CENTER FOR DRUG EVALUATION AND RESEARCH

APPLICATION NUMBER:

021196Orig1s000

PHARMACOLOGY REVIEW(S)

MEMORANDUM

To: File, NDA 21-196

Through: Robert Temple, M.D., ODE I Office Director
Russell Katz, M.D., Division Director, Neuropharmacologic Drug Products
Barry Rosloff, Ph.D., Pharmacology Supervisor, HFD-120
Anna Marie Hommonay R.Ph., Project Manager, HFD-120

From: Jeri El-Hage, Ph.D., ODE I Associate Director for Pharmacology/Toxicology

Subject: NDA 21-196, Xyrem®, sodium oxybate (sodium gamma hydroxybutyrate)
Tertiary Review of Pharmacology/Toxicology Data

Date: April 8, 2002

The preclinical pharmacology and toxicology data submitted in support of the approval of Xyrem suggest that the chronic administration of gamma hydroxybutyrate (GHB) to animals was associated minimal systemic toxicity. Therefore, I concur with Dr Rosloff's recommendation that the NDA is approvable.
Preclinical studies submitted in support of the NDA include complete genotoxicity and reproductive toxicity batteries and 6-month rat and 12-month dog oral toxicity studies with GHB. The published results of 2-year oral carcinogenicity studies conducted by the National Toxicology Program (NTP) with gamma-butyrolactone (GBL) in mice and rats were also provided . GBL is extensively converted to GHB *in vivo*. A separate 2-year rat carcinogenicity study with GHB has been recently completed and the results will be submitted as a Phase 4 commitment.

The genotoxic potential of GHB was evaluated in an Ames test, an *in vitro* chromosomal aberration assay in Chinese hamster ovary cells, and an *in vivo* rat micronucleus assay. GHB tested negative in all three assays. The NDA review (p.102) suggests that higher doses of GHB could have been utilized in the rat micronucleus assay. However, the high dose of 2000 mg/kg/day is considered the maximum required dose for testing in the *in vivo* micronucleus assay. Therefore, the completed genotoxicity battery is adequate.

The only drug-related adverse effects observed in the chronic oral toxicity studies were hypoactivity and mild decreases in food consumption and body weight gain in high dose rats and high dose dogs. The high doses tested, namely 1000 mg/kg/day in rats and 600/900 mg/kg/day in dogs, produce exposures comparable to (1-2 times) therapeutic exposures with the maximum recommended human dose of 9 grams/day. The animal to human exposure ratios (safety margins) are comparable regardless of whether the comparison is based on body surface area (mg/M^2) or actual pharmacokinetics (AUC) .

The effects of GHB on fertility, reproductive performance, embryo-fetal and postnatal development were evaluated in the standard battery of studies which included a rat fertility study, rat and rabbit embryo-fetal toxicity studies, and a rat pre/postnatal development study.
No compound-related reproductive or developmental adverse effects were observed in any of the studies. Similar to the oral toxicity studies, the highest doses evaluated in the reproductive toxicity studies produce drug exposures in animals comparable to human therapeutic exposures. The high dose of 1200 mg/kg/day utilized in the rabbit teratology study was associated with decreased food consumption and significant decreases in maternal weight gain supporting the

adequacy of the doses tested in the rabbit. Data were not provided in the NDA review to demonstrate that the 1000 mg/kg/day high dose evaluated in the rat studies was adequately high (i.e., associated with minimal maternal toxicity).

The rodent carcinogenicity studies conducted by the NTP evaluated gamma butyrolactone(GBL), which is extensively converted to GHB *in vivo*.
2-Year Study in B6C3F1 mice: Doses evaluated were 0, 262, and 525 mg/kg/day in both sexes. (50/sex/dose). There was no evidence of carcinogenic potential associated with chronic oral administration of GBL.
The high dose of 525 mg/kg/day GBL exceeded the maximum tolerated dose (MTD) in male mice since significant mortality was observed (76% at HD vs. 30% in controls). The high dose of 525 mg/kg/day GBL also represented the MTD in female mice since the final mean body weights were reduced 14-17% in HD female mice. A separate study was conducted to determine the plasma GHB exposures (AUC) after direct dosing with the MTD of GHB in mice (1000 mg/kg/day) or the high dose of 525 mg/kg/day GBL tested in the 2-year mouse CA study (see NDA review, p. 93). This was performed to determine adequacy of the completed mouse study with GBL to assess the carcinogenic potential of GHB. This evaluation demonstrated that GHB exposures after dosing with the high dose of 525 mg/kg/day GBL in mice were approximately 50% in males and 70% in females of those achieved after direct dosing with gamma hydroxybutyrate at the MTD. Therefore, it was concluded that the mouse study with GBL could be considered an adequate carcinogenicity assessment since it was conducted at GHB exposures in mice equivalent to 50% of those attained with the maximum tolerated dose of gamma hydroxybutyrate. (Carcinogenicity studies conducted at half the MTD are generally accepted as adequate).

2-Year Study in F344 rats: Doses evaluated were 0, 112, 225 mg/kg/day in males; 0, 225, 525 mg/kg/day in females (50/sex/dose). GBL produced no increases in neoplastic or non-neoplastic lesions in this study. However, doses of GBL evaluated in the rat did not represent a maximum tolerated dose (MTD) since they were not associated with excess mortality, decreased mean body weight, or any significant increase in tissue pathology or tumors.
In addition, the GHB exposures in rats after administration of the high doses of GBL were only fractions of the AUC exposure to GHB associated direct dosing of the maximum tolerated dose of GHB to rats (8% in males, 35% in females; NDA review p. 96). It was concluded that the rat study with GBL was not an adequate assessment of the carcinogenic potential of GHB. A 2-year rat study with GHB was conducted at FDA request. The 2-year rat carcinogenicity study with GHB has been completed and the sponsor has stated that no evidence of carcinogenic potential was observed. The Division has agreed to accept the results of the 2- year rat study with GHB as a Phase 4 commitment.

Assessments of carcinogenic potential are generally required prior to approval. The Division's decision to allow post-approval submission of the rat carcinogenicity study results for GHB appears reasonable based on the other available data suggesting a minimal carcinogenic risk. These supportive data include:
1) the absence of evidence of genotoxic potential
2) the absence of tissue proliferative effects in chronic toxicity studies (i.e., no evidence for potential carcinogenic effects via non-genotoxic mechanisms)
3) no evidence of carcinogenic potential in a 2-year mouse study with GBL (at GHB exposures half the MTD for GHB).

A labeling review has been conducted (NDA review pp. 103 and 104) and accurately represents the study findings.

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

/s/

Jeri El Hage
4/8/02 04:08:34 PM
PHARMACOLOGIST

Department of Health and Human Services
Public Health Service
Food and Drug Administration

Request for Consultation

Date: 4/18/2002

To: HFD-710/Jin/Kelly

From: Neuropharmacology, HFD-120

IND/NDA No. NDA 21-196

Drug Name sodium oxybate
Trade Name Xyrem
Sponsor Orphan Medical
Indication cataplexy

Type of Document post-NDA submission

Date of Document 4/3/2002

Reason for Request

Orphan Medical has submitted the requested Phase IV carcinogenicty study. Dr. Kathy Haberny is the HFD-120 assigned pharm/tox reviewer. She has requested a stat consult of the carc study results. I believe the submission is in the electronic document room under NDA 21-196.

Thank You,

Anna Marie Homonnay
X4-5535

Signature of Requester	Method of Delivery (Check One) ( ) Mail ( ) Hand
Signature of Receiver	Signature of Deliverer

Barry N. Rosloff, Ph.D.
2/2/01

PHARMACOLOGIST REVIEW OF NDA 21-196

ORIGINAL SUMMARY

SPONSOR: Orphan Medical
13911 Ridgedale Drive
Minnetonka, Minnesota 55305

DRUG: sodium gamma hydroxybutyrate (GHB) (Xyrem™)

(See attached page for other names and structure)

CATEGORY: endogenously-occurring compound; CNS depressant at pharmacologic doses; proposed here for narcolepsy

RELATED IND: IND (b)(4) (Companion to present NDA)

7.1.2 Description, Including Physical and Chemical Characteristics and Stability

a. Nomenclature

Chemical Names: Sodium Oxybate
Sodium Gamma Hydroxybutyrate
4-hydroxybutanoic acid (b)(4) sodium salt

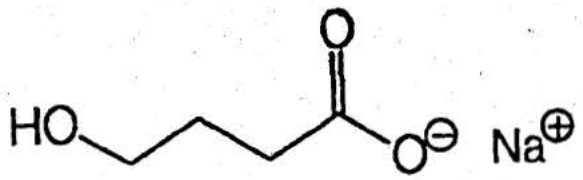
CAS Number: 502-85-2

Abbreviation: GHB

Trade Name: Xyrem™

Generic Name: Sodium Gamma Hydroxybutyrate

b. Chemical Structure



GHB

M.W. (Na salt) = 126.09

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6 MONTH P.O. TOXICITY IN RATS

A) Dosage

20/sex at 0, 150, 350, or 1000 mg/kg/day, by gavage.

Strain: Sprague – Dawley Crl: CD BR

Laboratory:

(b) (4)

Drug Lot # : 9501

B) Results

1) Observed signs

"Hypoactivity" seen in 11 HD M and 14 HD F. This was seen quite sporadically, i.e. on 1-6 days each among HD M and on 1-11 days each among HD F. It was seen only in the first 2 (M) or 3 (F) months of the study.

2) Mortality

One control F, 3 LD F, and 1 HD F died, not considered drug-related.

3) Bodyweight gain

Weights at HD were below controls beginning weeks 3-4; mean weights near end of study were ~ 90% of control. Weight gain was slightly less than controls in LD and MD M; this was not dose-related and mean weights were not statistically significantly different from controls.

4) Food Consumption

Decreased at HD periodically over most of the treatment period. Overall consumption 97% and 92% of control in HD M and HD F, respectively.

5) Ophthalmoscopic exam
(Done pre-study and during week 26)

No drug effects

6) Hematology
(Done at termination)

1) Total WBC, and numbers of lymphocytes and segmented neutrophils, were slightly decreased in HD M; mean ~ 75% of control. A slight/ equivocal decrease in total WBC and lymphocytes seen in HD F.

2) RBC, Hb, and HCT were very slightly below controls in all M groups, not clearly D-R.

3) Other parameters measured: platelets, PT, APTT, rest of differential.

7) Clinical chemistry
(Done at termination)

No clear/pronounced drug effects. Very slight/equivocal effects included decreased total protein, albumin, and potassium in HD M, and decreased inorganic phosphorus in HD F.

Other parameters measured: ALT, AST, AP, GGT, total bilirubin, glucose, urea N, creatinine, globulin, amylase, Ca, Na, Cl.

8) Urinalysis
(Done at termination)

Text states the results were "generally unremarkable and comparable between the groups." Summary tables were not presented; from the individual animal data it is evident that urine pH was greater than controls in all M groups and in HD F; mean values were approximately 1-1.5 units above control at HD.

Other parameters measured: volume, SG, appearance, glucose, ketones, protein, occult blood, microscopic exam of sediment.

9) Organ weights

No clear drug effects aside from changes in absolute or relative weights at HD likely secondary to decreased bodyweights.

10) Gross pathology

No drug effects

11) Microscopic pathology

(Routine exam in controls and HD only. Gross lesions, lung, liver, and kidney were examined in all groups).

No drug effects.

12) Plasma drug levels

Samples taken during week 26 at 0.5, 1, 2, 4, and 24 hours post-dose; N=3/time point. Results are shown in the attached tables. AUCs increased proportionally to dose between LD and MD F, but greater than proportionally in other cases. Levels in M and F were similar.

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RAT - 26 WEEK

Text Table 2
Mean Concentration (μg/mL) at Each Time Point per Group and Sex

		Males		Females	
Group	Hour	Mean	SD	Mean	SD
2	0.5	39.700	5.667	102.067	24.154
2	1.0	19.873	14.508	18.267	3.121
2	2.0	0.000	0.000	2.877	3.401
2	4.0	0.000	0.000	0.723	1.253
2	24.0	0.000	0.000	0.000	0.000
3	0.5	97.500	11.550	155.033	104.078
3	1.0	111.500	33.775	107.500	12.816
3	2.0	43.633	10.130	14.600	4.729
3	4.0	0.000	0.000	0.000	0.000
3	24.0	0.000	0.000	0.000	0.000
4	0.5	262.333	99.047	344.333	37.820
4	1.0	333.667	57.622	479.000	76.922
4	2.0	369.667	23.544	123.833	57.110
4	4.0	21.927	21.988	28.700	12.857
4	24.0	0.000	0.000	0.000	0.000

SD = Standard Deviation

Text Table 3
Experimental Values of Pharmacokinetic Parameters and Results of Statistical Analyses

Group	Dose	Sex	AUC_{0-24}	$AUMC_{0-24}$	MRT_{0-24}	C_{max}	T_{max}	AUC_{0-24}/Dose	C_{max}/Dose
2	150	M	34.755	24.830	0.7144	39.70	0.5	0.2317	0.2647
3	350	M	197.825	238.900	1.2076	111.50	1.0	0.5652	0.3186
4	1000	M	1177.110	2389.607	2.0301	369.67	2.0	1.1771	0.3697
2	150	F	77.005	79.673	1.0347	102.07	0.5	0.5134	0.6805
3	350	F	180.042	163.183	0.9064	155.03	0.5	0.5144	0.4430
4	1000	F	1032.867	2079.633	2.0135	479.00	1.0	1.0329	0.4790
Statistical Results of ANOVA									
Group p-value					.0599		.1875	.0784	.7797
Sex p-value					.9969		.2254	.8440	.1621

12 MONTH P.O. TOXICITY IN DOGS:

A) Dosage

4/sex at 0, 150, 350, and 600 → 900 mg/kg/day, by oral intubation.

The dosage increase at HD began week 32.

Strain: Beagle

Laboratory: (b) (4) (b) (4)

Drug Lot # : 9501

B) Results

1) Observed signs

a) All HD F had hypoactivity and ataxia on a very few occasions, mainly near the beginning of the study and/or after the dosage was increased to 900 mg/kg at week 32. One HD F also had prostration week 1 and another had slight convulsions week 33. One HD M had ataxia on 3 occasions near the beginning of treatment.

b) Increased incidence/frequency of diarrhea in MD and HD M and HD F.

c) Emesis was seen in most animals; frequency was greater than controls in 1 MD F and 1 HD F.

d) Thin appearance seen in 1 MD M, 1 HD M, and 2 HD F near the beginning of the study; decreased appetite seen sporadically in most of these.

2) Mortality

None

3) Bodyweight

Decreased gain in MD and HD M and HD F early in the study. Weights in M remained below control throughout the study (final weights 95% and 91% of control in MD M and HD M, resp.); weights in HD F returned to control level by week 14. (One HD F lost a relatively large amount of weight, 1.7 kg, shortly after increase in dosage, but rebounded rapidly).

4) Food consumption

Decreased in HD M (~ 60-75% of control, mainly due to a large effect in 1 dog) and HD F (~30-60% of control) during first 3-4 weeks. The HD F mentioned above which transiently lost weight upon dosage escalation also had a transiently reduced food consumption at this time.

5) Ophthalmoscopic exam
(Done pre-study and week 52)

No drug effects

6) EKG
(Done pre-study and "during" weeks 26 and 52; the time relative to dosing when this was done was not stated).

It was stated that EKGs were within normal limits; no results were shown.

7) Hematology
(Done pre-study and weeks 13, 26, 39, and 52)

No clear drug effects.

Parameters measured: RBC, Hb, Hct, platelets, PT, APTT, WBC, differential

8) Blood chemistry
(Done pre-study and weeks 13, 26, 39, and 52)

No clear drug effects. Chloride was equivocally slightly decreased (mean 2 meq/L below control) in HD M at most times during treatment.

Other parameters measured: ALT, AST, total bilirubin, GGT, total protein, albumin, globulin, glucose, urea N, creatinine, Ca, Pi, Na, K

9) Urinalysis
(Done pre-study and weeks 13, 26, 39, and 52)

Text states that results were "generally unremarkable and comparable between the control and treated groups". Summary tables were not presented; from the individual animal data it is evident that urine pH was greater than controls in HD M and MD and HD F at all times during treatment; sporadic increases were also seen at the lower doses. Mean values averaged ~ 1-1.5 units above control. Also, urine SG was slightly below control in HD M weeks 39 and 52.

Other parameters measured: appearance, glucose, ketones, protein, bilirubin, occult blood, sediment exam.

10) Organ weights

Absolute and relative liver weights slightly increased in HD M. (Rel. wt. 1.24 x control). Relative kidney weight slightly increased in HD M (1.19 x control).

11) Gross pathology

Pale/dark raised area in lung seen in 1 MD M, 2 HD M, and 1 HD F. See below for histology.

12) Microscopic pathology
(Routine exam done in all animals)

a) Lung- Chronic active inflammation seen in the 4 dogs with gross lesions noted above. Two of these dogs (1 MD M and 1 HD M) also had pigmented macrophages. The text states that these lesions "could be related to the irritation caused by the aspirated test article."

b) Esophagus- atrophy of submucosal gland seen in 7/8 MD and 8/8 HD; none in other groups. "Moderate" or "moderately severe" in most cases.

c) Mandibular salivary gland- atrophy in 6/8 MD and 7/8 HD; none in other groups. Severity ranged from "minimal" to "moderately severe".

13) Plasma drug levels

(Samples taken weeks 4, 30, 32, and 52, at 0.25, 0.5, 1, 2, 4, 8, and 24 hr. post-dose [with the exception that no samples were taken at 8 hours post-dose at week 4]. The samples at week 32 were presumably taken on the first day of the dosage increase at HD to 900 mg/kg).

Results are shown in the attached tables. (Note that no results were shown for LD and MD at weeks 30 and 32; the study report is contradictory as to whether or not samples were taken at these times in these groups).

The following conclusions may be drawn regarding the AUC values; however, note that these cannot be said to be well-established in view of the small sample sizes and large inter-animal variation (e.g. see "Text Table 1"):

a) AUCs increased much greater than proportionally to dose between LD (150 mg/kg) MD (350 mg/kg), but equal to or less than proportionally between MD and HD (600/900 mg/kg).

b) At LD, AUCs were similar between weeks 4 and 52; at MD, AUC at 52 weeks was about 2/3 that at 4 weeks. At HD, AUC decreased by about half between weeks 4 and 30; when the dose was increased to 900 mg/kg at week 32, AUCs were similar to those seen at week 4 (at 600 mg/kg).

c) AUCs were roughly similar between sexes.

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1 year dog

(b) (4) 6627-118

Text Table 1 - AUC_{0-24} (µg×hour/mL)

Group	Sex	Animal	Week 4	Week 30	Week 32	Week 52
2	M	33507	231.275			313.325
2	M	33508	196.250			259.575
2	M	33509	233.450			471.550
2	M	33510	265.750			372.475
2	F	33511	192.875			434.300
2	F	33512	31.245			13.625
2	F	33513	0.833			74.050
2	F	33514	237.300			351.675
3	M	33515	3353.375			1962.000
3	M	33516	1401.525			2119.125
3	M	33517	1994.125			971.125
3	M	33518	3646.125			1933.700
3	F	33519	2051.375			466.650
3	F	33520	4138.625			3222.750
3	F	33521	2942.250			2538.875
3	F	33522	324.450			129.538
4	M	33523	4152.625	2705.300	4987.875	5321.875
4	M	33524	2675.625	2515.400	5708.250	2671.125
4	M	33525	3466.750	1093.150	3990.825	4611.625
4	M	33526	5733.375	2222.000	4630.250	847.575
4	F	33527	2705.250	1761.250	3084.625	4021.250
4	F	33528	2881.125	169.925	4803.125	620.025
4	F	33529	4911.625	2683.500	772.550	4703.625
4	F	33530	3388.750	1981.750	5050.925	5180.500

1 year dog

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Text Table 4 - C_{max} (µg/mL)

Group	Sex	Animal	Week 4	Week 30	Week 32	Week 52
2	M	33507	123.00			177.00
2	M	33508	138.00			207.00
2	M	33509	162.00			215.00
2	M	33510	164.00			182.00
2	F	33511	151.00			197.00
2	F	33512	98.10			10.60
2	F	33513	2.22			87.60
2	F	33514	159.00			192.00
3	M	33515	619.00			468.00
3	M	33516	216.00			662.00
3	M	33517	371.00			187.00
3	M	33518	522.00			404.00
3	F	33519	286.00			223.00
3	F	33520	536.00			658.00
3	F	33521	458.00			670.00
3	F	33522	190.00			62.70
4	M	33523	626.00	610.00	752.00	615.00
4	M	33524	294.00	725.00	710.00	553.00
4	M	33525	651.00	257.00	446.00	914.00
4	M	33526	862.00	670.00	777.00	250.00
4	F	33527	512.00	531.00	608.00	702.00
4	F	33528	579.00	128.00	776.00	267.00
4	F	33529	824.00	688.00	263.00	899.00
4	F	33530	472.00	651.00	980.00	1039.0

1 year dog

(b) (4) 6627-118

Text Table 9 - Mean and Standard Deviation (SD) by Group, Sex, and Week

G	S	WK		T_{max}	C_{max}	AUC_{0-24}	$AUMC_{0-24}$	MRT_{0-24}	λ	$t_{1/2}$	AUC_{0-24}/D	C_{max}/D
2	M	4	MEAN	0.62500	146.750	231.68	263.81	1.13830	0.80958	0.88083	1.54454	0.97833
2	M	4	SD	0.25000	19.755	28.40	42.44	0.11543	0.15603	0.17078	0.18932	0.13170
2	M	52	MEAN	0.87500	195.250	354.23	521.75	1.43211	0.81895	0.99501	2.36154	1.30167
2	M	52	SD	0.25000	18.590	90.79	226.90	0.41317	0.44873	0.36670	0.60528	0.12393
2	F	4	MEAN	0.43750	102.580	115.56	119.38	0.74949	2.28894	0.52239	0.77042	0.68387
2	F	4	SD	0.12500	72.157	117.00	133.44	0.39269	2.25357	0.34288	0.78003	0.48105
2	F	52	MEAN	0.87500	121.800	218.41	284.81	1.03801	0.89468	1.17005	1.45608	0.81200
2	F	52	SD	0.25000	89.663	205.87	299.18	0.39452	0.74439	0.75620	1.37245	0.59775
3	M	4	MEAN	1.00000	432.000	2598.79	7970.96	3.07913	0.35369	2.01314	5.77508	0.96000
3	M	4	SD	0.70711	176.490	1074.77	3272.15	0.12724	0.07211	0.35015	2.38837	0.39220
3	M	52	MEAN	1.25000	430.250	1746.49	4454.98	2.56812	0.33084	3.76643	3.88108	0.95611
3	M	52	SD	0.50000	195.783	523.30	1275.31	0.19789	0.23576	3.78294	1.16290	0.43507
3	F	4	MEAN	1.37500	367.500	2364.18	7461.66	2.66719	0.46566	1.66154	5.25372	0.81667
3	F	4	SD	0.75000	157.830	1606.36	5683.14	1.06868	0.20037	0.54543	3.56969	0.35073
3	F	52	MEAN	1.00000	403.425	1589.45	4247.69	2.03961	0.42975	1.68741	3.53212	0.89650
3	F	52	SD	0.00000	307.960	1523.28	4815.95	0.90855	0.09557	0.44985	3.38506	0.68435
4	M	4	MEAN	1.25000	608.250	4007.09	12201.22	3.05301	0.34762	2.08426	6.67849	1.01375
4	M	4	SD	0.50000	234.723	1299.49	4013.65	0.20233	0.08747	0.48785	2.16581	0.39121
4	M	30	MEAN	1.75000	565.500	2133.96	5220.28	2.47508	0.54435	2.78359	3.55660	0.94250
4	M	30	SD	0.50000	210.961	721.79	1813.91	0.29685	0.31231	3.56355	1.20299	0.35160
4	M	32	MEAN	1.87500	671.250	4829.30	21664.28	4.49537	0.24300	3.02648	5.36589	0.74583
4	M	32	SD	1.54785	152.690	716.56	3861.13	0.52571	0.06079	0.93687	0.79617	0.16966
4	M	52	MEAN	1.75000	583.000	3363.05	12755.51	3.26292	0.52170	1.81803	3.73672	0.64778
4	M	52	SD	0.50000	272.258	2016.79	10510.67	1.22156	0.33535	1.15643	2.24088	0.30251
4	F	4	MEAN	0.75000	596.750	3471.69	10039.39	2.87534	0.39364	1.88514	5.78615	0.99458
4	F	4	SD	0.28868	157.800	1002.74	3174.57	0.16350	0.10689	0.62174	1.67124	0.26300
4	F	30	MEAN	1.62500	499.500	1649.11	3617.45	1.90275	0.75489	1.08978	2.74851	0.83250
4	F	30	SD	0.75000	256.573	1061.63	2599.65	0.61072	0.34756	0.53536	1.76938	0.42762
4	F	32	MEAN	1.50000	656.750	3427.81	12511.69	3.25202	0.42243	1.73218	3.80867	0.72972
4	F	32	SD	0.57735	303.385	1974.35	8537.63	0.92982	0.10968	0.47713	2.19372	0.33709
4	F	52	MEAN	1.37500	726.750	3631.35	12544.98	3.01627	0.33830	2.31475	4.03483	0.80750
4	F	52	SD	0.75000	336.230	2063.15	7875.31	1.09191	0.10986	1.09524	2.29239	0.37359

G = Group
S = Sex
WK = Week
AUC_{0-24}/D = AUC_{0-24}/Dose
C_{max}/D = C_{max}/Dose

SEGMENT I REPRODUCTION IN RATS:

A) Dosage

20/sex at 0, 150, 350, or 1000 mg/kg/day, by gavage.

Males were treated for 10 weeks beginning 4 weeks pre-mating. Females were treated from 2 weeks pre-mating through day 13 of gestation, at which time they were sacrificed/ caesarian sectioned. Mating ratio = 1:1.

Strain: Sprague-Dawley Crl : CD BR

Laboratory: [redacted] (b) (4)

Drug Lot #: 9501

B) Results

1) Observed signs

Five HD M and 6 HD F had "hypoactivity"; in 9 of these rats this was seen only on a single day; in the other 2 rats it was seen on 2 days.

2) Bodyweight

Decreased weight gain in HD M (weights 90-95% of control). No clear effects in F. (Weights in HD F were slightly below controls during treatment, but initial weights were lower to a similar degree).

3) Food Consumption

Decreased in HD M (~92% of control).

4) Male organ weights

No effect on weight of testes, epididymis, seminal vesicles, prostate.

5) Sperm analysis
(10/sex/group)

No drug effect on sperm count, motility, or morphology

6) Mating

No drug effect on estrus cycling, mating performance, or successful matings.

7) Caesarian section data

No drug effects. (See attached table).

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TABLE 11
STUDY OF FERTILITY AND EARLY EMBRYONIC DEVELOPMENT TO IMPLANTATION IN RATS
SUMMARY OF CESAREAN SECTION DATA

		DOSE LEVEL	GROUP 1 0 MG/KG/DAY	GROUP 2 150 MG/KG/DAY	GROUP 3 350 MG/KG/DAY	GROUP 4 1000 MG/KG/DAY
Pregnant at C-section		N	20	19	19	19
Resorptions:	Total	MEAN	0.7	1.3	0.6	1.3
		S.D.	0.7	1.5	0.9	1.3
		N	20	19	19	19
		TOTAL	14	24	12	24
		MEAN%	9.7	11.1	3.8	9.9
		S.D.	22.6	22.3	5.3	15.8
	Early	MEAN	0.7	1.3	0.6	1.3
		S.D.	0.7	1.5	0.9	1.3
		N	20	19	19	19
		TOTAL	14	24	12	24
		MEAN%	9.7	11.1	3.8	9.9
		S.D.	22.6	22.3	5.3	15.8
	Late	MEAN	0.0	0.0	0.0	0.0
		S.D.	0.0	0.0	0.0	0.0
		N	20	19	19	19
		TOTAL	0	0	0	0
		MEAN%	0.0	0.0	0.0	0.0
		S.D.	0.0	0.0	0.0	0.0
Dead Fetuses		TOTAL	0	0	0	0
Postimplantation Loss		MEAN%	9.7	11.1	3.8	9.9
		S.D.	22.6	22.3	5.3	15.8

SIGNIFICANTLY DIFFERENT FROM CONTROL: * = $P \leq 0.05$; ** = $P \leq 0.01$.

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TABLE 11
STUDY OF FERTILITY AND EARLY EMBRYONIC DEVELOPMENT TO IMPLANTATION IN RATS
SUMMARY OF CESAREAN SECTION DATA

	DOSE LEVEL	GROUP 1 0 MG/KG/DAY	GROUP 2 150 MG/KG/DAY	GROUP 3 350 MG/KG/DAY	GROUP 4 1000 MG/KG/DAY
Pregnant at C-section	N	20	19	19	19
Live Fetuses	MEAN	14.4	14.6	16.4	14.0
	S.D.	4.6	4.0	1.8	3.3
	N	20	19	19	19
	TOTAL	289	277	312	266
	MEAN%	90.3	88.9	96.2	90.1
	S.D.	22.6	22.3	5.3	15.8

SIGNIFICANTLY DIFFERENT FROM CONTROL: * = $P \leq 0.05$; ** = $P \leq 0.01$.

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EGMENT II REPRODUCTION IN RATS:

A) Dosage

25 F at 0, 150, 350, or 1000 mg/kg/day, by gavage, days 6-17 of gestation

Dams sacrificed day 20 of gestation. All fetuses examined externally. One half of fetuses processed for visceral exam by Wilson technique. Remaining fetuses processed for skeletal exam by Alizarin Red S staining method.

Strain: Sprague-Dawley Crl: CD BR

Laboratory: (b) (4)

Drug Lot # : 9501

B) Results

1) Observed signs

"Hypoactivity" seen in 5 HD F (on 1 day each in 3; on 2 days each in 2). Ataxia was also seen in 2 of the above on 1 day each. Prostration was seen in an additional HD on a single day.

2) Body weight

No drug effect

3) Food consumption

Equivocal slight decrease at HD during first week of treatment;
mean values 6-8% below controls, generally not statistically significant

4) Gravid uterine weight

No drug effects

5) Reproductive parameters

No drug effects on resorptions, live or dead fetuses, or post-implantation loss.

Fetal weights were slightly greater than controls in all drug groups, not clearly dose-related

6) Fetal exams

Results shown in attached tables.

There were no clear drug effects with the possible exception of an increase in "wavy/bent rib(s)" (fetal incidence 0, 1.7, 4.2, and 4.1% in control, LD, MD, and HD, resp.; litter incidence = 0, 8, 14, and 21%, resp.). (Historical control values not given, although it was stated that the skeletal variations "were generally of the type and frequency commonly noted in this strain of rat.").

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Table 9A
Summary of Fetal External Variations
Rat Developmental Toxicity Study

TABLE 9A
RAT DEVELOPMENTAL TOXICITY STUDY
SUMMARY OF FETAL EXTERNAL VARIATIONS

DOSE LEVEL		GROUP 1 0 MG/KG/DAY	GROUP 2 150 MG/KG/DAY	GROUP 3 350 MG/KG/DAY	GROUP 4 1000 MG/KG/DAY
Litters Evaluated	N	23	25	22	24
Fetuses Evaluated	N	333	331	293	332
Live	N	333	331	293	332
Dead	N	0	0	0	0
TOTAL FETAL EXTERNAL VARIATIONS					
Fetal Incidence	N	0	0	0	0
	%	0.0	0.0	0.0	0.0
Litter Incidence	N	0	0	0	0
	%	0.0	0.0	0.0	0.0

N = Number

(53)

Table 9B
Summary of Fetal External Malformations
Rat Developmental Toxicity Study

TABLE 9B
RAT DEVELOPMENTAL TOXICITY STUDY
SUMMARY OF FETAL EXTERNAL MALFORMATIONS

	DOSE LEVEL	GROUP 1 0 MG/KG/DAY	GROUP 2 150 MG/KG/DAY	GROUP 3 350 MG/KG/DAY	GROUP 4 1000 MG/KG/DAY
Litters Evaluated	N	23	25	22	24
Fetuses Evaluated	N	333	331	293	332
Live	N	333	331	293	332
Dead	N	0	0	0	0
ANOPHTHALMIA					
Fetal Incidence	N	0	1	0	1
	%	0.0	0.3	0.0	0.3
Litter Incidence	N	0	1	0	1
	%	0.0	4.0	0.0	4.2
BRACHYGNATHIA					
Fetal Incidence	N	0	1	0	1
	%	0.0	0.3	0.0	0.3
Litter Incidence	N	0	1	0	1
	%	0.0	4.0	0.0	4.2
NOSE MALFORMED					
Fetal Incidence	N	0	1	0	0
	%	0.0	0.3	0.0	0.0
Litter Incidence	N	0	1	0	0
	%	0.0	4.0	0.0	0.0
COCCYCEPHALY					
Fetal Incidence	N	0	0	0	1
	%	0.0	0.0	0.0	0.3
Litter Incidence	N	0	0	0	1
	%	0.0	0.0	0.0	4.2
ASTOMIA					
Fetal Incidence	N	0	0	0	1
	%	0.0	0.0	0.0	0.3
Litter Incidence	N	0	0	0	1
	%	0.0	0.0	0.0	4.2

N = Number

TABLE 9B
RAT DEVELOPMENTAL TOXICITY STUDY
SUMMARY OF FETAL EXTERNAL MALFORMATIONS

	DOSE LEVEL	GROUP 1 0 MG/KG/DAY	GROUP 2 150 MG/KG/DAY	GROUP 3 350 MG/KG/DAY	GROUP 4 1000 MG/KG/DAY
Litters Evaluated	N	23	25	22	24
Fetuses Evaluated	N	333	331	293	332
Live	N	333	331	293	332
Dead	N	0	0	0	0
TOTAL FETAL EXTERNAL MALFORMATIONS					
Fetal Incidence	N	0	1	0	1
	%	0.0	0.3	0.0	0.3
Litter Incidence	N	0	1	0	1
	%	0.0	4.0	0.0	4.2

N = Number

Table 10A
Summary of Fetal Soft Tissue Variations
Rat Developmental Toxicity Study

TABLE 10A
RAT DEVELOPMENTAL TOXICITY STUDY
SUMMARY OF FETAL SOFT TISSUE VARIATIONS

DOSE LEVEL		GROUP 1 0 MG/KG/DAY	GROUP 2 150 MG/KG/DAY	GROUP 3 350 MG/KG/DAY	GROUP 4 1000 MG/KG/DAY
Litters Evaluated	N	23	25	22	24
Fetuses Evaluated	N	166	159	149	163
Live	N	166	159	149	163
Dead	N	0	0	0	0
DILATATION OF LATERAL VENTRICLE(S)					
Fetal Incidence	N	7	1	3	0
	%	4.2	0.6	2.0	0.0
Litter Incidence	N	4	1	2	0
	%	17	4.0	9.1	0.0
INCREASED RENAL PELVIC CAVITATION					
Fetal Incidence	N	3	4	5	5
	%	1.8	2.5	3.4	3.1
Litter Incidence	N	3	4	3	4
	%	13	16	14	17
DILATED URETER(S)					
Fetal Incidence	N	0	3	0	3
	%	0.0	1.9	0.0	1.8
Litter Incidence	N	0	3	0	3
	%	0.0	12	0.0	13
TOTAL FETAL SOFT TISSUE VARIATIONS					
Fetal Incidence	N	9	7	7	7
	%	5.4	4.4	4.7	4.3
Litter Incidence	N	5	7	3	6
	%	22	28	14	25

N = Number

Table 10B
Summary of Fetal Soft Tissue Malformations
Rat Developmental Toxicity Study

TABLE 10B
RAT DEVELOPMENTAL TOXICITY STUDY
SUMMARY OF FETAL SOFT TISSUE MALFORMATIONS

DOSE LEVEL		GROUP 1 0 MG/KG/DAY	GROUP 2 150 MG/KG/DAY	GROUP 3 350 MG/KG/DAY	GROUP 4 1000 MG/KG/DAY
Litters Evaluated	N	23	25	22	24
Fetuses Evaluated	N	166	159	149	163
Live	N	166	159	149	163
Dead	N	0	0	0	0
INTERNAL HYDROCEPHALY					
Fetal Incidence	N	1	1	0	0
	%	0.6	0.6	0.0	0.0
Litter Incidence	N	1	1	0	0
	%	4.3	4.0	0.0	0.0
TOTAL FETAL SOFT TISSUE MALFORMATIONS					
Fetal Incidence	N	1	1	0	0
	%	0.6	0.6	0.0	0.0
Litter Incidence	N	1	1	0	0
	%	4.3	4.0	0.0	0.0

N = Number

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Table 11A
Summary of Fetal Skeletal Variations
Rat Developmental Toxicity Study

TABLE 11A
RAT DEVELOPMENTAL TOXICITY STUDY
SUMMARY OF FETAL SKELETAL VARIATIONS

DOSE LEVEL		GROUP 1 0 MG/KG/DAY	GROUP 2 150 MG/KG/DAY	GROUP 3 350 MG/KG/DAY	GROUP 4 1000 MG/KG/DAY
Litters Evaluated	N	23	25	22	24
Fetuses Evaluated	N	167	172	144	169
Live	N	167	172	144	169
Dead	N	0	0	0	0
INCOMPLETE/UNOSSIFIED HYOID BODY					
Fetal Incidence	N	39	52	29	41
	%	23	30	20	24
Litter Incidence	N	17	14	12	14
	%	74	56	55	58
INCOMPLETE OSSIFICATION OF SKULL					
Fetal Incidence	N	21	25	20	22
	%	13	15	14	13
Litter Incidence	N	12	9	9	9
	%	52	36	41	38
FONTANELLE ENLARGED					
Fetal Incidence	N	0	0	0	1
	%	0.0	0.0	0.0	0.6
Litter Incidence	N	0	0	0	1
	%	0.0	0.0	0.0	4.2
INCOMPLETE OSSIFICATION OF VERTEBRAL ARCH(ES)					
Fetal Incidence	N	48	40	40	39
	%	29	23	28	23
Litter Incidence	N	17	13	18	13
	%	74	52	82	54
LESS THAN FOUR CAUDAL VERTEBRAE OSSIFIED					
Fetal Incidence	N	79	44	43	49
	%	47	26	30	29
Litter Incidence	N	23	16	17	13
	%	100	64	77	54
UNOSSIFIED VERTEBRAL CENTRUM(A)					
Fetal Incidence	N	3	2	0	3
	%	1.8	1.2	0.0	1.8
Litter Incidence	N	3	2	0	3
	%	13	8.0	0.0	13

N = Number

TABLE 11A
RAT DEVELOPMENTAL TOXICITY STUDY
SUMMARY OF FETAL SKELETAL VARIATIONS

DOSE LEVEL		GROUP 1 0 MG/KG/DAY	GROUP 2 150 MG/KG/DAY	GROUP 3 350 MG/KG/DAY	GROUP 4 1000 MG/KG/DAY
Litters Evaluated	N	23	25	22	24
Fetuses Evaluated	N	167	172	144	169
Live	N	167	172	144	169
Dead	N	0	0	0	0
BIPARTITE VERTEBRAL CENTRUM(A)					
Fetal Incidence	N	2	2	3	1
	%	1.2	1.2	2.1	0.6
Litter Incidence	N	2	2	3	1
	%	8.7	8.0	14	4.2
HEMICENTRUM(A)					
Fetal Incidence	N	0	0	0	1
	%	0.0	0.0	0.0	0.6
Litter Incidence	N	0	0	0	1
	%	0.0	0.0	0.0	4.2
25 PRESACRAL VERTEBRAE					
Fetal Incidence	N	1	0	0	0
	%	0.6	0.0	0.0	0.0
Litter Incidence	N	1	0	0	0
	%	4.3	0.0	0.0	0.0
5TH STERNEBRA UNOSSIFIED					
Fetal Incidence	N	93	87	66	76
	%	56	51	46	45
Litter Incidence	N	22	25	20	21
	%	96	100	91	88
5TH/6TH STERNEBRA(E) INCOMPLETE OSSIFICATION					
Fetal Incidence	N	140	141	115	146
	%	84	82	80	86
Litter Incidence	N	23	25	22	24
	%	100	100	100	100
OTHER STERNEBRA(E) INCOMPLETE OSSIFICATION					
Fetal Incidence	N	8	10	5	11
	%	4.8	5.8	3.5	6.5
Litter Incidence	N	5	4	4	8
	%	22	16	18	33

N = Number

TABLE 11A
RAT DEVELOPMENTAL TOXICITY STUDY
SUMMARY OF FETAL SKELETAL VARIATIONS

DOSE LEVEL		GROUP 1 0 MG/KG/DAY	GROUP 2 150 MG/KG/DAY	GROUP 3 350 MG/KG/DAY	GROUP 4 1000 MG/KG/DAY
Litters Evaluated	N	23	25	22	24
Fetuses Evaluated	N	167	172	144	169
Live	N	167	172	144	169
Dead	N	0	0	0	0
STERNEBRA(E) ASYMMETRICALLY OSSIFIED					
Fetal Incidence	N	10	5	5	7
	%	6.0	2.9	3.5	4.1
Litter Incidence	N	7	5	5	7
	%	30	20	23	29
OTHER STERNEBRA(E) UNOSSIFIED					
Fetal Incidence	N	5	1	2	1
	%	3.0	0.6	1.4	0.6
Litter Incidence	N	2	1	2	1
	%	8.7	4.0	9.1	4.2
6TH STERNEBRA UNOSSIFIED					
Fetal Incidence	N	6	5	1	2
	%	3.6	2.9	0.7	1.2
Litter Incidence	N	3	3	1	2
	%	13	12	4.5	8.3
5TH/6TH STERNEBRA(E) BIPARTITE					
Fetal Incidence	N	0	1	0	0
	%	0.0	0.6	0.0	0.0
Litter Incidence	N	0	1	0	0
	%	0.0	4.0	0.0	0.0
OTHER STERNEBRA(E) BIPARTITE					
Fetal Incidence	N	0	0	0	1
	%	0.0	0.0	0.0	0.6
Litter Incidence	N	0	0	0	1
	%	0.0	0.0	0.0	4.2
14TH RUDIMENTARY RIB(S)					
Fetal Incidence	N	0	2	2	0
	%	0.0	1.2	1.4	0.0
Litter Incidence	N	0	2	2	0
	%	0.0	8.0	9.1	0.0

N = Number

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TABLE 11A
RAT DEVELOPMENTAL TOXICITY STUDY
SUMMARY OF FETAL SKELETAL VARIATIONS

DOSE LEVEL		GROUP 1 0 MG/KG/DAY	GROUP 2 150 MG/KG/DAY	GROUP 3 350 MG/KG/DAY	GROUP 4 1000 MG/KG/DAY
Litters Evaluated	N	23	25	22	24
Fetuses Evaluated	N	167	172	144	169
Live	N	167	172	144	169
Dead	N	0	0	0	0
13TH RUDIMENTARY RIB(S)					
Fetal Incidence	N	7	4	4	3
	%	4.2	2.3	2.8	1.8
Litter Incidence	N	4	4	4	3
	%	17	16	18	13
INCOMPLETE OSSIFICATION OF RIB(S)					
Fetal Incidence	N	1	1	5	2
	%	0.6	0.6	3.5	1.2
Litter Incidence	N	1	1	2	2
	%	4.3	4.0	9.1	8.3
WAVY/BENT RIB(S)					
Fetal Incidence	N	0	3	6	7
	%	0.0	1.7	4.2	4.1
Litter Incidence	N	0	2	3	5
	%	0.0	8.0	14	21
7TH CERVICAL RIB(S)					
Fetal Incidence	N	2	0	0	1
	%	1.2	0.0	0.0	0.6
Litter Incidence	N	2	0	0	1
	%	8.7	0.0	0.0	4.2
LESS THAN FOUR METATARSALS OSSIFIED					
Fetal Incidence	N	1	0	0	1
	%	0.6	0.0	0.0	0.6
Litter Incidence	N	1	0	0	1
	%	4.3	0.0	0.0	4.2
UNOSSIFIED PUBIS(ES)					
Fetal Incidence	N	3	4	2	1
	%	1.8	2.3	1.4	0.6
Litter Incidence	N	3	2	2	1
	%	13	8.0	9.1	4.2

N = Number

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TABLE 11A
RAT DEVELOPMENTAL TOXICITY STUDY
SUMMARY OF FETAL SKELETAL VARIATIONS

DOSE LEVEL		GROUP 1 0 MG/KG/DAY	GROUP 2 150 MG/KG/DAY	GROUP 3 350 MG/KG/DAY	GROUP 4 1000 MG/KG/DAY
Litters Evaluated	N	23	25	22	24
Fetuses Evaluated	N	167	172	144	169
Live	N	167	172	144	169
Dead	N	0	0	0	0
UNOSSIFIED ISCHIUM(A)					
Fetal Incidence	N	0	0	1	0
	%	0.0	0.0	0.7	0.0
Litter Incidence	N	0	0	1	0
	%	0.0	0.0	4.5	0.0
INCOMPLETE OSSIFICATION OF ISCHIUM(A)					
Fetal Incidence	N	0	0	1	3
	%	0.0	0.0	0.7	1.8
Litter Incidence	N	0	0	1	3
	%	0.0	0.0	4.5	13
TOTAL FETAL SKELETAL VARIATIONS					
Fetal Incidence	N	156	157	131	155
	%	93	91	91	92
Litter Incidence	N	23	25	22	24
	%	100	100	100	100

N = Number

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Table 11B
Summary of Fetal Skeletal Malformations
Rat Developmental Toxicity Study

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TABLE 11B
RAT DEVELOPMENTAL TOXICITY STUDY
SUMMARY OF FETAL SKELETAL MALFORMATIONS

DOSE LEVEL		GROUP 1 0 MG/KG/DAY	GROUP 2 150 MG/KG/DAY	GROUP 3 350 MG/KG/DAY	GROUP 4 1000 MG/KG/DAY
Litters Evaluated	N	23	25	22	24
Fetuses Evaluated	N	167	172	144	169
Live	N	167	172	144	169
Dead	N	0	0	0	0
ABSENT BONE(S) IN SKULL					
Fetal Incidence	N	0	0	0	1
	%	0.0	0.0	0.0	0.6
Litter Incidence	N	0	0	0	1
	%	0.0	0.0	0.0	4.2
VERTEBRAL ANOMALY WITH/WITHOUT ASSOCIATED RIB ANOMALY					
Fetal Incidence	N	0	0	0	1
	%	0.0	0.0	0.0	0.6
Litter Incidence	N	0	0	0	1
	%	0.0	0.0	0.0	4.2
EXTRA RIB(S)					
Fetal Incidence	N	0	0	0	1
	%	0.0	0.0	0.0	0.6
Litter Incidence	N	0	0	0	1
	%	0.0	0.0	0.0	4.2
TOTAL FETAL SKELETAL MALFORMATIONS					
Fetal Incidence	N	0	0	0	2
	%	0.0	0.0	0.0	1.2
Litter Incidence	N	0	0	0	2
	%	0.0	0.0	0.0	8.3

N = Number

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SEGMENT II REPRODUCTION IN RABBITS

A) Dosage

20 F at 0, 300, 600, or 1200 mg/kg/day, by gavage, days 7-20 of gestation. An additional 3 F in LD and HD were used for TK data.

Does sacrificed day 29 of gestation. All fetuses examined externally, for soft tissue abnormalities using a modification of Staples' technique, for contents of cranium using a midcoronal slice of the head, and for skeletal abnormalities using Alizarin Red S staining method.

Strain: Hra: (NZW) SPF

Laboratory: (b) (4)

Drug lot #: 9501

B) Results

1) Observed signs/mortality

Increased incidence of few or no feces at HD; incidence = 16/23 (seen on 1-14 days each). (Incidence in controls = 1/20, seen on 2 days).

One LD, 2 MD, and 1 HD were found dead; cause of death was not established. One MD and 1 HD aborted; the latter was on day 29 of gestation.

2) Bodyweight

No clear drug effects.

(Text notes that weight gain at HD was about 25% that of controls over the treatment period. However, the amount of gain in all groups was only a small % of the starting bodyweights. There was no difference in bodyweights across groups).

3) Food Consumption

Decreased at HD during the treatment period. (Mean ~75% of control).

4) Gravid uterine weight

No drug effect

5) Reproductive parameters

No drug effect on resorptions, live or dead fetuses, post-implantation loss, or fetal weight

6) Fetal exams

Results shown in attached tables.

There were no clear drug effects. (It is noted that there was a very low incidence [but greater than control] of angulated hyoid wings at HD [see Table "11A"]; 2 of the 5 affected fetuses also had incomplete/unossified hyoid wings. This was not discussed in the text; the text stated that the skeletal variations seen "were of the type and frequency commonly noted in this strain of rabbit").

7) Plasma drug levels

Samples were taken from 3 LD and 3 HD at 15, 30, and 60 minutes post-dose on gestation days 7 and 20, and analyzed for GHB. Results are shown in attached table (following fetal exam results). Levels at 15 min. were roughly proportional to dose; levels at later times were less than proportional. Levels on day 20 were generally greater than those on day 7. However, note that the inter-animal variation was large, thus bringing into question the accuracy of these values and comparisons. Also note that levels in all animals were greatest at the 1 hour measurement, thus neither Cmax nor AUC are obtainable from these data.

Table 9A
Summary of Fetal External Variations
Rabbit Developmental Toxicity Study

TABLE 9A
RABBIT DEVELOPMENTAL TOXICITY STUDY
SUMMARY OF FETAL EXTERNAL VARIATIONS

	DOSE LEVEL	GROUP 1 0 MG/KG/DAY	GROUP 2 300 MG/KG/DAY	GROUP 3 600 MG/KG/DAY	GROUP 4 1200 MG/KG/DAY
Litters Evaluated	N	19	18	16	19
Fetuses Evaluated	N	170	135	138	171
Live	N	170	135	138	170
Dead	N	0	0	0	1
DOME-SHAPED HEAD					
Fetal Incidence	N	0	0	2	0
	%	0.0	0.0	1.4	0.0
Litter Incidence	N	0	0	1	0
	%	0.0	0.0	6.3	0.0
TOTAL FETAL EXTERNAL	VARIATIONS				
Fetal Incidence	N	0	0	2	0
	%	0.0	0.0	1.4	0.0
Litter Incidence	N	0	0	1	0
	%	0.0	0.0	6.3	0.0

N = Number

(43)

Table 9B
Summary of Fetal External Malformations
Rabbit Developmental Toxicity Study

TABLE 9B
RABBIT DEVELOPMENTAL TOXICITY STUDY
SUMMARY OF FETAL EXTERNAL MALFORMATIONS

	DOSE LEVEL	GROUP 1 0 MG/KG/DAY	GROUP 2 300 MG/KG/DAY	GROUP 3 600 MG/KG/DAY	GROUP 4 1200 MG/KG/DAY
Litters Evaluated	N	19	18	16	19
Fetuses Evaluated	N	170	135	138	171
Live	N	170	135	138	170
Dead	N	0	0	0	1
TOTAL FETAL EXTERNAL MALFORMATIONS					
Fetal Incidence	N	0	0	0	0
	%	0.0	0.0	0.0	0.0
Litter Incidence	N	0	0	0	0
	%	0.0	0.0	0.0	0.0

N = Number

Table 10A
Summary of Fetal Soft Tissue Variations
Rabbit Developmental Toxicity Study

TABLE 10A
RABBIT DEVELOPMENTAL TOXICITY STUDY
SUMMARY OF FETAL SOFT TISSUE VARIATIONS

DOSE LEVEL		GROUP 1 0 MG/KG/DAY	GROUP 2 300 MG/KG/DAY	GROUP 3 600 MG/KG/DAY	GROUP 4 1200 MG/KG/DAY
Litters Evaluated	N	19	18	16	19
Fetuses Evaluated	N	170	135	138	171
Live	N	170	135	138	170
Dead	N	0	0	0	1
INTERMEDIATE LOBE OF LUNG SMALL/MISSING					
Fetal Incidence	N	7	8	6	4
	%	4.1	5.9	4.3	2.3
Litter Incidence	N	4	4	4	3
	%	21	22	25	16
GALL BLADDER SMALL					
Fetal Incidence	N	0	1	0	0
	%	0.0	0.7	0.0	0.0
Litter Incidence	N	0	1	0	0
	%	0.0	5.6	0.0	0.0
TOTAL FETAL SOFT TISSUE VARIATIONS					
Fetal Incidence	N	7	9	6	4
	%	4.1	6.7	4.3	2.3
Litter Incidence	N	4	5	4	3
	%	21	28	25	16

N = Number

Table 10B
Summary of Fetal Soft Tissue Malformations
Rabbit Developmental Toxicity Study

TABLE 10B
RABBIT DEVELOPMENTAL TOXICITY STUDY
SUMMARY OF FETAL SOFT TISSUE MALFORMATIONS

DOSE LEVEL		GROUP 1 0 MG/KG/DAY	GROUP 2 300 MG/KG/DAY	GROUP 3 600 MG/KG/DAY	GROUP 4 1200 MG/KG/DAY
Litters Evaluated	N	19	18	16	19
Fetuses Evaluated	N	170	135	138	171
Live	N	170	135	138	170
Dead	N	0	0	0	1
INTERNAL HYDROCEPHALY					
Fetal Incidence	N	0	0	2	0
	%	0.0	0.0	1.4	0.0
Litter Incidence	N	0	0	1	0
	%	0.0	0.0	6.3	0.0
KIDNEY(S) MALPOSITIONED					
Fetal Incidence	N	1	0	1	0
	%	0.6	0.0	0.7	0.0
Litter Incidence	N	1	0	1	0
	%	5.3	0.0	6.3	0.0
TOTAL FETAL SOFT TISSUE MALFORMATIONS					
Fetal Incidence	N	1	0	2	0
	%	0.6	0.0	1.4	0.0
Litter Incidence	N	1	0	1	0
	%	5.3	0.0	6.3	0.0

N = Number

Table 11A
Summary of Fetal Skeletal Variations
Rabbit Developmental Toxicity Study

TABLE 11A
RABBIT DEVELOPMENTAL TOXICITY STUDY
SUMMARY OF FETAL SKELETAL VARIATIONS

DOSE LEVEL		GROUP 1 0 MG/KG/DAY	GROUP 2 300 MG/KG/DAY	GROUP 3 600 MG/KG/DAY	GROUP 4 1200 MG/KG/DAY
Litters Evaluated	N	19	18	16	19
Fetuses Evaluated	N	170	135	138	171
Live	N	170	135	138	170
Dead	N	0	0	0	1
INCOMPLETE/UNOSSIFIED HYOID BODY					
Fetal Incidence	N	1	0	0	1
	%	0.6	0.0	0.0	0.6
Litter Incidence	N	1	0	0	1
	%	5.3	0.0	0.0	5.3
ANGULATED HYOID WING(S)					
Fetal Incidence	N	1	0	1	5
	%	0.6	0.0	0.7	2.9
Litter Incidence	N	1	0	1	4
	%	5.3	0.0	6.3	21
ACCESSORY BONE(S) IN SKULL					
Fetal Incidence	N	0	0	0	1
	%	0.0	0.0	0.0	0.6
Litter Incidence	N	0	0	0	1
	%	0.0	0.0	0.0	5.3
INCOMPLETE/UNOSSIFIED HYOID WING(S)					
Fetal Incidence	N	0	0	0	2
	%	0.0	0.0	0.0	1.2
Litter Incidence	N	0	0	0	2
	%	0.0	0.0	0.0	11
INCOMPLETE OSSIFICATION OF SKULL					
Fetal Incidence	N	0	0	2	0
	%	0.0	0.0	1.4	0.0
Litter Incidence	N	0	0	1	0
	%	0.0	0.0	6.3	0.0
26 PRESACRAL VERTEBRAE					
Fetal Incidence	N	25	31	29	31
	%	15	23	21	18
Litter Incidence	N	10	10	13	11
	%	53	56	81	58

N = Number

TABLE 11A
RABBIT DEVELOPMENTAL TOXICITY STUDY
SUMMARY OF FETAL SKELETAL VARIATIONS

DOSE LEVEL		GROUP 1 0 MG/KG/DAY	GROUP 2 300 MG/KG/DAY	GROUP 3 600 MG/KG/DAY	GROUP 4 1200 MG/KG/DAY
Litters Evaluated	N	19	18	16	19
Fetuses Evaluated	N	170	135	138	171
Live	N	170	135	138	170
Dead	N	0	0	0	1
24 PRESACRAL VERTEBRAE					
Fetal Incidence	N	0	0	1	1
	%	0.0	0.0	0.7	0.6
Litter Incidence	N	0	0	1	1
	%	0.0	0.0	6.3	5.3
LESS THAN 16 CAUDAL VERTEBRAE OSSIFIED					
Fetal Incidence	N	7	4	4	6
	%	4.1	3.0	2.9	3.5
Litter Incidence	N	4	3	4	3
	%	21	17	25	16
HEMICENTRUM(A)					
Fetal Incidence	N	0	0	0	1
	%	0.0	0.0	0.0	0.6
Litter Incidence	N	0	0	0	1
	%	0.0	0.0	0.0	5.3
5TH/6TH STERNEBRA(E) INCOMPLETE OSSIFICATION					
Fetal Incidence	N	19	22	10	28
	%	11	16	7.2	16
Litter Incidence	N	9	11	9	10
	%	47	61	56	53
5TH STERNEBRA UNOSSIFIED					
Fetal Incidence	N	12	13	5	11
	%	7.1	9.6	3.6	6.4
Litter Incidence	N	7	6	3	5
	%	37	33	19	26
6TH STERNEBRA UNOSSIFIED					
Fetal Incidence	N	1	4	5	5
	%	0.6	3.0	3.6	2.9
Litter Incidence	N	1	3	4	4
	%	5.3	17	25	21

N = Number

TABLE 11A
RABBIT DEVELOPMENTAL TOXICITY STUDY
SUMMARY OF FETAL SKELETAL VARIATIONS

DOSE LEVEL		GROUP 1 0 MG/KG	GROUP 2 300MG/KG	GROUP 3 600MG/KG	GROUP 4 1200MG/K
Litters Evaluated	N	19	18	16	19
Fetuses Evaluated	N	170	135	138	171
Live	N	170	135	138	170
Dead	N	0	0	0	1
STERNEBRA(E) ASYMMETRICALLY OSSIFIED					
Fetal Incidence	N	3	5	2	3
	%	1.8	3.7	1.4	1.8
Litter Incidence	N	3	4	2	3
	%	16	22	13	16
MINOR FUSION OF STERNEBRAE					
Fetal Incidence	N	3	3	5	0
	%	1.8	2.2	3.6	0.0
Litter Incidence	N	3	3	2	0
	%	16	17	13	0.0
5TH/6TH STERNEBRA(E) BIPARTITE					
Fetal Incidence	N	1	2	2	2
	%	0.6	1.5	1.4	1.2
Litter Incidence	N	1	2	2	2
	%	5.3	11	13	11
STERNEBRAE EXTRA OSSIFICATION SITE(S)					
Fetal Incidence	N	1	3	0	1
	%	0.6	2.2	0.0	0.6
Litter Incidence	N	1	3	0	1
	%	5.3	17	0.0	5.3
13TH RUDIMENTARY RIB(S)					
Fetal Incidence	N	39	32	38	34
	%	23	24	28	20
Litter Incidence	N	14	14	14	17
	%	74	78	88	89
13TH FULL RIB(S)					
Fetal Incidence	N	52	59	49	72
	%	31	44	36	42
Litter Incidence	N	13	13	14	16
	%	68	72	88	84

N = Number

TABLE 11A
RABBIT DEVELOPMENTAL TOXICITY STUDY
SUMMARY OF FETAL SKELETAL VARIATIONS

DOSE LEVEL		GROUP 1 0 MG/KG	GROUP 2 300MG/KG	GROUP 3 600MG/KG	GROUP 4 1200MG/K
Litters Evaluated	N	19	18	16	19
Fetuses Evaluated	N	170	135	138	171
Live	N	170	135	138	170
Dead	N	0	0	0	1
THICKENED RIB(S)					
Fetal Incidence	N	0	1	0	2
	%	0.0	0.7	0.0	1.2
Litter Incidence	N	0	1	0	2
	%	0.0	5.6	0.0	11
7TH CERVICAL RIB(S)					
Fetal Incidence	N	1	0	0	0
	%	0.6	0.0	0.0	0.0
Litter Incidence	N	1	0	0	0
	%	5.3	0.0	0.0	0.0
UNOSSIFIED TALUS(I)					
Fetal Incidence	N	1	0	0	2
	%	0.6	0.0	0.0	1.2
Litter Incidence	N	1	0	0	2
	%	5.3	0.0	0.0	11
UNOSSIFIED PUBIS(ES)					
Fetal Incidence	N	2	0	0	1
	%	1.2	0.0	0.0	0.6
Litter Incidence	N	1	0	0	1
	%	5.3	0.0	0.0	5.3
TOTAL FETAL SKELETAL VARIATIONS					
Fetal Incidence	N	114	104	107	129
	%	67	77	78	75
Litter Incidence	N	18	18	16	19
	%	95	100	100	100

N = Number

Table 11B
Summary of Fetal Skeletal Malformations
Rabbit Developmental Toxicity Study

TABLE 11B
RABBIT DEVELOPMENTAL TOXICITY STUDY
SUMMARY OF FETAL SKELETAL MALFORMATIONS

DOSE LEVEL		GROUP 1 0 MG/KG/DAY	GROUP 2 300 MG/KG/DAY	GROUP 3 600 MG/KG/DAY	GROUP 4 1200 MG/KG/DAY
Litters Evaluated	N	19	18	16	19
Fetuses Evaluated	N	170	135	138	171
Live	N	170	135	138	170
Dead	N	0	0	0	1
VERTEBRAL ANOMALY WITH/WITHOUT ASSOCIATED RIB ANOMALY					
Fetal Incidence	N	2	2	0	1
	%	1.2	1.5	0.0	0.6
Litter Incidence	N	1	2	0	1
	%	5.3	11	0.0	5.3
MAJOR FUSION OF STERNEBRAE					
Fetal Incidence	N	2	0	0	1
	%	1.2	0.0	0.0	0.6
Litter Incidence	N	2	0	0	1
	%	11	0.0	0.0	5.3
FORKED/FUSED RIB(S)					
Fetal Incidence	N	0	0	0	1
	%	0.0	0.0	0.0	0.6
Litter Incidence	N	0	0	0	1
	%	0.0	0.0	0.0	5.3
TOTAL FETAL SKELETAL MALFORMATIONS					
Fetal Incidence	N	4	2	0	3
	%	2.4	1.5	0.0	1.8
Litter Incidence	N	3	2	0	3
	%	16	11	0.0	16

N = Number

Text Table 1
Mean Plasma Data

Postdose timepoints:		0.25 hour	0.5 hour	1 hour
300 mg/kg/day				
Day 7	mean	24.83	55.67	115.43
	S.D.	17.12	17.10	30.51
	n	3	3	3
Day 20	mean	34.91	132.7	212.0
	S.D.	34.05	60.12	46.87
	n	3	3	3
1200 mg/kg/day				
Day 7	mean	85.97	171.0	317.3
	S.D.	29.65	85.14	54.05
	n	3	3	3
Day 20	mean	140.6	256.7	454.3
	S.D.	64.49	121.60	138.5
	n	3	3	3

PRE- AND POSTNATAL DEVELOPMENT STUDY IN RATS

A) Dosage

25 F at 0, 150, 350, or 1000 mg/kg/day, by gavage, from day 6 of gestation through day 21 PP.

Pregnant females were allowed to deliver and raise their young to day 21 PP. F_1 evaluations performed are indicated under "Results," below.

Strain: Sprague – Dawley Crl : CD BR

Laboratory: [redacted] (b) (4)

Drug lot # : 9501

B) Results

1) Observed signs in Fo dams

a) "Hypoactivity" seen at HD, sporadically during gestation (N=7; 6 of these on 1 day only) and more consistently during lactation (N=21, on 1-7 days each).

b) Ataxia seen in 5 HD (on 1 day each) during lactation

2) Bodyweights in Fo dams

Weight gain was decreased at HD during the first few days of treatment; weights remained slightly below controls (~3-4%) for the rest of the study. (Weights at MD were similar to those at HD during most of the study, although there was no period when weight gain was statistically significantly decreased at MD. Also, pre-treatment weights at MD were slightly below those at HD).

3) Food consumption in Fo dams

Slightly decreased at HD, primarily during the first week of treatment (92-95% of control); overall consumption during days 6-20 of gestation was 96% of control. These decreases were generally not statistically significant. Slight decreases during gestation were seen at MD but pre-treatment values were below controls to a similar degree. Food consumption was not measured during the lactation period.

4) F_1 - Preweaning assessment

Parameters measured and results obtained shown in attached table.

The number of stillborn pups was increased at HD (11/381 vs 1/367 in controls) but this was not statistically significant. Pup survival through day 4 PP was very slightly decreased at HD (94% vs 99% in controls) but this was not statistically significant (and mainly due to effects in a single litter).

Pup weights were below controls (~ 5-8%) at HD beginning day 4 PP. Pup weights at MD were sporadically very slightly below controls but never statistically significantly.

There were no drug effects on gross organ exam of pups which were culled or which died. (Not shown in attached table).

5) F_1 – Postweaning assessment

No effects on overall weight gain. (Weights at HD were slightly below controls, although the relationship to drug is not clear since this was also seen at LD [but not MD]).

No effects on time of sexual maturation (cleavage of balanopreputial gland and vaginal opening), development of pupillary reflex, open field locomotor activity (day 22 and week 5 PP), learning/memory (water "M" maze, weeks 6-7 PP), or reproductive performance (fertility, duration of gestation, pup weight at birth, pup survival through day 1 PP).

Table 6
Natural Delivery Data and Litter Data
Study of Effects of Pre- and Postnatal Development, Including Maternal Function, in the Rat
F_0 Generation

Note: Gestation Index (Parturition Index) = $\frac{\text{number of females delivering live pups}}{\text{number of pregnancies}} \times 100$

TABLE 6
STUDY FOR EFFECTS OF PRE AND POSTNATAL DEVELOPMENT, INCLUDING
MATERNAL FUNCTION, IN THE RAT
NATURAL DELIVERY DATA AND LITTER DATA -- SUMMARY

DOSE LEVEL		GROUP 1 0 MG/KG/DAY	GROUP 2 150 MG/KG/DAY	GROUP 3 350 MG/KG/DAY	GROUP 4 1000 MG/KG/DAY
Females: Mated	N	25	25	25	25
Pregnant	N	24	23	24	25
	%	96	92	96	100
Delivering	N	24	23	24	25
	%	96	92	96	100
Duration of Gestation:	MEAN	22.0	22.0	22.0	22.2
RT	S.D.	0.2	0.2	0.4	0.4
	N	24	23	24	25
Females with Liveborn Pups	N	24	23	24	25
Gestation Index	%	100	100	100	100
With Stillborn Pups	N	1	1	2	6
	%	4.2	4.3	8.3	24
Females with no Liveborn Pups	N	0	0	0	0
	%	0.0	0.0	0.0	0.0
Females with no Pups Delivered	N	0	0	0	0
	%	0.0	0.0	0.0	0.0
Pups Delivered	TOTAL	367	347	352	381
	MEAN	15.29	15.09	14.67	15.24
	S.D.	2.20	2.09	2.55	1.79
	N	24	23	24	25
Liveborn	TOTAL	365	346	350	367
Stillborn	TOTAL	1	1	2	11
Uncertain	TOTAL	1	0	0	3
Implantation Sites	TOTAL	389	371	388	408
	MEAN	16.21	16.13	16.17	16.32
	S.D.	2.13	2.10	2.18	1.65
	N	24	23	24	25

SIGNIFICANTLY DIFFERENT FROM CONTROL: * = $P \leq 0.05$; ** = $P \leq 0.01$.

N = Number of Females or Litters.
TOTAL = Number of Pups or Implants.

RT - Rank transformation used in test for significance on data.

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TABLE 6
STUDY FOR EFFECTS OF PRE AND POSTNATAL DEVELOPMENT, INCLUDING
MATERNAL FUNCTION, IN THE RAT
NATURAL DELIVERY DATA AND LITTER DATA -- SUMMARY

DOSE LEVEL		GROUP 1 0 MG/KG/DAY	GROUP 2 150 MG/KG/DAY	GROUP 3 350 MG/KG/DAY	GROUP 4 1000 MG/KG/DAY
Pup Survival Indices					
Livebirth Index (Number born alive/number born)	MEAN%	99	100	99	96
Viability Index (Number alive Day 4 precull/ number liveborn)	MEAN%	99	99	97	94
Weaning Index (Number alive at weaning/ number alive at Day 4 postcull)	MEAN%	100	99	98	99
Pup Disposition					
Culled day 4	TOTAL	169	159	149	147
Killed	TOTAL	0	0	0	0
Died	TOTAL	4	3	9	13
Cannibalized	TOTAL	0	0	0	0
Missing	TOTAL	1	1	3	11
Pups Surviving at 21 days	TOTAL	192	183	189	196
Pups Dying, Killed, Missing, and/or Cannibalized					
days 0-4	TOTAL	4	3	9	22
days 5-21	TOTAL	0	1	3	2
Entire Litter Died, Killed, Missing, and/or Cannibalized					
days 0-4	N	0	0	0	0
days 5-21	N	0	0	0	0
Total Number and Mean Males Percent by Litter					
day 0	TOTAL	175	172	164	167
	MEAN%	48	50	47	46
day 4 precull	TOTAL	172	171	161	155
	MEAN%	47	50	47	45
day 21	TOTAL	95	91	92	98
	MEAN%	49	50	49	50

SIGNIFICANTLY DIFFERENT FROM CONTROL: * = $P \leq 0.05$; ** = $P \leq 0.01$.

N = Number of Litters.
TOTAL = Number of Pups or Implants.

TABLE 6
STUDY FOR EFFECTS OF PRE AND POSTNATAL DEVELOPMENT, INCLUDING
MATERNAL FUNCTION, IN THE RAT
NATURAL DELIVERY DATA AND LITTER DATA -- SUMMARY

DOSE LEVEL		GROUP 1 0 MG/KG/DAY	GROUP 2 150 MG/KG/DAY	GROUP 3 350 MG/KG/DAY	GROUP 4 1000 MG/KG/DAY
Live Pups/Litter with Live Pups					
day 0	MEAN	15.21	15.04	14.58	14.68
	S.D.	2.26	2.10	2.55	1.84
	N	24	23	24	25
day 4 - Precull	MEAN	15.04	14.91	14.21	13.80
	S.D.	2.27	2.02	2.64	2.47
	N	24	23	24	25
day 4 - Postcull RT	MEAN	8.00	8.00	8.00	7.92
	S.D.	0.00	0.00	0.00	0.40
	N	24	23	24	25
day 7 RT	MEAN	8.00	7.96	7.96	7.84
	S.D.	0.00	0.21	0.20	0.80
	N	24	23	24	25
day 14	MEAN	8.00	7.96	7.88	7.84
	S.D.	0.00	0.21	0.61	0.80
	N	24	23	24	25
day 21	MEAN	8.00	7.96	7.88	7.84
	S.D.	0.00	0.21	0.61	0.80
	N	24	23	24	25
Pup Weight/Litter (grams)					
day 0 MALES	MEAN	6.36	6.32	6.41	6.32
	S.D.	0.42	0.32	0.48	0.61
	N	24	23	24	25
Covariate Adjusted	MEAN	6.39	6.32	6.37	6.34
day 0 FEMALES	MEAN	5.96	6.03	5.95	5.99
	S.D.	0.42	0.26	0.51	0.52
	N	24	23	24	25
Covariate Adjusted	MEAN	5.98	6.03	5.91	6.01
day 4 MALES - Precull	MEAN	10.45	10.30	10.21	9.84
	S.D.	1.07	0.76	1.15	1.42
	N	24	23	24	25
Covariate Adjusted	MEAN	10.55	10.38	10.16	9.71*

SIGNIFICANTLY DIFFERENT FROM CONTROL: * = $P \leq 0.05$; ** = $P \leq 0.01$.
N = Number of Litters.
RT - Rank transformation used in test for significance on data.

TABLE 6
STUDY FOR EFFECTS OF PRE AND POSTNATAL DEVELOPMENT, INCLUDING
MATERNAL FUNCTION, IN THE RAT
NATURAL DELIVERY DATA AND LITTER DATA -- SUMMARY

DOSE LEVEL		GROUP 1 0 MG/KG/DAY	GROUP 2 150 MG/KG/DAY	GROUP 3 350 MG/KG/DAY	GROUP 4 1000 MG/KG/DAY
day 4 FEMALES - Precull	MEAN	9.84	9.91	9.44	9.31
	S.D.	1.04	0.66	1.21	1.45
	N	24	23	24	25
Covariate Adjusted	MEAN	9.93	9.99	9.39	9.19*
day 4 MALES - Postcull	MEAN	10.44	10.27	10.26	9.89
	S.D.	1.12	0.84	1.00	1.42
	N	24	23	24	25
Covariate Adjusted	MEAN	10.54	10.35	10.21	9.76
day 4 FEMALES - Postcull	MEAN	9.96	9.92	9.47	9.33
	S.D.	1.01	0.69	1.20	1.47
	N	24	23	24	25
Covariate Adjusted	MEAN	10.06	10.00	9.42	9.20*
day 7 MALES	MEAN	17.02	16.81	16.52	15.81
	S.D.	1.70	1.35	1.73	2.30
	N	24	23	24	25
Covariate Adjusted	MEAN	16.91	16.78	16.49	15.99
day 7 FEMALES	MEAN	16.12	16.08	15.34	14.99
	S.D.	1.58	1.15	1.99	2.23
	N	24	23	24	25
Covariate Adjusted	MEAN	16.01	16.05	15.31	15.18
day 14 MALES	MEAN	34.31	34.36	34.01	31.74
	S.D.	3.34	2.73	2.44	3.48
	N	24	23	24	25
Covariate Adjusted	MEAN	34.15	34.28	34.09	31.90*
day 14 FEMALES	MEAN	32.99	33.10	32.18	30.42
	S.D.	2.85	2.17	2.54	3.46
	N	24	23	24	25
Covariate Adjusted	MEAN	32.83	33.02	32.26	30.56*
day 21 MALES	MEAN	56.28	55.39	56.15	53.23
	S.D.	5.39	5.06	3.99	5.95
	N	24	23	24	25
Covariate Adjusted	MEAN	56.10	55.30	56.24	53.41

SIGNIFICANTLY DIFFERENT FROM CONTROL: * = $P \leq 0.05$; ** = $P \leq 0.01$.

N = Number of Litters.

TABLE 6
STUDY FOR EFFECTS OF PRE AND POSTNATAL DEVELOPMENT, INCLUDING
MATERNAL FUNCTION, IN THE RAT
NATURAL DELIVERY DATA AND LITTER DATA -- SUMMARY

	DOSE LEVEL	GROUP 1 0 MG/KG/DAY	GROUP 2 150 MG/KG/DAY	GROUP 3 350 MG/KG/DAY	GROUP 4 1000 MG/KG/DAY
day 21 FEMALES	MEAN	53.67	53.64	52.60	50.81
	S.D.	4.59	4.29	4.21	5.60
	N	24	23	24	25
	Covariate Adjusted MEAN	53.53	53.57	52.67	50.95

SIGNIFICANTLY DIFFERENT FROM CONTROL: * = $P \leq 0.05$; ** = $P \leq 0.01$.

N = Number of Litters.

GENOTOXICITY

A) Ames Test

Performed by [redacted] (b) (4) "Lot # 3-7, GHB Lot No. 019183"

Tester strains: Salmonella typhimurium TA 98, TA 100, TA 1535, and TA 1537, and E. coli WP2 uvr A. With and without liver microsomes derived from Aroclor treated rats. Plate incorporation method.

Na-GHB was negative up to the highest concentration tested (5000 ug/plate, which caused no cytotoxicity).

B. Chromosomal Aberrations in CHO Cells In Vitro

Performed by [redacted] (b) (4) Drug lot # 9501.

Assays were conducted with a 20 hour harvest time in the initial trial and 20 and 44 hour harvest times in a confirmatory trial. (Drug treatment times for the 20 hour harvest assays were 17.8 and 3 hours in the absence and presence, resp., of S9. Drug treatment times for the 44 hour harvest assays were 41.8 and 3 hours in the absence and presence, resp., of S9).

Na-GHB was negative up to the highest concentration tested (5000 ug/ml). (Cytotoxicity [reduced mitotic index] was seen across the dosage range tested [1250-5000 ug/ml]; however this was not always seen and was usually not dose-related; values [% reduction in mitotic index] ranged up to 67 % but were generally much lower).

C. In Vivo Rat Micronucleus Assay

Performed by [redacted] (b) (4) "Lot # 3-7 GHB, Lot. No. 019183."

Doses were based on a rangefinding study in which doses of 1400-2000 mg/kg (by gavage) caused "hypoactivity". The hypoactivity was of greater severity in females; however only males were used in the main study. Doses in the main study were 500, 1000, and 2000 mg/kg, by gavage. Animals were sacrificed at 24 hours post-dose; additional groups given HD and vehicle controls were sacrificed at 48 hours post-dose.

Toxic signs in the main study consisted of "hypoactivity" in 7 of the 12 HD (also seen in 1/6 LD); this was described as "slight" in all but 1 animal. There was no drug-related bone marrow toxicity (PCE:NCE ratio) or micronucleated PCEs. (5 rats/group/time point were evaluated; at least 2000 PCEs per animal were evaluated).

SUMMARY AND EVALUATION

A) Pharmacodynamics/ADME

No animal studies in these areas were performed by the sponsor (aside from plasma GHB measurements done in conjunction with some toxicity and reproduction studies; results are shown in the sections on the individual studies in this review); literature articles were submitted.

GHB is a normal constituent of human brain (and other organs). It is formed by reduction of succinic semialdehyde (which in turn is formed from GABA by GABA transaminase). It has been hypothesized that GHB may be a neurotransmitter, based on its brain distribution and demonstration of specific binding sites, active uptake system, depolarization – induced release, and post-synaptic responses in brain; however to date no neuronal pathways using GHB as a transmitter have been demonstrated. In pharmacologic doses GHB is a CNS depressant in animals and man. It has been used since the early 1960s (primarily abroad) as an anesthetic adjunct (at i.v. doses of 4-6 grams). In addition to sleep induction, pharmacological effects seen in animals include anticonvulsant (although a proconvulsant effect, possibly analogous to myoclonus or petit mal seizures, has also been seen), analgesic, hypothermic, and anti-ischemia/hypoxia. Several of its effects are antagonized by naloxone. Potentiations between GHB and other CNS depressants have been reported.

The mechanism of action of GHB is unknown. It decreases cerebral glucose utilization and causes a shift in intermediary metabolism toward the pentose shunt. It produces a decrease in dopaminergic firing rate and an increase in dopamine levels in brain. GHB may be converted to GABA in vivo, although this pathway has not been clearly shown to be involved in the pharmacological effects of GHB; on the other hand some effects of GHB may be produced by interaction with GABA receptors.

Metabolic pathways of GHB include tranformation to succinic semialdehyde (with further metabolism through the Krebs cycle) and beta-oxidation.

B) Chronic Toxicity

A 6 month rat study and a 12 month dog study were performed

In the rat study, daily doses were 150, 350, and 1000 mg/kg, by gavage. The only drug-related observed sign was "hypoactivity" (not further described) which occurred very sporadically during the first 2-3 months. Bodyweight gain and food consumption were decreased at HD (final weights ~ 90 % of control). There were no drug effects on ophthalmoscopic exams. There were no pronounced effects on lab tests; slight effects included decreased total WBC, lymphocytes, and segmented neutrophils at HD, decreased RBC, Hb, and Hct in all M groups (not D-R), decreased total protein, albumin, and K in HD M (slightly decreased protein and albumin also seen in a 3 month rat study at the same dose), and increased urine pH in all M groups and HD F. There were no drug effects on gross or microscopic organ exams. (In a 3 month study, at the same doses, some animals were formalin perfused and brain was examined [with H & E] at 4 levels; no drug effects were said to have been found, although no specific results were given).

In the dog study, daily doses were 150, 350, and 600 → 900 mg/kg, by oral intubation. The dosage increase at HD began at week 32. All HD F had sporadic "hypoactivity" and ataxia mainly near the beginning of the study and after the dosage increase. One HD F also had prostration at week 1, and another had slight convulsions week 33. One HD M had ataxia on 3 occasions near the beginning of treatment. The incidence/frequency of diarrhea was increased in MD and HD M and HD F. The frequency of emesis was increased in 1 MD F and 1 HD F. Thin appearance/decreased appetite was seen in 1 MD M, 1 HD M, and 2 HD F near the beginning of the study. Bodyweight gain was decreased early in the study in MD and HD M and HD F ; final weights in MD M and HD M were 95 % and 91 % of control, respectively; weights in HD F returned to control level by week 14. Food consumption was decreased early in the study at HD. There were no drug effects on ophthalmoscopic or EKG exams (no results shown for the latter). There were no pronounced drug effects on lab tests; slight effects included decreased blood chloride in HD M, increased urine pH in MD and HD F and HD M (also sporadically at lower doses), and decreased urine SG in HD M. Absolute and relative liver weights were slightly increased in HD M but there was no associated histopathology. Pale/dark raised area in lung was seen in 1 MD M, 2 HD M, and 1 HD F; histology showed chronic active inflammation in these dogs, and two also had pigmented macrophages. The text of the report stated that these lesions "could be related to the irritation caused by the aspirated test article". Other drug-related histopathological effects included atrophy of submucosal gland of esophagus ("moderate" or "moderately severe") and atrophy of mandibular salivary gland (ranging from "minimal" to "moderately severe") in MD and HD. (The esophageal and salivary gland pathology were also seen in a 3 month dog study at the HD of 600 mg/kg).

C) Carcinogenicity

Carcinogenicity studies in rats and mice were performed by the National Toxicology Program (NTP) with the compound gamma-butyrolactone (GBL), which is extensively converted to GHB in vivo. The abstract and tumor incidence tables from these studies are attached. MTDs were reached in these studies based on toxic signs and/or bodyweight and/or mortality effects occurring in the 2 year studies or at higher doses used in rangefinding studies. Percent survival in the 2 year studies was as follows:

		RATS	MICE
Control	M	48	70
LD	M	54	60
HD	M	64	**24**
Control	F	56	76
LD	F	54	68
HD	F	56	76

Bodyweights over the 2nd year of the two year studies were as follows (expressed as % of control):

	RATS	MICE
LD M	100	89
HD M	98	88
LD F	97	85
HD F	85	88

The NTP report concludes that there was "no evidence of carcinogenic activity" in rats of either sex or in female mice, and that there was "equivocal evidence of carcinogenic activity" in male mice. (Definitions of these criteria are shown in the attached abstract). The conclusion of equivocal evidence in male mice is based on the finding of "marginally increased incidences of adrenal medulla pheochromocytomas and hyperplasia in the low dose group." The incidence values in male mice were as follows:

	C	LD	HD
Benign pheochromocytoma	1/48	5/50	1/50
Malignant pheochromocytoma	1/48	1/50	0/50
Adrenal medulla hyperplasia	2/48	9/50	4/50

The increase in pheochromocytomas (benign, malignant, or combined) was not statistically significant (both by trend test and pairwise comparison, survival-adjusted), but at LD the combined (malignant or benign) incidence (12%) was above the historical range (mean incidence 3%; range 0-6%). The increase in hyperplasia at LD adds biological plausibility to a tumorigenic effect in this group. It was stated that the lack of a dose response relationship "may be related to the reduced survival" at HD. However, although overall survival was clearly reduced at HD (24%) compared to control (70%), it appears to this reviewer that there were enough HD male mice surviving long enough to allow a tumorigenic effect to become manifest. The benign pheochromocytomas were detected late in the study: the one tumor in controls was detected at week 83, all 5 tumors at LD were detected at termination (week 104), and the 1 tumor at HD was detected week 91. The approximate (estimated from survival curves) numbers of animals alive at week 83 (the time of the first detection of this tumor type) were 44, 36, and 25 in controls, LD, and HD, respectively. The numbers surviving until termination were 34, 30, and 12, respectively.

The NTP report also notes lower bodyweights in HD M mice as a possible reason for a lack of an increase in pheochromocytomas in this group; this is a possibility although it is noted that weight gain in LD M was reduced to a similar extent.

Although the rat carcinogenicity study with GBL was said to be negative, the following results are noted, particularly in view of the above. The incidence of malignant pheochromocytomas in males was 0/48 in controls and 5/49 at HD. (LD not routinely examined). The incidence of benign pheochromocytomas was not different between control M (15/48) and HD M (14/49). In F, no malignant pheochromocytomas were seen in controls or HD. The incidence of benign pheochromocytoma was 1/50 in control F and 4/49 in HD F. (LD not routinely examined; 1 malignant and 0 benign pheochromocytomas were seen among the 25 LD examined). These results

were not mentioned in the text of the report. Historical control values were not given. Historical control values (for gavage corn oil studies) on the current NTP WEB page showed, for malignant pheochromocytoma in male rats, a mean of 2% and a range of 0-6%; thus the incidence of 5/49 in HD M in the present study is slightly above this range. (Benign pheochromocytoma is a very common tumor in male Fisher rats; the historical control values are similar to the values seen in the present study). The incidence of benign pheochromocytoma in HD F in the present study (4/49) was near the upper end of the historical range (mean 4.5%, range 0-10%).

In sum, there is a slight/equivocal signal for an effect on pheochromocytoma incidence in the studies conducted with GBL; the currently ongoing carcinogenicity study of GHB being performed in rats by the sponsor should help determine the relevance of these results for GHB.

what?

(The text of the NTP report discusses a few tumor types whose incidences were increased in the rat GBL study but it was concluded that these were not drug-related because they were not statistically significant and/or within the historical range and/or **not dose-related.** These tumor types included keratoacanthomas, basal cell adenoma/carcinomas, and mesotheliomas [see p. 33 of the NTP report (NDA volume 1.18, p. 261) for details]. The results of the ongoing rat carcinogenicity study of GHB should help clarify the relevance of these results for GHB).

Since there is evidence that GBL is rapidly and extensively converted to GHB in vivo, the sponsor had suggested that the NTP carcinogenicity studies of GBL adequately assessed the carcinogenic potential of GHB, and requested a waiver of carcinogenicity studies of GHB per se. As amply documented in the IND file, we agreed to this provided it could be shown that exposure to GHB in the GBL studies was adequate as defined by either of the following criteria: (1) plasma levels (AUC) of GHB reached at the high dose of GBL were at least as high as those reached with administration of an MTD of GHB, or (2) plasma levels (AUC) of GHB reached at the high dose of GBL were sufficiently greater (at least 25 x, in accordance with ICH guidelines) than those reached in humans receiving the maximum recommended dose. The sponsor performed bridging studies to determine if either of the above criteria were met. My IND review of 9/13/99, attached, discusses the nature and results of these studies. Briefly, it was concluded that the first criterion was met for mice but not for the rats. (The second criterion was not met for either species). Therefore, it was concluded that a mouse carcinogenicity study with GHB did not have to be performed. A rat study is underway, and it was agreed that this could be submitted post-approval.

20 pages(s) have been withheld for NTP TR 406 abstract. Please refer to http://ntp.niehs.nih.gov/ntp/htdocs/lt_rpts/tr406.pdf#search=butyrolactone

Barry N. Rosloff, Ph.D.
9/13/99

Pharmacologist Review of IND 49, 641
Submissions of 3/15/99, 4/15/99, 5/18/99, and 7/30/99

SPONSOR: Orphan Medical, Inc.
13911 Ridgedale Drive
Suite 475
Minnetonka, MN 55305

DRUG: GHB

CATEGORY: narcolepsy

SUMMARY:

The following summarizes the issue of whether the rat and mouse carcinogenicity studies which have been performed (by NTP) with GBL (gamma-butyrolactone), a compound which is converted to GHB in vivo, can serve as an adequate evaluation of the carcinogenicity of GHB. Briefly, we suggested that the GBL studies would be acceptable if the sponsor could demonstrate, by appropriate "bridging" studies, that exposure to GHB in the GBL studies was adequate (as defined below). We have worked extensively with the sponsor in the design of such bridging studies, as documented in several previous reviews and meeting/telecon minutes. The submission of 3/15/99 contains the results of the bridging studies; the other submissions noted above contain clarifications and answers to questions concerning these studies which we had previously transmitted to the sponsor.

There is a belief, based on some rather old (and not always satisfying) literature studies, that GBL is rapidly and extensively converted to GHB, which is primarily responsible for its pharmacological effects. If it were true, for example, that the in vivo conversion of GBL to GHB were virtually instantaneous and total, carcinogenicity studies of GBL would be near perfect surrogates for those of GHB, possibly only failing to evaluate potential effects of GHB on the G.I. tract if the conversion occurred post-absorption. A less than instantaneous and total conversion raises questions of possible differences in the plasma level profile of GHB when given as GHB vs. when formed from GBL, possible failure to reach adequate plasma levels of GHB after GBL administration (e.g., if the highest testable dose of GBL were limited by toxicity of the GBL molecule), possible differences in tissue distribution of GHB, and possible pro- or anticarcinogenic effects of the GBL molecule. At any rate, it the interest of avoiding unnecessary duplication of effort and use of animals, we proposed that the GBL carcinogenicity studies would acceptable if the sponsor could demonstrate that the exposure to GHB achieved in

these studies was adequate as defined by either of the following two criteria; (1) plasma levels (AUC) of GHB reached at the high dose of GBL were at least as high as those reached with administration of an MTD of GHB, or (2) plasma levels (AUC) of GHB reached at the high dose of GBL are sufficiently greater (e.g., 25x according to ICH guidelines) than those reached in humans receiving the maximum recommend daily dose. (The latter criterion was not met, as discussed later; thus the following discussion focuses on the former).

In order to estimate exposure to GHB achieved in the GBL carcinogenicity studies, bridging studies were performed in which mice and rats (same strain as in GBL carcinogenicity studies) received GBL, at the same high dose and in the same vehicle as in the GBL carcinogenicity studies, for 14 days. (Doses of GBL were 525 mg/kg in male and female mice, 225 mg/kg is male rats, and 450 mg/kg in female rats). The resultant plasma levels of GHB were compared with those achieved after a dose of 1000 mg/kg GHB, a dose estimated to be that which would have been used as an MTD in carcinogenicity studies of GHB in both mice and rats. Levels of GBL after dosing with GBL were also to be measured, in part to assess exposure to GBL, but more importantly because of the nature of the GHB assay, i.e. GHB is first converted to GBL prior to GC-MS measurement, and thus any GBL present prior to this conversion must be known and subtracted from the total.

The results (GHB levels) of the 14-day bridging studies are shown on the attached pages taken from the submission. (Times of measurements indicated in the two "Table 1"s; N=4/sex/time point). The following summarizes the results expressed as the % of the GHB AUC seen after dosing with 1000 mg/kg GHB (i.e., the estimated MTD of GHB) that was achieved after dosing with GBL given at the HD which was used in the GBL carcinogenicity studies:

	MICE	RATS
M	47 %	8 %
F	72 %	35 %

However, these values need to be modified since levels of GBL were only measured at a single time point (0.5 hr. post-dose); at other time points the GBL level is unknown and thus its contribution to the measured "GHB" (which, as noted above, must be converted to GBL before measurement) is also unknown. Since levels of GBL at 0.5 hr. were negligible (as were levels at 1 hr. determined in a separate single dose study), it was assumed that levels beyond this time were zero. We requested that the sponsor re-calculate the GHB AUCs (after dosing with GBL) with the assumption that all of the measured "GHB" before 0.5 hr. was really GBL; this represented a "worst case" scenario. The re-calculation was submitted (although for mice only) 7/30/99; results are attached. (Calculations were also made assuming that 50% of the "GHB" before 0.5 hours was GBL, to represent an intermediate situation). This "worst case" re-calculation lowered the above percentages from 47% to 36% in male mice, and from 72% to 53% in female mice; the true values probably lie within these ranges.

Note that the validity of the above approach depends on the correctness of the assumption that the 1000 mg/kg dose of GHB used would be an adequate HD in a carcinogenicity study of GHB. This dose was determined in mice in a 10-day rangefinding study (briefly described in submission of 7/30/99). The dose range was 1000-4000 mg/kg/day. Dose-related CNS depression (prostration, lack of righting reflex, labored breathing, decreased activity) were seen for 2-5 hr. post-dose on each day of the study, with "no evidence of tolerance." There was no drug effect on bodyweight. It was concluded that 1000 mg/kg "may be considered the MTD of sodium GHB administered orally in the mouse that could reasonably be expected to be utilized in a chronic dosing regimen". In the 14-day bridging study in mice, animals receiving both GHB and GBL were said to have had sternal recumbency and hypoactivity on each day. The situation in rats is less clear. The 1000 mg/kg dose of GHB was based on previously conducted subacute and chronic toxicity studies, in which this dose caused signs of CNS depression and decreased weight gain. (The CAC [meeting 4/27/99] concurred with the use of this dose in an ongoing rat carcinogenicity study of GHB, although, due to the possible development of tolerance to the decreased weight gain, a possible increase in dose after one year was considered). However, the rat strain used in these toxicity studies (SD) was different from that used in the GBL carcinogenicity study and in the bridging study (F334). No toxic signs or decreased weight gain was seen in the rat bridging study with either GHB or GBL.

Another caveat in the interpretation of the bridging studies is the possible conversion of GBL to GHB in plasma between the time of sampling and the time of assay (up to ½ hour). (We had previously asked that the sponsor investigate this, but this was not done). The sponsor stated (meeting of 5/21/99) that although such conversion occurs rapidly in plasma at room temperature, it was unlikely to occur at the 5° C at which the samples were kept. This seems plausible in view of the fact that the conversion of GBL to GHB takes place enzymatically (Roth and Giarman, Biochem. Pharmacol. 14:177-8, 1965).

Another area of possible concern is whether steady state plasma levels were reached at 14 days. The answer to this appears to be yes, since plasma levels of GHB in single dose studies (submission of 3/15/99) were similar to (rats) or slightly greater than (mice) these in the 14 day studies (both after GHB or GBL administration), and no GHB was present at pre-dosing on days 12-14 of the 14 day study. These results are expected based on the short T 1/2 (1-3 hr.) of GHB.

(As noted earlier, the possibility existed that the acceptability of the GBL carcinogenicity studies as surrogates for those of GHB could be based on an adequate GHB exposure margin, e.g. 25x, compared to humans. However, using a value of 500 ug. hr/ml [submission of 8/9/99, p. 66] for a 9 g/day dose in humans [the currently proposed maximum daily dose], this criterion was not met; in fact the AUC values for GHB after GBL administration were less than those in humans [60-80% of human for mice and female rats; 15% for male rats]).

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MICE

Table 2: Selected GHB Pharmacokinetic Parameters Following the Repeated Oral Administration of Either 1000 mg/kg of Sodium GHB or 525 mg/kg GBL to Male and Female Mice Daily for 14 Days

Parameter	Sodium GHB		GBL	
	Male (1000 mg/kg)	Female (1000 mg/kg)	Male (525 mg/kg)	Female (525 mg/kg)
T_{max} (hr)	0.75	0.33	0.17	0.17
C_{max} (µg/mL)	387.25	306.00	306.45	274.25
$AUC_{0\text{-}Last}$ (hr-µg/mL)	804.01	423.92	378.87	303.98
$AUC_{0\text{-}\infty}$ (hr-µg/mL)	809.60	431.67	383.45	305.57
$T_{1/2}$ (hr)	1.17	1.51	1.11	1.88

Table 3: Ratio of Selected GHB Pharmacokinetic Parameters Following the Repeated Oral Administration of Either 1000 mg/kg of Sodium GHB or 525 mg/kg GBL to Male and Female Mice Daily for 14 Days

Ratio (as %)	Male	Female
C_{max} GBL/GHB	79.13	89.62
$AUC_{0\text{-}Last}$ GBL/GHB	47.12	71.71
	47.36	$AUC_{0\text{-}\infty}$ GBL/GHB

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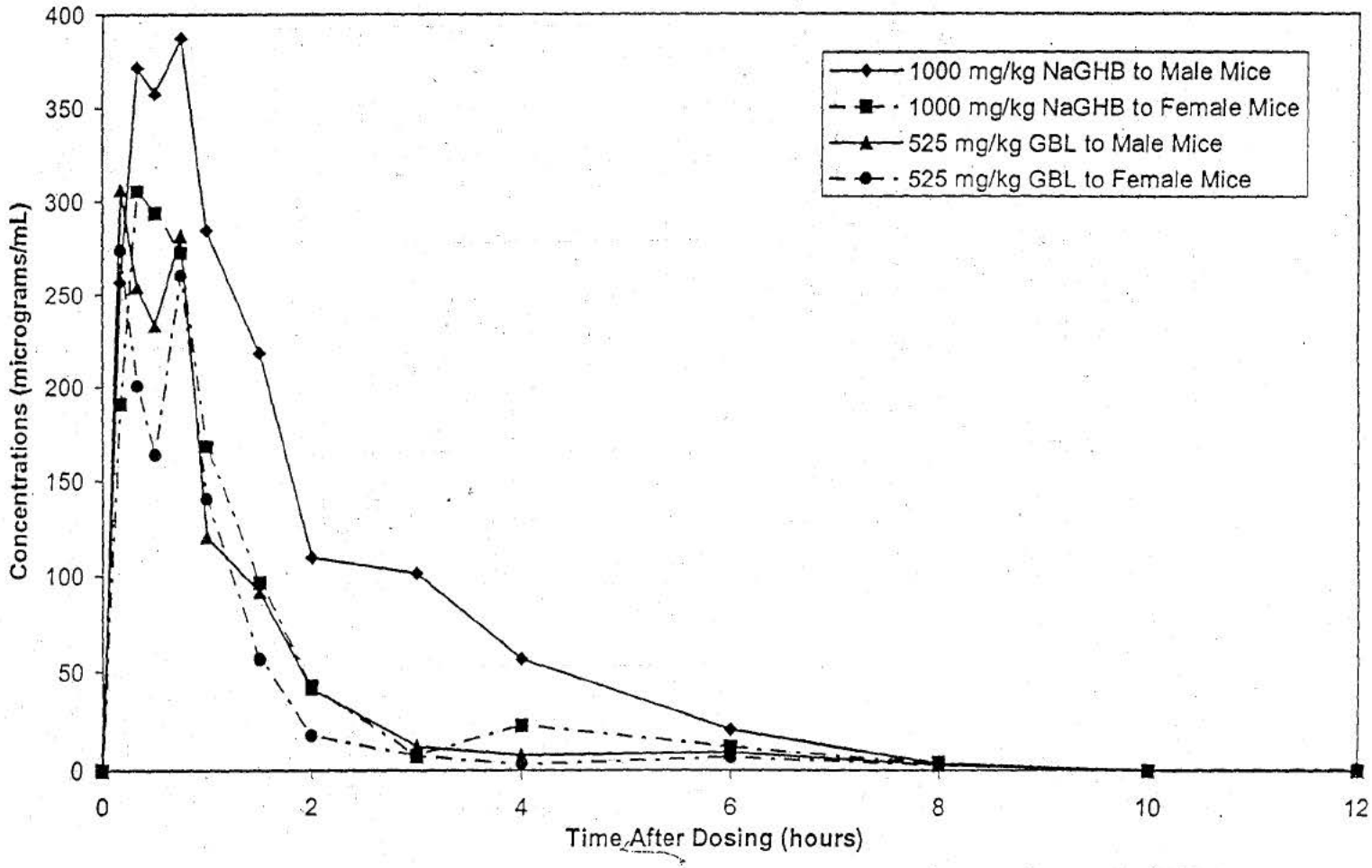
Figure 1
Plasma Concentrations of GHB Following the Daily Administration of Either 1000 mg/kg NaGHB or 525 mg/kg GBL to Male and Female Mice for 14 Days



MICE

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MICE

units?

Table 1: Plasma Concentrations of GHB Following the Repeated Oral Administration of Either 1000 mg/kg Sodium GHB or 525 mg/kg GBL to Male and Female Mice Daily for 14 Days

Time (Hours)	Sodium GHB		GBL	
	Male (1000 mg/kg)	Female (1000 mg/kg)	Male (525 mg/kg)	Female (525 mg/kg)
0[a]	0	0	0	0
0[b]	0	0	0	0
0[c]	0	0	0	0
0.17	257.25	190.75	306.45	274.25
0.33	371.50	306.00	254.50	200.60
0.5	357.75	294.25	233.50	163.75
0.75	387.25	273.00	282.00	260.50
1	285.00	168.00	120.18	140.23
1.5	218.25	96.55	91.65	56.85
2	109.43	42.91	41.39	17.24
3	101.13	7.54	11.88	7.56
4	56.88	22.53	7.80	3.39
6	20.26	11.78	9.21	6.93
8	3.31	3.57	2.85	2.55
10	0	0	0	0
12	0	0	0	0.59

[a] Pre-Dose, Day 12

[b] Pre-Dose, Day 13

[c] Pre-Dose, Day 14

N = 4 | sex | time point

RATS

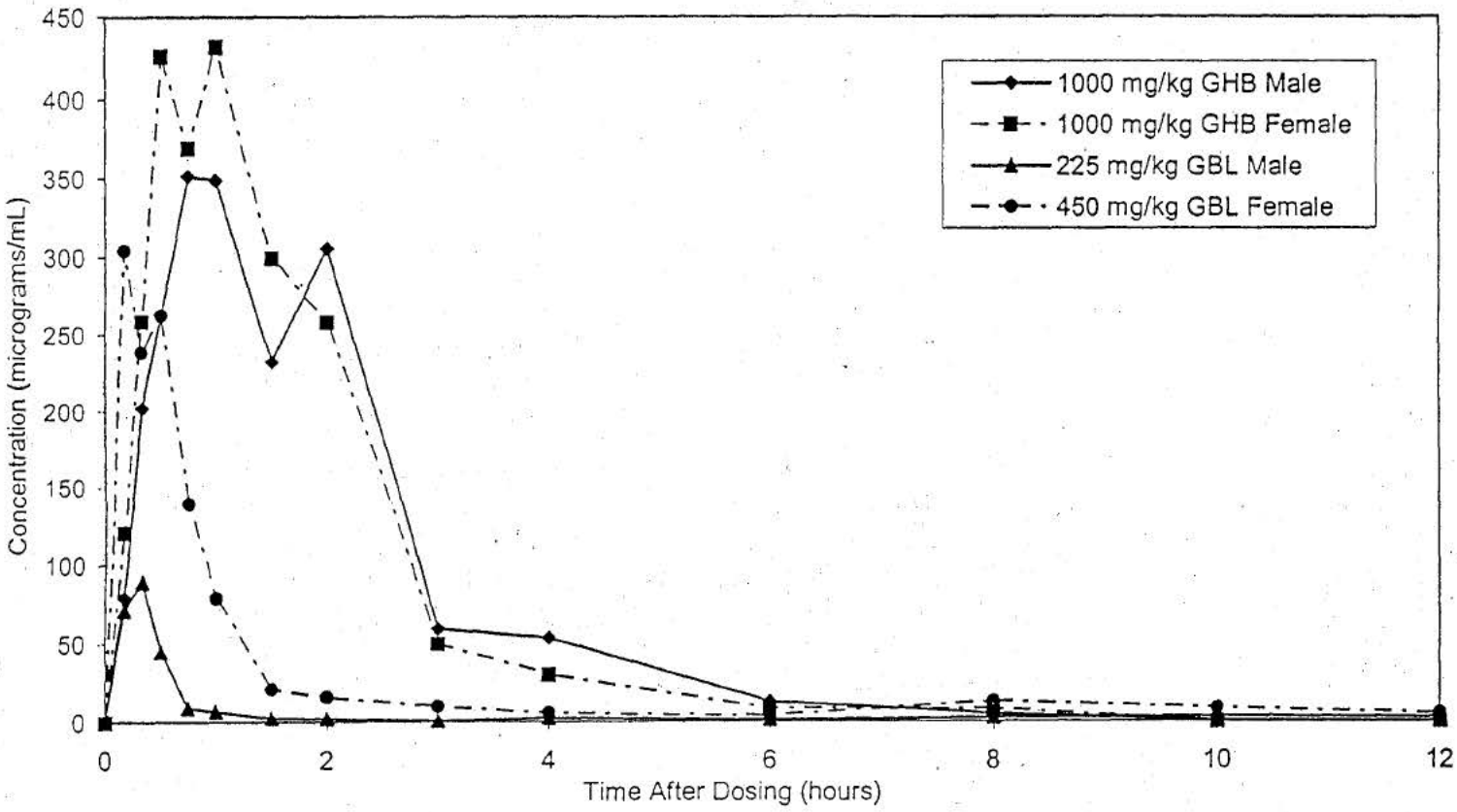
Table 2: Selected GHB Pharmacokinetic Parameters Following the Repeated Oral Administration of Either 1000 mg/kg of Sodium GHB to Male and Female Rats or 225 mg/kg of GBL to Male Rats or 450 mg/kg of GBL to Female Rats, Daily for 14 Days

Parameter	Sodium GHB		GBL	
	Male (1000 mg/kg)	Female (1000 mg/kg)	Male (225 mg/kg)	Female (450 mg/kg)
T_{max} (hr)	0.75	1	0.33	0.5
C_{max} (µg/mL)	351.50	431.75	89.73	304.25
$AUC_{0\text{-}Last}$ (hr-µg/mL)	841.07	869.66	64.34	303.95
$AUC_{0\text{-}\infty}$ (hr-µg/mL)	841.80	882.77	75.87	321.96
$T_{1/2}$ (hr)	1.01	1.12	3.30	2.72

Table 3: Ratio of Selected GHB Pharmacokinetic Parameters Following the Repeated Oral Administration Daily for 14 Days of Either 1000 mg/kg of Sodium GHB to Male and Female Rats or 225 mg/kg of GBL to Male Rats or 450 mg/kg of GBL to Female Rats

Ratio (as %)	Male	Female
C_{max} GBL/GHB	25.53	70.47
$AUC_{0\text{-}Last}$ GBL/GHB	7.65	34.95
$AUC_{0\text{-}\infty}$ GBL/GHB	9.01	36.47

Figure 1
Mean Plasma GHB Concentrations Following the Daily Administration of Either Sodium Gamma-hydroxybutyrate or Gamma-butyrolactone to Male and Female Rats for 14 Days



RATS

Table 1: Plasma Concentrations of GHB Following the Repeated Oral Administration of 1000 mg/kg Sodium GHB to Male and Female Rats or 225 mg/kg GBL to Male Rats and 450 mg/kg GBL to Female Rats, Daily for 14 Days

Time (Hours)	Sodium GHB		GBL	
	Male (1000 mg/kg)	Female (1000 mg/kg)	Male (225 mg/kg)	Female (450 mg/kg)
0[a]	0	0	0	0
0[b]	0	0	0	0
0[c]	0	0	0	0
0.17	79.45	120.50	71.80	304.25
0.33	202.33	258.75	89.73	238.45
0.5	262.50	426.25	44.85	263.00
0.75	351.50	369.25	9.25	139.25
1	348.75	431.75	6.76	79.23
1.5	232.00	299.25	2.40	20.98
2	305.50	258.00	2.07	16.03
3	59.75	50.03	0.93	9.76
4	53.70	30.28	2.43	5.76
6	12.65	8.32	1.24	3.40
8	4.78	8.15	2.27	12.70
10	0.51	0	3.34	8.50
12	0	0	2.42	4.59

[a] Pre-Dose, Day 12

[b] Pre-Dose, Day 13

[c] Pre-Dose, Day 14

N = 4/sex/time point

MICE (RE-CALCULATIONS)

Comparison of AUC $_{0-\infty}$ Values Following Administration of 1000 mg/kg NaGHB to Mice under 3 Different Assumptions: as Measured, if 50% response due to GBL, if 100% response due to GBL						
Data Below Represents As Measured Response						
Dose	Test Article	Gender	Actual AUC Value	50% GBL	100% GBL	Ratio (%)
1000 MPK	NaGHB	Female	431.67			
1000 MPK	NaGHB	Male	809.60			
525 MPK	GBL	Female	305.57			70.79
525 MPK	GBL	Male	383.45			47.36
Data Below Represents 50% Response Due to GBL in the First 30 Minutes Post-dose						
525 MPK	GBL	Female		266.39		61.71
525 MPK	GBL	Male		337.54		41.69
Data Below Represents 100% Response Due to GBL in the First 30 Minutes Post-dose						
525 MPK	GBL	Female			227.22	52.64
525 MPK	GBL	Male			291.14	35.96

The assumptions chosen here are extremely exaggerated, since it assumes the half-life of GBL conversion to GHB *in vivo* at 37°C is much slower than *in vitro* at 20-25°C. The half-life of GBL in plasma at room temperature was 7 minutes and the first sample wasn't drawn until 10 minutes, assuming the half-life *in vivo* was the same as that at room temperature (it is more likely to be faster at the higher *in vivo* temperature); you

CONCLUSIONS:

It is concluded that the carcinogenicity study of GBL in mice can serve as an adequate evaluation of the carcinogenicity of GHB in this species. The exposure to GHB in the GBL study was 36-47% (males) and 53-72%. (females) of the estimated exposure that would have occurred at an MTD of GHB. On the other hand, the exposure in the rat study was lower, especially in males. (8% and 35% of target in males and females, resp.; the actual values are probably lower due to GBL interference as discussed above, although the rat AUCs have as yet not been re-calculated to correct for this). In addition, as discussed above it is not clear that the dose of GHB used (1000 mg/kg) in the bridging study is an MTD in F334 rats; use of a higher dose of GHB would lower the above percentages further. The sponsor had already begun a 2 year rat carcinogenicity study with GHB.

Barry Rosloff

Barry N. Rosloff, Ph.D.

CC: IND 49, 641, submissions of 3/15/99 and 7/30/99, and division file
Rosloff, Fitzgerald, Homonnay
9/18/99

D) Reproduction

The following animal reproduction studies were performed (daily gavage doses in mg/kg in parentheses):

1) Segment I rat (150, 350, 1000) (both sexes treated)

2) Segment II rat (150, 350, 1000)

3) Segment II rabbit (300, 600, 1200)

4) Pre- and post-natal rat (150, 350, 1000) (treatment from day 6 of gestation – day 21 PP).

In the segment I study parental effects were minimal: observed signs consisted of only very isolated occurrences of "hypoactivity" in a few HD of both sexes, and a slight decrease in bodyweight gain and food consumption was seen in HD M. There were no drug effects on the usual reproductive parameters.

In the segment II study in rats, which used the same doses as above, effects on the dams were also minimal, consisting of only very sporadic occurrences of "hypoactivity" in a few HD; 2 of these also had ataxia on 1 day each, and an additional HD was prostrate on a single day. Food consumption was equivocally slightly decreased HD during the first week of treatment; there were no drug effects on maternal bodyweight. There were no clear drug effects on reproductive parameters or fetal exams with the possible exception of a slight increase in "wavy/bent rib(s)" at MD and HD.

In the segment II study in rabbits, there was an increased incidence of few or no feces among HD does. Food consumption was decreased at HD, although there was no clear drug effect on doe bodyweight. (All groups, including controls, lost bodyweight during the first 2 days of treatment. This loss was slightly greater at HD compared to the other groups; however the amount of loss was very small in relation to bodyweights). There were no clear drug effects on reproductive parameters or fetal exams.

In the pre- and post-natal study in rats, which used the same doses as in the segment I and II rat studies, maternal effects were, as above, minimal, with observed signs consisting of hypoactivity (sporadic during gestation, more consistent during lactation) and sporadic ataxia at HD. Dam food consumption (measured during gestation period only) was slightly decreased at HD, mainly during the first week of treatment. Dam bodyweight gain was slightly decreased at HD during the first few days of treatment; weights remained slightly below controls (3-4%) for the rest of the study. The number of stillborn pups was higher at HD (11/381) than in controls (1/367) but this was not statistically significant. Pup survival through day 4 PP was slightly less at HD (94%) than in controls (99%) but this was not statistically significant and was mainly due to effects in a single litter. Pup weights were below controls (5-8%) at HD beginning day 4 PP. There were no drug effects on F_1 weight gain, developmental parameters, or reproductive performance.

In view of the minimal parental effects noted in these reproduction studies, the question of dosage adequacy arises. No specific rangefinding studies were apparently performed in rats. However, 1000 mg/kg (the HD in rat reproduction studies) appeared to be an MTD with longer term treatment; in the 6 month rat study bodyweights at this dose were below controls from weeks 3-4 onward; weights near the end of the study were ~ 90% of control. (In addition, increased mortality in males at 1000 mg/kg in an ongoing rat carcinogenicity study was reported [submission of 8/8/00 to IND 49,641], although the relation of this to drug is unclear at this time). Plasma drug levels were not measured in the rat reproduction studies; AUC values measured at various times (day 1, weeks 13 and 26) in rat toxicity studies at 1000 mg/kg were about 2x that in humans receiving the currently proposed maximum recommended dose. (Human AUC was 518 ug.hr/mL after two 4.5 gram doses given 4 hours apart, as indicated in volume 1.1, p. 175)

In rabbits, a rangefinding study was done (in pregnant animals) which used an HD of 1800 mg/kg; although a concurrent control group was not used, it appeared that this dose had effects similar to those seen at 1200 mg/kg (the HD) in the main rabbit study, and thus it is not clear that 1800 mg/kg or higher could not have been used in the main study. Plasma drug levels were measured in the main rabbit study; however this was done only in 3 LD and 3 HD on gestation days 7 and 20, and levels were only measured at 15, 30, and 60 minutes post dose. Since levels were highest at the latter time point , neither Cmax or AUC can be calculated; however it may be noted that the mean levels seen at 60 minutes in HD rabbits (data shown earlier in this review) were 2-3x the mean human Cmax of 142 ug/mL (obtained after the second of two 4.5 gram doses given 4 hours apart, as shown in vol. 1.1, p. 175).

Overall, although it is likely that higher doses could have been used in these reproduction studies, it is concluded that these studies were adequate.

E) Genotoxicity

GHB was negative in an Ames test, an in vitro chromosomal aberration study in CHO cells, and a rat micronucleus assay. (The doses used in the latter could probably have been higher; only slight "hypoactivity" [not further described] was seen at the highest dose of 2000 mg/kg p.o. On the other hand, as noted earlier, a dose of 1000 mg/kg was shown to be an MTD with chronic treatment in rats and resulted in plasma AUCs about 2x those seen in humans at the currently recommended maximum dose. In view of the fact that GHB was negative in the in vitro genotoxicity assays and the fact that GBL does not appear to be carcinogenic [and assuming that the ongoing rat study with GHB also shows a lack of carcinogenicity], further genotoxicity testing does not appear to be warranted at this time).

LABELING

A) Clinical Pharmacology/Mechanism of Action section

This section as proposed by the sponsor is too long, and contains information of little or no practical use to the prescriber. The first paragraph should read:

(b) (4)

The following paragraph, discussing effects of oxybate on sleep architecture, could remain pending determination by the clinical reviewer of its accuracy and relevance to clinical outcome.

B) Carcinogenicity, Mutagenicity, Impairment of Fertility section

The first paragraph should be re-written as follows:

Oral carcinogenicity studies have been conducted in rats and mice with gamma-butyrolactone, a compound which is metabolized to oxybate *in vivo,* with no clear evidence of carcinogenic potential. Plasma levels (AUC) of oxybate achieved in these studies were estimated to be approximately 1/2 (mice and female rats) and 1/10 (male rats) those seen in humans receiving the maximum recommended daily dose of oxybate. (b) (4)

(b) (4)

The second paragraph should be re-written as follows:

Oxybate was negative in the Ames microbial mutagen test, an *in vitro* chromosomal aberration assay in CHO cells, and an *in vivo* rat micronucleus assay.

The third paragraph should be re-written as follows:

Oxybate did not impair fertility in rats at doses up to 1000 mg/kg (approximately equal to the maximum recommended human daily dose on a mg/m^2 basis).

C) Pregnancy section

This section should be re-written as follows:

Pregnancy Category B: **Reproduction studies conducted in pregnant rats at doses up to 1000 mg/kg (approximately equal to the maximum recommended human daily dose on a mg/m^2 basis) and in pregnant rabbits at doses up to 1200 mg/kg (approximately 3 times the maximum recommended human daily dose on a mg/m^2 basis) revealed no evidence of teratogenicity. In a study in which rats were given oxybate from day 6 of gestation through day 21 post-partum, slight decreases in pup and maternal weight gains were seen at 1000**

mg/kg; there were no drug effects on other developmental parameters. There are, however, ... (No changes to remainder of paragraph).

APPEARS THIS WAY ON ORIGINAL

RECOMMENDATIONS

This NDA is approvable.

As noted above, a rat carcinogenicity study is currently underway and should be submitted post-marketing.

Barry Rosloff

Barry N. Rosloff, Ph.D.