CENTER FOR DRUG EVALUATION AND RESEARCH

APPLICATION NUMBER:

21-348

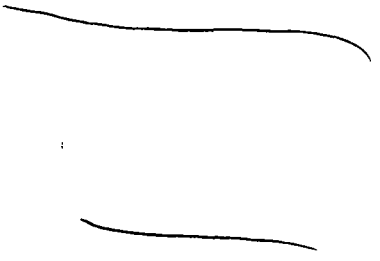
PHARMACOLOGY REVIEW

PHARMACOLOGY/TOXICOLOGY COVER SHEET

IND number: 60197.
Review number: 3; NDA 21-348.
Sequence number/date/type of submission: # 008SX; November 5, 2001; Dose Selection Study and Carcinogenicity study protocol.
Information to sponsor: Yes ( ) No (x)
Sponsor and/or agent: Oxford GlycoSciences, The Forum, 86 Milton Park, Abingdon Oxon OX14 4RY, UK.
Manufacturer for drug substance: G. D. Searle and Company, 4901 Searle Parkway, Skokie, IL 6007.

Reviewer name: John Colerangle
Division name: Metabolic & Endocrine Drug Products.
HFD #: 510.
Review completion date: February 13, 2003.

Drug:
Trade name: Zavesca™.
Generic name (list alphabetically): Miglustat.
Code name: OGT 918, SC-48334.
Chemical name: 1,5 (Butlyimino)-1,5-dideoxy-D-glucitol
CAS registry number: 72599-27-0
Mole file number: N/A.
Molecular formula/molecular weight: $C_{10}H_{21}NO_4$; 219.28
Structure:



Relevant INDs/NDAs/DMFs: [redacted] Oxford GlycoSciences.

Drug class: Glycoslytransferase inhibitor (An imino sugar)
Indication: Gaucher Disease (Lysosomal glycolipid storage disease)
Clinical formulation:

Ingredient	Content
OGT 918	50/100 mg
Sodium starch glycollate	[redacted]
Povidone (K30)	[redacted]
Magnesium sterate	[redacted]
[redacted]	[redacted]

Route of administration: Oral.

Disclaimer: Some tabular and graphical information is taken from sponsor's submission.

Studies reviewed within this submission:

- 2-Week study in mice with administration by gavage three times daily.
- 13-Week study in mice with administration by gavage three times daily.

Studies not reviewed within this submission:

None.

Introduction:

These studies in mouse are submitted in support of dose selection for a 2-year carcinogenicity study as partial fulfillment of a post-approval requirement for NDA 21-348.

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APPEARS THIS WAY
ON ORIGINAL

I. GENERAL TOXICOLOGY

STUDY TITLE: 2-WEEK TOXICITY STUDY IN MICE WITH ADMINISTRATION BY GAVAGE THREE TIMES DAILY

Key study findings:

- Due to marked weight loss in HD (2400 mg/kg/d) animals, all remaining animals in the group (9/10 HD males) and (9/10 HD females) were sacrificed on Day 9. The cause of death could not be determined. 1/10 HD males and 1/13 HD females died of gavage error. 3 HD females (TK group) were also sacrificed on Day 9.
- Treatment-related clinical signs include hunched posture, piloerection and pale skin mainly in the HD group.
- Erythrocyte parameters RBC (males only), HCT, HGB and MCH (females only) decreased slightly and dose-dependently achieving statistical significance in the MD group. This is suggestive of anemia. Dose-dependent decreases in WBC (41% at MD) and Lymphocytes (42%↓ at MD) were observed in treated males. The decrements were significant in MD males. Basophils and large unstained cells were significantly decreased in MD males by 50% and 40% respectively. Platelets decreased in a dose dependent manner achieving statistical significance at doses ≥ LD. Eosinophils were significantly decreased by 65% in MD females.
- Slight but significant increase in AST was observed in MD males (1.5-fold↑) and females (1.8-fold↑) relative to control. ALT was slightly but significantly increased in MD females (1.4-fold↑). This may correlate with the hepatocyte vacuolation observed. AG ratio increased slightly and dose-dependently achieving statistical significance in MD males. Alkaline phosphatase decreased significantly and in a dose-dependent but the significance of this change is not clear.
- Absolute heart weight was significantly decreased by 19% in HD males (due to decreased body wt.) relative to control. Weights of the liver and spleen were slightly but significantly increased in the HD group. The increased weight of the spleen correlates with the megakaryocytosis noted. Weight of the thymus was significantly decreased in the HD group by 50% (males) and 66% (females). This correlates with the lymphocytolysis observed. Weight of the uterus was significantly decreased (55%↓) in HD females with no correlative histopathology.
- The target organs of toxicity include the stomach (gastritis), spleen (megakaryocytosis), thymus (lymphocytolysis) and liver (vacuolation). NOAEL could not be established because only tissues from control and HD groups were examined for the most part. For the tissues examined from all dose groups (spleen, thymus and liver), NOAEL could not be established due to histopathology findings in the spleen and thymus at the LD.

Study no: 455853
Volume #, and page #: Vol. 1, pg. 1.
Conducting laboratory and location: [redacted]
Date of study initiation: September 5, 2001.
GLP compliance: Yes (UK).
QA report: Yes (X) no ( )
Drug, lot #, radiolabel, and % purity: Batch # 004, 100% pure.
Formulation/vehicle: A solution of OGT 918 in sterile water.

Methods (unique aspects):

Dosing: Animals were dosed by gavage with OGT 918 at 240, 1200 and 2400 mg/kg/d administered as 3 equal doses 6 hr apart.
Species/strain: Mouse/Crl:CD-1^{TM}(ICR)BR.
#/sex/group or time point (main study): 10/sex/group.
Satellite groups used for toxicokinetics or recovery: 20/sex/group for TK.
Age: 8 weeks at initiation of study.
Weight: 22-25g (M); 19-22g (F).
Doses in administered units: 240, 1200 and 2400 mg/kg/d (total dose).
Route, form, volume, and infusion rate: Oral (gavage), 5ml/kg.

Observations and times:
Clinical signs: Daily.
Body weights: Weekly.
Food consumption: Weekly.
Ophthalmoscopy: Not conducted.
EKG: Not conducted.
Hematology: Blood samples were taken from all surviving animals on Day 15 for routine hematology evaluation (HD animals were sacrificed on Day 9).
Clinical chemistry: Blood samples were taken from all HD animals on Day 8. Animals in the remaining dose groups were bled on Day 15.
Urinalysis: Not conducted.
Gross pathology: Organs/Tissues isolated for gross examination are indicated in the list of addendum.
Organs weighed: Organs weighed are indicated in the list of addendum.
Histopathology: Tissues isolated for histopathology examination are indicated in the list of addendum.
Toxicokinetics: Blood samples were obtained on Days 1 and 14 (with the exception of high dose animals which were bled on Day 1 only) at 0, 0.5, 1, 2, 3 and 6 hr postdose.

Results:
Mortality: Due to mortality in HD animals, all remaining animals in the group (9/10 HD males) and (9/10 HD females) were sacrificed on Day 9. The cause of death could not be determined. 1/10 HD males and 1/10 HD females died of gavage error. 3 HD females (TK group) were also sacrificed on Day 9.

Animal #	Day of Sacrifice	Cause of Death	Gross/Histopathology Findings
77 (M)	7	Gavage error	Esophagus: rupture, myositis; Lung: adhesions, congestion/hemorrhage, pleuritis. Heart: inflammatory cell infiltration; Lymph nodes (bronchial, mandibular, mesenteric): lymphoid atrophy; Spleen: megakaryocytosis, lymphoid atrophy; Thymus: lymphocytolysis.
177 (F)	3	Gavage error	Esophagus: inflammation, myositis, food present in muscle layer; Lung: reddened, congestion/hemorrhage, pleuritis; Spleen: congestion; Thym -us: lymphocytolysis, inflammation.
71–76 M) 78–80(M)	9	Undetermined	Lung: congestion; Spleen: megakaryocytosis; sternum: megakaryocytosis; Thyroid: follicular cell hypertrophy; Thymus: lymphocytolysis; Liver: hepatocyte vacuolation;
171-76(F) 178-92(F)	9	Undetermined	Esophagus: fibrosis, myositis, Liver: hepatocyte vacuolation; Spleen: megakaryocytosis; Sternum: megakaryocytosis; Thymus: lymphocytolysis; Thyroid: follicular cell hypertrophy; Heart: inflammatory cell infiltration.

9/10 HD males and 12/13 HD females died of undetermined cause. 1/10 HD males and 1/13 HD females died of gavage error.

Clinical signs:

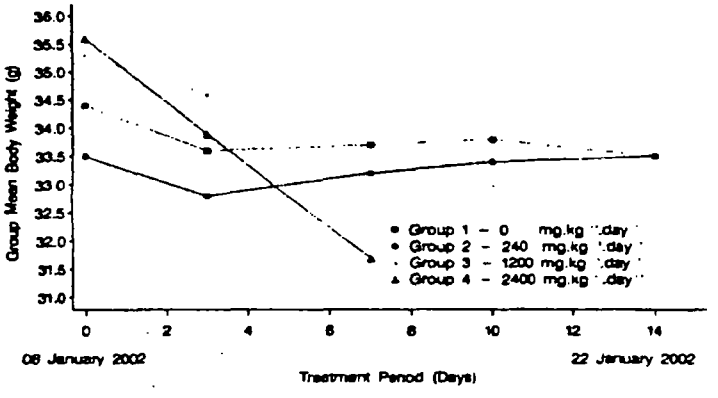
Dose (mg/kg/d)	0		240		1200		2400	
Observations/Findings	**M**	**F**	**M**	**F**	**M**	**F**	**M**	**F**
Hunched posture				1/10		1/10	4/10	7/10
Piloerection						2/10	3/10	7/10
Slow respiration							1/10	1/10
Irregular respiration							1/10	
Labored respiration								2/10
Shallow respiration								2/10
Subdued							1/10	3/10
Pale skin							2/10	5/10
Weight loss							1/10	1/10

Empty cells = zero incidence

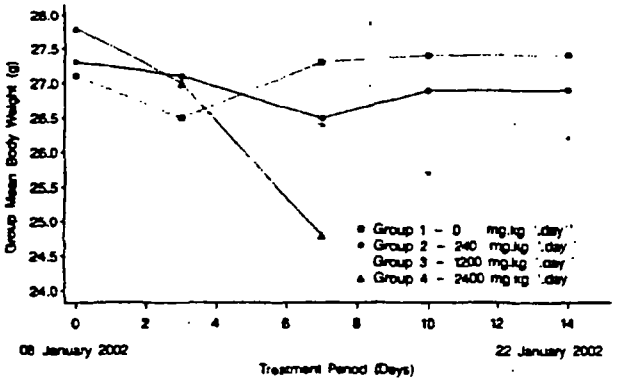
Body weights: (g)

MALES	Day 0	Day 14	% Decrease in Actual B. Wt. (Day 14)
0	34.4	33.5	0%
240	33.5	33.5	0%
1200	35.3	33.0	1%
2400	35.6	No data	No data
FEMALES	**Day 0**	**Day 14**	**% Decrease in Actual B. Wt. (Day 14)**
0	27.1	27.4	0%
240	27.3	26.9	2%
1200	27.3	26.2	4%
2400	27.8	No data	No data

Group Mean Body Weight: Males



Group Mean Body Weight: Females



Food consumption: No treatment-related changes.

Ophthalmoscopy: No data.
Electrocardiography: No data.
Hematology:

MALES	Dose (mg/kg/d)	0	240	1200	2400
Hemoglobin (g/dl)		14.4	14.3	13.0*** (10%↓)	
RBC		9.2	9.3	8.4** (9%↓)	
HCT		0.44	0.44	0.40** (9%↓)	
WBC		8.3	7.5	4.9**(41%↓)	
Lymphocytes		6.9	6.2	4.0** (42%↓)	
Basophils		0.02	0.02	0.01* (50%↓)	
Large unstained cells		0.05	0.04	0.03* (40%↓)	
Platelets (x 10^9/l)		1368	1101** (20%↓)	830*** (39%↓)	
FEMALES	**Dose (mg/kg/d)**	**0**	**240**	**1200**	**2400**
Hemoglobin (g/dl)		15.0	14.8	13.7** (9%↓)	
HCT		0.46	0.46	0.42* (9%↓)	
MCH (g/dl)		16.7	15.9*** (5%↓)	15.9** (5%↓)	
Eosinophils		0.17	0.26	0.06** (65%↓)	
Platalet (x 10^9/l)		1067	914* (14%↓)	613*** (43%↓)	

*** p<0.05; ** p<0.01; *** p<0.001**

Clinical chemistry: Mean/SD : SD included to clarify significant differences.

MALES	Dose (mg/kg/d)	0	240	1200	2400
AST		91/63	79/27	138/35**	123/61
Alkaline Phosphatase		191/59	174/28	107/30***	109/63
AG Ratio		1.7/0.1	1.8/0.3	2.0/0.2***	1.7/0.5
FEMALES	**Dose (mg/kg/d)**	**0**	**240**	**1200**	**2400**
AST		93/23	77/14	172/26***	202/180
ALT		41/11	37/12	56/19*	105/120
Alkaline Phosphatase		261/65	186/35**	96/38***	66/21

*** p<0.05; ** p<0.01; *** p<0.001**

Urinalysis: No data.
Organ weights: (g)

MALES	Dose (mg/kg/d)	0	240	1200	2400
Heart		0.20	0.21	0.21	0.17*
Liver		1.78	1.80	1.81	2.29**
Spleen		0.09	0.08	0.09	0.12***
Thymus		0.04	0.04	0.03**	0.02***
FEMALES	**Dose (mg/kg/d)**	**0**	**240**	**1200**	**2400**
Liver		1.45	1.37	1.52	1.83**
Spleen		0.10	0.12	0.11	0.15**
Thymus		0.06	0.06	0.06	0.02***
Uterus		0.18	0.17	0.18	0.08***

*** p<0.05; ** p<0.01; *** p<0.001**

Gross pathology: Tissues from all dose groups were examined.

Dose (mg/kg/d)	0		240		1200		2400	
Sex	**M**	**F**	**M**	**F**	**M**	**F**	**M**	**F**
Adrenal gland								
Pale							1/10	1/10
Dark							1/10	
Duodenum								
Pale								2/10
Liver								
Pale							5/10	4/10
Prominent lobulation							1/10	1/10

Gross Pathology Contd.

Dose (mg/kg/d)	0		240		1200		2400	
Sex	**M**	**F**	**M**	**F**	**M**	**F**	**M**	**F**
Lung								
Pale								1/10
Dark focus			1/10				2/10	
Adhesions							1/10	
Reddened								1/10
Bronchial lymph node								
Enlarged				1/10	1/10		1/10	
Reddened							1/10	
Mandibular lymph node								
Enlarged							1/10	
Mediastinal lymph node								
Enlarged				1/10				
Esophagus								
Lesions					1/10			1/10
Rupture							1/10	

Empty cells = zero incidence

Histopathology: Spleen, thymus, liver and gall bladder from all dose groups were examined. For the remaining tissues, only tissues from control and HD groups were examined.

Dose (mg/kg/d)	0		2400	
Sex	**M**	**F**	**M**	**F**
Lung				
Alveolar macrophage accumulation			1/10(1)	1/10(2)
Pleuritis			1/10(3)	1/10(3)
Congestion			2/10 1/10(1) 1/10(2)	1/10(1)
Mandibular lymph node				
Lymphadenitis				1/10(3)
Lymphoid atrophy			1/10(1)	1/10(1)
Kidney				
Hypertrophy, tubular			1/10(1)	
Esophagus				
Myositis			1/10(4)	1/13(2)
Inflammation				1/13(1)
Rupture		1/10X		
Fibrosis	4/10 2/10(1) 1/10(2) 1/10(3)	1/10(1)	1/10(2)	4/13 2/10(1) 2/10(2)
Stomach, glandular				
Gastritis				1/13(3)
Stomach, nonglandular				
Gastritis				1/13(2)
Inflammatory cell foci			2/10(2)	1/13(1)
Sternum			3/10	
Megakaryocytosis			2/10(1) 1/10(2)	1/13(1)

1 = minimal; 2 = mild; 3 = moderate; 4 = marked; X = present; Empty cells = zero incidence

Histopathology: Tissues examined from all dose groups

Dose (mg/kg/d)	0		240		1200		2400	
Sex	**M**	**F**	**M**	**F**	**M**	**F**	**M**	**F**
Spleen Megakaryocytosis	1/10(1)	4/10 3/10(1) 1/10(2)		8/10 4/10(1) 2/10(2) 2/10(3)	4/10 3/10(1) 1/10(2)	8/10 3/10(1) 3/10(2) 2/10(3)	8/10 1/10(1) 4/10(2) 3/10(3)	11/13 7/13(1) 3/13(2) 1/13(3)
Lymphocytosis							1/10(3)	1/13(2)
Congestion								1/13(3)
Lymphoid atrophy							1/10(2)	
Thymus **Lymphocytolysis**		3/10(1)	1/10(1)	4/10(1)	8/10 6/10(1) 2/10(2)	10/10 5/10(1) 4/10(2) 1/10(3)	9/10 1/10(1) 4/10(2) 2/10(3) 2/10(4)	13/13 2/13(2) 5/13(3) 6/13(4)
Liver **Hepatocyte vacuolation**		4/10(2)			3/10 2/10(1) 1/10(2)	2/10(1)	6/10 3/10(2) 3/10(3)	9/13 7/13(2) 2/13(3)

1 = minimal; 2 = mild; 3 = moderate; 4 = marked; X = present; Empty cells = zero incidence

Toxicokinetics:

		TK Parameters Males			TK Parameters Females		
Dose (mg/kg/d)	**Day**	**$AUC_{(0-\infty)}$ (ng.h/ml)**	**$AUC_{(0-t)}$ (ng.h/ml)**	**C_{max} (obs) (ng.h/ml)**	**$AUC_{(0-\infty)}$ (ng.h/ml)**	**$AUC_{(0-t)}$ (ng.h/ml)**	**C_{max} (obs) (ng.h/ml)**
240	1	22366	21939	25467	25868	23861	
	14	26536	25280	27833	34622	33305	
1200	1	151703	103832	77533	202846	118843	
	14	148349	136971	72167	181444	157381	
2400	1	N/C	167963	127400	N/C	179100	
	14	NS	NS	NS	NS	NS	NS

NS = No samples taken from HD group on Day 14; NC = Not possible to calculate this estimate from concentration data available. Clinical dose is 100 mg TID (AUC_{0-6} = 8911 ng.hr/ml).

Summary of individual study findings:

Four groups of 10 male and 10 female mice were dosed with OGT 918 by gavage TID for 14 days at 240, 1200 and 2400 mg/kg/d (total dose). Due to marked weight loss in HD (39X the clinical dose - mg/m^2) animals, all remaining animals in the group (9/10 HD males) and (9/10 HD females) were sacrificed on Day 9. The cause of death could not be determined. 1/10 HD males and 1/13 HD females died of gavage error. 3 HD females (TK group) were also sacrificed on Day 9. Erythrocyte parameters (RBC-males only), HCT, HGB and MCH-females only) decreased slightly and dose-dependently achieving statistical significance in the MD (5X and 6X the clinical dose for males and females respectively - AUC) group. This is suggestive of anemia. Dose-dependent decreases in WBC (41% at MD) and Lymphocytes (42%↓ at MD) were observed in treated males. The decrements were significant in MD males. Basophils and large unstained cells were significantly decreased in MD males by 50% and 40% respectively. Platelets decreased in a dose dependent manner achieving statistical significance at doses ≥ LD (1X the clinical dose for males and females - AUC). Eosinophils were significantly decreased by 65% in MD females. Slight but significant increase in AST was observed in MD males (1.5-fold↑) and females (1.8-fold↑) relative to control. ALT was slightly but significantly increased in MD females (1.4-fold↑) only. This may correlate with the hepatocyte vacuolation observed.

Absolute heart weight was significantly decreased by 19% in HD males (due to decreased body wt.) relative to control. Weights of the liver and spleen were slightly but significantly increased in the HD group. The increased weight of the spleen correlates with the megakaryocytosis noted.

Weight of the thymus was significantly decreased in the HD group by 50% (males) and 66% (females). This correlates with the lymphocytolysis observed. Weight of the uterus was significantly decreased (55%↓) in HD females with no correlative histopathology. The target organs of toxicity include the stomach (gastritis), spleen (megakaryocytosis), thymus (lymphocytolysis) and liver (vacuolation). NOAEL could not be established because only tissues from control and HD groups were examined for the most part. For the tissues examined from all dose groups (spleen, thymus and liver), NOAEL could not be established due to histopathology findings in the spleen and thymus at the LD.

STUDY TITLE: 13-WEEK STUDY IN MICE WITH ADMINISTRATION BY GAVAGE THREE TIMES DAILY

Key study findings:

- Dose-dependent decrease in platelets was observed in all treated mice. This achieved statistical significance at doses ≥ MD. Slight but significant decreases in hemoglobin (10%↓) and MCHC (10%↓) were observed in HD females. Platelet count decreased dose-dependently and significantly by 19% and 28% in MD and HD males respectively. In females, plate count was significantly decreased at the MD and HD by 31% and 30% respectively. PT was not measured.
- AST was significantly increased by 2-fold in both HD males and females. Alkaline phosphatase and total protein were significantly decreased by 2-fold and 1-fold respectively in HD females relative to control.
- Weights of the heart, kidney and salivary gland were slightly but significantly decreased by 14%, 17% and 14% respectively in HD males. Heart weight was also significantly decreased by 18% in MD males. Liver weight was significantly increased in HD females by 32% relative to control. Brain weight was slightly but significantly increased by 6% and 8% in MD and HD females respectively. Except for the kidney (inflammatory cell foci) there was no correlative histopathology associated with these weight changes.
- The target organs of toxicity include the spleen (lymphoid depletion), axillary lymph node (inflammation), thymus (lymphocytolysis), Kidney (inflammatory cell foci, basophilic tubules - males), liver (vacuolation - females), spinal cord (vacuolation, mineralization) and brain (vacuolation).
- NOAEL could not be established because of brain, liver and thymus histopathology at the LD.

Study no: 455869
Volume #, and page #: Vol. 2, pg. 1.
Conducting laboratory and location:
Date of study initiation: September 5, 2001.
GLP compliance: Yes (UK).
QA report: Yes (X) no ( )
Drug, lot #, radiolabel, and % purity: Batch # 004, 100% pure.
Formulation/vehicle: A solution of OGT 918 in sterile water.

Methods (unique aspects):
Dosing: Oral (gavage), with OGT 918 administered TID at total doses of 100, 420 and 840 mg/kg/d.
Species/strain: Mouse/Crl: CD-1™(ICR) BR.
#/sex/group or time point (main study): 10/sex/group.
Satellite groups used for toxicokinetics or recovery: 20/sex/group for TK.

Age: 8 weeks at study initiation.
Weight: 20-22g (M); 17-19g (F).
Doses in administered units: 100, 420 and 840 mg/kg/d.
Route, form, volume, and infusion rate: Oral (gavage), 5 ml/kg.
Observations and times:
Clinical signs: Daily.
Body weights: Weekly.
Food consumption: Weekly.
Ophthalmoscopy: Not conducted.
EKG: Not conducted.
Hematology: Blood samples were collected during week 13 of treatment for routine hematology evaluation.
Clinical chemistry: Blood samples were collected during week 13 of treatment for routine clinical chemistry evaluation.
Urinalysis: Not conducted.
Gross pathology: Organs/Tissues isolated for gross examination are indicated in the list of addendum.
Organs weighed: Organs weighed are indicated in the list of addendum.
Histopathology: Tissues isolated for histopathology examination are indicated in the list of addendum.
Toxicokinetics: Blood samples were obtained from 3/sex/TK animals on Days 1 and 90 at 0, 0.5, 1, 2, 4 and 6 hr post dose.
Results:
Mortality: Sponsor attributed the demise of all animals to gavage error.

Animal No./Group (Dose Level)	Week of death/status	Clinical signs prior to death	Necropsy Findings
No. 8/Gp 1 Male (0)	7/FD	NAD	Organs autolysed
No. 111/Gp 2 Female (100)	8/KP	Irregular and crackling respiration	Intestine distended by contents
No. 42/Gp 3 Male (420)	1/KP	Slow and irregular respiration, subdued, hard area on ventral neck	Mass in thoracic cavity
No. 46/Gp 3 Male (420)	7/KP	Irregular and gasping respiration, swollen ventral neck	Spleen enlarged, mass on thoracic region
No. 148/Gp 3 Female (420)	1/KP	Slow respiration, subdued, rolling gait, hunched posture	Eyes opaque, lungs reddened
No. 76/Gp 4 Male (840)	1/KP	Irregular respiration, subdued	Oesophagus ruptured
No. 175/Gp 4 Female (840)	1/KP	Markedly subdued, rolling gait, hunched posture, weight loss, extremities cold and pale	Lungs dark

FD = found dead; KP = killed prematurely; NAD = no abnormalities detected

Dose (mg/kg/d)	Incidence of Deaths
0	1/20 F: FD
100	1/20 F: KP
420	3/20 (2/20 M; 1/20 F): KP
840	2/10 (1/20 M: 1/20 F): KP

Clinical signs: n = 20 (10/sex/group)

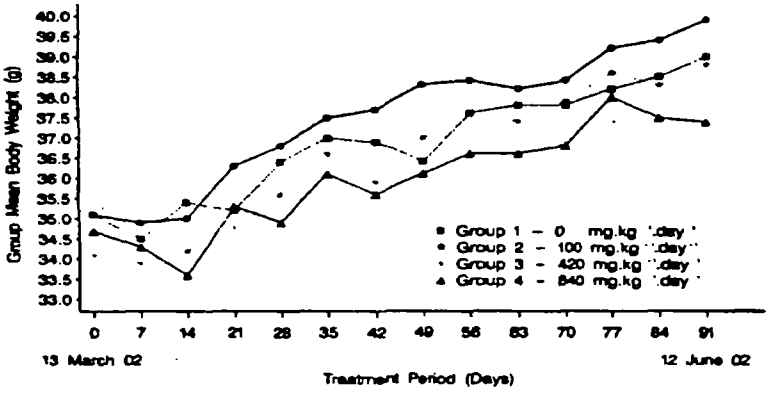
Observation/Finding	Dose (mg/kg/d)			
	0	100	420	840
Subdued		1/20	4/20	3/10
Rolling gait		1/20	3/20	1/20
Piloerection		1/20	4/20	4/20
Weight loss		1/20	3/20	2/20
Hunched posture	1/20		4/20	6/20
Slow/irregular/gasping/crackling respiration		1/20	3/20	1/20

Empty cells = zero incidence

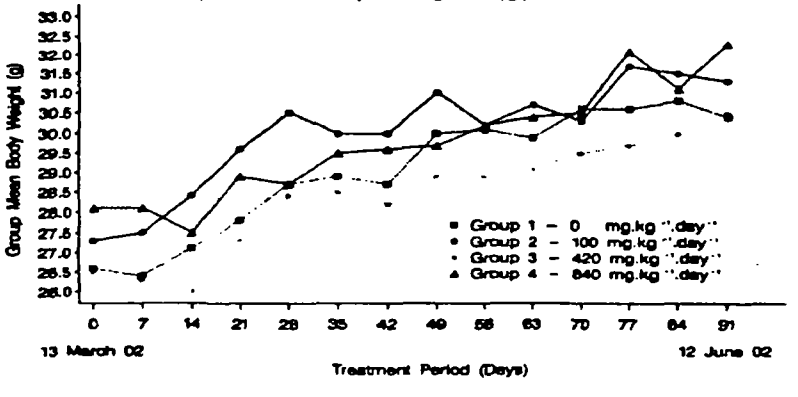
Body weights: (g)

MALES	Week 1	Week 13	Wt. Gain	Decrement	% Decrement in B. Wt. Gain	% Decrease in Actual B. Wt.
0	35.1	39.0	3.9	0	0%	0%
100	35.1	39.9	4.8	+ 0.9	+ 23%	0%
420	34.1	38.8	4.7	+ 0.8	+ 21%	0%
840	34.7	37.4	2.7	- 1.2	- 31%	4%
FEMALES	**Week 1**	**Week 13**	**Wt. Gain**	**Decrement**	**% Decrement in B. Wt. Gain**	**% Decrease in Actual B. Wt.**
0	26.6	30.4	3.8	0	0%	0%
100	27.3	31.3	4.0	+ 0.2	+ 5%	0%
420	26.5	30.5	4.0	+ 0.2	+ 5%	0%
840	28.1	32.3	4.2	+0.4	+ 11%	0%

Group Mean Body Weights (g): Males



Group Mean Body Weights (g): Females



Food consumption: Unremarkable

Ophthalmoscopy: No data.
Electrocardiography: No data.
Hematology:

MALES	**0 mg/kg/d**	**100 mg/kg/d**	**420 mg/kg/d**	**840 mg/kg/d**
Platelets (x 10^9/l)	1246	1120	1013* (19%↓)	892** (28%↓)
FEMALES	**0 mg/kg/d**	**100 mg/kg/d**	**420 mg/kg/d**	**840 mg/kg/d**
Hemoglobin (g/dl)	13.9	14.2	14.0	12.5* (10%↓)
MCHC (g/dl)	32.2	32.0	32.2	31.0* (4%↓)
Platalet (x 10^9/l)	1113	1156	769** (31%↓)	779** (30%↓)

*** p<0.05; ** p<0.01**

Clinical chemistry:

MALES	**0 mg/kg/d**	**100 mg/kg/d**	**420 mg/kg/d**	**840 mg/kg/d**
AST (IU/L)	69	64	86	112*** (2X↑)
FEMALES	**0 mg/kg/d**	**100 mg/kg/d**	**420 mg/kg/d**	**840 mg/kg/d**
AST (IU/L)	93	79	105	150*** (2X↑)
AP (IU/L)	179	160	139	74*** (2X↓)
TP (g/l)	54	53	52	47** (2X↓)

*** p<0.05; ** p<0.01; *** p<0.001**

Urinalysis: No data.
Organ weights: (g)

MALES	**0 mg/kg/d**	**100 mg/kg/d**	**420 mg/kg/d**	**840 mg/kg/d**
Heart (g)	0.22	0.22	0.18* (18%↓)	0.19* (14%↓)
Kidneys (g)	0.60	0.63	0.56	0.50* (17%↓)
Salivary glands (g)	0.22	0.23	0.21	0.19* (14%↓)
FEMALES	**0 mg/kg/d**	**100 mg/kg/d**	**420 mg/kg/d**	**840 mg/kg/d**
Brain (g)	0.49	0.51	0.52** (6%↑)	0.53*** (8%↑)
Liver (g)	1.37	1.42	1.43	1.81** (32%↑)

*** p<0.05; ** p<0.01; *** p<0.001**

Gross pathology:

Dose (mg/kg/d)	**0**		**100**		**420**		**840**	
	M	**F**	**M**	**F**	**M**	**F**	**M**	**F**
Lymph node (axillary) Enlarged – R								1/10
Lymph node (Lumbar) Enlarged							1/10	
Lymph node (mesent –eric) Enlarged							1/10	
Esophagus Ruptured							1/10	

Empty cells = zero incidence

Histopathology: Tissues from all groups were examined.

Dose (mg/kg/d)	**0**		**100**		**420**		**840**	
	M	**F**	**M**	**F**	**M**	**F**	**M**	**F**
Trachea Inflammation								2/10(1)
Lung Inflammation								2/10 1/10(1) 1/10(2)
Congestion/hemorrhage							1/10(2)	2/10(2)
Lymph node (axillary) Inflammation								1/10(2)
Spleen Increased hemopoiesis			1/10(1)				1/10(1)	2/10(1)
Lymphoid depletion					1/10(4)	1/10(3)	1/10(4)	1/10(1)

Histopathology contd.

Dose (mg/kg/d)	0		100		420		840	
	M	F	M	F	M	F	M	F
Thymus ↑ Lymphocytolysis		3/10(1)	1/10(2)	7/10 5/10(1) 2/10(2)	6/10 2/10(1) 4/10(2)	9/10 5/10(1) 3/10(2) 1/10(3)	8/10 2/10(1) 5/10(2) 1/10(3)	8/10 3/10(1) 6/10(2)
Testis Vacuolation							2/10(1)	
Kidney Inflammatory cell foci	3/10(1)	6/10 5/10(1) 1/10(2)		1/10(1)			7/10 6/10(1) 1/10(2)	5/10 4/10(1) 1/10(2)
Basophilic tubules	1/10(1)	1/10(1)					2/10(1)	1/10(1)
Esophagus Inflammation						1/10(1)	2/10 1/10(1) 1/10(3)	2/10 1/10(1) 1/10(2)
Stomach Dilated glands							1/10(1)*	
Cecum Goblet cell hyperplasia							1/10(1)*	
Colon Goblet cell hyperplasia							1/10(1)*	
Rectum Goblet cell hyperplasia							1/10(1)*	
Liver Hepatocyte vacuolation		1/10(1)		1/10(1)		1/10(1)		2/10 1/10(1) 1/10(2)
Spinal cord Vacuolation							2/10 1/10(1) 1/10(2)	
Mineralization							1/10(1)	
Brain Vacuolation	3/10(1)	2/10(1)		1/10(1)	2/10 1/10(1) 1/10(2)	1/10(2)	4/10 2/10(1) 2/10(2)	

Empty cells = zero incidence; 1 = minimal; 2 = mild; 3 = moderate, 4 = marked, * same animal

Toxicokinetics:

Pharmacokinetic Parameter Estimates (n=3) Day 1

Dose Level ($mg.kg^{-1}.bid^{-1}$)	Sex	Tmax (obs) h	Cmax (obs) $ng.ml^{-1}$	T½el h	Kel 1/h	CL/F $ml.h^{-1}.kg^{-1}$	AUC (0-t) $ng.h.ml^{-1}$	AUC (0-∞) $ng.h.ml^{-1}$	Rsq
100	Male			1.59	0.4351	3449	9464	9664	0.95
	Female			0.95	0.7297	2819	11699	11825	0.99
420	Male			2.90	0.2390	2480	48692	56460	0.98
	Female			2.07	0.3354	2204	57355	63516	0.99
840	Male			4.35	0.1594	2159	95907	129668	0.60
	Female			5.14	0.1349	1765	114283	158637	0.69

Pharmacokinetic Parameter Estimates (n=3) Week 13

Dose Level ($mg\ kg^{-1}.bid^{-1}$)	Sex	Tmax (obs)	Cmax (obs)	T½el h	Kel 1/h	CL/F $ml.h^{-1}.kg^{-1}$	AUC (0-t) $ng.h.ml^{-1}$	AUC (0-∞) $ng.h.ml^{-1}$	Rsq
100	Male			2.02	0.3437	3573	9328	10121	1.00
	Female			1.10	0.6327	3240	10287	10505	0.99
420	Male			15.45	0.0449	2832	49443	118251	0.12
	Female			3.02	0.2292	1872	74796	92741	0.98
840	Male			1.65	0.4203	2526	110864	117523	0.85
	Female			2.31	0.3006	1491	187856	227781	0.95

Clinical dose is 100 mg TID (AUC_{0-6} = 8911 ng.hr/ml).

Summary of individual study findings:
Four groups of 10 male and 10 female CD-1 mice were dosed with OGT 918, by gavage three times daily for 13 consecutive weeks at doses of 100, 420 and 840 mg/kg/day (total dose). Dose-dependent decrease in platelets that achieved statistical significance at doses ≥ MD (2X and 3X the clinical dose for males and females respectively - AUC) was observed in all treated mice. Slight but significant decreases in hemoglobin (10%↓) and MCHC (10%↓) were observed in HD (7X the clinical dose - AUC) females. AST was significantly increased by 2-fold in both HD males (4X the clinical dose - AUC) and females. Alkaline phosphatase and total protein were significantly decreased by 2-fold and 1-fold respectively in HD females relative to control. Weights of the heart, kidney and salivary gland were slightly but significantly decreased by 14%, 17% and 14% respectively in HD males (4X the clinical dose - AUC). Heart weight was also significantly decreased by 18% in MD males (2X the clinical dose - AUC). Liver weight was significantly increased in HD females by 32% relative to control. Brain weight was slightly but significantly increased by 6% and 8% in MD and HD females respectively. Except for the kidney (inflammatory cell foci) there was no correlative histopathology associated with these weight changes. The target organs of toxicity include the spleen (lymphoid depletion), axillary lymph node (inflammation), thymus (lymphocytolysis), Kidney (inflammatory cell foci, basophilic tubules - males), liver (vacuolation - females), spinal cord (vacuolation, mineralization) and brain (vacuolation). NOAEL could not be established because of brain, liver and thymus histopathology at the LD (1X the clinical dose - AUC).

Toxicology summary:
Four groups of 10 male and 10 female CD-1 mice were dosed with OGT 918 by gavage TID for 14 days at 240, 1200 and 2400 mg/kg/d (total dose) and at 100, 420 and 840 mg/kg/d (total dose) for 13 weeks. In the 2-week study, due to marked weight loss in HD (39X the clinical dose - mg/m^2) animals, all remaining animals in the group (9/10 HD males) and (9/10 HD females) were sacrificed on Day 9. 3 HD females (TK group) were also sacrificed on Day 9. There were no drug-related deaths in the 13 -week study. In the 2 week study, erythrocyte parameters (RBC-males only), HCT, HGB and MCH-females only) decreased slightly and in a dose-dependent manner achieving statistical significance in the MD (5X and 6X the clinical dose for males and females respectively - AUC) group. Slight but significant decreases in hemoglobin (10%↓) and MCHC (10%↓) were observed in HD (7X the clinical dose - AUC) females following the 13 weeks exposure. Significant and dose-dependent decreases in WBC (41% at MD) and Lymphocytes (42%↓ at MD) were observed in treated males in the 2-week study. Eosinophils were significantly decreased by 65% in MD females. Basophils and large unstained cells were significantly decreased in MD males by 50% and 40% respectively after the 2-week exposure. The WBC, lymphocytes, basophils, eosinophils and large unstained cells were not affected in the 13-week study. Dose-dependent decrease in platelets that achieved statistical significance at doses ≥ MD (2X and 3X the clinical dose for males and females respectively - AUC) was observed in all mice following 13 weeks of treatment. Similarly, platelets decreased in a dose dependent manner achieving statistical significance at doses ≥ LD (1X the clinical dose for males and females - AUC) following 2-weeks of treatment.

In the 2-week study, slight but significant increase (2-fold↑) in AST was observed in MD males and females whereas ALT was slightly but significantly increased only in MD females (1.4-fold↑). In the 13 week study, AST was significantly increased by 2-fold in HD males and females (4X and 7X the clinical dose for males and females respectively - AUC). This may correlate with the hepatocyte vacuolation observed in both studies.

Absolute heart weight was significantly decreased by 19% in HD (39X the clinical dose - mg/m^2) males (due to decreased body wt.) in the 2-week study. Weights of the liver and spleen (megakaryocytosis) were slightly but significantly increased in the HD group. Weight of the thymus was significantly decreased in the HD group by 50% (males) and 66% (females). This correlates with the lymphocytolysis observed. Weight of the uterus was significantly decreased (55%↓) in HD females with no correlative histopathology. In the 13-week study, weights of the heart and kidney were slightly but significantly decreased by 14%, and 17% respectively in HD males (4X the clinical dose - AUC). Heart weight was also significantly decreased by 18% in MD males (2X the clinical dose - AUC). Liver weight was significantly increased in HD (7X the clinical dose - AUC) females by 32%. Brain weight was slightly but significantly increased by 6% and 8% in MD and HD females respectively. Except for the kidney (inflammatory cell foci) there was no correlative histopathology associated with the weight changes observed in the 13-week study.

Target organs of toxicity common to both the subacute and subchronic studies were the thymus (lymphocytolysis), liver and spleen. Toxicity of the spleen manifested as megakaryocytosis and lymphoid depletion in the subacute and subchronic studies respectively. While hepatocyte vacuolation was observed in both males and females of the subacute study, this occurred only in females of the subchronic study. In addition, toxicity of the axillary lymph node (inflammation), Kidney (inflammatory cell foci, basophilic tubules - males), spinal cord (vacuolation, mineralization) and brain (vacuolation) were observed in the subchronic study. NOAEL could not be established in the subchronic study because of brain, liver and thymus histopathology at the LD (< 1X the clinical dose - AUC). The target organ affected in the subacute study but not in the subchronic study is the stomach (gastritis). NOAEL could not be established for the subacute study as well because only tissues from control and HD groups were examined for the most part. For the tissues examined from all dose groups (spleen, thymus and liver), NOAEL could not be established due to histopathology findings in the spleen and thymus at the LD (1X the clinical dose for males and females - AUC). The NOAEL decreases with increased duration of dosing.

Toxicology conclusions:
The HD of 840 mg/kg/d was tolerated for the duration of the 13-week study. In the subacute study, toxicity of the spleen (megakaryocytosis) and thymus (lymphocytolysis) occurred at 1X the clinical dose in males and females - AUC. In the subchronic study, toxicity of the brain (vacuolation), liver (vacuolation) and thymus (lymphocytolysis) occurred at < 1X the clinical dose - AUC. These toxicities occurred at low multiples of the clinical dose. However, histopathology findings at the 840 mg/kg/d were mostly minimal to mild in severity after 13 weeks of exposure to OGT 918. Reviewer believes 840 mg/kg/d would be an appropriate HD for the 2-year carcinogenicity study.

Histopathology Inventory for IND # 60,197

Study	455853	455869
Species	Mouse	Mouse
Adrenals	X*	X*
Aorta	X	X
Bone Marrow smear		
Bone (femur)		
Brain	X*	X*
Cecum	X	X
Cervix		
Colon	X	X
Duodenum	X	X
Epididymis	X*	X*
Esophagus	X	X
Eye		
Fallopian tube		
Gall bladder	X*	X
Gross lesions	X	X
Harderian gland		
Heart	X*	X*
Ileum	X	X
Injection site		
Jejunum	X	X
Kidneys	X*	X*
Lachrymal gland		
Larynx		
Liver	X*	X*
Lungs		
Lymph nodes, cervical		
Lymph nodes, mandibular		
Lymph nodes, mesenteric	X	X
Mammary Gland		
Nasal cavity		
Optic nerves	X	X
Ovaries	X*	X*
Pancreas	X	X
Parathyroid	X*	X*
Peripheral nerve		
Pharynx		
Pituitary	X*	X*
Prostate	X*	X*
Rectum	X	X
Salivary gland	X*	X*
Sciatic nerve	X	X
Seminal vesicles	X	X
Skeletal muscle	X	X
Skin	X	X
Spinal cord	X	X
Spleen	X*	X*
Sternum	X	X
Stomach	X	X
Testes		
Thymus	X*	X*
Thyroid	X*	X*
Tongue	X	X
Trachea	X	X
Urinary bladder	X	X
Uterus	X*	X*
Vagina	X	X
Zymbal gland		

X, histopathology performed
*, organ weight obtained

II. GENETIC TOXICOLOGY

Study title: An Evaluation of the Mutagenic Potential of SC-48334 in the Ames Salmonella/Microsome Assay.

Key findings: Valid, Negative

Study title: An Evaluation of the Mutagenic potential of SC-48334 (NBDG Route) in the Ames Salmonella/Microsome assay.

Key findings: Valid, Negative

Study title: Bacterial Reverse Mutation Test

Key findings: Valid, Negative

Study title: An Evaluation of the Mutagenic Potential of SC-48334 in the CHO/HGPRT Mutation Assay.

Key findings: Valid, Negative

Study title: In Vitro Mammalian Cell Cytogenetic Test: Human Lymphocytes

Key findings: Valid, Negative

Study title: An Evaluation Of The Potential Of SC-48334 To Induce Micronucleated Polychromatic Erythrocytes In The Bone Marrow Cells Of Mice.

Key findings: Valid, Negative

Reviewer's Signature:

/S/

.....

John Colerangle, DVM, Ph.D. Date

Team Leader's Signature:

/S/

.....

Karen Davis-Bruno, Ph.D. Date

cc: IND Arch
HFD 510
HFS 510/Colerangle/Davis-Bruno/Wu
Review Code: ND
File Name: ECAC60197.doc

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

/s/

John Colerangle
2/27/03 11:14:41 AM
PHARMACOLOGIST
P/T 3 = MOUSE CARCINOGENICITY DOSE SELECTION STUDY

Karen Davis-Bruno
2/27/03 11:17:47 AM
PHARMACOLOGIST

PHARMACOLOGY/TOXICOLOGY COVER SHEET

NDA number: 21-348
Review number: 2
Sequence number/date/type of submission: AZ/February 7, 2003/NDA Amendment.
Information to sponsor: Yes ( ) No (X)
Sponsor and/or agent: Oxford Glycosciences, The Forum, 86 Milton Park, Abingdon, Oxon, OX14 4RY, U.K.
Manufacturer for drug substance: Galen Ltd., Seagoe Industrial Estate, Craigavon, BT65 5UA, UK.

Reviewer name: John Colerangle
Division name: Division of Metabolic and Endocrine Drug Products (DMEDP).
HFD #: 510
Review completion date: May 28, 2003.

Drug:
Trade name: Zavesca
Generic name (list alphabetically): Miglustat, N-butyl-deoxynojirimycin.
Code name: OGT 918, SC48344, [OGT 924 also known as SC49483 (prodrug) is a perbutyrated derivative of OGT 918 used in some toxicity studies].
Chemical name: 1, 5 (Butlyimino)-1,5-dideoxy-D-glucitol.
CAS registry number: 72599-27-0.
Mole file number: N/A.
Molecular formula/molecular weight: $C_{10}H_{21}NO_4$,/219.28

Structure:

Relevant INDs/NDAs/DMFs: IND 60,197 (0GS),

Drug class: An imino sugar (Glucoslytransferase inhibitor)
Indication: Treatment of Type 1 Gaucher Disease.

Clinical formulation:

Ingredient	Content
Miglustat (OGT 918) – active ingredient	100 mg
Sodium starch glycollate	
Povidone (K30)	
Magnesium sterate	
Capsule	1 capsule

The proposed clinical dose is 100 mg TID or 300 mg/day.

Executive Summary

I. Recommendations

A. Recommendation on Approvability
Pharmacology/Toxicology recommends approval of this drug for the proposed indication.

B. Recommendation for Nonclinical Studies
The preclinical studies are adequate to support the safety of the 100 mg TID dose. A rat carcinogenicity study was accepted on 8/4/99 as a Phase IV commitment. ECAC reviewed the rat dose selection on 12/18/01.

C. Recommendations on Labeling

2 page(s) of revised draft labeling has been redacted from this portion of the review.

II. Summary of Nonclinical Findings

A. Brief Overview of Nonclinical Findings

Based on the concern over neurological adverse events observed in clinical trials, the Division recommended re-evaluation of brain, spine and nerve histopathology in the chronic monkey study (SA 4078). Use of special stains for neurological tissue, neuroanatomical sectioning, and ultrastructural assessments were recommended. The sponsor was asked to determine plasma and/or tissue concentrations of ceramide and glucosylceramide in these monkey tissues if possible.

The Division recommended that the sponsor determine whether the adverse effects on the male reproductive function in dogs, rats and monkeys dosed with miglustat are relevant to humans through semen analysis. The specific nature, severity and reversibility of any abnormalities were to be assessed.

Sponsor stated that the 52-week monkey study with the pro-drug (P30S4078) showed no treatment-related neuropathology. The number and location of the brain sections examined were adequate and to the industry standard for a regulatory GLP toxicity study in monkeys. Therefore, it is the view of OGS that this study provides convincing support for there being no neurotoxicity attributable to miglustat even after 52 weeks of exposure (to the pro-drug) in a non-human primate. Limited amounts of formalin fixed tissues remain from this study due to the extensive pathological examination originally conducted. In addition, the study was conducted between August 1993 and October 1994 and the condition of any remaining tissues is not likely to be suitable for further analysis after such a long period of time. There are no plasma samples remaining from this study.

Due to the limited and/or unsuitable nature of remaining monkey tissues for further analysis and the EMEA's request for further neurotoxicity evaluation in rat, the sponsor has agreed to conduct a 13-week study in rats as a post approval commitment to address the neurotoxic concerns. The draft protocol for the 13-week rat study, include investigation of neurological parameters including a Full Functional Observation Battery. The sponsor stated that analysis of ceramide content of tissues would be problematic because at present there are no validated quantitative assays for such studies. Moreover, these lipids exist as normal cellular components in tissues therefore precise and accurate quantification as well as interpretation of data generated would be difficult.

The proposed protocol for the 13-Week neurotoxicity study in rats will be a GLP study. The doses (180, 360 and 420 mg/kg/d – total dose: to be administered in split doses TID, 6 hr apart) have been selected based on previous studies conducted in the rat. 5 animals/sex/control and HD groups will be used for evaluation of reversibility of drug effects. A functional observation battery (FOB) will be carried out. Tissue will be collected and processed for microscopic evaluation. Overall, the protocol seems adequate to address the neurotoxic concerns.

The sponsor stated that the 52-week monkey study with the pro-drug (SA4078) showed no treatment-related neuropathology. This is contrary to the Reviewer's observation. Histopathology findings in monkey brain including vascular mineralization (also spinal cord) and necrosis and mineralization of the white matter were observed at exposures of 4X (750 mg/kg/day) therapeutic dose based on AUC, in the absence of clinical signs.

Vacuolization of white matter was noted in rats at exposures 6X (180 mg/kg/day) therapeutic based on mg/m^2. CNS effects in dogs included tremor and absent corneal reflexes were observed at exposures 10X (105 mg/kg/day) therapeutic based on mg/m^2. Ataxia, diminished/absent pupillary, palpebral or patellar reflexes were observed in the dog at exposures 50X (495 mg/kg/day) the therapeutic exposure based on mg/m^2. Weanling female rats given ≥ 20 mg/kg/d had marked vacuolation of brain and sciatic/tibial nerves. Both males and females exhibited head tilting when dosed post natal days 12 –70.

In a recent 13-week toxicity study in mice to address EMEA's concern for neurotoxic effects of miglustat, signals of neurotoxicity characterized by vacuolation and mineralization (spinal cord) were observed in male mice at 12X the clinical dose (AUC). Mineralization of the brain was observed in some control group mice and all treated mice except LD males and HD females. The brain signals seem questionable.

Subchronic toxicology study (13-week) was recently conducted in male rats (180, 340 and 420 mg/kg/d; 6X, 11X and 14X the clinical dose – mg/m^2) to evaluate the effects of the drug on male reproductive function, specifically sperm parameters and the reversibility of effects following 16 weeks recovery period.

Reversible decreases in sperm parameters were observed in treated rats. Mean total sperm count decreased by 61% at 14X the clinical dose (mg/m^2). Percent motile sperm decreased dose dependently achieving statistical significance at doses ≥ 11X the clinical dose (mg/m^2). Average path velocity (VAP), straight line velocity (VSL) and curvilinear velocity (VCL) were decreased by 53%, 49% and 47% respectively at 14X the clinical dose (mg/m^2). Abnormal sperm was significantly increased by 49% at 14X the clinical dose (mg/m^2) relative to 0.3% for controls. Testicular staging data (stages XII = XIII) indicate that 19% of spermatids were retained in the seminiferous epithelium of control rats. In treated rats, spermatid retention decreased to 2% at 6X the clinical dose (mg/m^2) and zero at doses ≥ 11X the clinical dose (mg/m^2) indicative of treatment-related delayed spermiation. At the end of the recovery period, spermatid retention in treated rats was similar to that of control. This is indicative of reversibility of the drug-induced effects on sperm.

Previous studies submitted to the initial NDA showed that drug adverse effects were observed on sperm morphology and sperm parameters (concentration, motility) of rats and monkeys. 4-week studies in rats showed decreased spermatogenesis (testes), hypospermia (epididymis), degeneration of germinal epithelium (testes & epididymis) and atrophy (seminal vesicle) at ≥ 6X the therapeutic dose based on mg/m^2. Following 13-week studies in rats, desquamated germ cells (testes & epididymis), seminiferous tubule atrophy (testes) were observed at doses ≥ 0.3X the therapeutic dose based on AUC. In another study of similar duration, atrophy and degeneration (testes) that increased in incidence and severity with dose was observed at doses ≥ 3X the therapeutic dose based on AUC and dystrophy (testes) was observed at 22X the therapeutic dose based on AUC. In a 26-week rat study, testicular atrophy/degeneration that showed no recovery was observed at doses ≥ 3X the therapeutic dose based on AUC. Sperm motility, concentration and number of normal sperm were all decreased at doses ≥ 3X the therapeutic dose based on AUC and showed partial recovery. Chronic studies in rats (52 weeks) also showed hypospermia (epididymis), atrophy of seminiferous tubules, aspermatogenesis, edema, multinucleated giant cells and

hyperplasia of interstitial cells (testes) at doses ≥ 5X the therapeutic dose based on AUC. These lesions showed little or no recovery after 4 weeks. A 52-week study in monkeys did not demonstrate any effects on the testes or epididymis. Mineralization of the seminal vesicle was observed at doses ≥ 4X the therapeutic dose based on AUC. Sperm concentration decreased, whereas number of amorphous sperms increased in a dose dependent manner. These effects also occurred at doses ≥ 4X the therapeutic dose based on AUC.

B. Pharmacologic Activity

Zavesca is an inhibitor of the enzyme glucosylceramide synthase, a glucosyl transferase enzyme responsible for the first step in the synthesis of most glycolipids. Glucosylceramide synthase catalyses the transfer of glucose from a UDP-glucose donor to a ceramide acceptor to form the product glucosylceramide (GlcCer) which is the building block of all glucosphingolipids. Glucosphingolipids play an important role in cell signalling, development, differentiation and host-pathogen interactions. They are important constituents of mammalian cells. Gaucher disease is caused by a failure to degrade glucosylceramide, resulting in the lysosomal storage of this material within tissue macrophages and widespread pathology. Glucosylceramide synthase is ubiquitous in tissues, hence this explains the multi-tissue nature of toxicity observed and relates to the pharmacologic activity of OGT 918.

C. Nonclinical Safety Issues Relevant to Clinical Use

The neurotoxic potential of miglustat has been investigated in mice, rats, dogs and monkeys. While neurotoxic signals were observed in these species, their occurrence is sporadic and their incidences is not dose related. Because of the concern over neurotoxic effects (tremor, paresthesia or numbness) that were seen in patients in the clinical trials, the sponsor has agreed to conduct a 13-week study in rats as a post approval commitment to address these issues.

Miglustat had adverse effects on sperm in rats and monkeys studied. However, these sperm effects are reversed upon termination of dosing.

Administrative

/S/

A. Reviewer signature: _____

/S/

B. Supervisor signature: Concurrence - _____

Non-Concurrence - _____
(see memo attached)

C. cc: list: NDA Arch
HFD 510
HFD 510/Colerangle/Davis-Bruno/Madara

TABLE OF CONTENTS - PHARMACOLOGY/TOXICOLOGY REVIEW

I. GENERAL TOXICOLOGY

STUDY TITLE: 2-WEEK TOXICITY STUDY IN MICE WITH ADMINISTRATION BY GAVAGE THREE TIMES DAILY

Key study findings:

- Due to marked weight loss in HD (2400 mg/kg/d) animals, all remaining animals in the group (9/10 HD males) and (9/10 HD females) were sacrificed on Day 9. The cause of death could not be determined. 1/10 HD males and 1/13 HD females died of gavage error. 3 HD females (TK group) were also sacrificed on Day 9.
- Treatment-related clinical signs include hunched posture, piloerection and pale skin mainly in the HD group.
- Erythrocyte parameters RBC (males only), HCT, HGB and MCH (females only) decreased slightly and dose-dependently achieving statistical significance in the MD group. This is suggestive of anemia. Dose-dependent decreases in WBC (41% at MD) and Lymphocytes (42%↓ at MD) were observed in treated males. The decrements were significant in MD males. Basophils and large unstained cells were significantly decreased in MD males by 50% and 40% respectively. Platelets decreased in a dose dependent manner achieving statistical significance at doses ≥ LD. Eosinophils were significantly decreased by 65% in MD females.
- Slight but significant increase in AST was observed in MD males (1.5-fold↑) and females (1.8-fold↑) relative to control. ALT was slightly but significantly increased in MD females (1.4-fold↑). This may correlate with the hepatocyte vacuolation observed. AG ratio increased slightly and dose-dependently achieving statistical significance in MD males. Alkaline phosphatase decreased significantly and in a dose-dependent but the significance of this change is not clear.
- Absolute heart weight was significantly decreased by 19% in HD males (due to decreased body wt.) relative to control. Weights of the liver and spleen were slightly but significantly increased in the HD group. The increased weight of the spleen correlates with the megakaryocytosis noted. Weight of the thymus was significantly decreased in the HD group by 50% (males) and 66% (females). This correlates with the lymphocytolysis observed. Weight of the uterus was significantly decreased (55%↓) in HD females with no correlative histopathology.
- The target organs of toxicity include the stomach (gastritis), spleen (megakaryocytosis), thymus (lymphocytolysis) and liver (vacuolation). NOAEL could not be established because only tissues from control and HD groups were examined for the most part. For the tissues examined from all dose groups (spleen, thymus and liver), NOAEL could not be established due to histopathology findings in the spleen and thymus at the LD.

Study no: 455853
Volume #, and page #: Vol. 1, pg. 1.
Conducting laboratory and location: [redacted]
Date of study initiation: September 5, 2001.
GLP compliance: Yes (UK).
QA report: Yes (X) no ( )
Drug, lot #, radiolabel, and % purity: Batch # 004, 100% pure.
Formulation/vehicle: A solution of OGT 918 in sterile water.

Methods (unique aspects):
Dosing: Animals were dosed by gavage with OGT 918 at 240, 1200 and 2400 mg/kg/d administered as 3 equal doses 6 hr apart.
Species/strain: Mouse/Crl:CD-1™(ICR)BR.
#/sex/group or time point (main study): 10/sex/group.
Satellite groups used for toxicokinetics or recovery: 20/sex/group for TK.
Age: 8 weeks at initiation of study.
Weight: 22-25g (M); 19-22g (F).
Doses in administered units: 240, 1200 and 2400 mg/kg/d (total dose).
Route, form, volume, and infusion rate: Oral (gavage), 5ml/kg.
Observations and times:
Clinical signs: Daily.
Body weights: Weekly.
Food consumption: Weekly.
Ophthalmoscopy: Not conducted.
EKG: Not conducted.
Hematology: Blood samples were taken from all surviving animals on Day 15 for routine hematology evaluation (HD animals were sacrificed on Day 9).
Clinical chemistry: Blood samples were taken from all HD animals on Day 8. Animals in the remaining dose groups were bled on Day 15.
Urinalysis: Not conducted.
Gross pathology: Organs/Tissues isolated for gross examination are indicated in the list of addendum.
Organs weighed: Organs weighed are indicated in the list of addendum.
Histopathology: Spleen, thymus, liver and gall bladder from all dose groups were examined. For the remaining tissues, only control and HD groups were examined.
Toxicokinetics: Blood samples were obtained on Days 1 and 14 (with the exception of high dose animals which were bled on Day 1 only) at 0, 0.5, 1, 2, 3 and 6 hr postdose.

Results:
Mortality: Due to marked weight loss (11% ↓ in body wt.) in HD animals, all remaining animals in the group (9/10 HD males) and (9/10 HD females) were sacrificed on Day 9. 1/10 HD males and 1/10 HD females died of gavage error. 3 HD females (TK group) were also sacrificed on Day 9.

Animal #	Day of Sacrifice	Cause of Death	Gross/Histopathology Findings
77 (M)	7	Gavage error	Esophagus: rupture, myositis; Lung: adhesions, congestion/hemorrhage, pleuritis. Heart: inflammatory cell infiltration; Lymph nodes (bronchial, mandibular, mesenteric): lymphoid atrophy; Spleen: megakaryocytosis, lymphoid atrophy; Thymus: lymphocytolysis.
177 (F)	3	Gavage error	Esophagus: inflammation, myositis, food present in muscle layer; Lung: reddened, congestion/hemorrhage, pleuritis; Spleen: congestion; Thym -us: lymphocytolysis, inflammation.
71–76 M) 78–80(M)	9	Marked wt. loss	Lung: congestion; Spleen: megakaryocytosis; sternum: megakaryocytosis; Thyroid: follicular cell hypertrophy; Thymus: lymphocytolysis; Liver: hepatocyte vacuolation;
171-76(F) 178-92(F)	9	Marked wt. loss	Esophagus: fibrosis, myositis, Liver: hepatocyte vacuolation; Spleen: megakaryocytosis; Sternum: megakaryocytosis; Thymus: lymphocytolysis; Thyroid: follicular cell hypertrophy; Heart: inflammatory cell infiltration.

Clinical signs: **Empty cells = zero incidence**

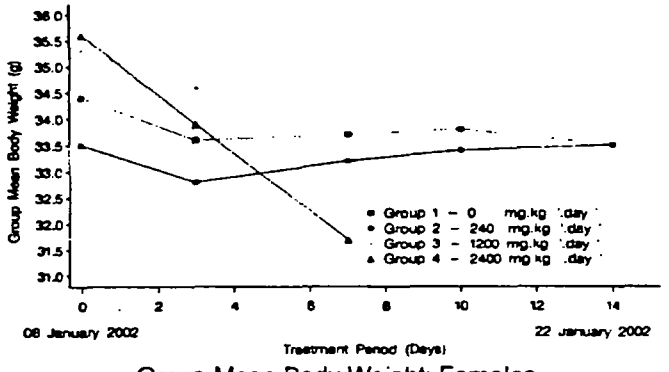
Dose (mg/kg/d)	0		240		1200		2400	
Observations/Findings	**M**	**F**	**M**	**F**	**M**	**F**	**M**	**F**
Hunched posture				1/10		1/10	4/10	7/10
Piloerection						2/10	3/10	7/10
Slow respiration							1/10	1/10
Irregular respiration							1/10	
Labored respiration								2/10
Shallow respiration								2/10
Subdued							1/10	3/10
Pale skin							2/10	5/10
Weight loss							1/10	1/10

Body weights: (g)

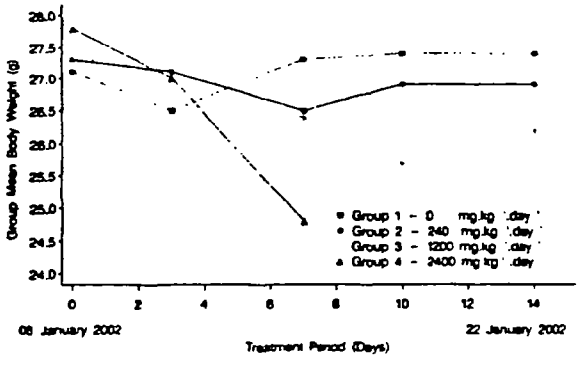
MALES	Day 0	Day 7	Day 14	% Decrease in Actual B. Wt. (Day 14)
0	34.4	33.7	33.5	0%
240	33.5	33.2	33.5	0%
1200	35.3	33.2	33.0	1%
2400	35.6	31.7 (11%↓)	No data	No data
FEMALES	**Day 0**	**Day 7**	**Day 14**	**% Decrease in Actual B. Wt. (Day 14)**
0	27.1	27.3	27.4	0%
240	27.3	26.5	26.9	2%
1200	27.3	26.4	26.2	4%
2400	27.8	24.8 (11%↓)	No data	No data

For the HD animals that were sacrificed (Day 9), body weight was decreased by 11% on Day 7 of treatment.

Group Mean Body Weight: Males



Group Mean Body Weight: Females



Food consumption: No treatment-related changes.
Ophthalmoscopy: No data.

Electrocardiography: No data.

Hematology:

MALES Dose (mg/kg/d)	0	240	1200	2400
Hemoglobin (g/dl)	14.4	14.3	13.0*** (10%↓)	
RBC	9.2	9.3	8.4** (9%↓)	
HCT	0.44	0.44	0.40** (9%↓)	
WBC	8.3	7.5	4.9**(41%↓)	
Lymphocytes	6.9	6.2	4.0** (42%↓)	
Basophils	0.02	0.02	0.01* (50%↓)	
Large unstained cells	0.05	0.04	0.03* (40%↓)	
Platelets (x 10^9/l)	1368	1101** (20%↓)	830*** (39%↓)	
FEMALES Dose (mg/kg/d)	**0**	**240**	**1200**	**2400**
Hemoglobin (g/dl)	15.0	14.8	13.7** (9%↓)	
HCT	0.46	0.46	0.42* (9%↓)	
MCH (g/dl)	16.7	15.9*** (5%↓)	15.9** (5%↓)	
Eosinophils	0.17	0.26	0.06** (65%↓)	
Platalet (x 10^9/l)	1067	914* (14%↓)	613*** (43%↓)	

*** p<0.05; ** p<0.01; *** p<0.001**

Clinical chemistry: Mean/SD : SD included to clarify significant differences.

MALES Dose (mg/kg/d)	0	240	1200	2400
AST	91/63	79/27	138/35**	123/61
Alkaline Phosphatase	191/59	174/28	107/30***	109/63
AG Ratio	1.7/0.1	1.8/0.3	2.0/0.2***	1.7/0.5
FEMALES Dose (mg/kg/d)	**0**	**240**	**1200**	**2400**
AST	93/23	77/14	172/26***	202/180
ALT	41/11	37/12	56/19*	105/120
Alkaline Phosphatase	261/65	186/35**	96/38***	66/21

*** p<0.05; ** p<0.01; *** p<0.001**

Urinalysis: No data.

Organ weights: (g)

MALES Dose (mg/kg/d)	0	240	1200	2400
Heart	0.20	0.21	0.21	0.17*
Liver	1.78	1.80	1.81	2.29**
Spleen	0.09	0.08	0.09	0.12***
Thymus	0.04	0.04	0.03**	0.02***
FEMALES Dose (mg/kg/d)	**0**	**240**	**1200**	**2400**
Liver	1.45	1.37	1.52	1.83**
Spleen	0.10	0.12	0.11	0.15**
Thymus	0.06	0.06	0.06	0.02***
Uterus	0.18	0.17	0.18	0.08***

*** p<0.05; ** p<0.01; *** p<0.001**

Gross pathology: Tissues from all dose groups were examined.

Day of Sacrifice	Day 14 (Terminal)						Day 9	
Dose (mg/kg/d)	0		240		1200		2400	
Sex	M	F	M	F	M	F	M	F
Adrenal gland								
Pale							1/10	1/10
Dark							1/10	
Duodenum								
Pale								2/10
Liver								
Pale							5/10	4/10
Prominent lobulation							1/10	1/10

Gross Pathology Contd.

Day of Sacrifice	Day 14 (Terminal)						Day 9	
Dose (mg/kg/d)	0		240		1200		2400	
Sex	M	F	M	F	M	F	M	F
Lung Pale								1/10
Dark focus			1/10				2/10	
Adhesions							1/10	
Reddened								1/10
Bronchial lymph node Enlarged				1/10	1/10		1/10	
Reddened							1/10	
Mandibular lymph node Enlarged							1/10	
Mediastinal lymph node Enlarged				1/10				
Esophagus Lesions					1/10			1/10
Rupture							1/10	

Empty cells = zero incidence

Histopathology: Only tissues from control and HD groups were examined.

Dose (mg/kg/d)	0 (Day 14)		2400 (Day 9)	
Sex	M	F	M	F
Lung Alveolar macrophage accumulation			1/10(1)	1/10(2)
Pleuritis			1/10(3)	1/10(3)
Congestion			2/10 1/10(1) 1/10(2)	1/10(1)
Mandibular lymph node Lymphadenitis				1/10(3)
Lymphoid atrophy			1/10(1)	1/10(1)
Kidney Hypertrophy, tubular			1/10(1)	
Esophagus Myositis			1/10(4)	1/13(2)
Inflammation				1/13(1)
Rupture		1/10X		
Fibrosis	4/10 2/10(1) 1/10(2) 1/10(3)	1/10(1)	1/10(2)	4/13 2/10(1) 2/10(2)
Stomach, glandular Gastritis				1/13(3)
Stomach, nonglandular Gastritis				1/13(2)
Inflammatory cell foci			2/10(2)	1/13(1)
Sternum Megakaryocytosis			3/10 2/10(1) 1/10(2)	1/13(1)

1 = minimal; 2 = mild; 3 = moderate; 4 = marked; X = present; Empty cells = zero incidence
Note: HD animals were sacrificed on treatment Day 9 due to 11% decrease in body weight.

Histopathology: Spleen, thymus, liver and gall bladder from all dose groups were examined.

Dose (mg/kg/d)	0 (Day 14)		240 (Day 14)		1200 (Day 14)		2400 (Day 9)	
Sex	**M**	**F**	**M**	**F**	**M**	**F**	**M**	**F**
Spleen	1/10(1)	4/10		8/10	4/10	8/10	8/10	11/13
Megakaryocytosis		3/10(1)		4/10(1)	3/10(1)	3/10(1)	1/10(1)	7/13(1)
		1/10(2)		2/10(2)	1/10(2)	3/10(2)	4/10(2)	3/13(2)
				2/10(3)		2/10(3)	3/10(3)	1/13(3)
Lymphocytosis							1/10(3)	1/13(2)
Congestion								1/13(3)
Lymphoid atrophy							1/10(2)	
Thymus					8/10	10/10	9/10	13/13
Lymphocytolysis		3/10(1)	1/10(1)	4/10(1)	6/10(1)	5/10(1)	1/10(1)	
					2/10(2)	4/10(2)	4/10(2)	2/13(2)
						1/10(3)	2/10(3)	5/13(3)
							2/10(4)	6/13(4)
Liver					3/10		6/10	9/13
Hepatocyte vacuolation					2/10(1)	2/10(1)		
		4/10(2)			1/10(2)		3/10(2)	7/13(2)
							3/10(3)	2/13(3)

1 = minimal; 2 = mild; 3 = moderate; 4 = marked; X = present; Empty cells = zero incidence
Note: HD animals were sacrificed on treatment Day 9 due to 11% decrease in body weight.

Toxicokinetics:

		TK Parameters Males			TK Parameters Females		
Dose (mg/kg/d)	**Day**	**$AUC_{(0-\infty)}$ (ng.h/ml)**	**$AUC_{(0-t)}$ (ng.h/ml)**	**C_{max} (obs) (ng.h/ml)**	**$AUC_{(0-\infty)}$ (ng.h/ml)**	**$AUC_{(0-t)}$ (ng.h/ml)**	**C_{max} (obs) (ng.h/ml)**
	1	22366	21939	25467	25868	23861	20800
240	14	26536	25280	27833	34622	33305	30233
	1	151703	103832	77533	202846	118843	94533
1200	14	148349	136971	72167	181444	157381	64033
	1	N/C	167963	127400	N/C	179100	120233
2400	14	NS	NS	NS	NS	NS	NS

NS = No samples taken from HD group on Day 14; NC = Not possible to calculate this estimate from concentration data available. Clinical dose is 100 mg TID (AUC_{0-t} = 8911 ng.hr/ml).

Summary of individual study findings:

Four groups of 10 male and 10 female mice were dosed with OGT 918 by gavage TID for 14 days at 240, 1200 and 2400 mg/kg/d (total dose). Due to 11% weight loss in HD (39X the clinical dose - mg/m^2) animals, all remaining animals in the group (9/10 HD males) and (9/10 HD females) were sacrificed on Day 9. 1/10 HD males and 1/13 HD females died of gavage error. 3 HD females (TK group) were also sacrificed on Day 9. Erythrocyte parameters (RBC-males only), HCT, HGB and MCH-females only) decreased slightly and dose-dependently achieving statistical significance in the MD (5X and 6X the clinical dose for males and females respectively - AUC) group. This is suggestive of anemia. Dose-dependent decreases in WBC (41% at MD) and Lymphocytes (42%↓ at MD) were observed in treated males. The decrements were significant in MD males. Basophils and large unstained cells were significantly decreased in MD males by 50% and 40% respectively. Platelets decreased in a dose dependent manner achieving statistical significance at doses ≥ LD (1X the clinical dose for males and females - AUC). Eosinophils were significantly decreased by 65% in MD females. Slight but significant increase in AST was observed in MD males (1.5-fold↑) and females (1.8-fold↑) relative to control. ALT was slightly but significantly increased in MD females (1.4-fold↑) only. This may correlate with the hepatocyte vacuolation observed.

Absolute heart weight was significantly decreased by 19% in HD males (due to decreased body wt.) relative to control. Weights of the liver and spleen were slightly but significantly increased in the HD group. The increased weight of the spleen correlates with the megakaryocytosis noted.

Weight of the thymus was significantly decreased in the HD group by 50% (males) and 66% (females). This correlates with the lymphocytolysis observed. Weight of the uterus was significantly decreased (55%↓) in HD females with no correlative histopathology. The target organs of toxicity include the stomach (gastritis), spleen (megakaryocytosis), thymus (lymphocytolysis) and liver (vacuolation). NOAEL could not be established because only tissues from control and HD groups were examined and the HD group was sacrificed on treatment Day 9. For the tissues examined from all dose groups (spleen, thymus and liver), NOAEL could not be established due to histopathology findings in the spleen and thymus at the LD.

STUDY TITLE: 13-WEEK STUDY IN MICE WITH ADMINISTRATION BY GAVAGE THREE TIMES DAILY

Key study findings:

- Dose-dependent decrease in platelets was observed in all treated mice. This achieved statistical significance at doses ≥ MD. Slight but significant decreases in hemoglobin (10%↓) and MCHC (10%↓) were observed in HD females. Platelet count decreased dose-dependently and significantly by 19% and 28% in MD and HD males respectively. In females, platelet count was significantly decreased at the MD and HD by 31% and 30% respectively. PT was not measured.
- AST was significantly increased by 2-fold in both HD males and females. Alkaline phosphatase and total protein were significantly decreased by 2-fold and 1-fold respectively in HD females relative to control.
- Weights of the heart, kidney and salivary gland were slightly but significantly decreased by 14%, 17% and 14% respectively in HD males. Heart weight was also significantly decreased by 18% in MD males. Liver weight was significantly increased in HD females by 32% relative to control. Brain weight was slightly but significantly increased by 6% and 8% in MD and HD females respectively. Except for the kidney (inflammatory cell foci) there was no correlative histopathology associated with these weight changes.
- The target organs of toxicity include the spleen (lymphoid depletion), axillary lymph node (inflammation), thymus (lymphocytolysis), Kidney (inflammatory cell foci, basophilic tubules - males), liver (vacuolation - females), spinal cord (vacuolation, mineralization) and brain (vacuolation).
- NOAEL could not be established because of brain, liver and thymus histopathology at the LD.

Study no: 455869
Volume #, and page #: Vol. 2, pg. 1.
Conducting laboratory and location: [redacted]
Date of study initiation: September 5, 2001.
GLP compliance: Yes (UK).
QA report: Yes (X) no ( )
Drug, lot #, radiolabel, and % purity: Batch # 004, 100% pure.
Formulation/vehicle: A solution of OGT 918 in sterile water.

Methods (unique aspects):
Dosing: Oral (gavage), with OGT 918 administered TID at total doses of 100, 420 and 840 mg/kg/d.
Species/strain: Mouse/Crl: CD-1™(ICR) BR.
#/sex/group or time point (main study): 10/sex/group.
Satellite groups used for toxicokinetics or recovery: 20/sex/group for TK.

Age: 8 weeks at study initiation.
Weight: 20-22g (M); 17-19g (F).
Doses in administered units: 100, 420 and 840 mg/kg/d.
Route, form, volume, and infusion rate: Oral (gavage), 5 ml/kg.
Observations and times:
Clinical signs: Daily.
Body weights: Weekly.
Food consumption: Weekly.
Ophthalmoscopy: Not conducted.
EKG: Not conducted.
Hematology: Blood samples were collected during week 13 of treatment for routine hematology evaluation.
Clinical chemistry: Blood samples were collected during week 13 of treatment for routine clinical chemistry evaluation.
Urinalysis: Not conducted.
Gross pathology: Organs/Tissues isolated for gross examination are indicated in the list of addendum.
Organs weighed: Organs weighed are indicated in the list of addendum.
Histopathology: Tissues isolated for histopathology examination are indicated in the list of addendum.
Toxicokinetics: Blood samples were obtained from 3/sex/TK animals on Days 1 and 90 at 0, 0.5, 1, 2, 4 and 6 hr post dose.
Results:
Mortality: Sponsor attributed the demise of all animals to gavage error.

Animal No./Group (Dose Level)	Week of death/status	Clinical signs prior to death	Necropsy Findings
No. 8/Gp 1 Male (0)	7/FD	NAD	Organs autolysed
No. 111/Gp 2 Female (100)	8/KP	Irregular and crackling respiration	Intestine distended by contents
No. 42/Gp 3 Male (420)	1/KP	Slow and irregular respiration, subdued, hard area on ventral neck	Mass in thoracic cavity
No. 46/Gp 3 Male (420)	7/KP	Irregular and gasping respiration, swollen ventral neck	Spleen enlarged, mass on thoracic region
No. 148/Gp 3 Female (420)	1/KP	Slow respiration, subdued, rolling gait, hunched posture	Eyes opaque, lungs reddened
No. 76/Gp 4 Male (840)	1/KP	Irregular respiration, subdued	Oesophagus ruptured
No. 175/Gp 4 Female (840)	1/KP	Markedly subdued, rolling gait, hunched posture, weight loss, extremities cold and pale	Lungs dark

FD = found dead; KP = killed prematurely; NAD = no abnormalities detected

Dose (mg/kg/d)	Incidence of Deaths
0	1/20 F: FD
100	1/20 F: KP
420	3/20 (2/20 M; 1/20 F): KP
840	2/10 (1/20 M: 1/20 F): KP

Clinical signs: n = 20 (10/sex/group); Empty cells = zero incidence

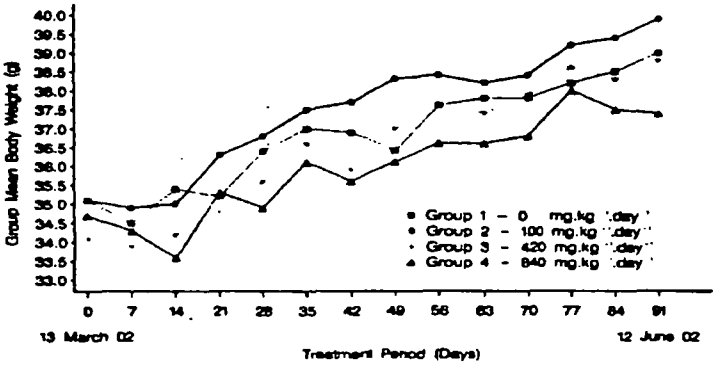
Observation/Finding	Dose (mg/kg/d)			
	0	100	420	840
Subdued		1/20	4/20	3/10
Rolling gait		1/20	3/20	1/20
Piloerection		1/20	4/20	4/20
Weight loss		1/20	3/20	2/20
Hunched posture	1/20		4/20	6/20
Slow/irregular/gasping/crackling respiration		1/20	3/20	1/20

Body weights: (g)

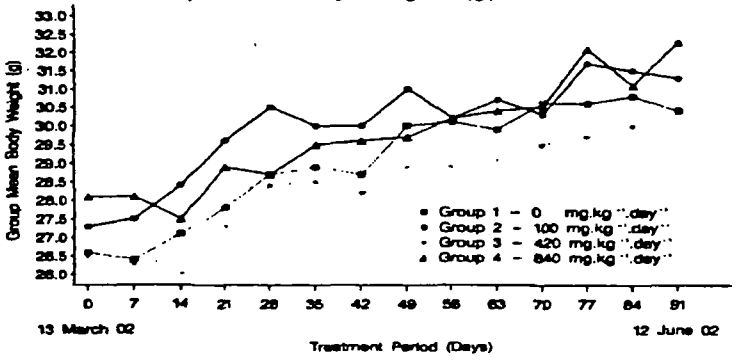
MALES	Week 1	Week 13	Wt. Gain	Decrement	% Decrement In B. Wt. Gain	% Decrease in Actual B. Wt.
0	35.1	39.0	3.9	0	0%	0%
100	35.1	39.9	4.8	+ 0.9	+ 23%	0%
420	34.1	38.8	4.7	+ 0.8	+ 21%	0%
840	34.7	37.4	2.7	- 1.2	- 31%	4%
FEMALES	**Week 1**	**Week 13**	**Wt. Gain**	**Decrement**	**% Decrement in B. Wt. Gain**	**% Decrease in Actual B. Wt.**
0	26.6	30.4	3.8	0	0%	0%
100	27.3	31.3	4.0	+ 0.2	+ 5%	0%
420	26.5	30.5	4.0	+ 0.2	+ 5%	0%
840	28.1	32.3	4.2	+0.4	+ 11%	0%

Except for the 840 mg/kg/d males, body weight decrease was not observed as in the 2-week study.

Group Mean Body Weights (g): Males



Group Mean Body Weights (g): Females



Food consumption: Unremarkable

Ophthalmoscopy: No data.

Electrocardiography: No data.

Hematology:

MALES	0 mg/kg/d	100 mg/kg/d	420 mg/kg/d	840 mg/kg/d
Platelets (x 10^9/l)	1246	1120	1013* (19%↓)	892** (28%↓)
FEMALES	**0 mg/kg/d**	**100 mg/kg/d**	**420 mg/kg/d**	**840 mg/kg/d**
Hemoglobin (g/dl)	13.9	14.2	14.0	12.5* (10%↓)
MCHC (g/dl)	32.2	32.0	32.2	31.0* (4%↓)
Platalet (x 10^9/l)	1113	1156	769** (31%↓)	779** (30%↓)

*** p<0.05; ** p<0.01**

Clinical chemistry:

MALES	0 mg/kg/d	100 mg/kg/d	420 mg/kg/d	840 mg/kg/d
AST (IU/L)	69	64	86	112*** (2X↑)
FEMALES	**0 mg/kg/d**	**100 mg/kg/d**	**420 mg/kg/d**	**840 mg/kg/d**
AST (IU/L)	93	79	105	150*** (2X↑)
AP (IU/L)	179	160	139	74*** (2X↓)
TP (g/l)	54	53	52	47** (2X↓)

*** p<0.05; ** p<0.01; *** p<0.001**

Urinalysis: No data.

Organ weights: (g)

MALES	0 mg/kg/d	100 mg/kg/d	420 mg/kg/d	840 mg/kg/d
Heart (g)	0.22	0.22	0.18* (18%↓)	0.19* (14%↓)
Kidneys (g)	0.60	0.63	0.56	0.50* (17%↓)
Salivary glands (g)	0.22	0.23	0.21	0.19* (14%↓)
FEMALES	**0 mg/kg/d**	**100 mg/kg/d**	**420 mg/kg/d**	**840 mg/kg/d**
Brain (g)	0.49	0.51	0.52** (6%↑)	0.53*** (8%↑)
Liver (g)	1.37	1.42	1.43	1.81** (32%↑)

*** p<0.05; ** p<0.01; *** p<0.001**

Gross pathology:

Dose (mg/kg/d)	0		100		420		840	
	M	F	M	F	M	F	M	F
Lymph node (axillary) Enlarged – R								1/10
Lymph node (Lumbar) Enlarged							1/10	
Lymph node (mesenteric) Enlarged							1/10	
Esophagus Ruptured							1/10	

Empty cells = zero incidence

Histopathology: Tissues from all groups were examined.

Dose (mg/kg/d)	0		100		420		840	
	M	F	M	F	M	F	M	F
Trachea Inflammation								2/10(1)
Lung Inflammation								2/10 1/10(1) 1/10(2)
Congestion/hemorrhage							1/10(2)	2/10(2)
Lymph node (axillary) Inflammation								1/10(2)
Spleen Increased hemopoiesis			1/10(1)				1/10(1)	2/10(1)
Lymphoid depletion					1/10(4)	1/10(3)	1/10(4)	1/10(1)
Thymus ↑ Lymphocytolysis		3/10(1)	1/10(2)	7/10 5/10(1) 2/10(2)	6/10 2/10(1) 4/10(2)	9/10 5/10(1) 3/10(2) 1/10(3)	8/10 2/10(1) 5/10(2) 1/10(3)	8/10 3/10(1) 6/10(2)
Testis Vacuolation							2/10(1)	
Kidney Inflammatory cell foci	3/10(1)	6/10 5/10(1) 1/10(2)		1/10(1)			7/10 6/10(1) 1/10(2)	5/10 4/10(1) 1/10(2)
Basophilic tubules	1/10(1)	1/10(1)					2/10(1)	1/10(1)

Esophagus Inflammation						1/10(1)	2/10 1/10(1) 1/10(3)	2/10 1/10(1) 1/10(2)
Stomach Dilated glands							1/10(1)*	
Cecum Goblet cell hyperplasia							1/10(1)*	
Colon Goblet cell hyperplasia							1/10(1)*	
Rectum Goblet cell hyperplasia							1/10(1)*	
Liver Hepatocyte vacuolation		1/10(1)		1/10(1)		1/10(1)		2/10 1/10(1) 1/10(2)
Spinal cord Vacuolation							2/10 1/10(1) 1/10(2)	
Mineralization							1/10(1)	
Brain Vacuolation	3/10(1)	2/10(1)		1/10(1)	2/10 1/10(1) 1/10(2)	1/10(2)	4/10 2/10(1) 2/10(2)	

Empty cells = zero incidence; 1 = minimal; 2 = mild; 3 = moderate, 4 = marked, * same animal

Toxicokinetics:

Toxicokinetic Parameter Estimates (n=3) Day 1

Dose Level ($mg\ kg^{-1}.bid^{-1}$)	Sex	Tmax (obs) h	Cmax (obs) $ng.ml^{-1}$	T½el h	Kel 1/h	CL/F $ml.h^{-1}.kg^{-1}$	AUC (0-t) $ng.h.ml^{-1}$	AUC (0-∞) ng.h.ml	Rsq
100	Male			1.59	0.4351	3449	9464	9664	0.95
	Female			0.95	0.7297	2819	11699	11825	0.99
420	Male			2.90	0.2390	2480	48692	56460	0.98
	Female			2.07	0.3354	2204	57355	63516	0.99
840	Male			4.35	0.1594	2159	95907	129668	0.60
	Female			5.14	0.1349	1765	114283	158637	0.69

Toxicokinetic Parameter Estimates (n=3) Week 13

Dose Level ($mg\ kg^{-1}.bid^{-1}$)	Sex	Tmax (obs)	Cmax (obs)	T½el h	Kel 1/h	CL/F $ml.h^{-1}.kg^{-1}$	AUC (0-t) $ng.h.ml^{-1}$	AUC (0-∞) ng.h.ml	Rsq
100	Male			2.02	0.3437	3573	9328	10121	1.00
	Female			1.10	0.6327	3240	10287	10505	0.99
420	Male			15.45	0.0449	2832	49443	118251	0.12
	Female			3.02	0.2292	1872	74796	92741	0.98
840	Male			1.65	0.4203	2526	110864	117523	0.85
	Female			2.31	0.3006	1491	187856	227781	0.95

Clinical dose is 100 mg TID (AUC_{0-8} = 8911 ng.hr/ml).

Summary of individual study findings:

Four groups of 10 male and 10 female CD-1 mice were dosed with OGT 918, by gavage three times daily for 13 consecutive weeks at doses of 100, 420 and 840 mg/kg/day (total dose). Dose-dependent decrease in platelets that achieved statistical significance at doses ≥ MD (6X and 8X the clinical dose for males and females respectively - AUC) was observed in all treated mice. Slight but significant decreases in hemoglobin (10%↓) and MCHC (10%↓) were observed in HD (21X the clinical dose - AUC) females. AST was significantly increased by 2-fold in both HD males (12X the clinical dose - AUC) and females. Alkaline phosphatase and total protein were significantly decreased by 2-fold and 1-fold respectively in HD females relative to control. Weights of the heart, kidney and salivary gland were slightly but significantly decreased by 14%, 17% and 14% respectively in HD males (12X the clinical dose - AUC). Heart weight was also significantly decreased by 18% in MD males (6X the clinical dose - AUC). Liver weight was

significantly increased in HD females by 32% relative to control. Brain weight was slightly but significantly increased by 6% and 8% in MD and HD females respectively. Except for the kidney (inflammatory cell foci) there was no correlative histopathology associated with these weight changes. The target organs of toxicity include the spleen (lymphoid depletion), axillary lymph node (inflammation), thymus (lymphocytolysis), Kidney (inflammatory cell foci, basophilic tubules - males), liver (vacuolation - females), spinal cord (vacuolation, mineralization) and brain (vacuolation). NOAEL could not be established because of brain, liver and thymus histopathology at the LD (1X the clinical dose - AUC).

Study title: 13 Week Oral (Gavage Administration) Toxicity Study in the Male Rat With A 16 Week Treatment Free Period.

Key study findings:

- Treatment-related clinical signs such as paddling, mouth rubbing and raised tail were observed in all treated rats relative to control. High gait was observed in all MD and HD rats. Dose-related increases in hunched posture, semi-closed eyes and liquid feces were observed. 4/10 rats in the HD group had swollen abdomen.
- There was a dose-dependent decrease in body weight gain at the end of the 13 week treatment period. However, at the end of the recovery period, body weight of the treated rats were comparable to those of control. The food consumption data does not explain the decrease in body weight gain.
- HDW and RDW showed dose dependent decreases that achieved statistical significance at doses ≥ LD. Platelets were significantly decreased by 14% (LD) and 19% (HD) relative to control. MPV increased dose dependently achieving statistical significance at doses ≥ MD. PDW increased dose dependently achieving statistical significance at the HD. WBC were slightly increased in all treated rats relative to control due neutrophilia that achieved statistical at the MD (74%) and HD (100%). Lymphocytes decreased dose dependently achieving statistical significance at doses ≥ MD. At the end of the recovery period Hb, RBC, PCV, and lymphocytes were slightly but significantly decreased at the HD. Reticulocytes were significantly increased in the HD rats by 27% relative to control.
- AST increased dose dependently achieving statistical significance at MD (2.4-fold↑) and HD (2.5-fold↑). ALT also increased dose dependently achieving statistical significance at HD (2-fold↑). Very slight and dose dependent increase in Ca was observed in treated rats. This achieved statistical significance at HD. At the end of the recovery period these parameters were similar to those of control.
- Weight of the spleen was significantly decreased by 18% at the MD (no correlative histopathology). Weight of the liver increased in a dose dependent manner achieving statistical significance at the HD (11%↑). There is no correlative histopathology. At the end of the recovery period liver weight was still significantly increased by 11% relative to control.
- Sperm count, average path velocity, straight line velocity and curvilinear velocity decreased dose dependently achieving statistical significance at the HD (61%↓). Percent of motile sperm decreased dose dependently achieving statistical significance ≥ MD. Abnormal sperm was significantly increased by 49% in HD rats relative to 0.3% for controls. At the end of the recovery period, there was no significant difference between control and treated rats.
- Tubular atrophy (bilateral) of the testis was observed in MD (1/10) and HD (2/10) rats. Oligospermia (epididymis) was observed in MD (1/10) and HD (2/10) rats. High incidence of cellular debris was observed in all treated rats in both the testis and epididymis relative to control. A high incidence of spermatid retention was observed in the testis of all treated rats relative to control.

- Testicular staging data (stages XII = XIII) indicate that 19% of spermatids were retained in the seminiferous epithelium of control rats. In the treated rats, spermatid retention decreased to 2% (LD) and zero for both MD and HD groups indicative of treatment-related delayed spermiation. Abnormal atrophic tubules (%) was increased (not dose dependent) in treated rats relative to control. At the end of the recovery period, there were no abnormal atrophic tubules in the treated rats and spermatid retention in treated rats was similar to that of control. This is indicative of reversibility of spermatic and testicular changes.
- NOAEL could not be established due to the testicular and epididymal changes (cellular debris) and spermatid retention observed at the LD.

Study no: 1514/08
Volume #, and page #: Vol. 6, pg. 1
Conducting laboratory and location:

Date of study initiation: May 2, 2001
GLP compliance: Yes (U.K.)
QA report: Yes (x) no ( )
Drug, lot #, radiolabel, and % purity: Batch # ZN-16049/0018/Batch 10; no information on purity.
Formulation/vehicle: A solution of OGT 918 in pre-sterilized water.

Methods (unique aspects):
Dosing: Animals were dosed three times daily, 6 hr apart at 180, 340 and 420 mg/kg/d (total dose) for 13 weeks.
Species/strain: Rat/Crl:CD® (SD)IGSBR
#/sex/group or time point (main study): 10 males/group.
Satellite groups used for toxicokinetics or recovery: 10 males/group for TK.
Age: 17 weeks at study initiation.
Weight: 488.8 – 521.5 g.
Doses in administered units: 180, 340 and 420 mg/kg/d (total dose) administered as split doses TID, 6 hr apart.
Route, form, volume, and infusion rate: Oral (gavage), 10 ml/kg.

Observations and times:
Clinical signs: Daily.
Body weights: Daily.
Food consumption: Daily.
Ophthalmoscopy: Not conducted.
EKG: Not conducted.
Hematology: Blood samples were collected at Weeks 12 from all study animals following overnight fasting and at week 29 from recovery animals (without overnight fasting) for routine hematology evaluation.
Clinical chemistry: Blood samples were collected at Weeks 12 from all study animals following overnight fasting and at week 29 from recovery animals (without overnight fasting) for routine clinical chemistry evaluation.
Urinalysis: Not conducted.
Gross pathology: Organs/Tissues isolated for gross pathology examination are indicated in the list of addendum.
Organs weighed: Organs weighed are indicated in the list of addendum.
Histopathology: Testes and epididymis from all dose groups were the only tissues examined. For testicular staging, sections were stained with both eosin and hematoxylin and PAS.