Lymph nodes with capsular fibrosis		1/3P				
Spleen Capsular fibrosis		1/3(2)			1/2(3)	
Depletion, lymphocytic, germinal centers	2/2 1/2(2) 1/2(3)				2/2 1/2(2) 1/2(4)	1/3(2)
Inflammation, fibrino-suppurative to granulomatous to chronic active	1/2(2)	1/3(4)			1/2(3)	
Lymphoreticular hyperplasia		1/3(2)				
Thymus Lymphocytic depletion		1/3(2)			1/2(4)	1/2(2)
Skin Edema		1/3(3)			1/2(3)	
Fibrosis, subcutaneous		1/3(3)				
Bone – Femur Hypocellular marrow						1/2(4)
Bone – sternum Lymphoid nodule, marrow		1/3(2)				
Brain Infiltrate, pigmented macrophage	1/2(1)	1/3(2)			1/2(2)	
Lungs Infiltrate, lymphohistiocytic	1/2(1)					
Fibrosis, pleural/subpleural	1/2(2)					
Inflammation, chronic active	1/2(1)	1/3(2)				
Leukocytes, increased, intravascular		1/3(1)				
Pigment	1/2(1)	2/3 1/3(1) 1/3(2)				
Trachea Acute inflammation			1/1(2)		1/2(2)	
Kidney Capsular fibrosis		1/3(2)			1/2(3)	
Infiltrate, lymphohistiocytic	2/2 1/2(1) 1/2(2)	3/3 2/3(1) 1/3(2)	1/1(1)		1/2(1)	
Mineralization, tubular		1/3(1)	1/1(1)		1/2(1)	1/2(1)
Hemorrhage	1/2(3)					
Inflammation, capsule	1/2(3)	1/3(4)				
Pigment, tubular	1/2(2)					
Urinary bladder Infiltrate, lymphohistiocytic	2/2(1)				1/2(1)	

1 = minimal, 2 = slight, 3 = moderate, 4 = marked, 5 = severe, P = present

Toxicokinetics: Please note that sponsor did not provide any [^{14}C]SC-49483 TK data, only OGT 918.

Dose (mg/kg)	Sex	AUC_{0-8h} (µg.h/ml)			C_{max} (µg/ml)			t_{max} (hours)		
		Day 1	Wk 26	Wk 52	Day 1	Wk 26	Wk 52	Day 1	Wk 26	Wk 52
750	M	19.7	38.9	34.6	4.05	6.28	6.17	1-3	1-3	1-3
	F	29.0	34.1	52.8	4.86	7.71	10.9	3-5	1	1
2000	M	34.0	49.8	57.8	5.85	9.08	12.0	3	1-3	0.5-1
	F	32.2	45.6	79.9	5.18	7.79	16.0	1-5	0.5-3	1

Summary of Study Findings:

In a 52-week chronic oral toxicity study in the cynomolgus monkey, OGT 924 was administered at doses of 0, 750 and 2000 mg/kg/day (as three equally divided doses/day). Clinical observations attributed to the test material were dose-dependent increases in fecal changes (white feces, yellowish substance in feces) during treatment. At the end of the recovery period, the fecal changes were still present in addition to red feces. Bodyweight gain was not affected

by treatment. No toxicologically meaningful changes were noted in respiration rates or rectal body temperatures. No test material-related differences in ECG, ophthalmic, or blood pressure parameters were observed. There did not appear to be any test material-related changes in hormone levels. Mildly lower red blood cell count, hemoglobin, hematocrit and total protein in HD animals, and mildly lower cholesterol in HD males. After 8 weeks of recovery, these findings were not completely reversed.

Mean sperm concentrations were highest for males in the control group (7.73×10^8), and decreased in LD and HD males (4.02×10^8 and 2.96×10^8, respectively). This corresponds to 48% and 62% decrease in the LD and HD males. The number of amorphous sperms was 1% in HD males compared to zero for control. The LD is 4x and 6x the maximum human dose based on $AUC_{0\text{-}6hr}$ for males and females respectively. The HD is 7x and 9x the maximum human dose based on $AUC_{0\text{-}6hr}$ for males and females respectively.

The target organs of toxicity include the GI tract – cecum, colon, stomach (pigmented macrophages, granulomatous inflammation), Liver (pigmented macrophages, vacuolation), Pancreas (↓ zymogen granule staining), adrenal gland (mineralization), thyroid gland (vacuolated macrophage), seminal vesicle (mineralization), lymph nodes – mesenteric & submandibular (granuloma, mineralized, pigmented macrophage), mammary gland (lymphocytic infiltrate), skin (acanthosis/hyperkeratosis), cervix (lymphocytic infiltrate), skeletal muscle (inflammation, necrosis/degeneration), brain (mineralization, necrosis), spinal cord (mineralization) and kidney (angiectasis-glomerulus). NOAEL could not be established because of lesions in the liver, pancreas, seminal vesicle, brain and skeletal muscle at the LD.

At the end of the recovery period, the following lesions were observed in the HD group – Pancreas (↓ zymogen granule staining), submandibular salivary gland (atrophy, mineralization), stomach (inflammation, fibrosis), heart, seminal vesicles, thyroid & parathyroid (lymphohistiocytic infiltrate), adipose tissue (fibrosis, inflammation, pigmented macrophage), cervix (mucopurulent exudate), spleen (fibrosis), skin (acanthosis/hyperkeratosis, fibrosis, crusting, ulceration), brain (mineralization), spinal cord (demyelination, axonal swelling) and kidney (tubular pigment, membranoproliferative glomerulopathy).

TOXICOLOGY SUMMARY

DOG:

OGT 918 was evaluated in dogs at total doses ranging from 20 mg/kg/d up to 825 mg/kg/d over periods ranging from 11 days to 4 weeks. Diarrhea, decreased body weight and decreased food consumption were common clinical signs observed at all doses tested. Severity of the diarrhea increased with dose and sometimes progressed to bloody diarrhea or black tarry stool. OGT 924 was also evaluated at 550 mg/kg/d for 2 weeks. All animals had diarrhea during the first week of dosing but subsided during the second week of dosing. Body weight loss was also negligible in OGT 924 treated animals. AST increased by 9 to 13 fold at doses ≥ 70 mg/kg/d (23x the therapeutic dose based on mg/m^2) and sometimes ALT was slightly but significantly increased at doses ≥ 105 mg/kg/d (34x the therapeutic dose based on mg/m^2). 1/4 dogs dosed at 105 mg/kg/d had liver histopathology (centrilobular hepatic vacuolation). The major target organ of toxicity is the GI tract: necrosis of crypts of epithelium with dilatation and plugging, necrosis of villus tips (≥ 85 mg/kg/d i.e. 27x the therapeutic dose based on mg/m^2); thymus (involution ≥ 40 mg/kg i.e. 13x the therapeutic dose based on mg/m^2) and atrophy (lymphoid depletion) of Peyer's patches in ileum (≥ 80 mg/kg/d i.e. 26x the therapeutic dose based on mg/m^2). Clinical signs suggestive of neurotoxicity (ataxia, diminished/absent pupillary, palpebral or patellar reflexes) were noted in animals treated with 495 or 825 mg/kg/d. These doses

represent 160x and 266x the human therapeutic dose (based on mg/m^2) respectively. Tremors and absent corneal reflexes were also observed at 105 mg/kg/d (34x the human therapeutic dose based on mg/m^2). No histopathologic lesions suggestive of neurotoxicity were observed in the dogs. Hematocrit, hemoglobin and RBC were significantly decreased at doses ≥ 70 mg/kg/d during the 4 week study. A NOAEL of 20 mg/mg/d (7x the human therapeutic dose on mg/m^2) following a 2-week study. NOAELs were not established for the other studies.

MONKEY:

In monkeys given either OGT 918 for 4 weeks, or OGT 924 for up to one year, there have been few toxic effects. A decrease in the number of zymogen granules in the glandular acini of the exocrine pancreas was noted in most animals treated with OGT 918 at doses ≥ 300 mg/kg (58x the therapeutic dose based on mg/m^2) or with OGT 924, at doses ≥ 750 mg/kg (5x to 8x the therapeutic dose based on AUC). Sponsor stated that the ↓ in zymogen granules was not associated with correlative functional changes and does not appear to be clinically significant.

While the Rhesus monkey showed GI toxicity similar to those observed in rats and dogs, the cynomolgus monkey had minimal GI lesions in the gut mucosa after doses up to 2000 mg/kg/day for 1 year with OGT 924.

In a 28 day study with OGT 918 (165, 495, 1650 mg/kg/d), the rhesus monkeys showed suppression of body weight gain accompanied by decreased appetite in some animals at doses ≥ 165 mg/kg (32x the therapeutic dose based on mg/m^2). This was partially reversed in HD animals after 10 days off drug. Platelets increased in the HD group (319x the therapeutic dose based on mg/m^2). Monkeys dosed by gavage with OGT 918 at the HD (1650 mg/kg/d i.e. 319x the therapeutic dose based on mg/m^2) or OGT 924 also at the HD (2000 mg/kg/d i.e. 7x to 9x the therapeutic dose based on AUC) showed a consistent decrease in hemoglobin and hematocrit. RBC was decreased only in studies where OGT 924 was administered. Monkeys dosed by intravenous infusion did not show any change in RBC parameters. Acute inflammation of the heart was noted in 1/10 MD (96x the therapeutic dose based on mg/m^2) and 2/10 HD dead rhesus monkeys dosed with OGT 918 for 28 days. Hepatic necrosis and inflammation was observed in the MD and HD rhesus monkeys. Hepatic vacuolation, which increased in incidence and severity with dose, was observed in all treated rhesus monkeys. AST was significantly increased in the MD and HD rhesus monkeys. The target organs of toxicity in the rhesus monkey include the GI tract (ulcer, inflammation, necrosis, hemorrhage – colon, cecum), mesenteric lymph node (lymphoid hyperplasia), liver (vacuolar change, necrosis, inflammation), kidney (infarct, hyaline casts) and adrenal (congestion). NOAEL could not be established because of histopathology observed at the LD.

In the cynomolgus monkeys that were dosed with 750 and 2000 mg/kg/d OGT 924 for a year, 10 animals (5 males and 5 females) died or were sacrificed moribund during this study. None of the deaths or unscheduled sacrifices was considered to be related to the test material. 8/10 unscheduled deaths were directly related to sequelae of the gastric catheterization, most commonly inflammation and infection at the skin or stomach implantation site and peritonitis. The remaining animals were both in the control group: one had significant inflammation of the large intestine, while the cause of moribundity of the other was undetermined. OGT 924 was associated with a low incidence (2/10) of diarrhea. Signals suggestive of neurotoxicity (vascular mineralization of brain and spinal cord, and mineralization and necrosis of the white matter) were noted at 750 and 2000 mg/kg/d (4x and 7x the human therapeutic dose respectively, based on AUC). Unlike the dogs, monkeys showed no clinical signs of neurotoxicity. RBC, HGB and HCT were slightly but statistically significantly decreased in HD females relative to control. Reticulocytes and prothrombin time were statistically significantly increased in HD females. At

the end of the recovery period, RBC was still slightly but statistically significantly decreased in HD females. Platelets were statistically significantly increased in HD males by 136% relative to control. Mean sperm concentrations were highest for males in the control group (7.73×10^8), and decreased for males given 750 and 2000 mg/kg/day (4.02×10^8 and 2.96×10^8, respectively). This corresponds to 48% and 62% decrease in the LD and HD males. The number of amorphous sperms was 1% in HD males compared to zero for control. OGT 924, produced toxicity as determined by clinical signs (fecal changes) and organ changes (decreased zymogen granules in the acinar cells of the pancreas) at dose levels of 750 and 2000 mg/kg/day when administered by gavage. T4 was statistically significantly decreased in LD females by 32% and in HD males and females by 41% and 37% respectively relative to control. At the end of the recovery period, T4 was still statistically significantly decreased in HD females. At the end of the recovery period, albumin was slightly but statistically significantly decreased in HD males whereas phosphate and bile acids were statistically significantly increased in HD females by 138% and 267% respectively relative to control. The increased bile acids may be suggestive of impaired liver function and cholestasis. The target organs of toxicity include the GI tract – cecum, colon, stomach (pigmented macrophages, granulomatous inflammation), liver (pigmented macrophages, vacuolation), pancreas (↓ zymogen granule staining), adrenal gland (mineralization), thyroid gland (vacuolated macrophage), seminal vesicle (mineralization), lymph nodes – mesenteric & submandibular (granuloma, mineralized, pigmented macrophage), mammary gland (lymphocytic infiltrate), skin (acanthosis/ hyperkeratosis), cervix (lymphocytic infiltrate), skeletal muscle (inflammation, necrosis/ degeneration), brain (mineralization, necrosis), spinal cord (mineralization) and kidney (angiectasis-glomerulus). Lesions in the HD group showed little or no recovery. NOAEL could not be established because of tissue pathology at LD.

For the cynomolgus monkeys that was treated for 28 days with OGT 918 (60, 300 & 600 mg/kg/d) by intravenous infusion, body weight was significantly decreased in MD (58x the human therapeutic dose based on mg/m^2) and HD (116x the human therapeutic dose based on mg/m^2) males. T4 was statistically significantly decreased in HD males. AST was also significantly increased in the HD group with no correlative histopathology. In addition to the decreased zymogen granules in the pancreas, severe lymphocyte depletion in the thymus was observed In the MD and HD groups. This correlates with the decreased relative weight of the thymus noted for the same dose groups. NOAEL was 60 mg/kg/d (12x the human therapeutic dose on mg/m^2).

RATS:

Rats were treated with OGT 918 or OGT 924 for periods ranging from 5 days to 52 weeks. The doses evaluated ranged from 20 to 4200 mg/kg/d (OGT 918) and 300 to 3830 mg/kg/d (OGT 924). Diarrhea was the most common clinical sign and tended to increase in incidence and severity with dose except for animals dose with OGT 924 that had low incidence/no diarrhea. The incidence of diarrhea was high during the first 12 weeks of dosing but subsided thereafter. Body weight, body weight gain and food consumption were all significantly decreased especially at the highest doses tested.

In a 4-week study where rats were administered OGT 918 at doses of 180, 840 and 4200 mg/kg/d, all the HD (470x the human therapeutic dose based on mg/m^2) animals were sacrificed between days 7 and 15 of treatment due to severe diarrhea. The incidence of diarrhea was 7/30-MD (81x the human therapeutic dose based on mg/m^2) and 30/30-HD (407x the human therapeutic dose based on mg/m^2). Body weight, body weight gain and food consumption were significantly decreased in the HD group. AST and ALT were significantly increased at doses ≥ MD with no correlative histopathology. The target organs of toxicity include the GI tract (↑ in the

mitotic figures in cecal epithelium, hemorrhage of stomach, depletion of goblet cells throughout the intestine, villous atrophy in the jejunum and ileum), prostate (atrophy), and lymphocyte depletion in the spleen, thymus and lymph nodes, pituitary (atrophy of the pars distalis), bone marrow (hypocellularity), testis (↓ spermatogenesis), epididymis (hypospermia), seminal vesicles (atrophy). Most of the target organs were observed in the MD and HD groups. In addition, hypospermia in the epididymis was observed in the LD (17x the human therapeutic dose based on mg/m^2). Based on these findings a NOAEL could not be determined. Another 4-week study with OGT 918 at slightly lower doses (420 and 1680 mg/kg/d) did not cause any deaths. Watery diarrhea and red stool were observed in the HD (163x the human therapeutic dose based on mg/m^2). Body weight, body weight gain and food consumption were significantly decreased in the HD group. AST and ALT were significantly increased at doses ≥ MD with no correlative histopathology. In addition to the target organs identified in the previous study, vacuolation of the white matter was observed (4/30 – control, 2/30 – LD, 2/30 – HD). While this observation did not show any dose-response, it is noteworthy. NOAEL could not be established due to tissue histopathology at the LD (41X the human therapeutic dose based on mg/m^2).

A 4-week study with OGT 924 (330, 1020, 3670 mg/kg/d) did not cause any deaths. The incidence of diarrhea was very low (0/20 – control, 1/20 – LD, 1/20 – MD, 1/20 – HD) compared to those observed in OGT 918 studies. These LD, MD and HD represent 3x, 10x and 30x the human therapeutic dose based on AUC). Mean body weight was significantly decreased by 44% and 37% in HD males and females respectively. Food consumption was also significantly decreased at the HD. Hemoglobin, hematocrit and platelets were significantly decreased at doses ≥ 10x the human therapeutic dose based on AUC). AST and ALT were significantly increased at the HD (30x the human therapeutic dose based on AUC). The target organs of toxicity include the pancreas (acinar cell vacuolation), salivary gland acinar cells (mucous cell atrophy/serous cell hypertrophy, prominent apoptosis), stomach (chief cell vacuolation), thyroid (follicular cell vacuolation), pituitary (intracytoplasmic eosinophilic droplets), spleen-thymus-mesenteric lymph node (lymphocyte depletion), uterus-prostate-seminal vesicle (atrophy), testes-epididymis (degeneration of germinal epithelium, hypospermia). Most lesions in the target organs were observed at the HD. NOAEL could not be established because of changes observed in urinalysis (urinary Ca and P excretion), serum chemistry (cholesterol, triglyceride levels in females), body weight, hematology (hemoglobin concentration), and histopathology (submandibular salivary gland, epididymis) at LD.

In a 13-week study, rats were dosed orally by gavage with OGT 918 at doses of 90, 180, 420 and 840 mg/kg/d. Dose-dependent increases in incidence of diarrhea and enlarged abdomen was observed at doses ≥ MD (5x the human therapeutic dose based on AUC). The diarrhea subsided upon termination of treatment. Body weight gain was decreased by 11% and 31% in HMD males (10x the human therapeutic dose based on AUC) and HD males (22x the human therapeutic dose based on AUC) and by 19% in HD females (16x the human therapeutic dose based on AUC) at the end of the treatment period. At the end of the recovery period, body weight gain was still decreased by 18% in HD males and females. Food consumption was decreased by 12% in HD males at the end of the treatment period but increased to control level at the end of the recovery period. The target organs of toxicity include the testes (atrophy/degeneration, dystrophy), kidney (dilatation-collecting tubule & pelvis, nephropathy), heart (degenerative cardiomyopathy), pancreas (acinar cell vacuolization), thymus (involution) and uterus (dilatation). NOAEL = 90 mg/kg/day (2x the human therapeutic dose based on AUC) based on histopathology.

In another 13-week study in rats, OGT 918 was administered by oral gavage at doses of 20, 60 and 180 mg/kg/d. Body weight gain was reduced by 12% in HD (2x the human therapeutic dose

based on AUC) males over the whole treatment period. At the end of the recovery period, % decrement in body weight gain was 52% (LD – 0.3x & MD males – 0.8x the human therapeutic dose based on AUC) and 61% (HD) males. Values for females are 42% (LD) and 25% (MD). HD females rather had an increase in body weight gain relative to control. The target organs of toxicity include the epididymides (desquamated germ cells, ↓ or no spermatozoa), kidney (pelvic dilatation, tubular basophilia, cortical tubular dilatation, corticomedullary dilatation), liver (hepatocyte necrosis), submandibular lymph node (lymphoid hyperplasia) and testes (desquamated germ cells, seminiferous tubular atrophy). At the end of the recovery period, the testicular and epididymal changes were still present but at a lower incidence suggesting partial recovery. The kidney changes were partially reversed. Histopathologic changes that were not present at the end of the treatment period but noted at the end of the recovery period included spleen (extramedullary hematopoiesis, hemosiderosis), submandibular lymph node (plasmacytosis) and uterus (luminal dilatation). NOAEL = 20 mg/kg/day (0.3x the human therapeutic dose based on AUC) based on the histopathology.

Chronic toxicity studies conducted in rats ranged from 26 weeks (OGT 924) to 52 weeks (OGT 918). In the 26-week rat study, animals were dosed orally by gavage with OGT 924 at 300, 600 and 1200 mg/kg/d. Dose-related increase in scaly tail was observed in both sexes (0/30-control; 1/48-LD i.e. 3x the maximum clinical dose of 100 mg TID based on $AUC_{0\text{-}6hr}$; 13/48-MD i.e. 5x the maximum clinical dose of 100 mg TID based on $AUC_{0\text{-}6hr}$); 44/48-HD i.e. 7x the maximum clinical dose of 100 mg TID based on $AUC_{0\text{-}6hr}$. Wart-like lesions on the tail also showed a dose-dependent increase in treated males but not in females (1/30-control; 8/48-LD; 9/48-MD; 19/48-HD). Mean body weight was decreased by 12% in MD males (6x the maximum clinical dose of 100 mg TID based on $AUC_{0\text{-}6hr}$) and by 23% and 9% in HD males (8x the human therapeutic dose based on AUC) and females (7x the human therapeutic dose based on AUC) respectively at the end of the treatment period. After the 4-week recovery period, mean body weight was still decreased by 10% in MD males and by 17% and 8% % in HD males and females respectively. Animals in the pair-fed control group also had decreased mean body weight (8%-10%) which was partially reversed to 6% at the end of the recovery period. Mean food consumption was decreased by 8% and 13% in MD and HD males and by 15% and 7% in the pair-fed control males and females respectively. At the end of the recovery period, mean food consumption showed partial recovery. Sperm motility, sperm concentration and the number of normal sperms were decreased in all treated males relative to control. Amorphous sperms and sperms with decapitated heads were increased in all treated males relative to control. At the end of the treatment-free period, partial recovery of the altered sperm parameters was observed. It is likely full recovery will occur with time. The target organs of toxicity include the GI tract – esophagus (serosal fibrosis), stomach (cytoplasmic vacuolation of chief cells), large intestine (mucosal necrosis), pancreas (acinal cell vacuolation), salivary gland (cytokaryomegaly – seromucus acinar cells), epididymides (hypospermia), testes (degeneration/atrophy), mesenteric lymph node (lymphoid depletion), skin of the tail (hyperkeratosis, acanthosis, pyogranulomatous dermatitis), eye (posterior synechia, cataract) and kidney (chronic progressive nephropathy). The incidence of testicular lesions was decreased at the end of the recovery period but the severity appear to have increased. The incidence of kidney lesions was decreased at the end of the recovery period. NOAEL could not be established because of the nephropathy and testicular lesions at the LD (3x the human therapeutic dose based on AUC).

In the 52-week chronic toxicity study in rats, OGT 918 was administered by oral gavage (30/sex/group) at doses of 0, 180, 420, 840 and 1680 mg/kg/day. Due to high mortality in the 1680 mg/kg/day animals, dosing was terminated in this group during Week 10 and sacrificed during week 20. Clinical findings judged to be treatment-related at the lower dose levels consisted of tail findings (exfoliation and segmenting) in the 180, 420 and 840 mg/kg/day groups

and soft stool in the 840 mg/kg/day group. Inhibition of bodyweight gain occurred in the 420, 840 and 1680 mg/kg/day groups throughout the treatment period. Inhibition of food consumption occurred in the 840 mg/kg/day males and 1680 mg/kg/day animals throughout the treatment period.

Mortality occurred at all doses. 44/60 animals in the HD group (no AUC data at week 52) were found dead or sacrificed moribund during SC-48334 administration. Of the 44/60 animals found dead or sacrificed moribund, the cause of death or moribundity was not ascertained in 35/60 animals. Death of the remaining animals was attributed to septicemia, chronic renal disease, mechanical trauma, gavage error and enteropathy. 15/60 HMD (10x and 11x the human therapeutic dose based on AUC for males and females respectively) animals were found dead or sacrificed moribund during the study. The cause of death was not ascertained for 7/60 HMD animals. Death of the remaining animals was attributed to chronic renal disease, gavage error, bleeding technique and pituitary tumor. 9/60 animals in the MD group (9x and 6x the human therapeutic dose based on AUC for males and females respectively) were found dead or sacrificed moribund. The cause of death was not ascertained in 2/60 animals. Death of the remaining animals was attributed to gavage error, chronic renal disease, pituitary tumor and septicemia. 7/60 LD (5x and 4x the human therapeutic dose based on AUC for males and females respectively) animals were found dead or sacrificed moribund during the study. The cause of death was not ascertained in 6/60 animals. Death of the remaining animal was attributed to gavage error. 3/60 purified diet control females were found dead during the study. They were all females and the cause of death was not ascertained. In the purina diet control group, 11/60 animals were found dead or sacrificed moribund. The cause of death was not ascertained in 5/60 animals. Death of the remaining animals was attributed to gavage error and leukemia. Sponsor stated that the cause of death was not ascertained for most animals. Reviewer believes that demise of most of these animals was due to GI toxicity (stomach-ulcer, hyperkeratosis; cecum-mucosal necrosis, inflammation, hemorrhage colon-dilatation of crypts, necrosis, edema, inflammation; ileum and jejunum-villous atrophy), renal toxicity (nephrosis, nephropathy, vacuolation of tubular epithelium, inflammation, protein casts and mineralization of tubular epithelium and pelvis), hepatotoxicity (necrosis, cytoplasmic vacuolation, hemorrhage and lymphocytic infiltrate) and cardiac toxicity (cardiomyopathy, inflammation and necrosis of myocardial fiber) based on the histopathylogy data provided. All or most of the animals found dead had undergone autolysis, This might have confounded the histopathologic evaluation.

A dose-dependent decrease in % mean body weight by 18% and 14% respectively for MD males and females, and by 32% and 26% for HMD males and females respectively was observed. Percent food consumption also showed a dose-dependent effect. Decrements of 13% and 5% respectively for MD males and females, and 26% and 9% for HMD males and females respectively were observed. Diarrhea was observed at doses ≥ HMD. The incidence of diarrhea was very high especially in the HD group during the first 12 weeks of treatment (45/60 - weeks 1-4; 46/60 - weeks 5-8; 21/60 - weeks 9-12). Treatment was terminated in the HD group at week 10 due to mortality. However, the diarrhea persisted till week 12 and thereafter subsided completely. In the HMD animals, the incidence of diarrhea was 26/60 during the first four weeks of treatment but subsided significantly from week 5 onwards.

RBC was slightly but statistically significantly decreased in LD and MD males and in MD and HMD females. MCH was significantly increased in MD males but decreased in LD females. While MCHC was slightly but statistically significantly decreased in all treated females, it was only decreased in HMD males. Platelets were dose-dependently and statistically significantly decreased in MD males and in HMD males and females. At the end of the recovery period, all affected hematology parameters had returned to normal limits.

At week 52, K, P, BUN and Ca were slightly but statistically significantly increased in all treated males relative to control. K was slightly but statistically significantly increased in all treated females. P was dose-dependently and slightly but statistically significantly increased in MD and HMD females. AST and ALT were significantly increased in males at doses ≥ MD and in HMD females with no correlative histopathology.

Equatorial cataracts were observed in treated males relative to controls. The incidence appears to be dose-related. In treated males the incidence are 1/28 (LD), 1/29 (MD) and 18/27 (HMD) at week 52. In treated females, the incidence at the HMD dose is 9/23 at week 52. After the 4 week recovery period, the incidence had decreased to are1/10 (LD), 1/9 (MD) and 5/9 (HMD) in treated males and 4/8 HMD females. In the HD group that was terminated at week 20, the incidence of cataracts was 9/9 (males) and 5/7 (females) at week 14.

Dose-dependent increases in raised areas on the tail were observed (2/30-control; 5/33-LD; 15/34-MD; 16/28-HMD). Microscopically these raised areas were areas of suppurative inflammation and/or abscesses.

The target organs of toxicity include the epididymides (hypospermia), heart (cardiomyopathy), kidney (nephropathy, lymphocyte infiltration, protein casts, hyperplasia and mineralization), testes (atrophy of seminiferous tubules, aspermatogenesis, edema, and hyperplasia of interstitial cells), mammary gland (galactocele, active secretion) and tail (suppurative inflammation). Most of these toxicities occurred at all dose levels and showed very little/no recovery at the end of the treatment free period. NOAEL could not be established since toxicities were observed at the LD tested.

MOUSE:

OGT 918 was administered daily by oral gavage for 2 weeks to mice (10 males and 5 females/group). Doses of 60, 300 and 600 mg/kg were given four times per day to give total daily doses of 240, 1200 and 2400 mg/kg/day. The doses represent 12x, 58x and 116x the human therapeutic dose based on mg/m^2). 4/15 control, 5/15 LD, 5/15 MD and 8/15 HD animals were found dead or sacrificed in extremis. For the majority of these animals, lesions observed at necropsy were indicative of gavage error. Inflammation of the thoracic organs or cervical musculature and alterations in lymphoid organs was observed in the dead animals. Inflammation was also present in several of the terminal sacrifice animals, but these lesions were less extensive compared to those seen in animals dying during the study. Sponsor stated that the inflammatory changes and subsequent changes in lymphoid organs occurred secondary to the trauma of restraint and test article administration and were not test article related. Diarrhea was observed in 5/15 MD and 7/15 HD mice. AST and ALT concentrations were increased in all OGT 918 treated groups. Alkaline phosphatase concentrations were decreased in MD females and HD animals; the difference was statistically significant in HD males. Vacuolar change of hepatocyte cytoplasm was observed in the livers of MD and HD animals and, at a lower incidence, in the livers of LD animals. This may correlate with the increased liver enzymes. The target organs include the liver (cytoplasmic vacuolation), mesenteric lymph node (lymphoid depletion, histiocytosis), pancreas (cytoplasmic vacuolation) and thymus (involution). Due to the histological changes in the liver at LD, NOAEL could not be defined.

TOXICOLOGY CONCLUSION

General Toxicology: Acute, subacute, subchronic and chronic toxicity studies were conducted in the rat, mouse, dog and monkey to evaluate the safety of OGT 918. The studies are adequate to support the safety of the 100 mg TID dose.

Genetic toxicology: Miglustat was not mutagenic or genotoxic in a battery of in vitro and in vivo assays including the bacterial reverse mutation (Ames), chromosomal aberration (in human lymphocytes), gene mutation in mammalian cells (Chinese hamster ovary), and mouse micronucleus tests.

Carcinogenicity: Carcinogenicity studies was waived as a Phase IV commitment on August 4, 1999 by Dr. Ron Steigerwalt, who was then the Supervisory Pharmacologist. Therefore the carcinogenic potential of the drug has not been investigated in animal studies.

Reproductive and developmental toxicology: OGT 918 affected normal morphology and motility of sperm, which was consistent with the observed reduction in fertility. However these effects are reversible. OGT 918 also had an adverse effect on fetal survival and weight gain as a result of maternal toxicity. In rats, OGT 918 caused embryo-fetal deaths, post-implantation loss and decreased number of implantations. These effects were statistically significant (MD & HD) and dose-dependent. Visceral malformation (absence of innominate artery) and skeletal malformations (wavy ribs, thoracic vertebra with one or more centra being hemicentric) were also increased in fetuses of HD treated females (not statistically significant). These fetal effects were observed at doses greater than the NOAEL (20 mg/kg/d = 2x the human dose based on mg/m^2). In a rat pre- and post-natal developmental toxicity study, post-natal survival of F1 animals was not affected, nor was their general or reproductive development and performance of males and females. However, body weight gain was statistically significantly decreased in HD males and females by 11% and 6% respectively. NOAEL for the F1 generation growth, development, behavior and reproductive performance toxicity was 20 mg/kg/d (2x the human dose based on mg/m^2).

In rabbit developmental toxicity studies, OGT 918 caused a statistically significant increase in runted fetuses (30 and 45 mg/kg/d), dose-dependent and statistically significant (all doses) increase aortic arch with additional blood vessels. NOAEL for fetal/developmental toxicity could not established. However, the LD (15 mg/kg/d) is 3x the human dose based on mg/m^2. A similar rabbit study evaluated the teratogenic potential of OGT 918 at doses of 3, 10 and 30 mg/kg/d. NOAEL for fetal/developmental toxicity could not be established because of the missing brachiocephalic, enlarged frontal fontanel and frontal fontanel with reduced size observed in the LD group. The LD tested is 0.5x the human dose based on mg/m^2.

Histopathology Inventory for NDA # 21-348

Organs	Study Species		Study Species		Study Species		
	Dog EX 3533	Rat WVC/001	Monkey SA 3965	Rat SA 4085	Monkey SA 4078	Rat WVC/012	Juv. Rat WVC0024
Adrenals		X*	X*	X*	X*	X	X*
Aorta			X		X	X	
Bone Marrow smear		X	X	X	X		X
Bone (femur)		X	X	X	X		X
Brain		X*	X*	X*	X*	X	X*
Cecum	X	X*	X	X*	X	X	X*
Cervix			X	X*	X*		X*
Colon	X	X*	X	X*	X	X	X*
Duodenum	X	X*	X*	X*	X*	X	X*
Epididymis		X*	X*	X*	X*	X	X*
Esophagus		X	X	X	X		X
Eye		X	X	X	X	X	X
Fallopian tube							
Gall bladder			X		X		
Gross lesions		X	X	X	X		X
Harderian gland				X			X
Heart		X*	X*	X*	X*	X	X*
Hyphophysis		X*	X*	X*	X*	X	X*
Ileum	X	X*	X*	X*	X*	X	X*
Injection site							
Jejunum	X	X	X*	X	X*	X	X
Kidneys		X*	X*	X*	X*	X	X*
Lachrymal gland				X			X
Larynx			X		X		
Liver	X	X*	X*	X*	X*	X	X*
Lungs	X	X	X	X	X	X	X
Lymph nodes, cervical							
Lymph nodes mandibular		X	X	X	X		X
Lymph nodes, mesenteric	X	X	X	X	X	X	X
Mammary Gland		X	X	X	X		X
Nasal cavity					X		
Optic nerves							
Ovaries		X*	X*	X*	X*		X*
Pancreas		X	X*	X	X*	X	X
Parathyroid		X	X*	X	X*	X	X
Peripheral nerve			X				
Pharynx			X				
Prostate		X*	X*	X*	X*	X	X*
Rectum		X	X	X	X	X	X
Salivary gland		X	X	X	X	X	X
Sciatic nerve		X	X	X	X	X	X
Seminal vesicles		X	X	X	X	X	X
Skeletal muscle		X	X	X	X	X	X
Skin		X	X	X	X	X	X
Spinal cord		X	X	X	X	X	X
Spleen	X	X*	X*	X*	X*	X	X*
Sternum		X	X	X	X		X
Stomach	X	X*	X*	X*	X*	X	X*
Testes		X*	X*	X*	X*	X	X*
Thymus	X	X*	X*	X*	X*	X	X*
Thyroid		X*	X*	X*	X*	X	X*
Tongue		X	X	X	X		X
Trachea		X	X	X	X	X	X
Urinary bladder		X	X	X	X	X	X
Uterus		X*	X*	X*	X*		X*
Vagina		X		X	X		X
Zymbal gland							

X histopathology performed; *organ weight obtained

IX. GENETIC TOXICOLOGY:

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

Key findings:

- No test article-related increases in the number of revertant colonies were observed in any of the tester strains either in the presence/absence of metabolic activation at any of the concentrations tested.
- SC-48334 is not mutagenic under the conditions of the test system.

Study no: PSA-89S-3397
Study type (if not reflected in title): In vitro prokaryotic mutagenesis assay.
Volume #, and page #: Vol. 48, pg. 54.
Conducting laboratory and location: G. D. Searle & Co., 4901 Searle Parkway, Skokie, IL. 60077.
Date of study initiation: December 6, 1988.
GLP compliance: Yes (UK)
QA reports: yes (X) no ( )
Drug, lot #, radiolabel, and % purity: Lot No. 88K024-301D; 99.5% Pure.
Formulation/vehicle: A solution of SC-48334 in distilled water.

Methods:
Strains/Species/Cell line: Salmonella typhimurium strains: TA1535, TA100, TA1538, TA98 and TA97.
Dose Selection Criteria: Based on toxicity: decrease in number of revertant colonies.
Basis of dose selection: Highest dose was chosen to either produce signs of toxicity or to be insoluble in aqueous medium.
Range finding studies: None.
Test Agent Stability: SC-48334 in aqueous medium at concentrations of 1 and 140 mg/ml was stable for at least 8 days at ambient conditions.
Metabolic Activation System: Arochlor 1254-induced rat liver S9 homogenate.
CONTROLS:
Vehicle: Distilled water.
Negative Controls: Distilled water.
Positive Controls:

TESTER STRAINS	-S9	+S9
TA1535	NaN_3 (1 µg/plate)	2-AA (1 µg/plate)
TA100	NaN_3 (1 µg/plate)	2-AA (1 µg/plate)
TA1538	2-NF (2.5 µg/plate)	2-AA (1 µg/plate)
TA98	2-NF (2.5 µg/plate)	2-AA (1 µg/plate)
TA97	ICR-191 acridine (0.5 µg/plate)	2-AA (1 µg/plate)

2-Aminoanthracene = 2-AA; 2-Nitrofluorene = 2-NF; Sodium Azide = NaN_3

Exposure Conditions: Plate incorporation method of Ames assay

The tube contents were mixed gently and then poured onto the minimal agar plates. The top agar was allowed to set, and the plates were incubated at approximately 37°C for 2 days.

Incubation and sampling times: The plates were incubated at approximately 37°C for 2 days.
Doses used in definitive study: 10, 50, 100, 500, 1000 and 5000 µg/plate.
Study Design: Plate incorporation method of Ames assay.
Analysis:
No. of replicates: 3 x 5 plates/dose; 3 x 2 plates/dose for 2-NF and NaN_3, 3 x 5 plates/dose for 2-AA and 3 x 1 plates/dose for ICR 191 acridine.
Counting method: Manually or with an automated colony counter.
Criteria for positive results: The assay was considered positive if the number of revertant colonies induced by the test article is at least 2-fold greater than the solvent negative control with an evident dose-related increase.

Summary of individual study findings:
Study Validity: A 'limit dose' of 5000 µg/plate was used. Positive controls showed appropriate increases in colony numbers. Control values were in the range of sponsor's historical values. The study was adequately performed.
Study Outcome: No test article-related increases in the number of revertant colonies were observed in any of the tester strains either in the presence/absence of metabolic activation at any of the concentrations tested. Hence, SC-48334 is not mutagenic under the conditions of the test system.

Study Title: Bacterial Reverse Mutation Test.

Key findings:
- Statistically significant increases in revertant numbers were detected in the plate incorporation test using strain WP2 uvrA at 8 µg/plate in the presence of S-9 and in the pre-incubation test using TA100 at 40 µg/plate without S-9. No dose response was associated with either of these increases. Hence, their biological significance is questionable.
- OGT-918 is therefore not mutagenic under the conditions of the test system.

Study no: WVC/002
Study type (if not reflected in title): In vitro prokaryotic mutagenesis assay.
Volume #, and page #: Vol. 48, pg. 1.
Conducting laboratory and location:

Date of study initiation: September 17, 1997
GLP compliance: Yes (UK)
QA reports: yes (X) no ( )
Drug, lot #, radiolabel, and % purity: Not provided.
Formulation/vehicle: A solution of SC-48334 in distilled water.

Methods:
Strains/Species/Cell line: S. typhimurium strains: TA1535, TA1537, TA98, TA100 and E. coli strain WP2 uvrA.
Dose Selection Criteria: Based on toxicity: thinning or disappearance of bacterial lawn and/or decrease in number of revertant colonies.
Basis of dose selection: "Limit dose" or highest dose was chosen as the dose at which no toxicity was observed.
Range finding studies: Range finding studies at 1.6, 8, 40, 200, 1000 and 5000 µg/plate.
Test Agent Stability: SC-48334 in aqueous medium at concentrations of 1 and 140 mg/ml was stable for at least 8 days at ambient conditions.

Metabolic Activation System: Was derived from livers of β-naphthoflavone and sodium pentobarbitone treated rats.
CONTROLS:
Vehicle: Distilled water.
Negative Controls: Distilled water.
Positive Controls:

TESTER STRAINS	-S9	+S9
TA1535	NaN_3 (1 µg/plate)	2-AA (2 µg/plate)
TA1537	9-AA (50 µg/plate)	2-AA (2 µg/plate)
TA98	2-NF (0.5 µg/plate)	2-AA (2 µg/plate)
TA100	NaN_3 (1 µg/plate)	2-AA (2 µg/plate)
WP2 uvrA	4-NQO (1 µg/plate)	2-AA (4 µg/plate)

4NQO = 4-Nitroquinoline-N- oxide; 9-Aminoacridine = 9-AA; 2-Nitrofluorene = 2-NF; Sodium Azide = NaN_3

Exposure Conditions: Both the Plate incorporation and pre-incubation methods of Ames test were used in separate experiments. For the plate incorporation method, the test article, tester strains and S9 mix (where appropriate) were rapidly mixed and poured onto Vogel Bonner agar plates. When the agar had set, the plates were inverted and incubated for approximately 66 hours at 37°C.

] When the top agar had set, the Petri dishes were inverted and incubated at 37°C for approximately 66 hours.
Incubation and sampling times: 66 hours incubation at 37°C.
Doses used in definitive study: 8, 40, 200, 1000 and 5000 µg/plate.
Study Design: Both Plate incorporation and pre-incubation methods of Ames Test.

Analysis:
No. of replicates: 2 plates/dose.
Counting method: Manually or with an automated colony counter.
Criteria for positive results: The assay was considered positive if the number of revertant colonies induced by the test article is at least 2-fold greater than the solvent negative control with an evident dose-related increase.

Summary of individual study findings:
Study validity: A 'limit dose' of 5000 µg/plate was used. Positive controls showed appropriate increases in colony numbers. Control values were in the range of sponsor's historical values. The study was adequately performed.
Study Outcome: Statistically significant increases in revertant numbers were detected in the plate incorporation test using strain WP2 uvrA at 8 µg/plate in the presence of S-9 and in the pre-incubation test using TA100 at 40 µg/plate without S-9. No dose response was associated with either of these increases. Hence, their biological significance is questionable. OGT-918 is therefore not mutagenic under the conditions of the test system.

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

Key findings:

- The average mutant frequency of the negative control was 5.7 mutant colonies per 10^6 clonable cells.
- The test article-treated groups yielded from 0 to 2.6 average mutant colonies per 10^6 clonable cells. None of these values were statistically significant relative to the negative control.
- The average mutant frequency of the positive control was 141.8 mutant colonies per 10^6 clonable cells.
- SC-48334 is not mutagenic in this assay system.

Study no: PSA-89S-3411
Study type (if not reflected in title): In vitro prokaryotic mutagenesis assay.
Volume #, and page #: Vol. 48, pg. 117.
Conducting laboratory and location: G. D. Searle & Co., 4901 Searle Parkway, Skokie, IL. 60077.
Date of study initiation: November 15, 1988.
GLP compliance: Yes (UK)
QA reports: yes (X) no ( )
Drug, lot #, radiolabel, and % purity: Lot No. 88K024-301D; 99.5% Pure.
Formulation/vehicle: A solution of SC-48334 in distilled water.

Methods:
Strains/Species/Cell line: Chinese Hamster Ovary cells: CHO-K1-B4.
Dose Selection Criteria: Sponsor did not discuss the criteria upon which doses were selected.
Basis of dose selection: Not discussed by sponsor.
Range finding studies: Doses ranging from 31.3, 62.5, 125, 250, 500, 750, 1000, 3000 and 5000 µg/ml (3 dishes/dose) were evaluated.
Test Agent Stability: SC-48334 in aqueous solution is stable for at least days at dosing concentrations of 1 to 500 mg/ml when stored at ambient conditions.
Metabolic Activation System: Commercially prepared liver homogenate form aroclor 1254-treated rats.
CONTROLS:
Vehicle: Water.
Negative Controls: Water.
Positive Controls:

CELL LINE	-S9	+S9
CHO-K1-BH4	ICR-191 Acridine (1 µg/ml)	3-Methylcholanthrene (5 µg/ml)

Exposure conditions:
Incubation and sampling times: Cells in complete F12 medium were exposed to the test article for 20 to 24 hours without activation. With the S9 activation system, the cells in serum-free medium containing S9 mix were exposed to the test article for approximately 4 hours. Following exposure, the medium was changed to complete F12 medium and the cultures were incubated for an additional 16 to 24 hours.
Doses used in definitive study: 362, 724, 2172 and 3620 µg/ml.
Study Design: Forward mutation assay.

Analysis:
No. of replicates: 3 plates/dose (2 plates/dose for positive controls).
Counting method: Not disclosed by sponsor.

Criteria for positive results: Test article is considered positive (mutagenic) if the mutation frequencies for two successive test article concentrations are at least 15 x 10^6 clonable cells and significantly higher than the negative control value.

Summary of individual study findings:
Study Validity: The study was adequately performed because acceptable controls (negative and positive), acceptable number of doses, and acceptable high doses (doses that reduce clonal survival to approximately 10% to 25% of the average of the negative control) were used.
Study Outcome: The average mutant frequency of the negative control was 5.7 mutant colonies per 10^6 clonable cells. The test article-treated groups yielded from 0 to 2.6 average mutant colonies per 10^6 clonable cells. None of these values were statistically significant when compared to the negative control. The average mutant frequency of the positive control was 141.8 mutant colonies per 10^6 clonable cells. Hence, SC-48334 is not mutagenic in this assay system.

Study Title: In Vitro Mammalian Cell Cytogenetic Test: Human Lymphocytes.

Key findings:
- At 2500 and 5000 µg/ml dose levels, statistically significant increases in aberration frequency was observed. This occurred in the first experiment in the absence of S-9. The numbers of aberrations observed were within the normal background range for this cell line and was not considered to be of any biological significance.
- The test article, OGT-918, is not a clastogen under the conditions of this test.

Study no: WCV/0008
Study type (if not reflected in title): In vitro analysis of chromosomal aberration (clastogenecity).
Volume #, and page #: Vol. 48, pg. 141.
Conducting laboratory and location:

Date of Study Initiation/completion: May 5, 1998/ July 1, 1998.
GLP Compliance: Yes (U. K.)
QA- Reports Yes (X) No ( ):
Drug Lot Number: Batch # 60609-03. Purity not provided.
Formulation/vehicle: A solution of SC-48334 in DMSO.

Methods:
Strains/Species/Cell line: Human peripheral blood lymphocytes.
Dose Selection Criteria: Dose selection was based on cytotoxicity (a decrease in MI of treated samples compared to negative controls).
Basis of dose selection: The highest dose for analysis was chosen as the dose that produces 50% reduction in MI (cytotoxicity).
Range finding studies: Cultures were treated with OGT-918 at concentrations of 8, 40, 200, 1000 and 5000 µg/ml. Exposure was for three hours. Harvesting was after approximately 1.5 cell cycle time when slides were prepared for the scoring of mitotic indices.
Test Agent Stability: No information was provided.
Metabolic Activation System: S9 was made from male Fischer 344 rats that had been induced with beta-naphthoflavone and sodium phenobarbitone.
Controls:
Vehicle: DMSO.
Negative Controls: DMSO.

Positive Controls: Mitomycin C (0.3 to 0.5µg/ml) was prepared without S9 and cyclophosphamide (12 to 15 µg/ml) was prepared in medium with S9.
Exposure conditions:

Doses used in definitive study: 8, 40, 200, 1000 and 5000 µg/ml.
Study Design:

- First Cytogenetic Experiment:

- Second Cytogenic Experiment: This experiment was conducted in a similar manner, but a second set of cultures were included for harvesting at a second harvest time 24 hours later than the first. Treatment in the absence of S-9 was continuous until the first harvest time and was conducted in the presence of 20%(v/v) serum. Dosing of second harvest cultures treated without S-9 was terminated at approximately 1.5 cell cycles by washing as for the cultures treated with S-9.

Analysis:
No. of replicates: 2 slides/culture.
Counting method:
Criteria for positive results: The test agent is considered positive (clastogenic) if it induces a dose response effect with at least one dose having statistically significant aberrations than the negative control.

Summary of individual study findings:
Study Validity: The positive control chemicals induced statistically significant increases in the number of aberrations scored in each experiment. This shows the cells were sensitive to the effects of a known clastogen and the metabolic activation system was capable of promutagen activation. The doses used in the study reached a maximum of 5000 µg/ml, at which level no significant cytotoxicity was observed.
Study Outcome: At 2500 and 5000 µg/ml dose levels, statistically significant increases in aberration frequency was observed. This occurred in the first experiment in the absence of S-9 but not in the second experiment. Aberration frequency in the presence of S9 was not significantly different from negative control. A the number of aberrations observed were within the normal background range for this cell line and was not considered to be of any biological significance. The test article, OGT-918, is not a clastogen under the conditions of this test.

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

Key findings:

- No test article-related increases in the number of revertant colonies were observed in any of the tester strains either in the presence/absence of metabolic activation at any of the concentrations tested.
- SC-48334 is not mutagenic under the conditions of the test system.

Study no: PSA-90S-3568
Study type (if not reflected in title): In vitro prokaryotic mutagenesis assay.
Volume #, and page #: Vol. 48, pg. 54.
Conducting laboratory and location: G. D. Searle & Co., 4901 Searle Parkway, Skokie, IL. 60077.
Date of study initiation: July 13, 1990.
GLP compliance: Yes (UK)
QA reports: yes (X) no ( )
Drug, lot #, radiolabel, and % purity: Lot No. 89K034-401J; 99.5% Pure.
Formulation/vehicle: A solution of SC-48334 in distilled water.

Methods:
Strains/Species/Cell line: Salmonella typhimurium strains: TA1535, TA100, TA1538, TA98 and TA97.
Dose Selection Criteria: Based on toxicity: decrease in number of revertant colonies.
Basis of dose selection: Highest dose was chosen to either produce signs of toxicity or to be insoluble in aqueous medium.
Range finding studies: Sponsor did not make mention of range finding studies.
Test Agent Stability: SC-48334 in aqueous medium at concentrations of 1 and 140 mg/ml was stable for at least 8 days at ambient conditions.
Metabolic Activation System: Arochlor 1254-induced rat liver S9 homogenate.
Controls:
Vehicle: Distilled water.
Negative Controls: Distilled water.
Positive Controls:

TESTER STRAINS	-S9	+S9
TA1535	NaN_3 (1 µg/plate)	2-AA (1 µg/plate)
TA100	NaN_3 (1 µg/plate)	2-AA (1 µg/plate)
TA1538	2-NF (2.5 µg/plate)	2-AA (1 µg/plate)
TA98	2-NF (2.5 µg/plate)	2-AA (1 µg/plate)
TA97	ICR-191 acridine (0.5 µg/plate)	2-AA (1 µg/plate)

2-Aminoanthracene = 2-AA; 2-Nitrofluorene = 2-NF; Sodium Azide = NaN_3

Exposure Conditions: Top agar, indicator organisms, test article in vehicle or vehicle alone, or the positive control, metabolic activation mixture or PBS , for tests with or without S9 respectively were mixed gently and incubated at approximately 37°C for 2 days.
Incubation and sampling times: The plates were incubated at approximately 37°C for 2 days. The total number of his^+ revertant colonies were then counted.
Doses used in definitive study: 10, 50, 100, 500, 1000 and 5000 µg/plate.
Study Design: Plate incorporation method of Ames assay.

Analysis:
No. of replicates: 3 x 5 plates/dose; 3 x 2 plates/dose for 2-NF and NaN_3, 3 x 5 plates/dose for 2-AA and 3 x 1 plates/dose for ICR 191 acridine.
Counting method: Manually or with an automated colony counter.

Criteria for positive results: The assay was considered positive if the number of his^+ revertant colonies induced by the test article is at least 2-fold greater than the vehicle control with an evident dose-related increase.

Summary of individual study findings:
Study Validity: A 'limit dose' of 5000 µg/plate was used. Positive controls showed appropriate increases in colony numbers. Control values were in the range of sponsor's historical values. The study was adequately performed.
Study Outcome: No test article-related increases in the number of revertant colonies were observed in any of the tester strains either in the presence/absence of metabolic activation at any of the concentrations tested. Hence, SC-48334 is not mutagenic under the conditions of the test system.

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

Key findings:
- Micronucleus frequencies of test article-treated groups were not significantly higher than the control group at any of the three dosage levels tested.
- SC-48334 did not induce micronucleated polychromatic erythrocytes in bone marrow cells of mice under the conditions of this assay.

Study no: PSA-89S-3455
Study type (if not reflected in title): In vivo assessment of DNA damage (clastogenicity).
Volume #, and page #: Vol. 48, pg. 190.
Conducting laboratory and location: G. D. Searle & Co., 4901 Searle Parkway, Skokie, IL. 60077.
Date of study initiation: January 10, 1989.
GLP compliance: Yes (UK)
QA reports: yes (X) no ( )
Drug, lot #, radiolabel, and % purity: Lot No. 88K021-301E; 99.5% Pure.
Formulation/vehicle: A solution of SC-48334 in distilled water.

Methods:
Strains/Species/Cell line: Six weeks old male and female mice: CD-1.
Dose Selection Criteria: Dose selected for the micronucleus assay were obtained from test results of a previous oral acute toxicity study of the test article in mice.
Basis of dose selection: Dose selection for the micronucleus assay was based upon test results from a previous oral acute toxicity study of the test article in mice.
Range finding studies: Conducted to identify the maximum dosage level of 5000 mg/kg/day and two lower dosage levels of 2500 and 1250 mg/kg/day used for the micronucleus assay.
Test Agent Stability: SC-48334 in aqueous solution is stable for at least days at dosing concentrations of 1 to 500 mg/ml when stored at ambient conditions.
Metabolic Activation System: Not applicable.
Controls:
Vehicle: Distilled water.
Negative Controls: Distilled water.
Positive Controls: Cyclophosphamide.
Exposure Conditions: In vivo exposure by gavage.

Incubation and sampling times: Mice were administered with the test or control articles by oral gavage on two successive days. Treatments were separated by approximately 24 hours with the bone marrow harvest occurring approximately 24 hours after the second treatment.
Doses used in definitive study: 2500, 1250 and 5000 mg/kg/day.
Study Design: Standard micronucleus assay.
Analysis:
No. of replicates: At least 3 slides/animal/dose.
Counting method: Slides were coded prior to examination and scored in a blind fashion. At least one thousand enucleate polychromatic erythrocytes (PCEs) were counted for each animal. Other observations included the number of PCEs with and without micronuclei and the number of normochromatic erythrocytes (NCEs) and micronucleated NCEs in a field containing at least one PCE. Micronucleated NCEs were scored as a safeguard against scoring artifacts. Artifacts were suspected if more than five micronucleated NCEs were counted and if the micronuclei were evenly distributed over both types of erythrocytes.
Criteria for positive results: The test article is considered positive (clastogenic) if it shows a statistically significant positive response (increase in MNPCE) relative to control for at least one dose level and a statistically significant dose-related response.

Summary of individual study findings:
Study Validity: The positive control induced statistically significant increase ($p<0.01$) in micronucleated PCEs in both sexes relative to vehicle controls which indicated that the test system was capable of detecting a known clastogen.
Study Outcome: Micronucleus frequencies of test article-treated groups were not significantly higher than the control group at any of the three dosage levels tested. Hence, SC-48334 did not induce micronucleated polychromatic erythrocytes in the bone marrow cells of mice under the conditions of this assay.

Study Title: An Evaluation of the Mutagenic Potential of SC-49483 in the Ames Salmonella/Microsome Assay.

Key findings:
- No test article-related increases in the number of revertant colonies were observed in any of the tester strains either in the presence/absence of metabolic activation at any of the concentrations tested.
- SC-49483 is not mutagenic under the conditions of the test system.

Study no: PSA-92S-3891.
Study type (if not reflected in title): In vitro prokaryotic mutagenesis assay.
Volume #, and page #: Vol. 48, pg. 221.
Conducting laboratory and location: G. D. Searle & Co., 4901 Searle Parkway, Skokie, IL. 60077.
Date of study initiation: November 13, 1991.
GLP compliance: Yes (UK)
QA reports: Yes (X) no ( )
Drug, lot #, radiolabel, and % purity: Lot No. CD419-113A; 99.5% Pure.
Formulation/vehicle: A solution of SC-49483 in DMSO.

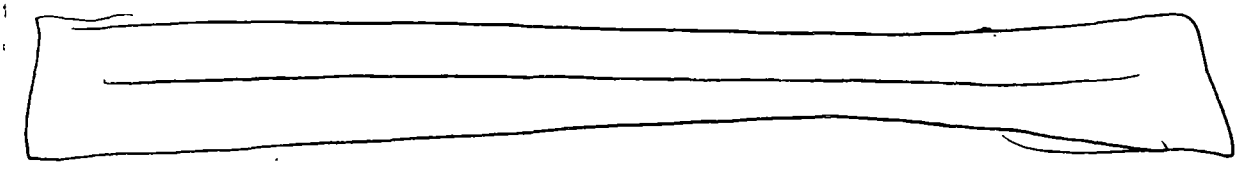
Methods:
Strains/Species/Cell line: Salmonella typhimurium strains: TA1535, TA100, TA1538, TA98 and TA97.
Dose Selection Criteria: Based on toxicity: decrease in number of revertant colonies.

Basis of dose selection: Highest dose was chosen to either produce signs of toxicity or to be insoluble in aqueous medium.
Range finding studies: None.
Test Agent Stability: Sponsor stated that the stability of the test agent was not determined (vol. 48, pg. 225)
Metabolic Activation System: Arochlor 1254-induced rat liver S9 homogenate.
CONTROLS:
Vehicle: DMSO.
Negative Controls: DMSO.
Positive Controls:

TESTER STRAINS	-S9	+S9
TA1535	NaN_3 (1 µg/plate)	2-AA (1 µg/plate)
TA100	NaN_3 (1 µg/plate)	2-AA (1 µg/plate)
TA1538	2-NF (2.5 µg/plate)	2-AA (1 µg/plate)
TA98	2-NF (2.5 µg/plate)	2-AA (1 µg/plate)
TA97	ICR-191 acridine (0.5 µg/plate)	2-AA (1 µg/plate)

2-Aminoanthracene = 2-AA; 2-Nitrofluorene = 2-NF; Sodium Azide = NaN_3

Exposure Conditions: Plate incorporation method of Ames assay.



Incubation and sampling times: The plates were incubated at approximately 37°C for 2 days.
Doses used in definitive study: 10, 50, 100, 500, 1000 and 5000 µg/plate.
Study Design: Plate incorporation method of Ames assay.
Analysis:
No. of replicates: 3 x 2 plates/dose for 2-NF and NaN_3, 3 x 5 plates/dose for 2-AA and 3 x 1 plates/dose for ICR 191 acridine.
Counting method: Manually or with an automated colony counter.
Criteria for positive results: The assay was considered positive if the number of revertant colonies induced by the test article is at least 2-fold greater than the solvent negative control with an evident dose-related increase.

Summary of individual study findings:
Study Validity: A 'limit dose' of 5000 µg/plate was used. Positive controls showed appropriate increases in colony numbers. Control values were in the range of sponsor's historical values. The study was adequately performed.
Study Outcome: No test article-related increases in the number of revertant colonies were observed in any of the tester strains either in the presence/absence of metabolic activation at any of the concentrations tested. Hence, SC-48334 is not mutagenic under the conditions of the test system.

Study Title: An Evaluation of the Potential of SC-49483 to induce Chromosome Aberrations in vitro in Chinese Hamster Ovary (CHO) cells.

Key findings:
- OGT-924 did not induce chromosome aberrations under the conditions of the study. Hence it is not a clastogen under the test conditions.

Study no: PSA-95S-4240.
Study type (if not reflected in title): In vitro prokaryotic mutagenesis assay.
Volume #, and page #: Vol. 48, pg. 248.
Conducting laboratory and location: G. D. Searle & Co., 4901 Searle Parkway, Skokie, IL. 60077.
Date of study initiation: April 14, 1994.
GLP compliance: Yes (UK)
QA reports: yes (X) no ( )
Drug, lot #, radiolabel, and % purity: Lot No. 93K003-B3C; 99.8% Pure.
Formulation/vehicle: A solution of SC-48334 in DMSO.

Methods:
Strains/species/cell line: CHO cell line.
Dose Selection Criteria: Dose selection was based on cytotoxicity (a decrease in MI of treated samples compared to negative controls).
Basis of dose selection: The highest dose for analysis was chosen as the dose that produces 50% reduction in MI (cytotoxicity).
Range finding studies: Approximately 0.3-0.5 x 10^6 cells/well were seeded in McCoy's complete media about 24 hours before exposure to the test article.

Test agent stability: Sponsor stated that test agent stability was not determined.
Metabolic activation system: S9 was prepared from Aroclor 1254 induced rat liver.
Controls:
Vehicle: DMSO.
Negative controls: DMSO.
Positive controls: Mitomycin C (0.5 µg/ml) used in cultures without S9, cyclophosphamide (5.0 µg/ml).
Comments: None.
Exposure conditions:
Incubation and sampling times: Please see study design.
Doses used in definitive study: 139, 278, 417, 833, 1250 µg/ml.
Study design:

TREATMENT		EXPOSURE	DOSES (µg/ml)
Short term	+ S9	4 hr, 37°C	417, 833, 1250
	- S9	4 hr, 37°C	417, 833, 1250
Continuous	-S9	24 hr, 37°C	17, 33

The 4 hour chromosomal aberration assay without activation had concurrent cell survival values ranging from 39% to 100%. The assay with activation had concurrent cell survival values ranging from 47% to 100%. Therefore, the test article concentrations scored for chromosome aberrations included 417, 833 and 1250 µg/ml with survival values ranging from 47% to 86% (with S9) and 39% to 41% (without S9).

Due to extreme cytotoxicity in the 24 hour assay without S9, a repeat assay was conducted at 17, 33, 50, 100 and 150 µg/ml. The repeat 24 hour chromosomal aberration assay without S9

had concurrent cell survival values ranging from 79% to 20%. Due to a lack of an ample number of metaphase cells, only the 17 and 33 µg/ml test article concentrations were analyzed for aberrations.

Analysis:
No. of replicates: 4 slides/dose level.
Counting method: By a trained scorer.
Criteria for positive results: For structural aberrations, including gaps and numerical aberrations, a score of < 5% was recorded as negative, ≥ 5% but < 10% was recorded as inconclusive /equivocal and scores ≥ 10% was recorded as positive.

Summary of individual study findings:
Study validity: The positive control chemicals caused significant increases in the number of aberrations scored in each experiment. This showed that the cells were sensitive to the effects of known clastogens and the metabolic activation system was capable of promutagen activation.
Study outcome: OGT-924 did not induce chromosome aberrations under the conditions of the study. Hence it is not a clastogen under the test conditions.

Study Title: An Evaluation of the Mutagenic Potential of SC-49483 in the CHO/HGPRT Mutation Assay.

Key findings:
- Without metabolic activation, relative cell survival ranged from 36% at 25 µg/ml to 20% at 250 µg/ml. Precipitation was observed at 100 and 250 µg/ml. The mutation responses of the OGT-924 treated groups ranged from 0.8 to 13.1 (control being 12.2) mutant colonies per 1 x 10^6 clonable cells.
- With metabolic activation, relative cell survival ranged from 110% at 50 µg/ml to 61% at 500 µg/ml. Precipitation was observed at 100, 250 and 500 µg/ml. The mutation responses of the OGT-924 treated groups ranged from 4.2 to 9.0 (control being 8.7) mutant colonies per 1 x 10^6 clonable cells.
- OGT-924 was not mutagenic under the conditions of the study.

Study no: PSA-92S-3893.
Study type (if not reflected in title)**:** In vitro prokaryotic mutagenesis assay.
Volume #, and page #: Vol. 48, pg. 286.
Conducting laboratory and location: G. D. Searle & Co., 4901 Searle Parkway, Skokie, IL. 60077.
Date of study initiation: December 106, 1991.
GLP compliance: Yes (UK)
QA reports: Yes (X) no ( )
Drug, lot #, radiolabel, and % purity: Lot No. GDS1609-053A; Purity was not provided.
Formulation/vehicle: A solution of SC-48334 in DMSO.

Methods:
Strains/species/cell line: CHO (subline K_1-BH_4).
Dose selection criteria: Based on cytotoxicity (relative cell survival).
Basis of dose selection: Dose levels for the mutation assays were selected based on the outcome of the range-finding assay.
Range finding studies: In a range-finding cytotoxicity test, test article concentrations of 31.3, 62.5, 125, 250, 500, 1000, 2500 and 5000 µg/ml were evaluated without and with metabolic activation. Without metabolic activation, cytotoxicity began to appear at 31.3 µg/ml and the

relative cell survival was 16% at 5000 µg/ml. Precipitation was observed at 125 µg/ml and higher concentrations. With metabolic activation, cytotoxicity began to appear at 250 µg/ml and the relative cell survival was 31% at 5000 µg/ml. Precipitation was observed at 125 µg/ml and higher concentrations. Therefore, the mutation test was conducted at 25, 50, 100 and 250 µg/ml without metabolic activation and 50, 100, 250 and 500 µg/ml with metabolic activation.
Test agent stability: Was not provided.
Metabolic activation system: S9 was prepared from Aroclor 1254 induced rat liver.
Controls:
Vehicle: DMSO.
Negative controls: DMSO.
Positive controls:

CELL LINE	-S9	+S9
CHO-K1-BH4	ICR-191 Acridine (1 µg/ml)	3-Methylcholanthrene (5 µg/ml)

Comments: None.
Exposure conditions: Please see study design below.
Incubation and sampling times: Please see study design below.
Doses used in definitive study: 25, 50, 100 and 250 µg/ml without metabolic activation and 50, 100, 250 and 500 µg/ml with metabolic activation.
Study design: The cells were exposed to the test article for 20 to 24 hours without metabolic activation at 37°C. For the assay with the metabolic activation system, the cells were exposed to the test article for approximately 4 hours 37°C.

Analysis:
No. of replicates: Triplicate (duplicate for positive controls)
Counting method: Manual counting.
Criteria for positive results: The test article is said to have a positive response if the mutation frequencies for two successive test article concentrations are at least 15 per 1 x 10^6 clonable cells and significantly higher than the solvent control value.
Summary of individual study findings:
Study validity: The assay was valid because acceptable controls were used. A positive control was included to ensure that the procedures used in this assay could detect mutagenic activity. Acceptable high doses were used (concentrations that reduce the clonal survival to ~ 10% to 25% of the average negative control).
Study outcome: Without metabolic activation, the relative cell survival ranged from 36% at 25 µg/ml to 20% at 250 µg/ml. Precipitation was observed at 100 and 250 µg/ml. The mutation responses of the OGT-924 treated groups ranged from 0.8 to 13.1 (control being 12.2) mutant colonies per 1 x 10^6 clonable cells. With metabolic activation, the relative cell survival ranged from 110% at 50 µg/ml to 61% at 500 µg/ml. Precipitation was observed at 100, 250 and 500 µg/ml. The mutation responses of the OGT-924 treated groups ranged from 4.2 to 9.0 (control being 8.7) mutant colonies per 1 x 10^6 clonable cells. Therefore OGT-924 was not mutagenic under the conditions of the study.

Genetic toxicology summary:
The sponsor covered the standard test battery for genotoxicity. The assays evaluated the genotoxic potential of both the prodrug (OGT 924 or SC-49483) and its active metabolite (OGT 918 or SC-48334).

OGT 918	
TESTS FOR GENE MUTATION IN BACTERIA	**OUTCOME**
An Evaluation of the Mutagenic Potential of SC-48334 in the Ames Salmonella-E. coli/Microsome Assay.	Negative
An Evaluation of the Mutagenic Potential of SC-48334 (NBDG Route) in the Ames Salmonella/Microsome Assay	Negative
An Evaluation of the Mutagenic Potential of SC-48334 in the Ames Salmonella-E. coli/Microsome Assay.	Negative
IN VITRO TEST WITH CYTOGENETIC EVALUATION OF CHROMOSOMAL DAMAGE IN MAMMALIAN CELLS	
An Evaluation of the Mutagenic Potential of SC-48334 in the CHO/HGPRT Mutation Assay.	Negative
In Vitro Mammalian Cell Cytogenetic Test: Human Lymphocyte.	Negative
IN VIVO TEST FOR CHROMOSOMAL DAMAGE USING RODENT HEMATOPOIETIC CELLS	
An Evaluation of the Potential of SC-48334 to Induce Micronucleated Polychromatic Erythrocytes in the Bone Marrow Cells of Mice.	Negative
OGT 924	
TESTS FOR GENE MUTATION IN BACTERIA	**OUTCOME**
An Evaluation of the Mutagenic Potential of SC-48334 (NBDG Route) in the Ames Salmonella/Microsome Assay	Negative
IN VITRO TEST WITH CYTOGENETIC EVALUATION OF CHROMOSOMAL DAMAGE IN MAMMALIAN CELLS	
An Evaluation of the Mutagenic Potential of SC-49483 to induce Chromosome aberrations in CHO cells.	Negative
An Evaluation of the Mutagenic Potential of SC-49483 in the CHO/HGPRT Mutation Assay.	Negative

Genetic toxicology conclusions: The prodrug and its active metabolite tested negative in all the genotoxicity assays. Hence the drug was neither mutagenic nor clastogenic under the conditions of the study.

Labeling recommendations: Miglustat was not mutagenic or genotoxic in a battery of in vitro and in vivo assays including the bacterial reverse mutation (Ames), chromosomal aberration (in human lymphocytes), gene mutation in mammalian cells (Chinese hamster ovary), and mouse micronucleus

VI. CARCINOGENICITY:

Carcinogenicity studies was waived as a Phase IV commitment on August 4, 1999 by Dr. Ron Steigerwalt, who was then the Supervisory Pharmacologist. ECAC reviewed the rat dose selection on 12/18/01 (see appendix).

Structural Alert Assessment: Sponsor stated that OGT 918 has been applied to two structural alert analysis screens to assess its carcinogenic potential. The first analysis employed the _____ which provides a qualitative prediction of the possible toxicity of chemical compounds, based on a knowledge-base of toxophores. A further assessment was also made using the _____

Sponsor concluded that OGT 918 did not resemble any known carcinogen or genotoxic structure in these studies.

IX. REPRODUCTIVE AND DEVELOPMENTAL TOXICOLOGY:

Study title: Rat Oral (gavage) Male Fertility Study

Key study findings:

- 1/20 LD males (# 78-subgroup B) and 1/15 HD males (# 198-subgroup C) were sacrificed in extremis. The LD male had malignant nephroblastoma of the left kidney that has metastasized to the adrenal gland, marked extramedullary hematopoiesis, slight white pulp hyperplasia (spleen) and moderate lymphoid hyperplasia (thymic lymph node). Histopathology revealed no abnormal findings the HD animal. Two other animals were sacrificed in extremis during recovery. Animal #s 147 and 190, both males in the MD and HD groups respectively. Macroscopically, the MD male was observed with firm masses on both left and right preputial glands. Histopathology revealed no abnormal findings the MD animal. Macroscopically, the HD male was observed with accentuated lobular pattern of the liver. Histopathology revealed no abnormal findings in the liver of this animal.
- Fertility index was slightly reduced in HD males of subgroups A (4 weeks of treatment) and B (13 weeks of treatment). This effect was reversed at the end of the recovery period.
- Both absolute and relative weights of the cauda epididymides were slightly but statistically significantly decreased in all treated males (subgroup B only) relative control.
- Sperm motility was statistically significantly decreased in MD males (subgroup A only) by 8% relative to control. Actual path velocity (VAP) was slightly but statistically significantly decreased in all treated males (subgroup A only) relative control. Increase in dose appears to increase VAP.
- Sperm morphology parameters were altered in subgroups A and B only but not C. In subgroup A, there was a slightly but statistically significant decrease in number of normal sperms in all treated males relative control. The decrement was not dose-dependent. The number of headless sperms and those with reduced hooks were both statistically significantly increased in all treated males relative control. Miscellaneous abnormalities were also statistically significantly increased in MD and HD males relative control. These increments were not dose-dependent. Animals in subgroup B had statistically significant increases in headless sperms and sperms with reduced hooks in all treated males relative control. These increases were dose-dependent. Miscellaneous abnormalities were only statistically significantly increased in HD males relative control.
- Untreated females mated with treated males in subgroup B had slight but statistically significant decreases in corpora lutes/female (LD only) and implantations/female (LD and MD only) relative control. Implantations/female and live embryos/female were statistically significantly decreased in all females mated with treated males in subgroup C relative control. The decrements are not dose-dependent. Corpora lutea/female was also slightly but statistically significantly decreased (not dose-dependent) in females mated with MD and HD treated males relative control. Pre-implantations/female was statistically significantly increased (not dose-dependent) in all females mated with treated males.
- NOAEL could not be established because of the decreased sperm concentration, decreased number of normal sperms, increased headless sperms and increased sperms with reduced hooks in LD males. These effects were also reflected in the fertility parameters of females mated with these males.
- However, the male reproductive effects were reversed within approximately 6 weeks of cessation of treatment. The fertility parameters of females mated with treated males in subgroup C that were allowed to recover did not show statistical significance at any dose level relative to control. This suggests that decreased live births and implantations in untreated females is a function of adverse male fertility effects. Data not included because none of the female fertility parameters were statistically significantly different from control.

Study no.: WVC/012.
Volume #, and page #: Vol. 43, pg. 152.

Conducting laboratory and location:

Date of study initiation: November 13, 1998.
GLP compliance: Yes (UK).
QA reports: Yes (X) no ( )
Drug, lot #, radiolabel, and % purity: OGT 918, Batch # 60609-03 and 60689-07.
Formulation/vehicle: A solution of OGT 918 in ultra high purity water.

Methods:
Species/strain: Rat/Sprague Dawley.
Doses employed: Animals were dosed TID to give total doses of 20, 60, 180 mg/kg/day.
Route of administration: Oral (gavage).
Study design:

Group number	Colour Code	Number of animals Males	Females	Animal identification Numbers Males	Females	Dose level (mg/kg/day) OGT 918
1 (a)	White	15	15	1-15	201-215	Control (vehicle)
1 (b)		20	20	16-35	216-235	
1 (c)		15	60	36-50	236-295	
2 (a)	Green	15	15	51-65	296-310	20
2 (b)		20	20	66-85	311-330	
2 (c)		15	60	86-100	331-390	
3 (a)	Yellow	15	15	101-115	391-405	60
3 (b)		20	20	116-135	406-425	
3 (c)		15	60	136-150	426-485	
4 (a)	Pink	15	15	151-165	486-500	180
4 (b)		20	20	166-185	501-520	
4 (c)		15	60	186-200	521-580	

The sub-groups were dosed as follows, with groups of untreated females being used for mating:

Sub-group A – Dosed for 2 weeks prior to mating, throughout mating (2 weeks) and until Necropsy (total of 4 weeks treatment).

Sub-group B – Dosed for 10 weeks prior to mating, throughout mating (3 weeks) and until Necropsy (total of 13 weeks treatment).

Sub-group C – Dosed for 10 weeks prior to and throughout mating (2 weeks). After mating, animals were maintained untreated for approximately 13 weeks prior to necropsy, with further 10 day mating periods at 4 weeks, 6 weeks and 8 weeks into recovery (total of 12 weeks treatment and 13 weeks recovery).

Mating was conducted on a one male to one female basis within dose groups. The males were killed and subjected to a macroscopic necropsy after the end of the mating period. The testes and epididymides (total and cauda) were weighed and sperm analysis performed. In addition, the numbers of homogenisation resistant spermatids were assessed.

The females were killed on or after Day 13 of pregnancy and the pregnancy status, number of corpora lutea, number and distribution of implantations assessed.
Number/sex/group: Please see study design.

Parameters and endpoints evaluated:
Clinical signs: Daily.
Mortality: Twice daily.
Body weight: Twice weekly.
Food consumption: Weekly.
Terminal examination: Animals were killed by CO_2 asphyxiation. Organs or tissues showing any macroscopic abnormalities were removed and fixed in neutral buffered formaldehyde.

Male necropsy: The males were killed after completion of their respective mating periods and a necropsy performed. In addition, the testes and epididymides were removed and weighed. Immediately after removal, the right cauda epididymis from all males were used for sperm examination. Samples of sperm were collected to assess motility and concentration using [redacted] (computer assisted sperm motility analysis) [redacted] For morphology, a smear was prepared and the sperm examined by light microscopy, which included examination for broken, decapitate, two-headed sperm and other gross defects. Following completion of the sperm assessment, the right testis was prepared and assessed for the numbers of homogenisation resistant spermatids. Sperm samples were collected at sacrifice, without interim evaluations.

Female Necropsy: The females were killed from Day 14 after mating, if mating was confirmed, or fourteen days after the end of the mating period, if not confirmed and a necropsy performed where the uterus was examined and the pregnancy status assessed.

For pregnant females killed on or after Day 13 of pregnancy, the pregnancy status, number of corpora lutea, number and distribution of implantations in uterine horns, classified as early resorptions, late resorptions, dead embryos or live embryos were observed.

Toxicokinetics: Not conducted.
Statistics:
Interval data: Group means and standard deviations were calculated for each observation time. Analysis of variance (ANOVA) was performed on all parameters, using treatment group as the factor in the analysis. Residuals from this preliminary analysis were examined for heterogeneity of variance using Levenes test. Other statistical analysis used included a Kruskal-Wallis ANOVA, and Shirley's non-parametric version of Williams test.
Nominal data: Fisher's Exact Test was used to compare each test article treated group with the vehicle control group.

Results:
In-life observations:
Mortality: Two males were prematurely sacrificed during the treatment period and a further two during the recovery period.
In sub-group B 1/20 males (animal # 78) receiving 20 mg/kg/day, was killed in extremis on Day 47 of dosing due to its poor clinical condition. Observations prior to sacrifice included a red colored penis, teeth abnormalities, pale extremities, cranial fur-staining and a hard, irregular, immobile mass in the lumber lateral region. 1/15 males in sub-group C (animal # 198) receiving 180 mg/kg/day, was killed in extremis on Day 12 of dosing due to its poor clinical condition. Observations noted prior to sacrifice included slow, irregular breathing, a cold and pale body, partially closed eyes, red urine and a purple scrotum. The animal was also prostrate.

There were two premature deaths during the recovery phase of the study. 1/15 males in the 60 mg/kg/day recovery group (animal # 147) was killed in extremis on Day 10 of recovery after

being observed with two abdominal masses, of which one had a green discharge. 1/15 males (# 190), from the group receiving 180 mg/kg/day /kg/day, was prematurely sacrificed on Day 78 of recovery. Observations prior to sacrifice included scabbing and desquamation of the tail and a dark protruding eye. Sponsor stated that the premature deaths of these animals were not considered treatment-related.

Clinical signs: Except for the clinical signs observed prior to the sacrifice of the 2 males, there were no other clinical signs that could be attributed to treatment.
Body weight: No treatment-related effects.
Food consumption: No treatment-related effects.
Toxicokinetics: No data.
Pre-coital interval: There was no treatment-related effect during treatment or recovery on the time course of mating.

MALE FERTILITY	0 mg/kg/d	20 mg/kg/d	60 mg/kg/d	180 mg/kg/d
SUB GROUP A				
Copulation index	87%	73%	73%	93%
Fertility index	92%	100%	100%	71%
SUB GROUP B				
Copulation index	90%	100%	100%	100%
Fertility index	94%	100%	90%	80%
SUB GROUP C				
Copulation index	100%	100%	88%	93%
Fertility index	93%	100%	100%	100%
SUB GROUP C (RECOVERY WEEK 8)				
Copulation index	93%	100%	93%	100%
Fertility index	93%	100%	100%	93%

Terminal and necroscopic evaluations:
Males: Organ weights: absolute wt. (g); Relative wt. in parenthesis (%).

Parameter	0 mg/kg/d	20 mg/kg/d	60 mg/kg/d	180 mg/kg/d
SUB GROUP A				
Cauda epididymides	0.43 (0.12)	0.42 (0.12)	0.41 (0.12)	0.45 (0.13)
Epididymides	1.12 (0.32)	1.11 (0.32)	1.13 (0.32)	1.17 (0.34)
Testes	3.64 (1.05)	3.72 (1.09)	3.69 (1.03)	3.77 (1.09)
SUB GROUP B				
Cauda epididymides	0.51 (0.12)	0.42*** (0.10***)	0.42*** (0.10***)	0.45*** (0.11***)
Epididymides	1.30 (0.30)	1.23(0.29)	1.27 (0.30)	1.31 (0.31)
Testes	3.84 (0.88)	3.67 (0.85)	3.70 (0.86)	3.78 (0.89)
SUB GROUP C				
Cauda epididymides	0.52 (0.11)	0.50 (0.10)	0.51 (0.10)	0.52 (0.11)
Epididymides	1.36 (0.28)	1.34 (0.28)	1.37 (0.27)	1.39 (0.28)
Testes	4.05 (0.82)	3.82 (0.80)	3.95 (0.78)	3.99 (0.82)

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

Semen analysis:

Parameter	0 mg/kg/d	20 mg/kg/d	60 mg/kg/d	180 mg/kg/d
SUB GROUP A				
Motility (%)	90	89	82*	91
Concentration (M/ml)	15	13	13	15
VAP (µm/s)	66	60**	62**	63*
VSL (µm/s)	18	16	16	19
SUB GROUP B				
Statistically, there was no significant differences between treated and control parameters.				
SUB GROUP C				
Statistically, there was no significant differences between treated and control parameters.				

*** = p <0.05; ** = p < 0.01; VAP = Actual path velocity; VSL = Straight line velocity.**

Sperm morphology:

Parameter	0 mg/kg/d	20 mg/kg/d	60 mg/kg/d	180 mg/kg/d
SUB GROUP A				
# normal	198	178***	171***	190***
# headless	0.87	13.40***	14.60***	3.67**
# with reduced hooks	0.73	7.80***	12.53***	3.87***
Miscellaneous abnormalities	0.33	0.67	1.47*	1.20**
SUB GROUP B				
# normal		NS	NS	NS
# headless	1.40	16.26**	13.80**	13.10*
# with reduced hooks	1.75	8.37**	8.40**	10.45***
Miscellaneous abnormalities	0.55	1.95	0.50	2.80*
SUB GROUP C				
Statistically, there was no significant differences between treated and control parameters.				

*** = p <0.05; ** = p < 0.01; *** = p < 0.001; NS = not significant**

Homogenization resistant spermatids: Statistically, there was no significant differences between treated and control parameters (average # spermatids, spermatids/ml and spermatids/testis) in any of the sub groups. Sub group C was evaluated at the end of recovery, not at the end of treatment.

Females: NS = Not significant statistically

Parameter	0 mg/kg/d	20 mg/kg/d	60 mg/kg/d	180 mg/kg/d
SUB GROUP A				
Corpora lutea/female		NS	NS	NS
Implantations/female		NS	NS	NS
Post implantation loss	10.8	2.8**	5.4**	2.7**
Live embryos/female		NS	NS	NS
SUB GROUP B				
Corpora lutea/female	15	13*	14	16
Implantations/female	14	10*	10*	13
Post implantation loss		NS	NS	NS
Live embryos/female		NS	NS	NS
SUB GROUP C				
Corpora lutea/female	16 ± 2.3	14 ± 3.5	12 ± 2.3*	14 ± 2.6*
Implantations/female	15	11*	6**	13**
Pre-implantation loss	9	30*	57*	13*
Post implantation loss		NS	NS	NS
Live embryos/female	14	10*	5**	12**
SUB GROUP C (RECOVERY WEEK 8)				
At the end of the recovery period, there was no significant difference between control and treated groups.				

*** = p <0.05; ** = p < 0.01; *** = p < 0.001; SD included only where it clarifies statistical significance.**

Histopathology of animals sacrificed in extremis:

Organ/Tissue	SUB GROUP B (Animal # 78-male) 20 mg/kg/d.
Adrenals: Malignant nephroblastoma	1/15(P)
Epididymal fat: Fat necrosis	1/15(4)
Neovascularization	1/15(2)
Kidney: Malignant nephroblastoma	1/15(P)
Spleen: Extramedullary hematopoiesis	1/15(4)
White pulp hyperplasia	1/15(2)
Thymic lymph node: Lymphoid hyperplasia	1/15(4)
SUB GROUP C (Animal # 198-male) HD group.	
Histopathology: no abnormalities were detected.	
ANIMALS SACRIFICED IN EXTREMIS DURING RECOVERY	
(Animal # 147-male) 60 mg/kg/d	
Firm masses – macroscopically	2 firm masses were observed on both left and right preputial glands. No abnormalities were detected microscopically.
(Animal # 190-male) 180 mg/kg/d	
Liver: accentuated lobular pattern – macroscopically	No abnormalities were detected microscopically.

1 = minimal, 2 = slight, 3 = moderate, 4 = marked

Review of the individual animal histopathology of surviving animals indicated that no abnormalities were detected.

Summary of Study Findings:
A male rat oral fertility study was conducted to investigate the effects of OGT 918 on the fertility of the male rat following administration for 14 (4 weeks treatment in total) or 70 (13 weeks in total) days prior to mating and during mating, and to look at reversibility during a subsequent 13 week recovery period. The dose levels used were 0, 20, 60 and 180 mg/kg/day. The males were killed after the end of the mating period and the females were killed on Day 13 of pregnancy.

There was no adverse effect of treatment on group mean bodyweight or bodyweight gain, food consumption or time course of mating. Fertility index was slightly reduced in HD (6x the maximum human based on mg/M^2) males of subgroups A (4 weeks of treatment) and B (13 weeks of treatment) but not C (12 weeks of treatment and 13 weeks of recovery). This effect was reversed at the end of the recovery period. Both absolute and relative weights of the cauda epididymides were slightly but statistically significantly decreased in all treated males (subgroup B only) relative control.

Sperm motility was statistically significantly decreased in MD (2x the maximum human dose based on mg/M^2) males (subgroup A only) by 8% relative to control. Actual path velocity (VAP) was slightly but statistically significantly decreased in all treated males (subgroup A only) relative control. Increase in dose appears to increase VAP. Sperm morphology parameters were altered in subgroups A and B only but not C. In subgroup A, there was a slightly but statistically significant decrease in number of normal sperms in all treated males relative control. The decrement was not dose-dependent. The number of headless sperms and those with reduced hooks were both statistically significantly increased in all treated males relative control. Miscellaneous abnormalities (not specified) were also statistically significantly increased in MD and HD males relative control. These increments were not dose-dependent. Animals in subgroup B had statistically significant increases in headless sperms and sperms with reduced hooks in all treated males relative control. These increases were dose-dependent. Miscellaneous abnormalities were only statistically significantly increased in HD males relative control.

Post implantation loss was statistically significantly decreased in all females (not dose-dependently) mated with treated males in subgroup A. Females mated with treated males in subgroup B had slight but statistically significant decreases in corpora lutea/female (LD only – 0.6x the maximum human dose based on mg/M^2) and implantations/female (LD and MD only) relative control. Implantations/female and live embryos/female were statistically significantly decreased in all females mated with treated males in subgroup C relative control. The decrements were not dose-dependent. Corpora lutea/female was also slightly but statistically significantly decreased (not dose-dependent) in females mated with MD and HD treated males relative control. Pre-implantations/female was statistically significantly increased (not dose-dependent) in all females mated with treated males.

NOAEL could not be established because of the decreased sperm concentration, decreased number of normal sperms, increased headless sperms and increased sperms with reduced hooks in LD males. These effects were also reflected in the fertility parameters of females mated with these males. However, the male reproductive effects were reversed within approximately 6 weeks of cessation of treatment. The fertility parameters of females mated with treated males in subgroup C that were allowed to recover did not show any significant difference

relative to control. Data not included because none of the female fertility parameters were statistically significantly different from control.

Study title: Male rat oral (gavage) fertility investigation study.

Due to the increased pre-implantation loss being recorded in the previous study (WVC/012), this study was conducted to further investigate the fertility of the male following administration of OGT 918 for approximately 42 days prior to mating and during mating.

Key study findings:

- Mean pre-coital interval (time course of mating) was slightly decreased (not statistically significant) during the treatment period compared to the pre-treatment period.
- Copulation index was not affected following treatment (i.e. the libido of males was not affected). Fertility index was reduced from 86% (pre-dose males) to 53% (post-dose males) following treatment suggesting that only 53% of males successfully sired litters.
- An increase in the mean number of unfertilized and fragmenting eggs was observed in females mated with treated males. There was a significant increase in the number of unfertilized eggs per female following treatment, and a corresponding decrease in the number of fertilized eggs. Although females were still capable of becoming pregnant, the number of fertilized eggs per female, post-dosing averaged five. Sponsor stated that in those females where pregnancy had occurred, however, the eggs developed normally with no signs of abnormalities.
- Sponsor stated that examination of the testes and epididymides did not reveal any major abnormalities (no data). There was no treatment-related effect on the absolute or relative weight of the testes.
- The 60 mg/kg/d dose decreased fertility index in treated males. This resulted in an increase in the mean number of unfertilized and fragmenting eggs in females mated with the treated males.

Study no.: WVC/020.
Volume #, and page #: Vol. 45, pg. 1.
Conducting laboratory and location:

Date of study initiation: July 22, 1999.
GLP compliance: Yes (UK).
QA reports: Yes (X) no ( )
Drug, lot #, radiolabel, and % purity: OGT 918, Batch #60689-07. Purity = 99.3%.
Formulation/vehicle: OGT 918 dissolved in ultra high purity water.

Methods:
Species/strain: Rat/Sprague Dawley, **Crl:CD (SD) BR (**
Doses employed: Animals were dosed TID to give a total dose of 60 mg/kg/d.
Route of administration: Oral (gavage).
Study design:

Group number	Colour Code	Number of Animals		Animal identification Numbers		Male dose level test article (mg/kg/day)
		Males	Females	Males	Females	
1	Yellow	20	40	1-20 61-63	21-40a 41-60b	60

a = females paired pre-male dosing; b = females paired aftermales dosed for 42 days.

One group of 20 male Sprague-Dawley rats were mated with untreated virgin females. Vaginal smears were taken daily until sperm was found in the smear. The male was removed on the day of mating and the female caged individually. The females were killed on approximately Day 4.5 after mating and the number of corpora lutea counted. Each uterine horn was flushed with saline and the eggs were collected and examined for the degree of development. Following confirmation of successful mating, the males commenced dosing for 42 days prior to a second mating. The males were dosed once daily, by oral gavage, with the test article, OGT 918 at a dose level of 60 mg/kg/day.

After the dosing period, each male was paired with a second virgin, untreated female. Vaginal smears were taken daily until sperm was found in the smear. The male was removed on the day of mating and the female caged individually. The females were killed on approximately Day 4.5 post-mating and the number of corpora lutea counted. The males were subject to macroscopic necropsy approximately one week after the end of the mating period.

Number/sex/group: 20/sex/group – pre-male dosing, 20/sex/group post-male dosing.

Parameters and endpoints evaluated:
Clinical signs: Daily.
Mortality: Daily.
Body weight: Twice weekly.
Food consumption: Weekly.
Terminal examination:
Male Necropsy: The males were killed and necropsied after completion of the second mating period and a necropsy performed. In addition, the testes and epididymides were removed and the testes were weighed. The testes and epididymides were fixed in Bouin's fluid for approximately 24 hours and then transferred to neutral buffered formaldehyde.

Female Necropsy: The females were killed on approximately Day 4.5 after mating, a necropsy performed and the number of corpora lutea counted for each ovary. Each uterine horn was flushed with approximately I ml of 0.9% sodium chloride. The eggs were collected and examined for the degree of development as follows:

- Fertilized egg: at this time unfertilized eggs show a degree of degeneration and fragmentation.
- Intact zona pellucida (i.e. had the zygote 'hatched'?)
- Degree of development – at this time the zygotes should have reached the late morulla/early blastocyst stage. Earlier stages recorded at this time-point were interpreted as disruption of normal development.

Toxicokinetics: Not conducted.
Statistics: ANOVA and William's test: Body wt., body wt. gains, number of corpora lutea, fertilized eggs.
Kruskal-Wallis and Shirley's test: Pre-implantation test.
Fisher exact test: Copulation index, fertility index.

Results:
Male Fertility
In-life observations:
Mortality: None.
Clinical signs: No treatment-related clinical signs.
Body weight: No treatment-related effect on body weight.

Food consumption: No data.
Toxicokinetics: No data.
Time Course of mating

Pre-Dose Males		Post-Dose Males	
Mean # of days taken to mate	3.1 ± 2.3	Mean # of days taken to mate	1.8 ± 1.1

Fertility and Mating Data

Pre-Dose Male	# Paired		# Mated		# Fertile		Copulation index		Fertility index	
	a	b	a	b	a	b	a	b	a	b
60 mg/kg/d	20	24	18	22	17	19	90	92	94	86
Post-Dose Male	**# Paired**		**# Mated**		**# Fertile**		**Copulation index**		**Fertility index**	
60 mg/kg/d	20		19		10		95		53	

a = not including test mates; b = including test mates

Terminal and necroscopic evaluations:
Males
Organ weight: Testes; n = 20.

Organ	Absolute organ weight (g)	Relative organ weight (%)
Day 1	3.45	0.88
Day 20	3.74	0.87

Relative wt. = relative to body wt.

Gross Pathology: Unremarkable. Sponsor stated that specific examination of the testes and epididymides did not reveal any major abnormalities (no data).
Females
In utero examination – Day 4.5 of gestation

Pre-Dose Males	# of corpora lutea		# with zona intact		# fertilized		Morula stage		Early blastocys-t stage		Late blastocys-t stage		# unfertiliz-ed		# fragmenti-ng	
	a	b	a	b	a	b	a	b	a	b	a	b	a	b	a	b
n	17	20	12	15	16	19	13	15	15	18	3	3	0	0	0	1
Mean	14	14	8	8	9	9	2	2	7	7	3	3	0	0	0	1
SD	3	3	4	4	4	4	1	1	3	3	0	0	0	0	0	0
Post-Dose Males	**# of corpora lutea**		**# with zona intact**		**# fertilized**		**Morula stage**		**Early blastocys-t stage**		**Late blastocys-t stage**		**# unfertiliz-ed**		**# fragmenti-ng**	
n	19		16		10		5		8		2		13		13	
Mean	15		8		5		2		4		2		7		7	
SD	4		4		3		1		2		1		4		4	

a = not including test mates; b = including test mates

Summary of Study Findings:
Due to the increased pre-implantation loss recorded in the previous study (WVC/012), this study was conducted to further investigate the fertility of the male following administration of OGT 918 for approximately 42 days prior to mating and during mating (WVC/020). One group of 20 male rats was mated with untreated virgin females. The females were killed on approximately Day 4.5 after mating and the number of corpora lutea counted. Each uterine horn was flushed with saline and the eggs were collected and examined for the degree of development. Following confirmation of successful mating, the males commenced dosing for 42 days prior to a second mating. The males were dosed once daily, by oral gavage, with OGT 918 at a dose level of 60 mg/kg/day. After the dosing period, each male was paired with a second virgin, untreated female. Again the females were killed on approximately Day 4.5 post-mating and the number of corpora lutea counted. Each uterine horn was flushed with saline and the eggs collected, and examined for the degree of development. The males were sacrificed and subject to macroscopic examination approximately one week after the end of the mating period.

Mean pre-coital interval (time course of mating) was slightly decreased (not statistically significant) during the treatment period compared to the pre-treatment period. Copulation index (libido of males) was not affected following treatment. Fertility index was reduced from 86% (pre-dose males) to 53% (post-dose males) following treatment suggesting that only 53% of males successfully sired litters.

An increase in the mean number of unfertilized and fragmenting eggs was observed in females mated with treated males. There was a significant increase in the number of unfertilized eggs per female mated with a treated male, and a corresponding decrease in the number of fertilized eggs. Although females were still capable of becoming pregnant, the number of fertilized eggs per female, post-dosing averaged five. Sponsor stated that in those females where pregnancy had occurred, however, the eggs developed normally with no signs of abnormalities. Sponsor further stated that examination of the testes and epididymides did not reveal any major abnormalities (no histopathology data). There was no treatment-related effect on the absolute or relative weight of the testes. The 60 mg/kg/d (2x maximum human dose based on mg/M^2) dose decreased fertility index in treated males. This resulted in an increase in the mean number of unfertilized and fragmenting eggs in females mated with the treated males.

Study title: Rat oral (gavage) female fertility study and embryonic development study.

Key study findings:

- Body weight of HD females was statistically significantly decreased on Gestation Days 17 and 20 by 4% and 10% respectively. Body weight gain was decreased by 29% in the HD females relative to control.
- Food consumption was slightly but statistically significantly increased in HD females during the 2nd – 3rd pre-mating week, and in MD females during Gestation days 17 to 20.
- There was no effect of treatment on mating performance or fertility at any dose level.
- Mean litter weight decreased in a dose-dependent manner achieving statistical significance in litters of HD females (48% decrease by relative to control). Mean fetal weight and mean fetal weight of males and females were slightly but statistically significantly decreased in fetuses of HD females relative to control. Mean placental weight was slightly but statistically significantly increased for fetuses of MD and HD females.
- Mean post-implantation loss increased in a dose-dependent manner achieving statistical significance in MD (4-fold) and HD females (15-fold). Concurrently, mean percent of implantations decreased in a dose-dependent manner achieving statistical significance in MD (9%) and HD females (42%).
- Number of early embryo fetal deaths also increased in a dose dependent manner achieving statistical significance in MD (2.4-fold) and HD females (15-fold).
- Group mean incidence of absent innominate artery appear to increase with dose at ≥ 60 mg/kg/d. The incidence in fetuses of HD females is 3-fold that of control and was greater than historical control mean. Group mean incidence of the misshapen ventricle is slightly higher than that of historical control mean but is within historical control range. Group mean incidence of increased pelvic cavitation of the kidney increased dose-dependently at ≥ 60 mg/kg/d and was 2-fold greater in fetuses of HD females relative to control. However, the group mean incidence is less than that of historical control mean. Group mean incidence of dilated ureter increased dose-dependently at ≥ 60 mg/kg/d and was 2-fold greater in fetuses of HD females relative to control. However, the group mean incidence is greater than that of historical control mean, but within the historical control range.
- For skeletal malformations, group mean incidence of wavy ribs is 7-fold greater than that of control at 180 mg/kg/d, and also greater than historical control mean and range. Group mean incidence of malformation of the sternum was slightly increased in fetuses of MD and

HD females relative to control but less than that of historical control mean. Group mean incidence of hemicentric thoracic vertebra and caudal vertebra with two or less centra and those with no neural arches were increased in fetuses of MD and HD females relative to control. Their incidence was also greater than the historical control means.

- For skeletal variations, group mean incidence of incompletely ossified occipital appear to increase dose-dependently and is 5-fold higher in fetuses of HD females relative to control and is greater than historical control mean. Group mean incidence of non-ossified centra of the thoracic vertebra is increased in fetuses of all treated females relative to control and is significantly greater than historical control mean. Group mean incidence of incomplete/non ossified sternebrae (2 – 5) and hindlimb metatarsals (≥ 1) were higher in females given 180 mg/kg/d. Values exceed the historical control mean and range. The delayed ossification (incomplete/absent) correlates with a dose associated with 29% decrement in body weight gain.
- NOAEL for maternal toxicity is 60 mg/kg/d based on the statistically significant decrease in body weight (10%) and decreased body weight gain (29%) at the HD. NOAEL for fetal/developmental toxicity is 20 mg/kg/d because of the statistically significant increase in early embryo-fetal deaths, post implantation loss in the 60mg/kg/d (MD) and 180 mg/kg/d (HD). Group mean % of implantations were also statistically significantly decreased in MD and HD females relative to control. Visceral malformation (absence of innominate artery) and skeletal malformations were also increased in fetuses of HD treated females (not statistically significant).

Study no.: WVC/013
Volume #, and page #: Vol. 45, pg. 80.
Conducting laboratory and location:

Date of study initiation: February 16, 1999.
GLP compliance: Yes (UK).
QA reports: Yes (X) no ( )
Drug, lot #, radiolabel, and % purity: OGT 918 Batch # 60689-07, 99.3% pure.
Formulation/vehicle: OGT 918 dissolved in ultra high purity water.

Methods:
Species/strain: Rat/Sprague Dawley.
Doses employed: Total doses of 20, 60 and 180 mg/kg/d were administered by orally (gavage) as three equally divided doses/day.
Route of administration: Oral (gavage).
Study design: Females were dosed for 15 days prior to mating, during mating and until Day 17 of pregnancy. Control females received vehicle (ultra high purity water). The males remained untreated throughout the study.
Number/sex/group: 25/sex/group.

Parameters and endpoints evaluated:
Clinical signs: Daily.
Mortality: Twice daily.
Body weight: Daily.
Food consumption: Weekly.
Terminal examination: Males were killed and discarded without examination after completing the mating period. All study females were killed on Day 20 after mating. For pregnant females, the number of corpora lutea, number and distribution of implantations in uterine horns, classified as early resorptions, late resorptions, dead fetuses or live fetuses. Live fetuses and their

plancentae were removed and the uterus and ovaries retained in neutral buffered formaldehyde. Plancental weights, fetal weights (live fetuses), fetal sex (live fetuses) and external fetal abnormalities were recorded.
Toxicokinetics: Not conducted.
Statistics: ANOVA and William's test (body wt., food consumption, number of corpora lutea, implantation sites, live fetuses, fetal body wt.)
Kruskal-Wallis and Shirley's test (sperm number and motility data, pre-implantation loss, post-implantation loss, % of male fetuses).
Kruskal-Wallis and Fisher Exact test (fetal abnormalities)
Fisher exact test (copulation index, fertility index).

Results:
In-life observations:
Mortality: None.
Clinical signs: No treatment-related clinical signs.
Body weight: (g)

Day: Pre-mating	0 mg/kg/d	20 mg/kg/d	60 mg/kg/d	180 mg/kg/d
1	222	226	226	225
11	233	237	238	239

Day of pregnancy	0 mg/kg/d	20 mg/kg/d	60 mg/kg/d	180 mg/kg/d
0	246	247	252	249
17	336	334	340	323*
20	385	380	385	348***
Gain (Day 20 –Day 0)	139	133	133	99
Decrement	0	6	6	40
% Decrement	0%	4%	4%	29%

* = p < 0.05; *** = p < 0.001, William's test

Food consumption: (g/rat/day)

Week: Pre-mating	0 mg/kg/d	20 mg/kg/d	60 mg/kg/d	180 mg/kg/d
1 to 2	18	18	19	18
2 to 3	18	19	19	20*

* = p < 0.05

Day of pregnancy	0 mg/kg/d	20 mg/kg/d	60 mg/kg/d	180 mg/kg/d
0 to 6	21	22	22	22
17-20	27	27	30**	26

** = p < 0.001, ANOVA

Toxicokinetics: No data.
Estrous Period: Estrous periods assessed during the 15 day pre-pairing period were similar in all groups.
Time Course of mating: There was no effect of treatment on the mean time taken to mate. Majority of females in all groups mated during the first four days of the pairing period.

For fertility studies:
In-life observations:
Female fertility and Mating data: No statistical significance.

Dose (mg/kg/d)	Sex	# paired	# mated	# fertile	Copulation index (%)	Fertility index (%)
0	F	25	25	23	100	92
20	F	25	25	22	100	88
60	F	25	25	23	100	92
180	F	25	25	25	100	100

Terminal and necroscopic evaluations:
Dams: Unremarkable
Female fertility

Dose (mg/kg/d)	0	20	60	180	HCD Mean & Range
# of early embryo fetal deaths	10	19	24*	153***	
Mean post-implantation loss %	3.4	6.1	12.0**	45.3***	4.3
Mean % of implantations	97	94	88*	55***	15.5
Mean litter weight (g)	50	47	45	26***	
Mean fetal weight (g)	3.62	3.55	3.50	3.30***	3.67
Mean fetal weight (Males) (g)	3.72	3.67	3.56	3.36***	
Mean fetal weight (Females) (g)	3.52	3.44	3.44	3.28**	
Mean placental weight (g)	0.58	0.59	0.62**	0.78***	

*** p < 0.05; ** p < 0.01; *** p < 0.001; Mean & range of historical control data (HCD) available**

Sponsor provided historical control data. The Sprague Dawley rat/(Crl: CD(SD) BR) was used. Study duration was from March 1993 to May 1997. Totals number of studies and number of animals used were not disclosed.

For embyrofetal *development* studies:
Terminal and necroscopic evaluations:

Fetal External Malformations: Unremarkable.

Fetal External Variations: Unremarkable.

Fetal Visceral Malformations: Sponsor did not conduct statistical analysis of data.

Dose (mg/kg/d)	0	20	60	180	HCD
Total # of fetuses examined	318	287	285	197	Mean & Range
Total # of litters examined	23	22	22	25	
Innominate artery: absent	6(1.8)	2(0.6)	5(1.8)	12(5.4)	0.09
Ventricle: misshapen	0(0.0)	0(0.0)	0(0.0)	1(0.3)	0.0
Lungs: reduced size	0(0.0)	0(0.0)	0(0.0)	1(0.3)	
Kidney: increased pelvic cavitation	5(1.8)	1(0.3)	6(2.1)	10(6.6)	10.63
Ureter: dilated	28(9.2)	29(9.6)	36(12.8)	34(18.6)	15.8

# of fetuses affected (group mean %); Mean & range of historical control data (HCD) available

Sponsor provided historical control data. The Sprague Dawley rat/(Crl: CD(SD) BR) was used. Study duration was from March 1993 to May 1997. Totals number of studies and number of animals used were not disclosed.

Fetal Skeletal Malformations: Sponsor did not conduct statistical analysis of data.

Dose (mg/kg/d)	0	20	60	180	HCD
Total # of fetuses examined	164	150	148	104	Mean & Range
Total # of litters examined	23	22	22	25	
Ribs: wavy	0(0.0)	1(0.6)	1(0.6)	5(6.5)	0.54
Pectoral girdle: scapula – malformed	0(0.0)	0(0.0)	0(0.0)	1(0.6)	
Sternum: one or more-bilobed, bipartite, misshapen, misaligned	2(1.2)	0(0.0)	3(1.7)	3(2.9)	3.65
Thoracic vertebra: ≥ 1 centra – hemicentric	0(0.0)	0(0.0)	1(0.6)	1(2.0)	0.11
Caudal vertebra: # of centra ≤ 2	1(0.5)	4(5.8)	2(1.1)	6(5.3)	0.88
Caudal vertebra: # of neural arches – 0	0(0.0)	1(2.3)	1(0.6)	1(4.0)	0.81

# of fetuses affected (group mean %); Mean & range of historical control data (HCD) available

Fetal Skeletal Variations: Sponsor did not conduct statistical analysis of data.

Dose (mg/kg/d)	0	20	60	180	HCD
Total # of fetuses examined	164	150	148	104	Mean & Range
Total # of litters examined	23	22	22	25	
Occipital: not ossified	0(0.0)	0(0.0)	0(0.0)	1(0.6)	0.02
Occipital: incomplete ossification	15(8.3)	16(13.3)	20(13.9)	39(44.8)	12.2(
Thoracic vertebra: ≥ 1 centra – not ossified	1(0.5)	7(7.7)	4(2.7)	17(23.1)	0.81
Thoracic vertebra: ≥ 1 centra – incomplete ossification	13(7.6)	9(7.2)	25(16.5)	29(30.1)	3.45
Sternum: 1st sternebra – incomplete ossification	0(0.0)	1(2.3)	2(1.3)	2(5.0)	0.89
Sternum: 2nd sternebra – not ossified	1(0.5)	5(4.7)	1(0.6)	13(12.8)	1.13
Sternum: 2nd sternebra – incomplete ossification	9(5.4)	14(10.4)	11(6.9)	28(27.0)	5.84(
Sternum: 3rd sternebra – incomplete ossification	2(1.1)	8(6.9)	1(0.6)	21(23.6)	0.93
Sternum: 4th sternebra – incomplete ossification	2(1.0)	11(6.9)	10(6.1)	28(31.2)	2.65
Sternum: 5th sternebra – not ossified	9(5.0)	19(15.1)	21(14.2)	47(49.9)	23.6(
Sternum: 5th sternebra – incomplete ossification	12(7.2)	10(6.4)	16(10.8)	23(21.1)	
Sternum: 6th sternebra – not ossified	1(0.5)	9(8.9)	3(1.9)	10(15.5)	6.51(
Sternum: 6th sternebra – incomplete ossification	3(1.6)	11(6.5)	11(7.9)	24(20.9)	
Pelvic girdle: Pubis – incomplete ossification	2(1.4)	9(7.2)	8(6.0)	17(20.8)	4.88
Fore limb: ≥ 1 metacarpal – incomplete ossification	0(0.0)	0(0.0)	0(0.0)	6(4.0)	
Fore limb: ≥ 1 metacarpal – not ossified	5(3.5)	4(2.4)	2(1.8)	10(13.3)	37.9
Fore limb: 5th metacarpal – not ossified	25(13.7)	44(31.5)	59(39.6)	65(60.6)	
Fore limb: ≥ 1 phalange ossified	119(74.1)	95(62.3)	77(52.6)	25(22.0)	10(
Hind limb: ≥ 1 metatarsal – not ossified	0(0.0)	6(7.0)	2(1.2)	12(12.9)	0.55
Hind limb: ≥ 1 metatarsal – incomplete ossification	0(0.0)	1(0.6)	0(0.0)	1(1.0)	

# of fetuses affected (group mean %); Mean & range of historical control data (HCD) available

Summary of Study Findings:

Groups of 25 female rats were treated by oral gavage with OGT 918 at dose levels of 0, 20, 60 and 180 mg/kg/day (WVC/013). Females were dosed for 15 days prior to mating, during mating and until Day 17 of pregnancy. The females were killed and subject to necropsy on Day 20 of gestation. At 180 mg/kg/day dose, statistically significant lower bodyweight gains were recorded from Day 17 of gestation.

There was no effect of treatment on mating performance or fertility at any dose level. Mean litter weight decreased in a dose-dependent manner achieving statistical significance in litters of HD (6x maximum human dose based on mg/m^2) females (48% ↓ relative to control). Mean fetal weight and mean fetal weight of males and females were slightly but statistically significantly decreased in fetuses of HD females relative to control. Mean placental weight was slightly but statistically significantly increased for fetuses of MD and HD females. Mean post-implantation loss was dose-dependently and statistically significantly increased in MD (2x maximum human dose based on mg/m^2) and HD females (6x maximum human dose based on mg/m^2) by 4- and 15-fold respectively. Concurrently, mean percent of implantations decreased in a dose-dependent manner achieving statistical significance in MD (9%) and HD females (42%). Number of early embryo fetal deaths also increased in a dose dependent manner achieving statistical significance in MD (2.4-fold) and HD females (15-fold).

Visceral malformations such as absent innominate artery, misshapen ventricle and lungs with reduced size and dilated ureter were observed in fetuses of HD females. Group mean incidence of these variations were greater than historical control means. For skeletal malformations, group mean incidence of wavy ribs and hemicentric thoracic vertebra were increased in fetuses of HD treated females relative to control, and were also greater than historical control means.

Fetal skeletal variations such as incompletely ossified occipital (HD), non-ossified centra of the thoracic vertebra, incompletely ossified centra of the thoracic vertebra, caudal vertebra with two or less centra and caudal vertebra with no neural arches (all dose levels) had group mean incidence that were increased as specified relative to control. The incidence of these skeletal variations were greater than those of historical control means. Group mean incidence of incompletely/not ossified 1st through 6th sternebrae was increased mostly in fetuses of HD treated females relative to control. The group mean incidence is greater than the historical control means in most cases. Group mean incidence of non-ossified 6th sternebrae is increased (not dose-dependently) in fetuses of all treated females relative to control. Only the incidences of LD and HD fetuses are greater than historical control mean. Other fetal skeletal variations such as incompletely ossified pubis and non ossified metatarsals had group mean incidence that was increased in fetuses of HD treated females relative to control, and are greater than historical control mean.

NOAEL for maternal toxicity is 60 mg/kg/d (2x maximum human dose based on mg/m^2) based on the statistically significant decrease in body weight (10%) and decreased body weight gain (29%) at the HD. NOAEL for fetal/developmental toxicity is 20 mg/kg/d (0.6x maximum human dose based on mg/m^2) because of the statistically significant increase in early embryo-fetal deaths, post implantation loss in the 60mg/kg/d (MD) and 180 mg/kg/d (HD). Group mean % of implantations were also statistically significantly decreased in MD and HD females relative to control. Visceral malformation (absence of innominate artery) and skeletal malformations were also increased in fetuses of HD treated females (not statistically significant).

Study title: Oral (gavage) Rabbit developmental toxicity study.

Key study findings:

- There were 6 premature deaths. Sponsor stated that the demise of 2/20 (MD) and 1/24 (HD) females may be treatment-related. Sponsor attributed the rest of the deaths [1/29 (control), 1/20 (LD) and 1/20 (MD)] to dosing accident (sponsor did not provide detailed gross/histopathology findings to justify these deaths).
- Body weight gain was statistically significantly decreased in LD (50%) and HD (44%↓) females but increased in MD females relative to control.
- Food consumption decreased in a dose-dependent manner achieving statistical significance in MD (31%↓) and HD (38%↓) females.
- Mean pre-implantation loss and early embryo/fetal deaths increased in a dose-dependent manner (not statistically significant from control).
- Fetal external variations revealed statistically significant increase in runted fetuses in MD and HD groups relative to control.
- Fetal visceral malformations showed a dose-dependent and statistically significant increase in group mean incidence of aortic arches with additional blood vessel.
- For fetal skeletal malformations, group mean incidence of fused zygomatic arch and maxilla was statistically significantly increased in MD fetuses relative to control.
- For fetal skeletal variations, group mean incidences of incompletely ossified maxilla and hyoid were statistically significantly increased in LD and HD fetuses relative to control. Group mean incidence of non-ossified 5th sternebrae increased dose-dependently achieving statistical significance in MD and HD fetuses relative to control. Group mean incidence of non-ossified epiphyses (forelimb) is statistically significantly increased in LD and HD fetuses relative to control. Group mean incidence of non-ossified metacarpal(s) is statistically significantly increased in MD and HD fetuses relative to control. Group mean incidences of non-ossified phalange(s) and incompletely ossified phalange(s) of the forelimb are statistically significantly increased in HD fetuses relative to control. Group mean incidences

of non-ossified astragalus and incompletely ossified phalange(s) of the hindlimb are statistically significantly increased in HD fetuses relative to control.

- NOAEL for maternal toxicity could not be established because of 50% reduction in body weight gain at LD. NOAEL for fetal/developmental toxicity could not be established because aortic arch anomaly (additional blood vessel) at all dose levels and some visceral skeletal variations observed in LD and HD fetuses.

Study no.: WVC/017
Volume #, and page #: Vol. 46, pg. 210.
Conducting laboratory and location:

Date of study initiation: May 19, 1999.
GLP compliance: Yes (UK).
QA reports: Yes (X) no ( )
Drug, lot #, radiolabel, and % purity: OGT 918 Batch # 60689-07, 99.3% pure.
Formulation/vehicle: OGT 918 dissolved in ultra high purity water.

Methods:
Species/strain:Rabbit/New Zealand white.
Doses employed: Total doses of 15, 30 and 45 mg/kg/day were administered as three equally divided doses.
Route of administration: Oral (gavage).
Study design: Females were dosed three times daily from Days 6 to 18 of pregnancy, inclusive. The total dosage was administered as three equally divided doses with an interval between administration of each dose of at least 6 hours. A constant dose volume of 2ml/kg bodyweight was used and individual doses were adjusted daily according to bodyweight.

Group number	Colour	Number of mated females	Animal identification numbers	Dose level (mg/kg/day) OGT 918
1	White	20	1 - 20	Control (vehicle)
2	Green	20	21 - 40	15
3	Yellow	20	41 - 60	30
4	Pink	24	61 - 84	45

Number/sex/group: Please see study design.

Parameters and endpoints evaluated:
Clinical signs: Daily.
Mortality: Daily.
Body weight: Daily.
Food consumption: Daily.
Terminal examination: All animals were sacrificed by an IV injection of sodium pentobarbitone solution and necropsied. Organs or tissues showing macroscopic abnormalities were removed, fixed and stored in neutral buffered formaldehyde.

For pregnant females, on Day 28 of pregnancy, the number of corpora lutea, fetal weights (live fetuses), placental weights, external abnormalities of live fetuses and number and distribution of implantation sites in the uterine horns were recorded. The implantations were classified as early deaths, late deaths, dead fetuses/live fetuses.