liver weights and rate of thymidine incorporation into liver in rats were observed. Neuropharmacological effects by LG1069 with doses up to 100 mg/kg/day was unremarkable.

PHARMACOKINETICS AND TOXICOKINETICS

1. Relative bioavailability of micronized LGD 1069 in sesame oil and in 10% PEG/Aqueous formulation in male rats (vol. 1.43, pp 207- , RR-845-96-003a)

method- male SD rats (4/group, bw 237-276g) received a single oral dose of 35 mg/kg LG1069 (lot# LG100069-000Z011) in sesame oil (micronized) or in PEG400/water vehicle (micronized or microparticulated LG1069 in 10% PEG400 (containing 0.5% Tween 80)/1% carboxymethylcellulose in water) by gavage (5 ml/kg). Blood samples (0.4 ml) were collected to determine pharmacokinetic parameters.

GLP statement- No

Results- Systemic exposure to LG1069 was highest with microparticulate form in PEG400/water vehicle, followed by sesame oil vehicle and micronized form in PEG400/water vehicle, suggesting a trend that reduced particle size enhances absorption (and bioavailability).

Parameter	Sesame Oil	PEG400/Water, micronized	PEG/Water, microparticulate
Cmax, uM	6.37 ± 1.10	5.16 ± 2.55	7.12 ± 0.25
Tmax, h	3	2	2.5
AUC ₀₋₆ , uM.h	25.4 ± 6.06	20.9 ± 8.28	31.5 ± 3.01

2. Single oral pharmacokinetics study in rats (vol. 1.44, pp 2- , RR-815-98-013)

method- SD rats (10/sex/group, M- 197.5-258.0g, F- 167.1-205.9g, approximately 5 weeks of age) received a single oral dose of 3, 30, 100 or 300 mg/kg LG1069 (lot# LG100069-000Z035) in vehicle containing PEG400, polysorbate 20, butylated hydroxyanisole and povidone, by gavage. Blood samples were collected at 9, 0.5, 1, 2, 3, 4, 6, 9 and 12 h post-dose.

GLP statement- Yes

Results- The systemic exposure to LG1069 was dose-proportional over the dose range of 3-300 mg/kg. Cmax and AUC in males were greater than those in females.

Dose, mg/kg	3		30		100		300	
	Male	Female	Male	Female	Male	Female	Male	Female
Cmax, ng/ml	488	441	2150	1840	6020	4250	8920	6930
Tmax, h	1	1	2	1	3	3	4	2
AUC ₀₋₁₂ , ng.h/ml	2470	1830	14100	12600	39000	24100	61000	52100

3. Single and repeat dose oral pharmacokinetics in rats (vol. 1.45, pp2-33, RR-845-98-007)

method- SD rats (3/sex/group) received oral daily doses of 0, 10, 30 or 100 mg/kg/day micronized LG1069 (lot# LG100069-000Z011) as a PEG400/water suspension by gavage for 14 days. On day 15, the vehicle control group received a single oral dose of 10, 30 or 100 mg/kg micronized LG1069 in PEG400/water. Blood samples (0.7-1 ml) were collected via the jugular vein at 0, 1, 2, 3, 6, 9 and 12 h post-dose.

GLP statement- No

Results- Cmax and AUC values of repeat daily dosing groups (both males and females) were significantly less than those of single dose groups. Cmax and AUC were similar between genders at all dose levels.

Dose, mg/kg	10		30		100	
	Single (M/F)	Repeated (M/F)	Single (M/F)	Repeated (M/F)	Single (M/F)	Repeated (M/F)
Cmax, uM	3.47/3.14	1.55/2.01	6.67/7.56	2.42/3.53	15.6/16.9	4.86/10.0
Tmax, h	2.4/1.6	1.8/1.7	2.4/1.3	2.4/1.4	3.7/2.6	3.2/2.1
T1/2, h	2.86/6.20	5.22/5.94	7.47/63	5.40/6.57	7.60/5.09	4.41/5.07
AUC ₀₋₂₄ , uM.h	25.0/22.5	14.1/15.8	71.2/69.2	22.6/31.3	205/169	44.6/82.1

4. Two and three-way crossover oral bioequivalence studies of two clinical formulations in dogs (vol. 1.46, pp4-163, RR-845-94-010/011)

method- Eight female beagle dogs received a single iv dose of 5 mg/kg LG1069 (lot#. LG100069-000Z010- crystalline) or received a single oral dose of 25 mg/kg LG1069 (lot#. LG100069-000Z015; formulation lot #. 9309-002 (non-micronized, capsules formulation SG2) and 9408-003 (micronized, capsules formulation SG3)) on two occasions separated by 1 week. On week 3, a single iv dose of 5 mg/kg LG1069 (lot#. 9408-003 (micronized)) was administered to all dogs. Serial blood samples (3 ml) were collected via a cephalic vein cannula or by jugular venipuncture prior to and at 0.5, 1, 2, 4, 6, 8, 12 and 24 h post-dose.

GLP statement- No

Results- Cmax and AUC values of a clinical formulation (SG3) containing micronized LG1069 was 7.6-fold and 6-fold greater than those values of a clinical formulation (SG2) containing non-micronized LG1069, respectively, in female dogs.

Formulation	Non-micronized	Micronized	Micronized	Micronized
Dose, mg/kg	25	25	5	5
Route of administration	PO, capsule	PO, capsule	IV	PO, capsule
Study No.	RR-845-94-011	RR-845-94-011	RR-845-94-010	RR-845-94-011
Cmax, uM	9.24	70.4	32.3	15.3
Tmax, h	2.38	1.5	0.5	1.13
T1/2, h	4.03	2.53	3.82	3.44
AUC _{0-∞} , uM.h	41.1	243	73.5	54.6
Cl, ml/min/kg			3.65	
Vd, l/kg			0.563	
Bioavailability (%)*	10.4	83.1		74.3

*bioavailability (%) - absolute oral bioavailability relative to a 5 mg/kg iv LG1069.

5. Five-way crossover oral study in female beagle dogs (vol. 1.46, pp 205-315, RR-845-98-014)

method- 8 female beagle dogs received a single oral dose of 35 mg/kg LG1069 (lot#. LG100069-000Z018/020/026/032) of each formulation (soft gelatin capsules (SG), soft gelatin capsules which formed pellicles during in vitro dissolution test (SG2), hard gelatin capsules (HG), tablet A, or tablet B) with one week washout period between dosings. Serial blood samples (2.5 ml) were collected from the cephalic vein prior to dosing, at 0.33, 0.67, 1, 1.5, 2, 3, 4, 6, 9 and 12 h post-dose. SG is a clinical formulation.

GLP statement- No

Results- Cmax and AUC values of formulations of a hard gelatin capsules and two tablets were significantly higher than those of soft gelatin capsules.

Formulation	Soft gelatin capsules, SG	Soft gelatin capsules, SG2	Hard gelatin capsules, HG	Tablet A	Tablet B
Dose, mg/kg	35	35	35	35	35
Cmax, uM	97.1	95.6	114.0	117.0	127.4
Tmax, h	1.5	1.5	1.8	1.8	2.0
T1/2, h	2.9	3.6	2.1	2.4	2.1
AUC _{0-∞} , uM.h	344	374	436	422	483

*HG and tablet A and B contain LG1069 50%, povidone K30 USP, sodium lauryl sulfate NF, corn starch NF, microcrystalline cellulose NF, lactose NF (none in tablet B), croscarmellose sodium NF (none in HG and tablet B), sodium starch glycolate NF (none in HG and tablet A), colloidal silicon dioxide NF and magnesium stearate NF (none in HG).

6. Tissue distribution of [¹⁴C]LGD 1069 in rats (vol. 1.47, pp 188- , RR-845-97-002b)

method- SD rats (3/sex/time point, bw 200-250g, 6-8 weeks of age) received a single oral dose of 100 mg/kg [¹⁴C]LG1069 (in sesame oil suspension; lot#. LG100069-914Z001 and LG100069-000Z004, 67.5 uCi/kg) by gavage (5 ml/kg). Animals were sacrificed 4, 8, 24 or 48 h post-dose. Blood was drawn from the abdominal aorta and tissues and excreta were collected. Radioactivity from urine, feces, blood and tissue homogenates was measured using a scintillation counter.

GLP statement- No

Results- Orally administered LG1069 was eliminated almost exclusively in the feces in rats. Radioactivity in tissues decreased with time (in which the highest level was observed at 4 h) and no apparent accumulation of radioactivity in tissues after 48 h was observed.

Sample	%Dose Recovered			
	4h (M/F)	8h (M/F)	24h (M/F)	48h (M/F)
Blood	0.64/0.67	0.27/0.29	0.07/0.13	0.01/0.70
Tissues*	13.2/7.6	4.8/5.3	1.4/3.7	0.1/0.1
GIT contents	80.1/75.6	85.4/85.0	20.7/65.8	0.3/0.7
Feces	0.001/0.006	0.17/2.52	76.1/27.5	91.4/108.6
Urine	0.004/0.005	0.04/0.06	0.12/0.33	0.14/0.59
Total activity recovered, 0-48h	94.0/83.7	90.7/93.2	98.4/97.5	91.7/110.7

*the liver, kidneys, adrenal glands, fat, mesenteric lymph node, pancreas, heart, salivary gland, gastrointestinal tract and ovaries

7. Effects on hepatic microsomal cytochrome P450 and in vitro metabolism (vol. 1.48, pp 2-20, RR-845-98-009)

method- in vivo effects- Male SD rats (3/group, bw approximately 250g) received oral doses of 0 (PEG400/water vehicle only) or 100 mg/kg/day LG1069 (in PEG400/water microparticulate suspension; lot# LG100069-000Z011) or, ip doses of 80 mg/kg/day phenobarbital, 50 mg/kg/day dexamethasone, 25 mg/kg/day 3-methylcholanthrene or oil vehicle by gavage (5 ml/kg) for 4 days. After the final dose, hepatic microsomes were prepared by differential centrifugation. Microsomes were examined spectrophotometrically for total cytochrome P450 and for P450 isozyme levels using Western blot analysis. In vitro metabolism- the microsomes obtained from the in vivo study were incubated with 100 uM LG1069 under conditions supporting either P450-mediated metabolism or glucuronyltransferase (GT)-mediated metabolism. Rates of LG1069 metabolism were determined by monitoring the formation of metabolites using HPLC-UV.

GLP statement- No

Results- LG1069 (100 mg/kg/day) induced increase in total P450 concentration. Among P450 isozymes, concentrations of CYP2B1/2B2, CYP3A and CYP4A increased significantly and concentrations of CYP1A2 and CYP2C11 decreased. Both oxidative metabolism (hydroxy- and oxo-LG1069) and glucuronidation of LG1069 by liver microsomes were induced in microsomes from rats treated with 100 mg/kg/day LG1069.

a. effects on P450 in hepatic microsomes

Treatment	Total hepatic P450 (pmol/mg protein)	Change from control
PEG400/water control	577	-
LG1069, 100 mg/kg/day	1102	91%
Oil vehicle control	596	-
Phenobarbital, 80 mg/kg/day	1283	112%
Dexamethasone, 50 mg/kg/day	1251	110%
3-methylcholanthrene, 25 mg/kg/day	1132	90%

b. relative concentration of P450 isozymes in hepatic microsomes

P450 Isozyme	Optical Density x mm/mg protein (and Change from Control)					
	PEG400/Water	LG1069	Oil Vehicle	Phenobarbital	Dexamethasone	3-MC
CYP1A2	3.1	0.3 (-90%)	4.1	1.2 (-70%)	0 (-100%)	285 (6851%)
CYP2B1/2B2	0.9	26.3 (2822%)	3.4	221 (6400%)	5.6 (65%)	5.4 (59%)
CYP2C11	5.7	3.4 (-41%)	-	-	-	-
CYP2E	67.0	80.9 (21%)	9.2	119 (1193%)	69.6 (657%)	27.8 (202%)
CYP3A	11.4	105 (821%)	143	97.2 (-32%)	405 (183%)	21.4 (-85%)
CYP4A	2.1	152 (7138%)	4.1	5.6 (37%)	2.6 (-37%)	1.6 (-61%)

c. rates of formation of LG1069 metabolites

Treatment	Rate of Metabolite Formation (mAU x s/min/mg protein)			
	P450-Mediated		GT-Mediated	
	Mean	Change from Control	Mean	Change from Control
PEG400/water vehicle	12.0	-	13.0	-
LG1069, 100 mg/kg/day	43.0	252%	23.0	77%
Oil vehicle	15.0	-	10.9	-
Phenobarbital, 80 mg/kg/day	27.0	80%	15.2	39%
Dexamethasone, 50 mg/kg/day	68.5	357%	9.0	-17%
3-MC, 25 mg/kg/day	7.7	-49%	11.1	2%

*Rates are expressed in terms of increase in metabolite (sum of hydroxy-LG1069 and oxo-LG1069) HPLC peak areas over time.

8. Metabolic fate in rats (vol. 1.48, pp 42-79, RR-845-98-001)

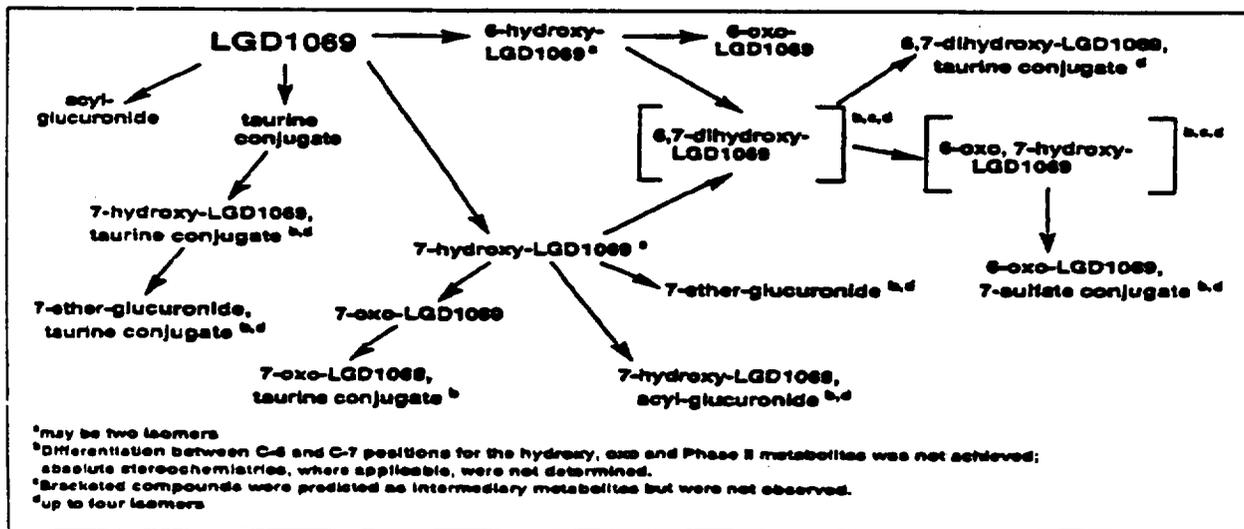
method- Metabolites of LG1069 (lot# LG100069-000Z007/Z011, 100 uM/incubation mixture for 4 h) were generated using rat liver slices (from a male SD rat), rat liver microsomes (from male SD rats) and by collecting bile from rats dosed with 100 mg/kg LG1069. Metabolite profiles were analyzed using mass spectrometry. Metabolites were identified using electron ionization and negative ion electron capture ionization mass spectrometry and gas chromatography/mass spectrometry.

GLP statement- No

Results- A number of metabolites were formed by rat liver slices incubated with LG1069 (putative metabolites were assigned numbers as peaks 2, 3, 4, 6, 7, 8, and 9 in analysis. peaks (metabolites presumed to be formed by P450) in liver microsomes from rats were peaks 2, 3, 4, 7/7' and 9/9'. Peaks from analysis of bile collected from a rat dosed with 100 mg/kg LG1069 were peaks 2, 3, 4, 6 and 8. Tentative structural assignments for major metabolites observed during analysis were made as below:

Peak	Identity
2	Ether glucuronide
3	Ether glucuronide
4	6- and/or 7-hydroxylated acyl glucuronide
6	taurine conjugate
7	6-hydroxy-LG1069
7'	7-hydroxy-LG1069
8	acyl glucuronide of LG1069
9	6-oxo-LG1069
9'	7-oxo-LG1069

Suggested Metabolic Pathway for LG1069 in rats:



9. Binding to human plasma protein (vol. 1.57, pp 135-136, RR-845-99-002)

method- Drug-free fasting plasma was obtained from 3 healthy male and female volunteers and pooled. The pooled human plasma was re-warmed to 37°C and pH was adjusted to 7.4. Plasma samples were spiked with [³H]LG1069 to achieve concentrations of 5, 10, 100, 1000 and 5000 ng/ml. After a 15 min incubation, plasma and PBS (dialysate) were added to respective sides of the dialysis cells of Spectra/Por Equilibrium Dialyzer with teflon cells and Spectra/Por 2 membranes and were incubated at 37°C for 4 h. At 4h, aliquots of plasma and dialysate were removed and analyzed for radioactivity by liquid scintillation counting and calculation of protein binding.

GLP statement- No

Results: Binding of LG1069 to human plasma proteins was very high (>99.8%), which was independent of LG1069 concentration. Free fractions ranged from 0.12% to 0.18%.

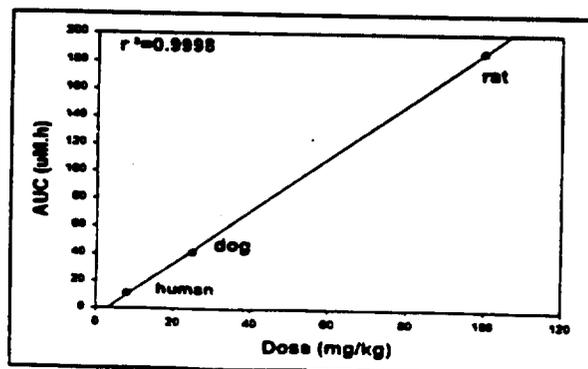
Plasma [³ H]LG1069 Concentration	Plasma Protein Binding (%)	Free Fraction (%)
5 ng/ml	99.87	0.13
10	99.88	0.12
100	99.82	0.18
1000	99.87	0.17
5000	99.85	0.15

Overall Pharmacokinetic Summary:

Pharmacokinetics	Rat (PEG/water vehicle)		Dog (capsule)	Human* (capsule)		
	Dose. mg/m ² mg/kg	600, single dose 100	600, multiple dose 100	500, single dose 25	300, single dose 8.1	300, multiple dose 8.1
Route		PO (Male/Female)	PO (Male/Female)	PO	PO	PO
Cmax. uM		15.6/16.9	4.8/10.0	70.4	2.6	3.2
Tmax. h		3.7/2.6	3.2/2.1	1.5		
T1/2. h		7.6/5.0	4.4/5.0	2.5	1-3	1-3
AUC _{0-∞} . uM.h		205/169	44.6/82.1	41.1	11.1	10.9
Bioavailability. %				83.1		
Protein binding		-	-	-	>99.8 (5-5000 ng/ml)	
Tissue Distribution in Rats		8h - GI (85.4%M/85.0%F) > tissues (4.8%M/5.3%F) > plasma (0.27%M/0.29%F) 24h- GI (20.7%M/65.8%F) > tissues (1.4%M/3.7%F) > plasma (0.01%M/0.7%F)				
Metabolism		CYP2B, CYP3A and/or CYP4A-mediated oxidation: 6-hydroxy LG1069, 7-hydroxy LG1069, 6-oxo-LG1069, 7-oxo-LG1069 Glucuronidation: acyl glucuronide, ether glucuronide Conjugation: taurine conjugate, 7-sulfate conjugate				
Excretion, Feces		91.1(M)/100(F)			0.04	
Urine		0.14 (M)/0.59(F)			-	

*human pharmacokinetic parameters from vol. 1.57 (pp104-107)

Cmax of rats and dogs cannot be directly extrapolated to that of humans (r²=0.13 on mg/m² basis, r²=0.1570 on mg/kg basis), while AUC of these animals may be directly extrapolated to humans (r²=0.75 on mg/m² basis, r²= 0.9998 on mg/kg basis). Dosing on mg/kg basis may provide much greater interspecies correlation of AUC than dosing on mg/m² basis.



In hepatic microsomes, cytochrome P450 CYP2B, CYP3A and CYP4A were markedly induced (821% - 7138%) by LG1069 (100 µM). LG1069 was almost exclusively eliminated via feces.

TOXICOLOGY

Multiple Doses

1. 28-Day oral toxicity study in rats (vol. 1.16, pp 2-409, RR-815-94-013)

Animal- male SD rats (15/group except 10/group in low dose, plus 4/group in mid and high dose groups for pharmacokinetics; B.W. 83.6 - 116.2 g, 3-5 weeks of age).

Treatment- animals received oral daily doses of 0, 3, 10, 30 or 100 mg/kg/day LG1069 (lot #. LG100069-000Z007, formulated in sesame oil) by gavage (10 ml/kg) for 28 days.

Observation- recovery period- 28 days, clinical signs/mortality- daily, body weight/food consumption- weekly, clinical pathology- d29, necropsy- d29 and d57 (animals in recovery), pharmacokinetics- 1 ml blood collected 2h post dose on days 1, 14 and 28 from 4/raits/group.

GLP statement- yes

Results: HNSTD and LD₁₀ were 3 mg/kg/day and 10 mg/kg/day, respectively. Major toxic targets included blood clotting system (hemorrhage in numerous tissues, prolongation of PT and aPTT, increased levels of fibrinogen), liver (hemorrhage, hepatocellular necrosis), pancreas (acinar cell necrosis), heart (cardiomyopathy), bone (tibial osteopathy), skin (dermatopathy), and lymphoid organs (lymphoid depletion in spleen, lymph nodes, thymus).

Dose, mg/kg/day	0	3	10	30	100
Mortality	0/15	0/10	2/19	7/19	14/19
Clinical signs					
urine stain	0	1	1	1	7
discharge	4	3	9	11	13
bleeding	0	0	2	3	7
pale mucous memb	0	0	0	5	9
B.W. gain, d-1/28, g	121	166	176	173	161
Food consumption week 4 (g)	140	171	177	173	168
Hematology					
RBC, x10 ⁶	8.86	8.96	8.92	7.72	6.07
hemoglobin, g/dl	16.4	16.4	16.0	13.8	11.0
WBC, x 10 ³	15.5	13.0	12.7	12.2	9.3
Lymphocytes, x 10 ³	13.6	11.57	11.14	9.70	5.66
PT, sec	15.6	29.1	53.4	77.2	111
APTT, sec	40.6	66.2	102.2	>120	>120
fibrinogen, mg/dl	331	441	450	578	911
Clinical chemistry					
Glucose, mg/dl	119	119	128	137	319
BUN, mg/dl	12	10	11	10	20
Cholesterol, mg/dl	76	92	102	98	153
Triglyceride, mg/dl	69	73	103	179	788
Phosphate, mg/dl	9.5	9.1	8.6	8.3	8.5
Potassium, mEq/l	6.3	5.7	5.2	54.9	5.3
ALT, U/l	29	40	56	53	197
AST, U/l	65	77	97	83	183
ALK, U/l	158	152	184	234	384
Gross pathology					
adrenal wt. g	N=10 0.053	N=10 0.075	N=8 0.063	N=5 0.066	N=3 0.076
liver wt. g	12.93	17.52	17.86	20.30	21.16
red foci/color*	UR	UR	UR	2/5	3/3
Histopathology					
Hemorrhage**	UR	UR	5/8	5/5	3/3
Lymphoid depletion					
Spleen	UR	UR	UR	4/5	1/3
Thymus	UR	UR	2/8	2/5	3/3

Mesenteric LN	UR	UR	UR	1/5	2/3
Mandibular LN	UR	UR	UR	0/5	2/3
Adrenal cortex Hypertrophy	UR	UR	1/8	2/5	2/3
Liver Cellular necrosis	UR	UR	UR	UR	1/3
Acinar pancreas Acinar necrosis	UR	UR	UR	2/5	2/3
Skin, dermatopathy	UR	1/10	4/8	4/5	3/3
Tibia, osteopathy	UR	UR	3/8	4/5	2/3
Heart, cardiomyopathy	UR	UR	5/8	3/5	2/3
Pharmacokinetics Plasma conc. ng/ml			d1/d14/d28 879/643/578	d1/d14/d28 1891/807/1129	d1/d14/d28 3677/2514/2837

UR- unremarkable; * red foci in esophagus, epididymides, pancreas, seminal vesicles, skeletal muscle, skin, thymus, and testes; ** hemorrhage- esophagus, forestomach, heart, kidney, liver, lung, mandibular lymph node, mesenteric lymph node, acinar pancreas, prostate, salivary gland, skeletal muscle, skin, seminal vesicles, thymus, testes;

2. 91-Day oral toxicity study in dogs (vol. 1.30, pp 105, to vol.1.32, RR-815-95-003)

Animal- beagle dogs (6/sex/group, M 8.3-10.2 kg, F 6.4-8.8 kg)

treatment- animals received oral daily doses of 0, 0.1, 0.3 or 1.5 mg/kg/day LG1069 (suspensions in babassu oil and filled in capsules; lot #. LG100069-00Z008) for 91 days.

Observation- recovery period- 28 days, clinical signs/mortality/food consumption- daily, body weight- weekly, ophthalmic/cardiovascular (ECG, blood pressure, heart rate) examinations - prior to treatment, during wk 6 and prior to necropsy, clinical pathology- d-1, wk 4 and 8, and at necropsy, urinalysis- prior to treatment and within 48h of necropsy, pharmacokinetics- 2.8 ml blood/sample collected, time 0, 0.5, 1,2,3,4,6,8 and 12 h postdose on days 1, 45 and 91.

GLP statement- yes

Results: HNSTD was 1.5 mg/kg/day. Toxic target organ was testes (tubular degeneration- nuclear pyknosis, vacuolization and loss of germinal epithelium, recovered at the end of recovery period- Labeling: infertility). After repeated dosing, AUC of LG1069 at day 91 after subtracting trough levels was reduced by 48% in males and 39% in females when compared to day 1.

Dose. mg/kg/day	0	0.1	0.3	1.5
Mortality	None	None	None	None
Clinical signs	UR	UR	UR	UR
B.W.	UR	UR	UR	UR
Food consumption	UR	UR	UR	UR
Ophthalmology	UR	UR	UR	UR
Cardiovascular exam.	UR	UR	UR	UR
Hematology	UR	UR	UR	UR
Clinical chemistry	UR	UR	UR	UR
Urinalysis	UR	UR	UR	UR
Gross pathology				
Testes wt. g. male	15.11	11.77	15.50	11.00
Adrenal wt. g. females	0.943	0.985	1.172	1.219
Histopathology				
Testes, male tubular degeneration	UR	1/4	1/4	3/6
Pharmacokinetics				d1/d91
Cmax. ng/ml				M F
Cmin. ng/ml				133/107 147/131
Tmax. h				ND/22.1 ND/28.4
AUC _{0-12h} . ng.hr/ml				4.8/3.3 5.5/2.6
AUC*. ng.hr/ml				1099/832 1174/1049
				N/A/572 N/A / 715

UR- unremarkable; *AUC- AUC after subtracting of predose trough levels

3. 6-Month oral toxicity study in rats (vol. 1. 21, pp 2-, to vol. 1.27, RR-815-98-004)

Animal- SD rats (42/sex/group; 10/sex/group for pharmacokinetics; B.W. M 204-244, F 150-184; 4 weeks of age)

Treatment- animals received oral daily doses of 0, 3, 30, 100 or 300 mg/kg/day LG1069 (suspensions containing PEG 400 NF, polysorbate 20 NF, butylated hydroxyanisole NF and povidone USP; 5 ml/kg) by gavage for 26 weeks except animals at 300 mg/kg/day group which received for 8 weeks because of severe dermal toxicities.

Observation- recovery period- 28 days; clinical signs/mortality- daily, B.W./food consumption/physical examination- weekly, ophthalmology/clinical pathology/necropsy- week 8 (300 mg/kg group only), week 12 (3-month interim sacrifice), 3-month interim sacrifice, 4-month recovery sacrifice, 6-month terminal sacrifice and 7-month recovery sacrifice, pharmacokinetics- days 28, 50-56, 84, 112, 141 and 178.

GLP statement- yes

Results- Significant increases in mortality were observed in 300 mg/kg/group. Majority of mortalities appeared as a result of gavage damage (e.g., perforation of esophagus, etc). LG 1069 was cataractogenic (lens opacity- Labeling) (30 mg/kg or higher). Toxicity targets included, blood (prolonged clotting time, decreased hemoglobin and RBC), eye (cataract), liver (liver enzymes, hypertrophy), skin (acanthosis), and stomach (acanthosis, hyperkeratosis).

Dose. mg/kg/day	0	3	30	100	300 (8 weeks only)
Mortality*	M 3/42 (d 88, 112, 142)	M 1/52 F 1/52 (d 79, 152)	M 7/52 F 3/52 (d 63-170)	M 8/52 F 3/52 (d 34-182)	M 13/52 F 3/52 (d 8-53)
Clinical signs					
Alopecia	5/42	3/42	20/40	36/39	22/22 (wk9)
Erythema	UR	UR	3/40	28/40	30/37 (wk 7)
Ocular opacity	UR	UR	2/28	1/27	UR
B.W. gain, g. M/F	434.1/189.4	499.7/246	454.2/188.5	392/154.4	-15%/-10% vs control at wk 8
Food consumption g/kg/day	M/F	M/F	M/F	M/F	M/F
wk 8	60.2/74.3	64.2/81.0	66.9/77.3	69.3/75.8	74.0/98.6
wk 26	44.9/59.0	46.2/61.5	48.6/65.9	54.4/71.4	-
Hematology, wk26	M/F	M/F	M/F	M/F	M/F (wk 8)
Hemoglobin, g/dl	15.0/14.7	15.1/13.9	14.2/12.8	13.7/11.7	13.6/12.5
RBC, 10 ⁶ /ul	8.95/8.15	8.91/8.12	8.63/7.81	8.30/7.30	7.90/7.19
Platelet, 10 ³ /ul	887/825	965/900	1105/1116	1114/1207	1404/1496
PT, sec	11.1/10.8	13.3/10.9	17.4/11.6	20.9/12.7	26.5/12.9
APTT, sec	12.7/15.5	20.2/16.9	24.6/23.0	33.5/19.4	79.1/31.9
Clinical chemistry	M/F	M/F	M/F	M/F	M/F (wk 8)
AST, U/l	112/107	130/101	136/123	160/132	152/166
ALT, U/l	46/58	59/42	91/74	115/94	143/108
Alk. P. U/l	82/31	129/55	273/152	364/295	446/681
CK, U/l	100/97	121/116	152/117	233/165	216/232
Cholesterol, mg/dl	70/77	108/118	76/125	81/156	84/200
Triglyceride, mg/dl	78/83	127/112	187/174	156/211	116/93
Ophthalmology	wk26/ wk30	wk26/ wk30	wk26/ wk30	wk26/ wk30	wk8/ wk12
Cataract incidence %	0/0	2/0	48/42	88/92	51/88
Gross pathology	M/F, wk 27	M/F, wk 27	M/F, wk 27	M/F, wk 27	M/F (wk 8)
Adrenal wt, g	0.067/0.078	0.080/0.098	0.089/0.096	0.093/0.109	0.096/0.088
Liver wt, g	17.90/10.23	22.99/14.71	24.71/16.42	27.53/17.08	21.44/16.19
Liver hypertrophy	UR	UR	UR	M 1/10, F UR	M 4/9, F 5/10
Eye opacity	UR	UR	M 2/13, F 1/17	M 1/12, 3/17	M UR, F 1/10
Histopathology	wk 27	wk27	wk27	wk27	Wk8
Eye cataract	UR	M UR, F 2/19	M 6/13, F 3/17	M 8/12, F 10/17	M 2/9, M 3/10
Skin acanthosis	UR	UR	M 1/13, F UR	M 1/12, F 8/17	M 4/9, F 4/10
Stomach acanthosis	UR	UR	UR	M 5/12, F 12/17	M 9/9, 10/10
hyperkeratosis	UR	UR	UR	M 5/12, F 9/17	M 9/9, F 10/10

Pharmacokinetics	M/F	M/F	M/F	M/F
<u>Day 1</u>				
Cmax, ng/ml	488/441	2150/1840	6020/4250	8920/6930
Tmax, h	1/1	2/1	3/3	4/2
AUC _{0-12h} , ng.h/ml	2470/1830	14100/12600	39000/24100	61000/52100
<u>Day 50-52</u>				
Cmax, ng/ml	249/779	1480/2290	2080/3380	4260/5840
Tmax, h	2/6	3/2	2/1	2/2
AUC _{0-12h} , ng.h/ml	1520/4040	9050/11400	13800/24200	27000/36600
<u>Day 178</u>				
Cmax, ng/ml	120/234	1530/1950	1890/3500	
Tmax, h	1/1	2/1	2/1	
AUC _{0-12h} , ng.h/ml	1650/2840	8990/11000	12400/21400	

UR- unremarkable; * mortality- gavage injury was the most frequent cause of death, in part, due to treatment-related increase in skin sensitivity and morphologic changes in the esophagus.

4. 6-Month oral toxicity study in dogs (vol. 1.33,pp 2- to vol. 1.36, RR-815-98-003a)

Animal- beagle dogs (6/sex/group, M 8.5-11.0 kg, F 7.2-9.8 kg, 7-9 months old)

Treatment- animals received oral daily doses of 0, 1, 3 or 10 mg/kg/day LG1069 (suspensions containing PEG 400 NF, polysorbate 20 NF, butylated hydroxyanisole NF and povidone USP, which was filled in capsules; lot #. LG100069-00Z035) for 26 weeks.

Observation- recovery period- 28 days, clinical signs/mortality/food consumption- daily, body weight- 2/wk, ophthalmic/ cardiovascular (ECG, blood pressure, heart rate) examinations- prior to treatment, during wks 1, 12, 26 (6 month) and 30 (recovery), clinical pathology- d-12, days 29, 57, 89, 119, 148, 182 (6 month) and 210 (recovery), urinalysis- days -14, 86, 181 (6 month) and 209 (recovery), pharmacokinetics- 2.8 ml blood/sample collected, time 0, 0.5, 1, 2, 3, 4, 6, 9 and 12 h postdose on days 1, 180 and 210 and 2 h post-dose on days 30, 58, 90, 120 and 149.

GLP statement- yes

Results: HNSTD was 10 mg/kg/day. Toxic targets include adrenal cortex (vacuolation), liver (enzymes, hepatocellular hypertrophy), eyes (cataract), ears (excessive ceruminous material), and platelets (increased). In female animals, body weight gain in 10 mg/kg group was lower than that in control animals, while food consumption in 10 mg/kg animals was greater than that in control group (no apparent reason was provided by the sponsor).

Dose, mg/kg/day	0	1	3	10
Mortality	None	None	None	None
Clinical signs	M/F	M/F	M/F	M/F
Skin reddening	2/5	4/6 (longer duration)	3/5 (longer duration)	3/6 (longer duration)
B.W. gain, kg, d1-d179	M 1.2 F 1.5	M 1.7 F 1.4	M 1.6 F 1.2	M 1.4 F 0.9
Food consumption g/kg/day, wk 26	M 24.9 F 27.7	M 27.5 F 28.4	M 29.8 F 30.7	M 27.2 F 34.8
Ophthalmology				
Cataract incidence	UR	UR	2/12	5/12 (M 4, F 1)
Cardiovascular exam.	UR	UR	UR	UR
Hematology, month 6	M/F	M/F	M/F	M/F
Platelet, 10 ³ /ul	265/490	242/251	386/343	479/490
Clinical chemistry, mo 6	M/F	M/F	M/F	M/F
AST, u/l	31/24	30/28	29/31	50/59
ALT, u/l	33/29	35/28	34/37	178/92
Alk. P, u/l	72/68	78/76	131/139	333/220
HDL, mg/dl	90.1/117.4	94.4/107.3	101.9/87.9	77.2/77.9
Urinalysis	UR	UR	UR	UR
Gross pathology, 4/sex/gr	M/F	M/F	M/F	M/F
Ear, abnormal content	UR	UR	UR/1	3/1
Liver, enlarged	UR	UR	1/2	2/2
Histopathology, 4/sex/gr	M/F	M/F	M/F	M/F
Liver, bile stasis	UR	UR	UR	2/2

hypertrophy, cellular	UR	UR	4/4	4/4			
Testes, degeneration	UR	UR	UR	2M			
Adrenal gland							
vacuolation, cortex cell	UR	UR	2/1	4/4			
Eyes, cataract	UR	UR	UR	2M			
Ears, ceruminous gland*	UR	UR	UR/1	2/1			
Pharmacokinetics		M F	M F	M F			
Cmax, ng/ml	day 1	462	301	1210	1460	7750	6810
	wk 26	275	454	717	804	1960	2410
Tmax, h	day 1	1.25	1.08	1.50	1.58	1.60	1.50
	wk 26	1.17	1.58	2.17	1.50	1.83	2.00
AUC _{0-12h} , ng.h/ml, day1		2070	1570	5540	5940	30400	24400
	wk 26	1500	1440	3390	3460	7590	7890

UR- unremarkable; * ceruminous gland- hyperplasia

Overall Toxicology Summary-

Toxicity Study	Study Report No.	Treatment	Toxicity Targets and Toxic Effects	Toxic Levels
Rat 28-Day	RR-815-94-013	3, 10, 30 or 100 mkd in sesame oil, po, for 28 days	Blood (hemorrhage in many tissues, prolongation of PT and aPTT, increased levels of fibrinogen), liver (hepatocellular necrosis), pancreas (acinar cell necrosis), heart (cardiomyopathy), bone (tibial osteopathy), skin (dermatopathy), lymphoid organs (lymphoid depletion in spleen, lymph nodes and thymus)	LD ₁₀ = 10 mkd HNSTD= 3 mkd
Dog 91-Day	RR-815-95-003	0.1, 0.3 or 1.5 mkd, po, for 91 days, in babassu oil in capsules	Testes (tubular degeneration)	HNSTD= 1.5 mkd
Rat 6-Month	RR-815-98-004	3, 30, 100 or 300 mkd (in aqueous suspension in PEG 400), po, for 6 months	Blood (prolonged clotting time, ↓hemoglobin and RBC), Eye (lens opacity- cataractogenic), liver (hypertrophy, ↑AST/ALT), skin (acanthosis), stomach (acanthosis, hyperkeratosis)	HNSTD=300 mkd*
Dog 6-Month	RR-815-98-003a	1, 3 or 10 mkd (in aqueous suspension in PEG 400), po, for 6 months	Adrenal cortex (vacuolation), liver (hepatocellular hypertrophy, ↑ALT/AST), eye (cataract), platelets (↑ in counts).	HNSTD= 10 mkd

* mkd- mg/kg/day; * increased incidence of gavage-related deaths at 300 mkd

Labeling Issues:

Infertility- LG1069 induced testes degeneration in dogs (1.5 mg/kg/day, 1/10th the recommended clinical dose on a mg/m² basis)

Cataract- Cataract development has been reported in association with the oral administration of LG1069 at 3 mg/kg/day in rats (1/17th the recommended clinical dose on a mg/m² basis) and 3 mg/kg/day in dogs (1/5th the recommended clinical dose on a mg/m² basis). The mechanism of LG1069-induced cataracts was not known.

Drug Interaction- LG1069 induced bleeding in rats (10 mg/kg/day, 1/5th the recommended clinical dose on a mg/m² basis), probably due to the prolonged PT and aPTT (3 mg/kg/day, 1/17th the recommended clinical dose on a mg/m² basis). The concurrent use of LG1069 with anticoagulant or drugs that prolong clotting time such as NSAIDs should be discouraged.

REPRODUCTIVE TOXICITY

1. Oral developmental toxicity study in rats (vol.1.38, pp 134- , RR-815-97-003b)

Animal- female SD rats (25/group, BW- 237-268 g, Age- approximately 65 days)

Treatment- animals received daily oral doses of 0, 1, 4 or 16 mg/kg/day LG1069 (lot # LG 100069-000Z020, suspension of PEG 400: Tween:carboxymethyl cellulose: water = 9.95:0.05:0.9:89.1) by gavage (dosage volume- 1.5 ml/kg) on gestation days 7 through 17.

Observation- mortality/clinical signs-2/day, body weight- daily, food consumption- d0,7,10,12,15,18 and 20, necropsy- d20
GLP statement- yes

Results- 4 mg/kg/day- increased fetal incidences of skeletal variations, 16 mg/kg/day- reduced embryo/fetal viability (increased resorptions), reduced fetal body weights, increased fetal incidences of variations and malformations (cleft palate, depressed eye bulges, microphthalmia, and incomplete or no ossifications). Body weight gains- reduced gains at doses of 4 and 16 mg/kg/day during the drug treatment.

Dose, mg/kg/day	0	1	4	16
Mortality	None	None	None	None
Clinical signs				
alopecia, limbs	UR	UR	1/25	6/25 (p<0.01)
Pregnancy rate, %	100	100	100	96
Body weight gain, g.				
d7-d17	89.6	83.4	80.6 (p<0.05)	67.2 (p<0.01)
d0-d20	166.1	160.1	156.8	140.2 (p<0.01)
Food consumption,				
d7-d17, g/day	26.4	26.0	25.7	22.7 (p<0.01)
d0-d20, g/day	25.3	24.9	24.9	23.1 (p<0.01)
Litter				
dams with viable fetuses	25	25	25	24
corpora lutea, #/animal	17.8	17.4	18.2	17.6
#implantation, #/animal	16.8	16.1	15.9	16.1
live fetuses, #/animal	15.9	15.2	15.1	14.2
dead fetuses, #/animal	0	0	0	0
sex ratio, % males/litter	53.4	49.0	52.6	49.5
resorption, early, #/animal	0.8	0.9	0.8	2.0
resorption, late, #/animal	0	0	0	0.4
dams with any resorptions, # (%)	9 (36.0)	14 (56.0)	14 (56.0)	15 (62.5)
%resorbed conceptuses/litter	5.2	5.0	4.7	11.5
mean fetal body wt., g/litter	3.32	3.26	3.22	2.81 (p<0.01)
litters with fetuses with any alteration, # (%)	14 (56)	15 (60)	24 (96) (p<0.01)	24 (100) (p<0.01)
fetal observation:				
fetus with any alteration observed, # (%)	24 (6)	39 (10.3)	88 (23.3)	229 (67.4) (p<0.01)
%fetuses with any alteration/litter	5.81	9.88	22.71 (p<0.01)	67.66 (p<0.01)
external alterations				
cleft palate litter incidence, # (%)	1 (4)	0	0	18 (75) (p<0.01)
fetal incidence, # (%)	1 (0.2)	0	0	94 (27.6) (p<0.01)
eyes, depressed litter incidence, # (%)	1 (4)	0	0	15 (62.5) (p<0.01)
fetal incidence, # (%)	2 (0.5)	0	0	51 (15) (p<0.01)
ears, small litter incidence, # (%)	0	0	0	3 (12.5) (p<0.01)
fetal incidence, # (%)	0	0	0	18 (5.3) (p<0.01)
visceral alterations				
cleft palate litter incidence, # (%)	1 (4)	0	0	14 (58.3) (p<0.01)
fetal incidence, # (%)	1 (0.5)	0	0	44 (27.2) (p<0.01)
eyes, microphthalmia litter incidence, # (%)	1 (4)	0	0	10 (41.7) (p<0.01)
fetal incidence, # (%)	1 (0.5)	0	0	22 (13.6) (p<0.01)

skeletal alterations					
skull		0	0	0	
palate, IO*	litter incidence, # (%)	0	0	0	15 (62.5) (p<0.01)
	fetal incidence, # (%)	0	0	0	49 (27.5) (p<0.01)
tympanic rings, NO*	litter incidence, # (%)	0	0	0	15 (62.5) (p<0.01)
	fetal incidence, # (%)	0	0	0	41 (23) (p<0.01)
IO,	litter incidence, # (%)	0	0	0	7 (29.2) (p<0.01)
	fetal incidence, # (%)	1	0	0	11 (6.2) (p<0.01)
sphenoid, IS*	litter incidence, # (%)	1	0	0	18 (75) (p<0.01)
	fetal incidence, # (%)	0	0	0	63 (35.4) (p<0.01)
IO,	litter incidence, # (%)	0	0	0	6 (25) (p<0.01)
	fetal incidence, # (%)	0	0	0	7 (3.9) (p<0.01)
eye, small socket,	litter incidence, # (%)	0	0	0	14 (58.3) (p<0.01)
	fetal incidence, # (%)	0	0	0	29 (16.3) (p<0.01)
squamosal, IO,	litter incidence, # (%)	0	0	0	4 (16.7) (p<0.01)
	fetal incidence, # (%)	0	0	0	13 (7.3) (p<0.01)
NO,	litter incidence, # (%)	0	0	0	3 (12.5) (p<0.01)
	fetal incidence, # (%)	0	0	0	6 (3.4) (p<0.01)
nasals, short,	litter incidence, # (%)	0	0	0	3 (12.5) (p<0.01)
	fetal incidence, # (%)	0	0	0	3 (1.7) (p<0.01)
cervical rib at 7 th cervical vertebra		1(4)	0	0	
	litter incidence, # (%)	1 (0.5)	0	0	23 (95.8) (p<0.01)
	fetal incidence, # (%)				71 (39.9) (p<0.01)
thoracic vertebrae, unilateral ossification		1 (4)	0	0	
	litter incidence, # (%)	1 (0.5)	0	0	4 (16.7) (p<0.01)
	fetal incidence, # (%)	9 (28)	11 (44)	22 (88) (p<0.01)	4 (2.2) (p<0.01)
sternal centra, IO,	litter incidence, # (%)	11 (5.3)	22 (11.2)	70 (35.7) (p<0.01)	24 (100) (p<0.01)
	fetal incidence, # (%)	7 (28)	7 (28)	13 (52) (p<0.01)	152 (85.3) (p<0.01)
pelvis, IO,	litter incidence, # (%)	7 (3.4)	18 (9.1)	27 (13.8) (p<0.01)	12 (50) (p<0.01)
	fetal incidence, # (%)				31 (17.4) (p<0.01)
ossification sites/fetus/litter					
hyoid		0.74	0.72	0.72	0.96 (p<0.01)
vertebrae	thoracic	13.02	13.08 (p<0.05)	13.30 (p<0.01)	13.95 (p<0.01)
	lumbar	5.98	5.92	5.7 (p<0.01)	5.10 (p<0.01)
sternum	sternal centers	3.53	3.54	3.55	2.68 (p<0.01)
	xiphoid	0.99	1.0	0.98	0.85 (p<0.05)
forelimb (per limb)	metacarpals	3.51	3.65	3.53	3.24 (p<0.01)
hindlimb (per limb)	metatarsals	4.0	4.0	3.99	3.87 (p<0.01)
	phalange	5.0	4.98	4.97	4.81 (p<0.01)

* IO- incompletely ossified, NO- not ossified, IS- irregularly shaped

2. Oral dose-range development toxicity study in rats (vol. 1.39, pp 2-260, RR-915-97-004a)
 Animal- female SD rats (8/group, 4/group for pharmacokinetics, B.W.- 258-292 g, Age- about 65 days)
 Treatment- animals received daily oral doses of 0, 3, 10, 30 or 65 mg/kg/day LG 1069 (lot # LG100069-000Z020 in suspension of PEG 400: tween:carboxymethyl cellulose: water = 9.95:0.05:0.9:89.1) by gavage (dosage volume- 5 ml/kg) on gestation days 7 through 17.
 Observation- mortality/clinical signs-2/day, body weight- daily, food consumption- d0,7,10,12,15,18 and 20, necropsy- d20, blood samples- d17, prior to dosing, 1, 2, 3 and 4 h post-intubation
 GLP statement- yes

Results- 30 and 65 mg/kg/day- significantly reduced body weight gain of dam during gestation period (in particular the treatment period), reduced embryo/fatal viability, reduced fetal body weights, increased fetal incidence of external alterations, and reduced male sex ratio (65 mg/kg/day group only).

Dose, mg/kg/day	0	3	10	30	65
Mortality	None	None	None	None	None
Clinical signs red perivaginal substance	UR	UR	UR	2/8	7/8
Body weight gains, g					
d7-18	88.6	90.5	83.9	27.8	3.4
d0-20	159.8	157.9	149.9	78.9	49.1
Food consumption, g/day					
d7-d18	25.5	25.4	23.8	16.5	13.5
d0-20	25.2	25.2	24.4	19.2	18.3
Pregnancy rate, %	100	100	100	100	100
Gross pathology					
Liver weight, g	17.26	16.64	17.34	17.45	15.95
Litter					
dams with viable fetuses (%)	100	100	100	87.5	42.8
corpora lutea, #/animal	17.0	18.1	19.4	17.1	18.0
#implantation, #/animal	16.4	16.1	17.2	14.9	16.6
live fetuses, #/animal	15.6	15.5	16.1	4.2	1.6
dead fetuses, #/animal	0	0	0	0	0
sex ratio, % males/litter	52.5	52.9	59.6	49.3	36.1
resorption, early, #/animal	0.8	0.6	1.0	8.9	13.7
resorption, late, #/animal	0	0	0.1	1.8	1.3
dams with any resorptions, # (%)	4 (50)	4 (50)	4 (50)	8 (100)	7 (100)
% resorbed conceptuses/litter	4.7	3.9	6.4	68.9	77.3
mean fetal body wt., g/litter	3.38	3.29	3.01	2.15	1.96
fetal observation:					
external alterations					
eyes, depressed	0	0	0	3 (42.8)	2 (66.7)
litter incidence, # (%)	0	0	0	9 (26.5)	10 (90.0)
fetal incidence, # (%)	0	0	0		
Toxicokinetics					
Cmax, ng/ml		387	1181	1613	1527
AUC _{0-4h} , ng·h/ml*		-	2650	4867	-
Tmax, h		1.7	1.7	2.3	1.7

* AUC = 154.8 x Dose (mg/kg/day) + 440.4 (r² = 0.9428)

Overall Reproductive Toxicity Summary- development study was conducted in rats only (i.e., not in rabbits). Embryo/fetotoxic- ≥ 10 mg/kg/day, Teratogenic- ≥ 4 mg/kg/day, NOAEL- ≤ 1 mg/kg/day, and Maternally toxic- ≥ 4 mg/kg/day.

Toxic Effect	1 mg/kg/day	4 mg/kg/day	10 mg/kg/day	16 mg/kg/day	30/65 mg/kg/day
Dams					
Body weight gains, decreased, d7-17	-	+(3%)	+(5%)	++ (-25%)	+++ (> 68%)
Food consumption, reduced, d7-d17	-	-	-	+(15%)	+(>35%)
Litter					
Implantation, reduced	-	-	-	-	+
Embryo/fetal viability, reduced	-	-	-	++	+++
Fetal body weight, decreased	-	-	+(10%)	+(15%)	++ (>36%)
Variations/Malformations	-	+	-	+++	+++
Sex ratio, Male, reduced	-	-	-	-	+(65 mg/kg only)

* Changes- + slight, ++ mild, +++ moderate

Reproduction Risk Integration:

Signal: dysmorphogenesis (skeletal and soft tissue malformations and alterations) and altered growth

Criteria	Effects/Relevance	Score
Signal Strength part A	- cross species concordance: ↔ LG1069 was tested only in the rat.) - multiplicity of effects: ↑ (effects were seen on skeletal, visceral, external structures)	+1
Signal Strength part B	- maternal toxicity: ↔ (dysmorphogenesis occurred in the presence of	+1

	maternal toxicity) - dose response: ↑ - rare events: ↑ (some effects such as eye microphthalmia were relatively rare)	
Pharmacodynamics	- Therapeutic Index: ↑ (LOAEL/Clinical Dose = (24 mg/m ² /day)/(300 mg/m ² /day) = 0.08, of which value is less than 5, i.e., concern is increased) - Similarity of pharmacological and toxicological mechanisms: ↑ (teratogenic effects of retinoids may be mediated by interaction with their nuclear receptors.	+1
Concordance between Test Species and Humans	- metabolism and drug distribution: ↑ - general toxicity profile: ↑	+1
Relative Exposure	- ↑ (AUC of 10 mg/kg in rats- which is available)/(AUC of clinical dose) = 2650/3797 = 0.69, of which value is less than 10, i.e., concern is increased	+1
Class Alerts	- retinoids as a class are dysmorphogens: ↑	+1
Total Score		+6
Assessment	High concern	

Draft Labeling Recommendations- Pregnancy Category- D.

Retinoids, as a class, have demonstrated their capability of inducing teratogenic effects in animals and humans. LG1069 is maternally toxic in rats when given orally at 4 mg/kg/day (1/12th the recommended clinical oral dose on a mg/m² basis). LG1069 caused developmental mortality and altered growth (≥ 10 mg/kg/day, 1/5th the recommended clinical dose on a mg/m² basis) and was dysmorphogenic (≥ 4 mg/kg/day, 1/12th the recommended clinical dose on a mg/m² basis). LG1069 induces testicular degeneration in dogs (1.5 mg/kg/day, 1/10th the recommended clinical dose on a mg/m² basis).

GENETIC TOXICOLOGY

1. Salmonella/E. coli mutation assays (vol. 1.39, pp262- /1.40, pp 2-65, RR-815-95-005/RR-815-95-004,

method: test strains of TA 98, TA 100, TA 1353, TA 1357 (for histidine reversion at GC sites) and WP2uvrA (for tryptophan reversion at AT sites) were used in the presence or absence of S9 fraction. A mixture (2 ml of molten top agar, 0.1 ml indicator organism (about 10⁸ bacteria), and 0.5 ml S9 or buffer, and LG1069 (lot # LG100069-000Z015)) was poured into a plate containing about 25 ml of minimal glucose agar supplemented with a trace of Oxoid nutrient broth and then incubated at 37°C for 48 h. The histidine or tryptophan independent revertant colonies on each plate were then counted.

GLP statement: yes

Results: Negative

Strains	Mean: Revertant colonies per Plate											
	TA 98		TA 100		TA 1353		TA 1357		TA1538		WP2uvrA	
S9 Fraction	-S9	+S9	-S9	+S9	-S9	+S9	-S9	+S9	-S9	+S9	-S9	+S9
Control, 100 ul DMSO	16	29	121	110	14	14	6	5	30	27	26	24
LG1069, ug												
5	29	27	121	118	11	9	7	6	27	29	22	22
10	20	28	129	137	8	13	9	6	27	24	19	22
50	23	25	121	126	10	12	7	8	20	26	24	19
100	17	24	121	128	11	12	9	5	20	20	23	17
500	20	35	110	121	13	10	10	6	20	21	16	17
1000	21	25	114	125	8	10	6	9	19	22	22	11
Positive control	2-NF	2-A	NaN ₃	2-A	NaN ₃	2-A	9-AA	2-A	2-NF	2-A	ENNG	2-A
control	1013	1017	1259	1163	1079	282	178	296	1591	1045	894	158

*2-anthramine (2-4 ug)- 2-A; N-ethyl-N'-nitro-N-nitrosoguanidine (5 ug)- ENNG; 2-nitrofluorene (5 ug)- 2-NF; 9-aminoacridine (50 ug)- 9-AA; sodium azide (5 ug)- NaN₃

2. CHO chromosome aberration assay (vol. 1.40, pp 66-141, RR-815-97-008)

Method- CHO-K1 Chinese hamster cells were exposed to 4.9, 19, 78, 312, and 1250 ug/ml (for cytotoxic assay), or, 1.25, 2.5, 5, 10, 20, 40 and 60 ug/ml (for chromosome aberration assay) LG1069 (lot # LGD 100069-000Z035) in a vehicle (1 % DMSO) in the presence or absence of S9 fraction (positive controls- methyl methane sulfonate (MMS 20 ug/ml) without S9 and cyclophosphamide (CP 12.5 ug/ml) with S9) for 3 h and further allowed to grow for 21 h and 45 h. Colcemid (0.2 ug/ml) was added 2.5 h before harvest to induce mitotic arrest of cells. End points: cytotoxic assay- mitotic index (% cells in mitosis) in at least 500 cells per culture, chromosome aberration assay- chromosome aberrations in 100 cells per culture and polyploidy (45 h harvest only) from a minimum of 100 mitosis.

GLP statement- yes

Results- Negative. The highest concentration (5 ug/ml) selected in the absence of S9 activation appears to be not adequate since the concentration did not cause at least 50% reduction in cell number or confluency, as recommended by ICH S2A.

Cytotoxicity Assay

Treatment	Time, h	Mitotic Index (%)		Relative Confluence (%)	
		-S9	+S9	-S9	+S9
LG 1069, ug/ml					
0	21	8.4	10.8	100	100
4.9	21	7.6	12.3	76-100	76-100
19	21	4.5	11.3	26-50	76-100
78	21	suppressed mitosis/toxicity	suppressed mitosis/tox	0-25	0-25
312	21	precipitation /toxicity	precipitation /toxicity	51-75	0-25
1250	21	precipitation /toxicity	precipitation /toxicity	51-75	0-25

Chromosome Aberration Assay (21 h exposure; 200-cell analyzed)

S9	-S9					+S9				
	Treatment	DMSO	LG1069			MMS	DMSO	LG1069		
Conc. ug/ml	1%	1.25	2.5	5	20	1%	20	40	60	12.5
Mitotic index (%)	8.4	6.0	7.1	5.0	4.6	10.2	7.8	6.4	4	4.3
%cells, structurally abnormal chromosome	2.5	3.0	1.5	1.5	30.3	2.0	1.0	2.5	5.5	36.0
%cells. chromosome exchanges	1.5	3.0	1.0	0	15.2	2.0	0.5	2.0	3.5	22.9
Aberrations (frequency/cell)										
Structural aberrations	0.03	0.04	0.02	0.02	0.44	0.02	0.01	0.03	0.07	0.74
Chromosome exchange	0.02	0	0.01	0.02	0.02	0	0.01	0.01	0.02	0
chromatid deletion	0.01	0.04	0.01	0	0.20	0.02	0.01	0.02	0.04	0.31
% cells. chromatic gaps	2.0	3.5	3.5	0.5	4.5	4.0	3.0	1.0	1.5	4.2

3. L5178Y mouse lymphoma cell tk+/tk- gene mutation assay (vol 1.40, pp 142- , RR-815-97-009,

Method- L5178Y mouse lymphoma cells (6×10^6 cells/culture of 10-ml RPMI 1640 medium supplemented with 0.1% Pluronic F68, 0.22 mg/ml sodium pyruvate and 5% heat-inactivated donor horse serum: clone 3.7.2C heterozygous at the tk locus) were exposed to LG1069 (13, 16, 21, 26, 32 and 40 ug/ml LG 1069, lot # LG100069-000Z035), or vehicle (1% DMSO) only, or a positive control (methyl methanesulfonate (MMS 5 ug/ml), ethyl methanesulfonate (EMA 200 ug/ml), or 20-methylcholanthrene (MCA 5 ug/ml)) for 4 h in the presence or absence of S9 fraction. (0.1 ml per 10-ml culture medium). After removing treatment solutions by centrifugations, the resuspended cell were rotated in a roller drum for 2 days for expression of any mutations. Approximately 3×10^6 cells from each culture were seeded in 100 ml of cloning medium supplemented with trifluorothymidine (TFT 5 ug/ml) for selection of TFT-resistant cells and a serial dilution containing approximately 600 c.f.u.s was seeded in 100 ml of nonselective cloning medium to determine the percentage of viable cells. Cloning medium with cells was poured into 100-mm Petri dishes (3 dishes/100 ml of culture medium). Cells were incubated for 12 days. At the end of the 12-day culture, the colonies of cells in each Petri dish were counted using an Artek Model 880 automatic colony counter with a standard 50-mm lens.

Criteria for positivity-

- a significant ($p < 0.05$) dose-related increase in the mutant frequency (frequency of TFT-resistant colonies) occurred,
- the mean mutant frequency of a set of duplicate cultures treated with one or more of the three highest acceptable concentrations of LG1069 was statistically significant ($p < 0.05$),
- at least one concentration induced an average absolute increase in mutant frequency greater than 70×10^{-6} , and
- the results were reproducible in a second experiment.

GLP statement- yes

Results- Negative

S9 Activation Treatment	-S9 Fraction		+S9 Fraction	
	Relative Cloning Efficiency (%)	Average Mutant Frequency (%)	Relative Cloning Efficiency (%)	Average Mutant Frequency (%)
Control. 1% DMSO	100	77	100	75
LG1069, ug/ml				
13	138	63	102	55
16	96	67	136	47
21	74	88	105	64
26	4.5	32	13	49
32	0	0	1.5	175
40	not cloned	not cloned	0	100
MMS. 5 ug/ml	97	318		
EMS. 200 ug/ml	105	488		
MCA. 5 ug/ml			76	569

4. In vivo bone marrow micronucleus assay in mice (vol. 1.41, pp 2-163, RR-815-97-010,

Method- Swiss-Webster mice (15/sex/group, M- 19.9-34.6g, F-20.2-29.6 g, 6-8 weeks of age) received a single oral dose of 0, 250, 500 and 1000 mg/kg LG1069 (in DMSO, lot # LG100069-000Z038) or positive control (urethane 30 mg/kg) by gavage (5 ml/kg). At 24, 48 or 72 h postdose, mice (5/sex/group per each sampling time) were euthanized, both femurs were removed, and 3 bone marrow slides were prepared. For cytological analysis, the number of polychromatic erythrocytes (PCE) among 200 erythrocytes per animal and the number of micronucleated PCE among a total of 2000 PCE per animal were determined.

Criteria for positivity-

- there was a statistically significant ($p < 0.05$) increase in micronucleated PCE,
- the increase was dose-related, and
- the micronucleated PCE frequency was greater than the mean historical micronucleus vehicle frequency + 2SD (e.g.. 0.19 + 0.46).

GLP statement- yes

Results- negative

Treatment	Number of PCE with micronucleus			Clinical Signs	Body weight gain (d1-4), g
	24 h (M/F)	48 h (M/F)	72 h (M/F)		
LG 1069, mg/kg					
0	25/15	28/15	37/14	Unremarkable (UR)	1.5/0.4
250	28/19	28/21	26/23	UR	0.2/0.6
500	16/22	26/20	31/23	UR	0.6/1.5
1000	24/22	32/19	36/21	UR	1.5/0.2
Urethane. 30 mg/kg	57	29	27	UR	1.3

Overall Genetic Toxicity Summary- LG1069 is not mutagenic in bacterial (Salmonella and E.coli) or mammalian cell (mouse lymphoma L5178Y tk^{+/+}) mutation assays, or clastogenic in vitro (CHO cells) or in vivo (micronucleus test in mice). Note that the CHO cell assay in the absence of S9 was not an adequate test due to the absence of cytotoxicity at the highest concentration tested. This result should therefore not be included in the label.