CENTER FOR DRUG EVALUATION AND RESEARCH

APPLICATION NUMBER:

204063Orig1s000

PHARMACOLOGY REVIEW(S)

Tertiary Pharmacology Review

By: Paul C. Brown, Ph.D., ODE Associate Director for Pharmacology and Toxicology, OND IO
NDA: 204063
Submission date: 2/27/2012
Drug: dimethyl fumarate
Applicant: Biogen Idec Inc.
Indication: Treatment of patients with relapsing forms of multiple sclerosis
Reviewing Division: Division of Neurology Products

Discussion:
The pharm/tox reviewer did not recommend approval of this NDA based on the nonclinical data. Renal toxicity in nonclinical species was the specific reason for this conclusion. The pharm/tox supervisor agreed that the nonclinical studies demonstrate the potential for renal toxicity. It is the pharm/tox supervisor's understanding that dimethyl fumarate has demonstrated efficacy for treatment of MS and that there is sufficient evidence of safety to support approval of DMF based on the human safety data for dimethyl fumarate and the postmarketing experience with a related drug (Fumaderm). The supervisor also noted that a postmarketing study will be conducted in patients with close monitoring for renal toxicity. The supervisor recommends approval with description of the nonclinical renal toxicity findings in labeling.

Carcinogenicity studies of dimethyl fumarate were conducted in mice and rats. These studies were reviewed by the Executive Carcinogenicity Assessment Committee. The studies were found to be acceptable. Neoplasms of the nonglandular stomach and kidney were initially identified in mice and rats. Interstitial cell adenoma of the testes was determined to be drug-related in rats. The sponsor subsequently submitted a re-examination of the microscopic slides of the rat kidneys. In the re-examination, there was no drug-related increase in renal neoplasms in rats.

Conclusions:
The pharmacology/toxicology reviewer and supervisor conducted a thorough evaluation of the nonclinical information submitted in support of this NDA. I agree that the results observed in the animal studies suggest a potential for renal toxicity. Additional studies in animals are probably not necessary at this point because the concern has already been identified. I agree that there are no clear drug-related renal neoplasms in rat. I agree that this NDA may be approved for the above indication based in part on an understanding that current and additional clinical data will be adequate to address the potential for renal toxicity in patients. I agree with the labeling changes suggested by the pharmacology/toxicology supervisor. No established pharmacologic class has

been proposed for labeling. This appears appropriate because the mechanism by which dimethyl fumarate is effective in MS is unknown.

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/s/

PAUL C BROWN
03/25/2013

MEMORANDUM

DEPARTMENT OF HEALTH & HUMAN SERVICES
Public Health Service
Food and Drug Administration

Division of Neurology Products (HFD-120)
Center for Drug Evaluation and Research

Date: February 8, 2013

From: Lois M. Freed, Ph.D.
Supervisory Pharmacologist

Subject: NDA 204-063 (BG-00012, dimethyl fumarate, TECFIDERA)

NDA 204-063 was submitted by the sponsor (Biogen Idec) on February 24, 2012 (received February 27, 2012) for dimethyl fumarate (DMF) for the treatment of patients with relapsing forms of multiple sclerosis (MS). A major amendment submitted on October 2, 2012 (received October 5, 2012) extended the original PUDFA goal date by three months, to March 27, 2013. Clinical development of DMF for MS was conducted under IND 73061.

The sponsor conducted a full battery of nonclinical studies in support of NDA 204-063, including chronic toxicity studies (rat, dog, monkey), reproductive and developmental toxicity studies (rat, rabbit), carcinogenicity studies (mouse, rat), and investigative studies in rat to further assess the nephrotoxicity observed in multiple species. These studies were reviewed by Dr. Banks-Muckenfuss (*cf. Pharmacology/Toxicology NDA Review and Evaluation, NDA 204063, Melissa K. Banks-Muckenfuss, Ph.D., 1/28/2013*). Based on that review, Dr. Banks-Muckenfuss recommends that the NDA not be approved "…based on renal toxicity at clinically relevant doses (toxicity in all nonclinical species, including tumors in rodents)." Dr. Banks-Muckenfuss acknowledges that nephrotoxicity was not observed in humans during clinical development, citing the review of clinical safety data (*Clinical Review NDA 204063, Gerard Boehm, MD, MPH, 1/4/13*), but states that "…it is not clear from the nonclinical data that the monitoring conducted would be able to detect the toxicity in humans (e.g. due to the marker used, sensitivity and/or the duration of the exposures)."

This memo will briefly summarize the nonclinical findings (full details are provided in the review by Dr. Banks-Muckenfuss) but will focus on selected toxicities, particularly the signal for nephrotoxicity in the nonclinical studies conducted by the sponsor.

Pharmacology

Following oral administration of DMF in animals and humans, plasma levels of DMF are low to undetectable, consistent with in vitro studies demonstrating rapid hydrolysis of DMF in intestinal cells. A hydrolysis product, mono-methyl fumarate (MMF), is detectable in plasma of animals and humans and was the drug-related compound quantitated to assess systemic drug exposure. The sponsor conducted a series of in vitro and in vivo studies to characterize the pharmacology of DMF and MMF; however, it is the pharmacology of MMF that is most relevant to this application.

The sponsor hypothesizes that the ability to activate the nuclear factor (erythroid-derived 2)-like 2 (Nrf2) antioxidant response pathway is the mechanism by which orally administered DMF exerts therapeutic (e.g., neuroprotective, anti-inflammatory) effects. MMF was shown to induce Nrf2 activation in vitro in cells transfected with rat Keap-1. Keap-1 is a protein which binds to Nrf2 and facilitates its proteolysis. By disrupting this binding, MMF inhibits proteolysis of Nrf2, resulting in translocation of Nrf2 to the nucleus and subsequent induction of antioxidant response genes. Treatment of cultured CNS cells (astrocytes, neurons, oligodendrocytes) with MMF was demonstrated to increase cell survival by increasing cellular Nrf2 levels and upregulating antioxidant stress genes. In in vivo studies, oral administration of DMF induced transcriptional markers of Nrf2 activation and demonstrated beneficial effects in a malonate-induced striatal lesion model in rat and in a rat EAE model. Whether or not activation of the Nrf2 pathway underlies the therapeutic effect of DMF in patients with multiple sclerosis (MS) is unclear. Although the pharmacodynamic effects of MMF in vitro and DMF in vivo were shown to be, to some extent, dependent on Nrf2, the sponsor notes that "Nrf2 contributes to, but is not required for" some of these effects and that other mechanisms may be involved. In addition, in vivo studies of oral DMF in rodent demonstrated increases in transcriptional markers of pharmacodynamic effect (through Nrf2) in various tissues (duodenum, jejunum, spleen, liver), which was tissue and dose dependent, but to a substantially less extent in CNS tissue (spinal cord, cerebellum, forebrain).

Therefore, the mechanism(s) by which oral DMF exerts its therapeutic effect in MS patients has not been established. Activation of the Nrf2 antioxidant response pathway may be involved; however, the pharmacological activity of DMF or MMF was not fully characterized. (Neither compound was tested in a battery of in vitro receptor binding assays.) Published studies report that MMF (but not DMF) is a potent nicotinic acid receptor agonist (Hanson J *et al*. *J Clin Invest* 120(8):2910-2919, 2010; Hanson J *et al*. *Pharmacol Therap* 136:1-7, 2012), which is consistent with the flushing commonly reported in humans during clinical development. DMF and/or MMF may have additional pharmacological activity that has not yet been identified.

Dr. Banks-Muckenfuss discusses the possibility that some of the toxicities observed in the nonclinical studies (e.g., forestomach lesions such as hyperkeratosis, renal toxicity including tumors) may be mediated through DMF/MMF-induced activation of the Nrf2 pathway. Although this possibility is supported by published literature (e.g., Slocum SL,

Kensler TW *Arch Toxicol* 85:273-284, 2011), the available data are not sufficient to identify a mode of action for the toxicities observed.

PK/ADME

As noted, DMF, when administered orally, is rapidly hydrolyzed prior to systemic absorption and exhibits little or no systemic exposure in animals or humans. MMF, an active metabolite detected in plasma of animals and humans, was quantitated to assess systemic drug exposure.

Tissue distribution of radioactivity following single doses of radiolabeled DMF to Long Evans rats indicated rapid absorption and extensive distribution into all tissues examined, except for "fat in the reproductive area." At 0.5 hours postdose, highest levels of radioactivity were detected in kidney, followed by stomach, liver, pancreas, and brain. At 72 hours postdose, radioactivity was still detectable in all tissues. However, since MMF is extensively metabolized in vivo to fumaric acid, citric acid, and glucose and considering that the major route of elimination was expired air (as $^{14}CO_2$), the extent to which MMF itself contributed to tissue radioactivity could not be addressed. Although drug-related radioactivity distributed extensively to kidney, urine was not a major route of elimination, accounting for only approximately 20% of dose in Long Evans rat.

Toxicology

The pivotal oral toxicity studies for DMF were conducted in CD-1 mouse (13-week + 4-week recovery), Sprague-Dawley rat (3- and 6-month, both with 4-week recovery), cynomolgus monkey (1-year + 4-week recovery), and Beagle dog (4-week + 14-day recovery, 11-month + 1 month recovery).

Mouse: In CD-1 mouse, forestomach and kidney were the primary target organs for toxicity. In the 13-week study (0, 50, 200, 400 mg/kg/day, by gavage), forestomach lesions (including hyperkeratosis, squamous cell hyperplasia, subacute and chronic inflammation, microabscess, ulceration) were observed at all doses in both males and females, although findings were minimal at the low dose. Splenic changes (extramedullary hematopoiesis) were minimal but noted at all doses in males and females. Kidney weight was increased at all doses in males (11, 27, and 37%, respectively) and at all but the low dose in females (15% at the MD and HD), but there were no histopathological correlates. Only forestomach remained affected in recovery animals (MD and HD).

In a 28-day dose-ranging study in B57BL/6 mouse (doses of 50, 100, 250, and 400 mg/kg/day, by gavage), changes in forestomach, spleen (increased extramedullary hematopoiesis), testes (tubular degeneration/hypocellularity, tubular giant cells), epididymides (sperm granuloma) were the primary findings. Effects on male reproductive organs were not observed in the 13-week study in CD-1 mouse.

In the carcinogenicity study (0, 25, 75, 200, 600/400 mg/kg/day, by gavage; HD lowered on Day 9), kidney and forestomach lesions were observed at all doses (discussed under

Carcinogenicity); an increased incidence and/or severity of retinal degeneration was detected in males and females at the two highest doses.

Rat: In rat, the primary target organs for toxicity were the forestomach, kidney, and male reproductive organs. Microscopic changes in pancreas (including acinar epithelial cell apoptosis and vacuolization) were only observed (but at all doses) in one 3-month study (P00012-04-01). Liver necrosis and/or bile duct hyperplasia were detected in the 6-month study, primarily in females. Kidney and forestomach findings were observed in the majority of studies in rat, including the 3- and 6-month studies. Forestomach lesions (similar to those in mouse) were observed at all doses in both 3-month studies and the 6-month study. (The glandular stomach was also affected [e.g., minimal to mild inflammation in the 6-month study] but to a lesser extent.) Forestomach tumors (squamous cell carcinoma, papilloma) were observed in one 3-month study (P00012-04-01; 1 M and 1 F at 250 mg/kg/day) and the 6-month study (1 MDM, 1 HDM).

Selected renal findings from the two 3-month studies are summarized below.

STUDY	RENAL FINDINGS	MALES				FEMALES			
		0	**50**	**100**	**250**	**0**	**50**	**100**	**250**
3-month P00012-04-01	BUN	--	11%↓	4%↓	6%↓	--	12%↓	8%↓	24%↓
	serum creatinine	--	14%↓	22%↓	19%↓	--	19%↓	--	29%↓
	kidney wt (A-R)	--	0-11%↑	19-21%↑	42-59%↑	--	12-17%↑	22-20%↑	24-38%↑
	proteinosis								
	minimal	0/10	2/9	1/9	2/10	0/10	1/10	1/10	1/10
	mild	0/10	0/9	0/9	2/10	0/10	0/10	0/10	0/10
	tubular basophilia								
	minimal	0/10	1/9	0/9	6/10	0/10	0/10	0/10	0/10
	mild	0/10	0/9	1/9	3/10	0/10	0/10	0/10	0/10
	tubular dilatation								
	minimal	0/10	0/9	0/9	2/10	0/10	0/10	0/10	0/10
	mild	0/10	0/9	0/9	1/10	0/10	0/10	0/10	0/10
	RECOVERY								
	proteinosis								
	minimal	0/5	--	--	0/5	0/5	--	--	1/5
	tubular basophilia								
	minimal	0/5	--	--	3/5[#]	0/5	--	--	0/5
3-month 19416-05	BUN	--	--		--	--	--		--
	serum creatinine	--	5%↓		14-16%↓	--	7%↓		10-12%↓
	kidney wt (A-R)	--	16-10%↑		40-52%↑	--	0-11%↑		20-18%↑
	histopathology	no renal findings							

[*]dosing stopped in HD animals after seven days of dosing and 5/sex were sacrificed (clinical chemistry prior to necropsy); 5/sex were maintained for 4 weeks of recovery (terminal data for these not included due to lack of relevant controls). [#]discrepancy in study report: narrative indicates 3/5, while tabulated data indicate 1/5.

Selected renal findings in the 6-month study are summarized below.

RENAL FINDINGS	MALES				FEMALES			
	0	25	100	200	0	25	100	200
BUN	no findings				--	--	12%↓	12%↓
serum creatinine	--	--	13%↓	11%↓	--	5%↓	9%↓	18%↓
kidney wt (A-R)	--	12-10%↑	50-53%↑	62-76%↑	--	0-2%↑	19-16%↑	31-34%↑
nephropathy								
minimal	7/15	9/15	5/14	5/15	7/15	6/15	7/15	8/15
mild	1/15	1/15	8/14	5/15	0/15	0/15	1/15	1/15
moderate	0/15	0/15	1/14	5/15	0/15	0/15	0/15	0/15
tubular dilatation								
minimal	4/15	7/15	7/14	6/15	4/15	3/15	4/15	3/15
mild	0/15	1/15	5/14	6/15	0/15	0/15	0/15	0/15
hyaline droplet, tubule								
minimal	0/15	4/15	6/14	7/15	0/15	0/15	3/15	3/15
mild	0/15	0/15	1/14	3/15	0/15	0/15	0/15	1/15
hypertrophy, Bowman's capsule								
minimal	1/15	7/15	6/14	3/15	0/15	0/15	1/15	0/15
mild	0/15	0/15	0/14	7/15	0/15	0/15	0/15	0/15
hypertrophy, tubule								
minimal	0/15	2/15	11/14	13/15	0/15	1/15	12/15	15/15
regeneration, segmental, tubule								
minimal	1/15	11/15	6/14	9/15	0/15	0/15	2/15	1/15
mild	0/15	1/15	7/14	6/15	0/15	0/15	0/15	0/15
RECOVERY								
BUN	no findings				--	--	--	41%↑
serum creatinine	--	--	--	13%↓	--	--	--	11%↓
kidney wt (A-R)	--	--	29-26%↑	34-39%↑	--	--	8-12%↑	16-42%↑
nephropathy								
minimal	5/5	5/5	2/5	0/5	1/5	1/5	2/5	2/4
mild	0/5	0/5	3/5	4/5	0/5	0/5	0/5	1/4
moderate	0/5	0/5	0/5	1/5	0/5	0/5	0/5	0/4
dilatation, tubular								
minimal	1/5	2/5	5/5	2/5	0/5	0/5	0/5	2/4
mild	0/5	0/5	0/5	3/5	0/5	0/5	0/5	0/4
hyaline droplet, tubule								
minimal	1/5	0/5	2/5	4/5	0/5	0/5	0/5	0/4
mild	0/5	0/5	0/5	0/5	0/5	0/5	0/5	1/4
hypertrophy, Bowman's capsule								
minimal	1/5	3/5	5/5	3/5	0/5	0/5	0/5	0/4
mild	0/5	0/5	0/5	1/5	0/5	0/5	0/5	0/4
hypertrophy, tubule								
minimal	2/5	1/5	5/5	5/5	0/5	2/5	5/5	4/4
regeneration, segmental, tubule								
minimal	1/5	3/5	3/5	2/5	0/5	0/5	0/5	1/4
mild	0/5	0/5	1/5	3/5	0/5	0/5	0/5	0/4

Kidney toxicity (table below) was also demonstrated in the male fertility study in rat; males were dosed for 70 days prior to mating and throughout mating until sacrifice.

STUDY	RENAL FINDINGS	DOSES (mg/kg)			
		0	75	250	375
male fertility	dilatation, cortical tubules				
	minimal	4/25	18/25	14/25	11/25
	mild	0/25	0/25	11/25	11/25
	moderate	0/25	0/25	0/25	2/25
	hyaline droplets, cortical tubules				
	minimal	7/25	15/25	17/25	10/25
	mild	0/25	2/25	3/25	2/25
	nephropathy				
	minimal	18/25	21/25	17/25	16/25
	mild	0/25	1/25	5/25	4/25
	moderate	0/25	0/25	0/25	2/25
	nuclear/cellular hypertrophy, cortical tubular				
	minimal	0/25	1/25	20/25	8/25
	mild	0/25	1/25	5/25	16/25
	regeneration, tubular segmental				
	minimal	1/25	5/25	14/25	17/25
	mild	0/25	3/25	7/25	3/25
	moderate	0/25	0/25	0/25	2/25

At the higher doses tested in this study, glandular stomach (as well as forestomach) was also affected; mucosal erosion, epithelial and glandular hyperplasia, and mixed cellular inflammation were observed only in MD and HD males, with the majority of HDM being affected.

In the carcinogenicity study (0, 25, 50, 100 and 150 mg/kg/day, by oral gavage), kidney and forestomach changes were observed at all doses in males and females; testicular/epididymal changes were evident primarily at doses >50 mg/kg/day (discussed under Carcinogenicity). Testicular effects were also observed in the male fertility study, i.e., a dose-related increase in incidence and severity of interstitial cell hyperplasia. Additional findings in the carcinogenicity study were dose-related increased severity (but not incidence) of cardiomyopathy, increased incidence and severity of atrial thrombosis, and increased incidence and severity of chronic active inflammation of the arteries in a number of organs (including kidney, testes, and epididymides).

Dog: In dog, DMF was administered at doses of 0, 50, 100, and 250 mg/kg/day (oral gavage) for 4 weeks and at doses of 0, 5, 25, and 75/50 mg/kg/day (oral capsule) for 11 months. In the 4-week study (interim sacrifice on Day 13-15 for HD animals [4/sex] only), microscopic changes in thymus (atrophy), bone marrow (hypocellularity), stomach/esophagus (hemorrhage, erosion), and kidney (vacuolation of tubular epithelium) were observed at the interim sacrifice; only the thymus effects appeared dose-related in main-study animals sacrificed at the end of the 4-week dosing period.

In the 11-month study, the primary target organs for toxicity were the kidney, testis, epididymis, and adrenal gland (hypertrophy of the zona fasciculata). The testicular effects consisted of epithelial degeneration (1/4, 0/4, 1/4, and 3/4 at 0, 5, 25, and 75/50 mg/kg/day, respectively), with increased severity at the MD and increased incidence and severity at the HD, and spermatid giant cells (only detected at the HD [2/4, both minimal]). Hypospermia (epididymis) occurred only at the HD (3/4, all of moderate severity). There was no evidence of GI irritation.

Selected kidney findings are provided in the following table (the HD was lowered on Day 7).

RENAL FINDINGS	MALES				FEMALES			
	0	**5**	**25**	**75/50**	**0**	**5**	**25**	**75/50**
UN	--	0-11%↓	12-30%↓	29-55%↓	--	7-18%↓	13-25%↓	31-59%↓
serum creatinine	--	0-12%↓	14-20%↓	18-30%↓	--	5-9%↓	10-23%↓	27-36%↓
kidney wt (A-R)		11-16%↑	41-38%↑	46-58%↑	--	2-10%↑	13-23%↑	54-55%↑
tubular hypertrophy								
minimal	0/4	1/4	2/4	2/4	0/4	0/4	0/4	0/4
mild	0/4	1/4	1/4	2/4	0/4	0/4	0/4	0/4
total	**0/4**	**2/4**	**3/4**	**4/4**	**0/4**	**0/4**	**0/4**	**0/4**
cortical tubule dilation								
minimal	0/4	1/4	0/4	3/4	0/4	1/4	0/4	3/4
mild	0/4	0/4	1/4	1/4	0/4	0/4	0/4	0/4
total	**0/4**	**1/4**	**1/4**	**4/4**	**0/4**	**1/4**	**0/4**	**3/4**
tubular regeneration								
minimal	0/4	0/4	0/4	1/4	0/4	0/4	0/4	0/4
mild	0/4	0/4	1/4	1/4	0/4	0/4	0/4	0/4
total	0/4	0/4	1/4	2/4	**0/4**	**0/4**	**0/4**	**0/4**
cortical parenchyma atrophy								
minimal	0/4	1/4	0/4	1/4	1/4	0/4	1/4	4/4
mild	0/4	0/4	1/4	2/4	0/4	0/4	0/4	0/4
total	**0/4**	**1/4**	**1/4**	**3/4**	**1/4**	**0/4**	**1/4**	**4/4**
infiltration, mixed cell, papilla								
mild	0/4	0/4	0/4	0/4	0/4	0/4	0/4	1/4
moderate	0/4	0/4	0/4	1/4	0/4	0/4	0/4	0/4
total	**0/4**	**0/4**	**0/4**	**1/4**	**0/4**	**0/4**	**0/4**	**1/4**
hyperplasia, papillary urothelium								
minimal	0/4	1/4	2/4	2/4	0/4	0/4	1/4	1/4
mild	0/4	0/4	0/4	2/4	0/4	0/4	2/4	3/4
total	**0/4**	**1/4**	**2/4**	**4/4**	**0/4**	**0/4**	**3/4**	**4/4**
RECOVERY								
UN	--	18%↑	--	24%↑	--	7%↑	7%↑	27%↑
creatinine	--	6%↓	--	10%↑	--	4%↑	4%↓	10%↑
kidney wt (A-R)	--	7-6%↑	8-13%↓	3-6%↓	--	26-30%↑	15-20%↑	31-34%↑
tubular hypertrophy								
minimal	0/4	2/4	0/4	0/4	0/4	0/4	0/4	0/4
mild	0/4	0/4	0/4	1/4	0/4	0/4	0/4	0/4
total	**0/4**	**2/4**	**0/4**	**1/4**	**0/4**	**0/4**	**0/4**	**0/4**
cortical tubule dilation								
minimal	0/4	0/4	1/4	1/4	0/4	0/4	0/4	0/4
cortical parenchyma atrophy								
minimal	1/4	1/4	0/4	1/4	0/4	0/4	0/4	0/4
mild	0/4	0/4	2/4	0/4	0/4	0/4	0/4	0/4
total	**1/4**	**1/4**	**2/4**	**1/4**	**0/4**	**0/4**	**0/4**	**0/4**
infiltration, mixed cell, papilla								
minimal	0/4	0/4	0/4	0/4	0/4	0/4	0/4	1/4
tubular hypertrophy								
minimal	0/4	0/4	1/4	1/4	0/4	0/4	1/4	0/4
mild	0/4	0/4	1/4	0/4	0/4	0/4	0/4	1/4
total	**0/4**	**0/4**	**2/4**	**1/4**	**0/4**	**0/4**	**1/4**	**1/4**

The study pathologist's [redacted] (b) (4) characterization of selected kidney findings:

- Hypertrophy of tubular epithelium: "…characterized by enlarged, cuboidal epithelial cells with abundant eosinophilic cytoplasm lining cortical tubules. Some of the cells also had enlarged nuclei. The altered cells appeared to involve primarily convoluted tubules in the deep cortex."
- Regeneration of tubular epithelium in cortex: "…characterized by short segments of columnar to cuboidal tubular epithelial cells with increased basophilia and nuclear crowding sometimes overlying a thickened basement membrane. Regeneration, epithelial hypertrophy and tubular dilation were often seen concurrently."
- Atrophy of cortical parenchyma: "…characterized by atrophic tubules with or without sclerotic/degenerate glomeruli usually with interstitial fibrosis and mononuclear infiltrates. This change sometimes had a linear distribution radiating outward from the inner cortex towards the capsular surface."
- Infiltration of mixed inflammatory cells in renal papillae: "…characterized by numerous mononuclear cells, especially macrophages, and fewer neutrophils within the intertubular interstitium at the tip of the distal papillae. Although this finding was observed in only one male and one female from the high-dose group…, it was considered to be treatment-related because it has not been seen as an incidental finding."
- Hyperplasia of papillary urothelial cells lining the renal pelvis: "…characterized by an increase in the number of cell layers and cell size. Distribution of this change was either locally extensive or multifocal involving one or both kidneys."

The TK (plasma MMF) data from the 11-month study (Day 330) are summarized in the table below.

PARAMETER	MALES			FEMALES		
	5	25	50	5	25	50
C_{max} (ng/mL)	2072 ± 837	10962 ± 4526	9662 ± 4080	1687 ± 385	9105 ± 1566	14548 ± 4476
AUC (ng*hr/mL)	5684 ± 1768	23998 ± 8800	52045 ± 30758	6969 ± 4084	27336 ± 7499	44785 ± 12233

A no-effect level for renal effects was not identified; however, findings at the LD were, for the most part, minimal. Plasma exposure at the LD was similar to (C_{max}) or approximately one-half (AUC) that in humans at the recommended human dose (480 mg/day; C_{max}: 2.24-2.4 µg/mL; AUC: 10-11.3 µg*hr/mL).

Monkey: the only pivotal toxicity study conducted in monkey was a one-year oral (gavage) study testing DMF at doses of 0, 5, 25, and 75 mg/kg/day. Kidney was the only identified target organ.

At necropsy, macroscopic findings in kidney were described by the study pathologist (b) (4)

"…mild bilateral pale discoloration in 4 of 4 males and 2 of 4 females, bilateral increased size in 4 of 4 females, and watery consistency in 2 of 4 females given 75 mg/kg Dimethyl Fumarate. The gross changes correlated microscopically with renal tubular epithelial regeneration."

(b) (4) also noted pale discoloration of the kidney, correlated with renal tubular epithelial regeneration, in one HD male and one HD female at the end of the recovery period. Increases in kidney weight in main-study animals were noted to be associated with "treatment-related moderate regeneration of tubular epithelial cells"; in recovery animals, increases in kidney weight were noted to be associated with "treatment-related tubular epithelial regeneration and interstitial fibrosis in both animals."

Selected clinical pathology and terminal findings are provided in the following table:

FINDING	MALES				FEMALES			
	0	**5**	**25**	**75**	**0**	**5**	**25**	**75**
BUN	--	--	23-38%↓	16-35%↓	--	--	15-40%↓	26-50%↓
serum creatinine	--	--	12-21%↓	0-10%↓	--	--	8-12%↓	4-27%↓
kidney wt	--	5%↑	7%↑	7%↑	--	13%↑	25%↑	92%↑
tubular necrosis, single cell								
minimal	0/4	0/3	1/4	3/4	0/4	0/4	2/5	0/4
mild	0/4	0/3	0/4	0/4	0/4	0/4	0/5	4/4
total	**0/4**	**0/3**	**1/4**	**3/4**	**0/4**	**0/4**	**2/5**	**4/4**
tubular regeneration								
minimal	0/4	0/4	0/4	1/4	0/4	0/4	2/5	1/4
mild	0/4	0/4	0/4	1/4	0/4	0/4	0/5	0/4
moderate	0/4	0/4	0/4	1/4	0/4	0/4	0/5	3/4
total	**0/4**	**0/4**	**0/4**	**3/4**	**0/4**	**0/4**	**2/5**	**4/4**
RECOVERY (W57)								
BUN	--	7%↑	11%↑	39%↑	--	28%↑	7%↓	5%↑
serum creatinine	--	13%↑	13%↓	27%↑	--	5%↓	16%↓	16%↓
kidney wt	--	28%↑	15%↑	100%↑	--	--	--	18↑
tubular necrosis, single cell								
minimal	0/2	0/2	0/2	1/2	0/2	0/2	1/1	0/2
tubular regeneration								
mild	0/2	0/2	0/2	0/2	0/2	0/2	1/2	1/2
moderate	0/2	0/2	0/2	2/2	0/2	0/2	0/2	1/2
total	**0/2**	**0/2**	**0/2**	**2/2**	**0/2**	**0/2**	**1/2**	**2/2**
fibrosis, interstitium								
mild	0/2	0/2	0/2	1/2	0/2	0/2	0/2	0/2
moderate	0/2	0/2	0/2	1/2	0/2	0/2	0/2	0/2
total	**0/2**	**0/2**	**0/2**	**2/2**	**0/2**	**0/2**	**0/2**	**0/2**
tubules, cortex, atrophy								
mild	0/2	0/2	0/2	1/2	0/2	0/2	0/2	0/2
moderate	0/2	0/2	0/2	1/2	0/2	0/2	0/2	0/2
total	**0/2**	**0/2**	**0/2**	**2/2**	**0/2**	**0/2**	**0/2**	**0/2**

(b) (4) characterization of the kidney findings in main-study and recovery animals:

In main-study animals: "…Single cell necrosis consisted of individual tubular

epithelial cells in the cortex that were eosinophilic, shrunken, and had pyknotic or karyorrhetic nuclei. Some affected cells remained adjacent to the tubular basement membrane, while others had become detached and were in the tubular lumen. The necrotic cells were in some cases widely scattered in the renal cortex, although there were occasionally more than one affected cell in a single tubular profile…Tubular regeneration affected entire tubular profiles in some cases, but only segments in others, and consisted of scattered individual or clumps of cortical tubules in which tubular epithelial cells had one or more of the following changes: increased or decreased size, cytoplasmic basophilia, cytoplasmic vacuolation, increased or decreased size of the nucleus, irregular shape of nucleus, mitotic figures. The nuclei within the affected tubules were often irregularly distributed, being sometimes clumped together and sometimes sparse. The lumen of some affected tubules appeared large while others were small due to decreased or increased size of affected epithelial cells. Small numbers of mononuclear inflammatory cells were often in the interstitium adjacent to affected tubules and probably represented an inflammatory response to the damaged tubular epithelium. Both single cell necrosis of tubular epithelium and regeneration of tubular epithelium were sometimes concentrated in the medullary rays."

In recovery animals: "Treatment-associated histologic findings…in the kidney…consisted of single cell necrosis and regeneration of cortical tubular epithelial cells and fibrosis in the interstitium associated with mild to moderate tubular atrophy. Single cell necrosis of cortical tubular epithelial cells was similar to that observed at the Terminal Necropsy…Diffuse moderate interstitial fibrosis affected 1 of 2 males given 75 mg/kg/Dimethyl Fumarate and mild multifocal interstitial fibrosis affected the other male in this dose group. The change in these 2 males consisted of increased fibrous connective tissue around and between tubules and was quantitatively significantly more severe than the small amounts of focal interstitial fibrosis associated with small focal cortical scars. Mild or moderate atrophy of tubules was associated with the interstitial fibrosis in these 2 males. Additional, minimal to mild fibrosis of Bowman's capsule and mild multifocal mononuclear infiltrates were secondary changes associated with the interstitial fibrosis…The…findings indicate that single cell necrosis and regeneration of renal cortical tubular epithelium did not resolve after a 4 week drug-free interval. However, there was a decrease in incidence and severity of single cell necrosis for both affected dose groups and a decrease in severity of tubular epithelial regeneration for the [HD] females consistent with a trend towards recovery for these changes in some dose groups. Additional, the 2 males of the 4 animals given [HD] Dimethyl Fumarate had significant multifocal or diffuse interstitial fibrosis, a morphologic indication of irreversible loss of tissue and function. Renal fibrosis in the most severely affected animal (4006) was associated with increased BUN and creatinine at weeks 38, 52, and 56."

Selected individual data for the two HD recovery males, both exhibiting renal fibrosis, are provided in the following table. As (b) (4) notes, BUN and creatinine were

increased in HDM-R 4006; however, HDM-R 4005 was also reported to have had loss of renal tissue and function but had no changes in these parameters.

PARAMETER	SAMPLING TIME	CONTROL	HDM-R 4005	HDM-R 4006
BUN	-7	20.2±4.0	20	26
	Day 1	23.0±3.3	20	26
	Week 2	19.7±2.7	14	19
	Week 6	18.8±2.6	18	17
	Week 12	23.8±2.8	16	17
	Week 24	23.2±4.2	12	27
	Week 38	23.7±3.7	16	46
	Week 52	23.7±2.3	13	37
	Week 56	22.0±4.2	18	43
creatinine	-7	0.72±0.72	0.9	0.9
	Day 1	0.82±0.10	0.8	1.0
	Week 2	0.75±0.08	0.9	0.9
	Week 6	0.68±0.12	0.7	0.8
	Week 12	0.63±0.08	0.6	0.7
	Week 24	0.65±0.12	0.5	0.9
	Week 38	0.70±0.06	0.6	1.4
	Week 52	0.70±0.06	0.5	1.1
	Week 56	0.75±0.07	0.6	1.3
kidney wt	Week 57	14.222 and 11.723 g	26.187 g	26.064 g

The TK (plasma MMF) data from the 1-year study (Week 52) are summarized in the following table:

PARAMETER	MALES			FEMALES		
	5	25	75	5	25	75
C_{max} (µ/mL)	2.03 ± 0.99	8.82 ± 2.39	23.92 ± 6.80	2.06 ± 0.39	11.79 ± 4.85	31.47 ± 12.30
AUC (µg*hr/mL)	2.32 ± 0.53	12.21 ± 2.31	47.42 ± 11.27	2.73 ± 0.34	15.58 ± 2.22	43.63 ± 11.41

A clear no-effect level for renal toxicity was not identified, based on increases in kidney weight at the LD; however, the most severe effects were observed at the HD. Plasma exposure at the MD was approximately 4 times higher (C_{max}) or similar (AUC) to that in humans at the RHD.

Investigative studies: Due to the evidence of kidney toxicity in multiple species, investigative (14-day [4-week], 14-week [+ 4-week recovery], and evaluation of time course) studies were conducted in Sprague-Dawley rat in an attempt to further characterize the effects of DMF on the kidney.

In the 14-day study in male rats (DMF doses of 0, 250 mg/kg QD, or 83 mg/kg TID, by oral gavage), DMF had no effect on K_i-67 labeling (a marker of cell proliferation) in the kidney, whereas gentamicin (50 mg/kg/SC) induced an almost 5-fold increase in labeling over control. Gentamicin induced clear renal tubular injury, whereas DMF did not.

However, DMF was associated with "minimal to mild nuclear hypertrophy in proximal tubular epithelium throughout the outer two thirds of the cortex." This finding was not detected in gentamicin-treated or control animals. Nuclear hypertrophy was also observed in the male fertility study (dosing for up to 14 weeks), in additional to a slight exacerbation of nephropathy and tubular regeneration. DMF had no notable effect on urinary protein but did induce a transient (Day 1 only) increase in KIM-1 and NAG when given QD (not TID).

In the 14-week study, kidney weight was increased in males at all doses and in HD females. Renal nephropathy and tubular/cortical regeneration were observed at all doses (0, 50, 100, 250 mg/kg/day) in males; nuclear hypertrophy of the proximal tubule was observed at all doses in males and females. The tubular findings were observed, with a similar pattern, at the end of a 4-week recovery period. These histopathology changes were associated with increased urinary albumin and K_i-67 immunostaining of renal (cortex, outer medulla) tissue in males. K_i-67 positive cells were increased in females at the MD and HD but to a lesser extent than in males. KIM-1 was significantly increased in MD and HD females through Day 84. KIM-1 was not elevated in males, although kidney histopathology was more notable in males than in females.

In the time course study, oral administration of DMF to males at a dose of 100 mg/kg for 75 days resulted in an increase in kidney weight and an increase in incidence and severity of nuclear hypertrophy of the renal tubule and hyaline droplet accumulation. (There was little evidence of nephropathy in DMF-treated or control animals.) These histopathology findings were associated with an increase in K_i-67 staining in kidney in 3 of 4 DMF-treated males at terminal necropsy. Urinary microalbumin levels were clearly elevated above control values in only one of ten DMF-treated males; levels remained elevated throughout the recovery period in this animal. At the end of the recovery period, histopathology findings were minimal and K_i-67 staining was similar between DMF-treated and control animals.

Comments: Regarding the potential for DMF-induced renal toxicity, the clear DMF-induced exacerbation of chronic progressive nephropathy (CPN, or nephropathy) in rat, particularly in the chronic studies, making interpretation of the renal histopathology findings difficult. Although exacerbation of CPN was also observed in mouse in the lifetime carcinogenicity study, the relationship between this effect and other renal toxicities (including tumorigenicity) is less clear in this species.

In mouse, the only kidney-associated finding in the 13-week dose-range finding study was an increase in kidney weight. Although this is suggestive of kidney injury, there were no histopathology correlates. In the carcinogenicity study, the primary findings were some exacerbation of CPN and a proliferative effect on renal tubules, including tubular hyperplasia and tumors (adenoma and carcinoma).

In rat, evidence suggestive of nephropathy was noted as early as 3 month. For one 3-month study, the study pathologist indicated that a direct effect could not be dismissed; however, histopathology findings (including tubular basophilia) consistent with CPN

were detected, primarily in HDM (not detected in control animals). In the 6-month study, nephropathy was reported in control animals but was increased in incidence and severity in treated males. However, tubular regeneration (suggestive of prior injury) and hypertrophy were also reported. In females, the incidence of tubular hypertrophy was greater than that of nephropathy. In males, the histopathology findings appeared consistent with exacerbation of CPN; the study pathology did not attempt to distinguish direct from indirect effects of DMF. In the 3- and 6-month studies, increases in kidney weight, but decreases in BUN and/or creatinine, were observed in treated males and females. In the carcinogenicity study, there was clear evidence of exacerbation of CPN in both males and females.

Overall, there was no evidence of acute nephrotoxic effects in mouse or rat, and the chronic studies in rat were confounded by a drug-induced exacerbation of CPN (particularly in the carcinogenicity study). The shorter-tem studies, including the special investigative studies, in rat suggest the possibility of a low level of direct kidney toxicity with greater than 14 days of daily dosing. The data suggest, but do not conclusively demonstrate, that urinary albumin might be a useful parameter for monitoring in humans. Few data were available for other biomarkers (KIM-1 and NAG), but these were either increased only acutely (Day 1) or not clearly correlated with histopathology findings.

The most compelling evidence of direct DMF-related renal toxicity was observed in the chronic toxicity studies in cynomolgus monkey and Beagle dog. (A 4-week dose-ranging study in dog demonstrated no clear effect on kidney; there were no shorter duration studies in monkey that included histopathology.) In both non-rodent species, there was evidence of low level chronic renal toxicity, which after approximately a year of dosing resulted in irreversible toxicity (interstitial fibrosis) in some animals. For monkey, the study pathologist stated that two recovery animals exhibited irreversible loss of kidney tissue and function. In both species, BUN and creatinine were decreased (as consistently observed in rodent studies) during the dosing period. In only one of the two most severely affected monkeys were BUN and creatinine increased, and only beginning at Week 38 of dosing. There were no urinalysis findings consistent with renal toxicity, and other biomarkers of renal injury were not assessed in either monkey or dog. The LD tested in monkey and dog, associated with relatively minimal renal toxicity, was associated with plasma MMF similar to (C_{max}) or less than (AUC) that in humans at the recommended human dose.

Fumaderm: Since clinical data are available for Fumaderm (marketed in Europe for treatment of psoriasis, apparently at oral doses providing DMF at daily doses higher than the RHD of Tecfidera), a combination of DMF (55.6%) and various mono-ethyl fumarate (MEF) salts, the available general toxicology studies of Fumaderm were briefly reviewed. The pivotal studies were 52-week toxicity studies in Sprague-Dawley rat and Beagle dog.

In rat, Fumaderm was administered orally (by gavage) at doses of 0, 65, 195, and 390 mg/kg/day (resulting in DMF doses of approximately 36-218 mg/kg/day), with a 4-week recovery period. The final study report was amended to include additional histopathology

data on kidney (LD and MD groups) and on stomach. No toxicokinetic data were collected. Kidney findings included increase in kidney weight and numerous histopathology findings (including increases in "chronic renal disease", i.e., CPN, cortical fibrosis, mixed cell infiltrates, and/or tubule dilatation) at all doses in males and females. Exacerbation of CPN was evident primarily at the HD in males and females. In addition to the kidney findings, forestomach and testicular toxicity was also evident. Forestomach findings were similar to those reported in the DMF studies and included squamous cell papillomas and carcinomas (18/30 and 6/30, respectively, in HDM and 16/30 and 2/30, respectively, in HDF). Testicular toxicity consisted of degeneration of germinal epithelial cells (HD) and Leydig cell tumors at the MD (12/25) and HD (20/25); these findings should be described in appropriate section(s) of labeling.

In dog, Fumaderm was administered orally (by gavage) at doses of 0, 30, 60, and 120 mg/kg (HD given BID; resulting in DMF doses of 16.8-67 mg/kg/day) for 52 weeks, with a 26-week interim sacrifice and a 4-week recovery period. No toxicokinetic data were collected. Creatinine clearance was significantly increased at 26 and 52 weeks, but all values were stated to be within the normal range. BUN and serum creatinine were consistently decreased; urinalysis parameters were unaffected. There was a dose-dependent (all doses) increase kidney weight at 52 weeks. Increased kidney weights were stated to be correlated with a histopathology finding of "cloudy swelling", indicative of renal fluid retention. All renal changes were stated to be reversible.

The Fumaderm studies confirm forestomach, kidney, and testes as target organs. However, the histopathological changes in kidney in dog were substantially less severe than those reported in the chronic study of DMF in dog. No plasma exposure data are available for either study of Fumaderm. PK studies comparing plasma exposures following single doses of DMF, MEF, and Fumaderm were conducted in both species, but these studies only provided C_{max} data and were not conducted at toxicologically relevant doses. The C_{max} data demonstrated fairly similar plasma MMF levels following Fumaderm and DMF in rat (8.99 and 7.24 µg/mL, respectively) but slightly higher plasma MMF levels following Fumaderm (7.17 µg/mL) than after DMF (5.05 µg/mL) in dog. On a mg/kg basis, the doses of Fumaderm in both rat and dog provided similar doses of DMF as used in the chronic studies of DMF. So, although direct comparisons between the Fumaderm and DMF studies cannot be made, effects of Fumaderm on kidney were less than for DMF in the dog but fairly similar in the rat.

Genetic Toxicology

A full battery of genetic toxicology studies was conducted on DMF and MMF. DMF was tested in the following assays: in vitro bacterial reverse mutation (Ames) assay, in vitro mammalian cell gene mutation (HPRT) assay, in vitro chromosomal aberration assays in human peripheral blood lymphocytes, and in vivo micronucleus assay in Sprague-Dawley rat. DMF was negative in the Ames assay and the in vivo micronucleus assay but positive in the in vitro clastogenicity assays, in the absence of metabolic activation. DMF was negative in the in vitro mammalian cell gene mutation assay; however, a fairly steep

concentration-response for cytotoxicity prevented assessment at sufficiently cytotoxic concentrations.

The genotoxic potential of MMF was assess in an in vitro Ames assay, an in vitro chromosomal aberration assay in human peripheral blood lymphocytes, and an in vivo micronucleus assay in Sprague-Dawley rat. MMF was negative in the Ames assay but positive in the in vitro clastogenicity assay, in the absence of metabolic activation. MMF was negative in the in vivo micronucleus assay; however, the study was inadequate as conducted (an insufficient number of cells was examined per animal).

Carcinogenicity

The sponsor conducted lifetime carcinogenicity studies of DMF in CD-1 mouse and Sprague-Dawley rat. Final study reports were discussed with the Executive CAC (*Minutes of September 25, 2012 Meeting, 10/1/2012*).

In mouse, DMF was administered orally by gavage at doses of 0, 25, 75, 200, and 600/400 mg/kg/day. The HD was reduced on Day 9, following a brief dosing holiday, due to deaths in HDM and HDF. Dosing was stopped prematurely (during Week 72 in HDM or Week 82 in HDF), and all surviving HD animals were sacrificed prematurely during Week 101. Tumor findings consisted of the following:

- Squamous cell carcinomas and papillomas of the forestomach in males and females at 200 and 400 mg/kg/day; leiomyosarcomas of the forestomach in males and females at 400 mg/kg.
- Renal tubule adenomas and carcinomas in males at 200 and 400 mg/kg/day and renal tubule adenomas in females at 400 mg/kg/day.

A re-examination of the slides of renal tissue by the sponsor's expert consultant (b) (4) May 11, 2012) resulted in only one additional renal tumor (a tubule adenoma in one control male) and, therefore, did not change the original conclusions regarding renal tumors. Selected data are provided in the following tables:

RENAL FINDINGS	MALES					FEMALES				
	0	**25**	**75**	**200**	**600/ 400**	**0**	**25**	**75**	**200**	**600/ 400**
tubular hyperplasia										
minimal	1/75	4/75	13/75	27/75	7/75	0/75	5/75	8/75	10/75	6/75
mild	0/75	3/75	3/75	13/75	7/75	0/75	2/75	0/75	2/75	7/75
moderate	0/75	0/75	0/75	0/75	1/75	0/75	0/75	0/75	1/75	0/75
total	**1/75**	**7/75**	**16/75**	**40/75**	**15/75**	**0/75**	**7/75**	**8/75**	**13/75**	**13/75**
tubular mineralization										
minimal	14/75	20/75	27/75	34/75	15/75	2/75	3/75	3/75	13/75	27/75
mild	2/75	2/75	1/75	6/75	8/75	0/75	0/75	1/75	0/75	3/75
moderate	0/75	0/75	0/75	0/75	1/75	0/75	0/75	0/75	1/75	1/75
total	**16/75**	**22/75**	**28/75**	**40/75**	**24/75**	**2/75**	**3/75**	**4/75**	**14/75**	**31/75**

RENAL FINDINGS	MALES					FEMALES				
	0	25	75	200	600/ 400	0	25	75	200	600/ 400
nephropathy										
minimal	18/75	14/75	8/75	3/75	2/75	13/75	34/75	14/75	21/75	13/75
mild	21/75	30/75	35/75	12/75	19/75	41/75	25/75	45/75	34/75	29/75
moderate	27/75	23/75	26/75	45/75	27/75	12/75	8/75	11/75	12/75	22/75
marked	2/75	2/75	3/75	12/75	10/75	3/75	3/75	2/75	2/75	2/75
total	**68/75**	**69/75**	**72/75**	**72/75**	**58/75**	**69/75**	**70/75**	**72/75**	**69/75**	**66/75**
tubular cyst (multifocal)										
minimal	3/75	5/75	6/75	4/75	1/75	2/75	6/75	2/75	8/75	8/75
mild	5/75	16/75	17/75	6/75	4/75	5/75	8/75	10/75	17/75	7/75
moderate	15/75	16/75	27/75	41/75	26/75	6/75	7/75	5/75	11/75	19/75
marked	3/75	2/75	3/75	8/75	8/75	0/75	0/75	0/75	1/75	0/75
total	**26/75**	**36/75**	**53/75**	**59/75**	**39/75**	**13/75**	**21/75**	**17/75**	**37/75**	**34/75**
adenoma, tubule	**1/75**	**2/75**	**0/75**	**5/75**	**3/75**	**0/75**	**0/75**	**0/75**	**2/75**	**4/75**
carcinoma, tubule	**0/75**	**0/75**	**2/75**	**4/75**	**3/75**	**0/75**	**0/75**	**0/75**	**0/75**	**1/75**
RE-EXAMINATION [redacted] (b) (4)										
nephropathy										
incidence	65/75	69/75	73/75	71/75	60/75	61/75	65/75	72/75	72/75	61/75
severity	2.0	1.9	2.4	3.0	2.8	1.5	1.7	2.1	2.2	2.4
atypical tubule hyperplasia	0/75	0/75	2/75	3/75	0/75	0/75	0/75	0/75	1/75	1/75
adenoma, tubule	**2/75**	**2/75**	**0/75**	**5/75**	**3/75**	**0/75**	**0/75**	**0/75**	**2/75**	**4/75**
carcinoma, tubule	**0/75**	**0/75**	**2/75**	**4/75**	**3/75**	**0/75**	**0/75**	**0/75**	**0/75**	**1/75**

FORESTOMACH FINDINGS	MALES					FEMALES				
	0	25	75	200	600/ 400	0	25	75	200	600/ 400
ulceration										
mild	0/74	0/75	0/75	1/75	5/75	0/75	0/75	0/75	1/75	2/75
moderate	0/74	0/75	0/75	0/75	1/75	0/75	0/75	0/75	0/75	1/75
marked	0/74	0/75	1/75	0/75	8/75	0/75	0/75	0/75	2/75	5/75
total	**0/74**	**0/75**	**1/75**	**1/75**	**14/75**	**0/75**	**0/75**	**0/75**	**3/75**	**8/75**
erosion										
mild	0/74	0/75	0/75	0/75	1/75	0/75	0/75	0/74	1/75	1/75
marked	0/74	0/75	0/75	0/75	0/75	1/75	0/75	0/74	0/75	0/75
total	**0/74**	**0/75**	**0/75**	**0/75**	**1/75**	**1/75**	**0/75**	**0/74**	**1/75**	**1/75**
necrosis										
marked	0/74	0/75	0/75	0/75	13/75	0/75	0/75	0/74	0/75	13/75
hyperplasia										
minimal	0/74	6/75	7/75	3/75	4/75	0/75	23/75	13/74	8/75	1/75
mild	1/74	5/75	31/75	23/75	16/75	0/75	8/75	29/74	24/75	8/75
moderate	1/74	1/75	11/75	42/75	40/75	0/75	2/75	13/74	36/75	44/75
marked	0/74	0/75	0/75	0/75	0/75	0/75	0/75	1/74	2/75	5/75
total	**2/74**	**12/75**	**49/75**	**68/75**	**60/75**	**0/75**	**33/75**	**56/74**	**70/75**	**58/75**
hyperkeratosis										
minimal	1/74	4/75	6/75	2/75	3/75	1/75	17/20	11/74	8/75	0/75
mild	4/74	9/75	26/75	22/75	11/75	2/75	20/75	22/74	17/75	9/75
moderate	1/74	2/75	31/75	45/75	49/75	2/75	2/75	29/74	48/75	48/75
marked	0/74	0/75	1/75	1/75	1/75	0/75	0/75	1/74	2/75	5/75
total	**6/74**	**15/75**	**64/75**	**70/75**	**64/75**	**5/75**	**39/75**	**63/74**	**75/75**	**62/75**

FORESTOMACH FINDINGS	MALES					FEMALES				
	0	**25**	**75**	**200**	**600/ 400**	**0**	**25**	**75**	**200**	**600/ 400**
squamous cyst										
mild	0/74	0/75	0/75	1/75	0/75	1/75	0/75	1/74	0/75	2/75
moderate	0/74	0/75	0/75	0/75	0/75	0/75	0/75	0/74	1/75	3/75
total	**0/74**	**0/75**	**0/75**	**1/75**	**0/75**	**1/75**	**0/75**	**1/74**	**1/75**	**5/75**
squamous papilloma	**0/74**	**1/75**	**3/75**	**12/75**	**14/75**	**0/75**	**0/75**	**3/74**	**6/75**	**16/75**
squamous cell carcinoma	**0/74**	**1/75**	**0/75**	**2/75**	**6/75**	**0/75**	**1/75**	**1/74**	**5/75**	**12/75**
leiomyosarcoma	**0/74**	**0/75**	**0/75**	**0/75**	**3/75**	**0/75**	**0/75**	**0/74**	**0/75**	**3/75**
fibrosarcoma	**0/74**	**0/75**	**0/75**	**0/75**	**1/75**	**0/75**	**0/75**	**0/74**	**0/75**	**2/75**

Plasma AUC in mouse at the highest dose not associated with increased tumors (≈10 µg*hr/mL at 75 mg/kg/day) is similar to that in humans (≈10 µg*hr/mL) at the RHD.

In rat, DMF was administered orally by gavage at doses of 0, 25, 50, 100, and 150 mg/kg/day. Dosing was suspended in males in the two highest dose groups (Week 88 and 80, respectively) due to reduced survival; these groups were terminated prematurely (Week 88 and 86, respectively). Tumor findings consisted of the following:

- Squamous cell carcinomas and papillomas of the forestomach in males and females at all doses tested.
- Renal tubule adenomas in males and carcinomas in females at 150 mg/kg/day.
- Testicular interstitial cell adenomas at 100 and 150 mg/kg/day.

Forestomach and testicular findings are summarized below.

FORESTOMACH FINDINGS	MALES					FEMALES				
	0	**25**	**50**	**100**	**150**	**0**	**25**	**50**	**100**	**150**
inflammation, chronic active	3/75	11/75	24/75	48/75	51/75	4/75	8/75	13/75	40/75	55/75
mineralization	1/75	1/75	9/75	9/75	17/75	0/75	0/75	1/75	1/75	8/75
ulcer (epithelium)	0/75	0/75	0/75	0/75	0/75	4/75	1/75	0/75	6/75	16/75
ulcer	0/75	1/75	0/75	4/75	15/75	1/75	0/75	1/75	0/75	1/75
erosion (epithelium)	1/75	1/75	3/75	12/75	14/75	0/75	4/75	8/75	9/75	20/75
hyperplasia (squamous epithelium)	3/75	71/75	75/75	75/75	75/75	7/75	73/75	74/75	75/75	75/75
hyperkeratosis	2/75	69/75	75/75	75/75	75/75	6/75	63/75	74/75	75/75	75/75
squamous cyst(s)	2/75	9/75	28/75	53/75	64/75	1/75	0/75	11/75	45/75	62/75
squamous cell papilloma	0/75	22/75	24/75	46/75	49/75	0/75	11/75	21/75	31/75	24/75
squamous cell carcinoma	0/75	5/75	18/75	51/75	58/75	0/75	1/75	4/75	30/75	48/75
squamous cell papilloma/carcinoma	**0/75**	**22/75**	**34/75**	**68/75**	**70/75**	**0/75**	**11/75**	**23/75**	**48/75**	**58/75**

TESTICULAR FINDINGS	0	25	50	100	150
artery-chronic active inflammation	10/75	17/75	17/75	26/75	31/75
atrophy	12/75	16/75	19/75	17/75	29/75
germinal epithelial degeneration	7/75	8/75	5/75	19/75	15/75
interstitial cell hyperplasia	0/75	2/75	2/75	6/75	10/75
interstitial cell adenoma	3/75	3/75	2/75	9/75	19/75

Regarding the renal tumors, a re-examination of the slides of renal tissue by the sponsor's expert consultant [redacted] (b) (4) May 11, 2012) resulted in substantial changes in the tumor incidence. Both the original and revised renal tumor (and nephropathy) data are provided below:

RENAL FINDINGS	MALES					FEMALES				
	0	**25**	**50**	**100**	**150**	**0**	**25**	**50**	**100**	**150**
Original										
nephropathy										
incidence	68/75	75/75	75/75	75/75	75/75	49/75	55/75	68/75	69/75	73/75
severity	2.04	2.71	3.16	3.52	3.53	0.95	1.32	1.84	2.44	3.24
adenoma, tubule	**0/75**	**0/75**	**1/75**	**1/75**	**4/75**	**1/75**	**0/75**	**0/75**	**0/75**	**2/75**
carcinoma, tubule	**0/75**	**0/75**	**0/75**	**0/75**	**0/75**	**0/75**	**0/75**	**0/75**	**2/75**	**4/75**
Re-examination										
nephropathy										
incidence	73/75	75/75	75/75	75/75	75/75	67/75	70/75	74/75	70/75	74/75
severity	4.5	5.8	6.5	6.9	7.0	2.9	3.5	4.1	5.5	6.6
adenoma, tubule	**0/75**	**0/75**	**1/75**	**1/75**	**0/75**	**1/75**	**0/75**	**1/75**	**0/75**	**2/75**
carcinoma, tubule	**0/75**	**0/75**	**0/75**	**0/75**	**0/75**	**0/75**	**0/75**	**0/75**	**0/75**	**1/75**

Based on [redacted] (b) (4) re-examination, the incidence of renal tubule adenomas and/or carcinomas is not increased at any dose in males and only minimally in females, at the HD. Therefore, only the forestomach and testicular tumors are considered drug-related.

There was no dose not associated with an increase in tumors; plasma AUC at the lowest dose tested (≈3 μg*hr/mL) are substantially lower than that in humans at the RHD.

Reproductive and developmental toxicity: the sponsor conducted a full battery of oral reproductive and developmental studies for DMF: separate fertility and general reproduction toxicity studies in male and female Sprague-Dawley rat, embryo-fetal development studies in Sprague-Dawley rat and New Zealand White rabbits, and a pre and postnatal development study in Sprague-Dawley rat.

In the fertility study in male rats, oral doses of 0, 75, 250, and 375 mg/kg/day were administered prior to and throughout the mating period. No effects on fertility were observed; however, increases in non-motile sperm were observed at 250 and 375 mg/kg/day. In the fertility study in female rats, oral doses of 0, 20, 100, and 250 mg/kg/day were administered prior to and during mating and continuing to gestation day 7. Disruption of the estrus cycle and increases in embryolethality were observed at 250 mg/kg/day. The NOAEL for adverse effects was 75 mg/kg/day in males and 100 mg/kg/day in females.

In the embryo-fetal development study in rat, oral doses of 0, 25, 100, and 250 mg/kg/day were administered throughout organogenesis; embryo-fetal toxicity (reduced fetal body weight and delayed ossification) were observed at 250 mg/kg/day. In the embryo-fetal development study in rabbit, oral doses of 0, 25, 75, and 150 mg/kg/day were administered throughout organogenesis; increased embryolethality was observed at 150

mg/kg. The NOAEL for adverse developmental effects was 100 mg/kg/day in rat and 75 mg/kg/day in rabbit.

In the pre and postnatal development study in rat, oral doses of 0, 25, 100, and 250 mg/kg/day were administered throughout organogenesis and lactation. Increased lethality, persistent reductions in body weight, delayed sexual maturation (male and female pups) and reduced testicular weight were observed in offspring at 250 mg/kg/day. Neurobehavioral impairment was observed at all doses tested. Therefore, a NOAEL for developmental toxicity was not identified.

Conclusions and Recommendations

The sponsor has conducted an adequate battery of nonclinical studies to support marketing of DMF for treatment of patients with relapsing forms of multiple sclerosis. Forestomach (mouse, rat), testes (mouse, rat, dog), and kidney (mouse, rat, dog, monkey) were the primary and most consistently observed DMF-related target organs. While the forestomach lesions and tumors were clearly drug-related and observed at all doses tested (i.e., a no-effect dose was not identified in either species), the relevance to humans is mitigated by the marked species differences in exposures between rodent and human. The forestomach is a storage organ and is, therefore, exposed to higher concentrations of drug than would be the esophagus or stomach in humans at the RHD and with the to-be-marketed formulation (capsule (b) (4)). DMF has been reported to induced severe contact dermatitis in humans (Lefranc A *et al*. *Arch Environ Occup Health* 66(4):217-222, 2011; Pastor-Nieto MA *et al*. *Contact Dermatitis* 68:117-128, 2013; Rantanen T *Brit J Dermatol* 159:218-221, 2008), so it certainly has potential to be irritating in humans, if, for example, the squamous mucosa of the esophagus (similar to that of the forestomach in rodent) or other portions of the GI tract are exposed to a sufficient concentration of DMF. (Upper abdominal pain is a common adverse effect reported in humans.) However, it is likely that rodent would over predict the risk of GI toxicity in humans at the RHD.

Testicular toxicity was observed in one strain of mouse and in rat and dog studies of DMF, and in a 52-week study of Fumaderm in rat, and should be described in appropriate sections(s) of labeling.

Dr. Banks-Muckenfuss has identified the renal toxicity of DMF as the basis for her recommendation to not approve the NDA. Dr. Banks-Muckenfuss acknowledges that there has been no signal for renal toxicity in humans during clinical development but is concerned that humans were not exposed for a sufficient duration or monitored using sensitive enough markers to rule out the potential for renal toxicity. Dr. Banks-Muckenfuss recommends that the sponsor provide additional information, i.e., "…a thoroughly-reasoned discussion of the potential mechanism of the toxicity…" and "…Conduct further mechanistic studies to elucidate potential for this mechanism to result in the observed tumors…"

I agree with Dr. Banks-Muckenfuss that the data, particularly in monkey and dog, demonstrate the potential for renal toxicity in humans. The data in these species suggest a low level of chronic injury and repair that results, with prolonged exposure, in irreversible injury. The clinical team acknowledges that monitoring during the clinical trials of DMF may not have been sufficient to rule out the risk of a similar injury in humans; however, DMF has demonstrated efficacy in humans with MS and the available safety data for DMF and the postmarketing experience with Fumaderm provide sufficient evidence of safety to support approval of DMF. In addition, there will be a postmarketing requirement (PMR) to conduct a large 5-year observational study in MS patients, with close monitoring for renal toxicity. Therefore, I have no objection to approval of the NDA but do recommend that the renal toxicity in animals be described in appropriate section(s) of labeling. I do not believe that the additional information or studies recommended by Dr. Banks-Muckenfuss are necessary, since it is unclear what, if any, clinical impact the data from such studies would have. The sponsor should, however, be required to conduct, as a PMR, a juvenile animal toxicology study to support clinical development of DMF for the pediatric population.

Labeling recommendations will be provided in a separate memo.

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/s/

LOIS M FREED
02/08/2013

DEPARTMENT OF HEALTH AND HUMAN SERVICES
PUBLIC HEALTH SERVICE
FOOD AND DRUG ADMINISTRATION
CENTER FOR DRUG EVALUATION AND RESEARCH

PHARMACOLOGY/TOXICOLOGY NDA REVIEW AND EVALUATION

Application number:	204063
Supporting document/s:	SDN1, 2/24/12; SDN49, 10/23/12
Applicant's letter date:	2/24/12
CDER stamp date:	2/27/12
Product:	BG-12
	a.k.a. BG00012
Indication:	Multiple Sclerosis, Relapsing-Remitting
Applicant:	Biogen Idec
Review Division:	DNP, HFD-120
Reviewer:	Melissa K. Banks-Muckenfuss, Ph.D.
Supervisor/Team Leader:	Lois M. Freed, Ph.D.
Division Director:	Russell G. Katz, M.D.
Project Manager:	Nicole L. Bradley, Pharm.D.

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1 Executive Summary

1.1 Introduction

Fumaric acid esters have been used in the treatment of psoriasis since 1959 (Ormerod & Mrowietz, 2004). Dimethyl fumarate (DMF) is one component of Fumaderm® (combination of fumaric acid esters [i.e., dimethyl fumarate and monoethylfumarate salts]), an immunomodulatory therapy for psoriasis that was approved in Germany in 1994. Fumaderm® is a systemic oral therapy; the most commonly reported side effects are flushing, GI disorders, and "mild forms of lymphopenia and leucopenia." It is not approved in the US. Biogen Idec is developing BG-12 (120 mg DMF/capsule), a second generation, single substance, fumaric acid ester with immunomodulatory properties, to improve on overall tolerability compared to Fumaderm®. It is also notable that DMF has been shown to be a severe irritant, and has been restricted from use as a fungicide on products in the EU.

1.2 Brief Discussion of Nonclinical Findings

The mechanism by which DMF acts to treat multiple sclerosis is not fully understood, though a number of mechanisms have been implicated, such as: activation of the Nuclear Factor (Erythroid-derived 2)-like (NFE2L2 or Nrf2) antioxidant response pathway, inhibition of NF-KB signaling, inhibition of cyclin-dependent kinase, promotion of anti-inflammatory cytokine expression, and cytoprotection of CNS cells. In safety pharmacology studies, DMF showed some liability for cardiovascular, but not respiratory, toxicity; CNS toxicity was not directly evaluated. Following oral administration, DMF is rapidly absorbed and drug-related material is widely distributed. DMF is rapidly hydrolyzed by esterases and enzymes involved in the TCA cycle, and is not generally observed in plasma. MMF is considered the "primary metabolite" (at ~5% of the circulating drug-related plasma exposures); MMF was measured in the nonclinical species and humans for the purpose of plasma exposure comparisons. Other metabolic products include glucose (~50%), and fumaric and citric acid (~30%). DMF and MMF are rapidly metabolized and are primarily eliminated in expired air; generally, renal elimination plays a minor role. Although the benign metabolic disposition of DMF might not suggest it, DMF was shown to cause many toxicities, including carcinogenicity and some reproductive toxicity. DMF-related toxicity was observed in a number of organs, including kidney, testes, stomach (nonglandular), pancreas, liver, thymus, lymphatic system, and eye (retina). In several of the standard toxicological studies, multiple species showed clear renal (rat, mouse, dog, and monkey) and testicular (rat and dog) toxicity. Although toxicity in the nonglandular stomach of rodents was striking, evidence of toxicity in the stomach (and/or esophagus) of other species was more limited.

Renal toxicity was observed as damage (e.g., single cell necrosis, atrophy, nephropathy leading to renal failure) and/or evidence of repair (e.g., regeneration) in multiple species. Renal tubule regeneration was observed across species, with the dose yielding toxicity decreasing as a function of increasing treatment duration (although often without clear, direct evidence of damage in shorter term studies), usually without

pronounced alterations in clinical pathology and other non/minimally-invasive methods. Generally, reductions in serum creatinine and BUN were observed in most nonclinical species, but urinary protein was only clearly increased in rats; investigative studies demonstrated increased urinary albumin/microalbumin. Rodents clearly demonstrated exacerbations of rodent CPN, which led to increased mortality in long-term studies. While a few of the renal alterations in nonrodents demonstrated some recovery, evidence of irreversible changes was observed in the chronic monkey study. Renal neoplasias were observed in the 2-yr bioassays in rodents. Renal tumors demonstrated in rats may reasonably be attributed in part to a species-related disorder (i.e., exacerbation of rodent CPN); however, the relationship between the neoplasias and exacerbation of the species-related disorder (CPN) in mice is less clear. Some tumors in mice were noted to arise from renal cysts, and unlike the tumors in the presence of CPN-related changes, it is not clear whether tumors present in these circumstances were related to an increased process of damage/repair.

Testicular seminiferous epithelium was a target tissue in both rats and dogs. The 2-year carcinogenicity bioassay in rats showed Leydig cell hyperplasia and tumors.

Rodents demonstrated severe forestomach effects at most doses; the dose yielding toxicity decreased as a function of treatment duration. In the 2-year bioassays in rodents, the severe damage to the nonglandular stomach was accompanied by tumors at all doses tested. And, although of lesser incidence, toxicity (e.g., degeneration) of the glandular stomach was observed in the 2-yr bioassay in rats (the sponsor attributed this, at least in part, to CPN-related renal failure and secondary hyperparathyroidism). Some stomach effects (e.g., erosion, hemorrhage, or mononuclear cells) were also seen in subchronic studies at relatively high doses (i.e., doses not tolerated in longer studies) in the dog. Although the nonglandular stomach tumors were striking, the relevance of these tumors, observed at below clinically relevant doses, is unclear; there is no direct anatomical correlate in humans and the concentrative function of the rodent organ combined with the irritancy of DMF could reasonably result in the toxicity. However, this severe irritancy could prove problematic in humans when considered in the context of a mechanism of action known to have potential tumor promotion effects (i.e., Nrf2 activation, see discussion below). It is possible that the irritancy could have effects in the exposed portions of the GI tract (esophagus and/or stomach, in particular), especially given the potential for decades of twice daily exposure in the treatment of this chronic disease. With regard to human experience, the clinical trials identified GI and upper GI discomfort/pain as a common side effect.

The demonstrated organ toxicities, and most importantly the carcinogenic effects, may be attributable to the pharmacology of the drug. Although the sponsor emphasized that DMF has a short half-life, the distribution study data using radiolabeled DMF demonstrated high exposures in tissues that showed toxicity. The primary mechanism of action of DMF proposed by the sponsor (i.e., Nrf2 activation through direct effects on KEAP1) is known to possess both protective and oncogenic effects, although the reason for this duality has not been fully elucidated. It is possible that the mechanism conferring a protective effect elicited by some stimuli (e.g., a genotoxic agent) may

cause tumor formation/promotion when the pathway is activated constitutively (such as that demonstrated by genetic knock-outs) or without elicitation by physiological demand. The sponsor demonstrated that DMF and MMF alter KEAP1, activating the Nrf2 pathway; this modification was described in literature as irreversible (e.g., Lin et al., 2012). It is known that increased intracellular fumarate levels (i.e., caused by a genetic mutation in the gene encoding fumarate hydratase [FH]; see Adam et al., 2011) leads to the disorder HLRCC, causing renal tumors, Leydig cell tumors (Renal Carvajal-Carmona et al., 2006), and leiomyomas. Renal FH conditional knockout (KO) mice develop renal cysts beginning at 13 weeks of age that is followed by ill health and/or death from renal failure at 50-65 weeks (Pollard et al, 2007; Adam et al., 2011). Also, it is notable that KEAP1 KO mice (resulting in constitutively hyperactive Nrf2 signaling) was shown to be lethal, generally within in 3 weeks of birth, due to "obstructive lesions mediated by hyperkeratotic outgrowth of the oesophageal and forestomach epithelial cells" (see Martin-Maltalvo et al., 2011, Wakabayashi et al., 2003).

Limited drug-related reproductive toxicity was observed in standard nonclinical studies. Some evidence suggested slight effects on fertility. Nonmotile sperm were slightly increased in males, and dams showed reduced estrous cycling and increased cohabitation times in the presence of maternal toxicity; however, these effects did not translate into clear effects on fertility, as measured. The embryofetal development (EFD) studies demonstrated maternal toxicity as well as effects on fetal viability and/or development. MMF crossed the placenta, and fetal plasma concentrations were about 10% those of the rabbit does and 40-60% those of rat dams.

1.3 Recommendations

1.3.1 Approvability

From a Pharmacology/Toxicology perspective, and based solely on the nonclinical data, this NDA is not recommended for approval at this time based on renal toxicity at clinically relevant doses (toxicity in all nonclinical species, including tumors in rodents). Renal toxicity was observed in multiple nonclinical species, with the dose yielding toxicity decreasing as a function of increasing treatment duration and without a clear pathway for clinical monitoring. While this recommendation is tempered by the fact that renal toxicity monitoring was performed in the clinical trials and no significant results were obtained (see the clinical safety review by Dr. Boehm, dated 1/9/13), it is not clear from the nonclinical data that the monitoring conducted would be able to detect the toxicity in humans (e.g. due to the marker used, sensitivity and/or the duration of the exposures).

While the toxicity of DMF at or below clinically relevant exposures is notable (e.g., stomach toxicity, renal toxicity, liver toxicity, testicular toxicity), neither the majority of the general toxicities nor the potential reproductive toxicity (i.e., effects on reproductive parameters not generally reflected in rodent fertility measures and few effects on fetal survival and development) would preclude approval because most approved therapies for multiple sclerosis have a number serious toxicities. However, the seeming lack of a

clear means to monitor for potential renal toxicity with longer durations of dosing is notable; renal toxicity was demonstrated in 4 species (monkey, dog, rat, and mouse), and showed a potential to cause irreversible tissue damage and loss of function in the 1-year monkey toxicity study at approximately 3 times the RHD (i.e., at clinically relevant exposures). Although increased urinary albumin/microalbumin was observed in rats (and thus considered an indicator of early renal tubule injury), increased urinary protein was not clearly observed in dogs or monkeys (urinary albumin was not specifically measured). DMF has also demonstrated carcinogenic potential in kidney and nonglandular stomach at or below clinically relevant exposures (based on BSA comparisons). In the 2-year carcinogenicity study in mice, the incidence of renal tubular adenomas and carcinomas, and nonglandular stomach leiomyosarcomas, squamous cell papillomas, and squamous cell carcinomas were increased at less than the RHD, on a mg/m^2 basis. In the 2-year study in rats, increased incidences of nonglandular stomach squamous cell papilloma and carcinoma (at less than the RHD, on a mg/m2 basis) and testicular interstitial (Leydig) cell adenomas (at 2 times the RHD, on a mg/m^2 basis) were seen, and renal tubular neoplasias (at 3 times the RHD, on a mg/m^2 basis) were suggested. Even in subchronic repeated dose studies in rats, nonglandular stomach papillomas and/or carcinomas were observed with dosing durations as short as 12-14 weeks (with dose-related incidence at mostly, although not exclusively, higher doses; 1.5-8x the RHD). The relevance of these findings to human risk is unclear.

1.3.2 Additional Non Clinical Recommendations

The observed renal toxicity in multiple species is substantial and the relevance to humans is unclear but reasonably predicted at clinically relevant exposures of MMF; further work to identify a sensitive method for detecting renal toxicity in humans should be conducted. For example, demonstration that increased urinary albumin/microalbumin occurs at early time points in monkeys would strengthen the validity of this biomarker in humans. Additionally, the relevance of DMF's, MMF's and fumarate's activity at the Nrf2 transcriptional pathway in the elicitation of the observed toxicities (especially tumors) for human use of BG-12 is unclear. In order to assist in the tumor risk determination in humans and to obtain the information necessary to adequately inform labeling, the sponsor should do the following:

- Provide a thoroughly-reasoned discussion of the potential mechanism of the toxicity, providing data to support conclusions;
- Conduct further mechanistic studies to elucidate potential for this mechanism to result in the observed tumors. This might include, for example, assessment of intracellular levels of the fumarates in relevant tissues or whether blockade of the Nrf2 transcriptional pathway can block the formation of the tumors.

1.3.3 Suggested Labeling

Final labeling requires discussion among the review team members, as well as with the sponsor. This suggested labeling is provided in case the application moves to approval, and represents the reviewer's current thoughts, but does not reflect final label wording.

HIGHLIGHTS OF PRESCRIBING INFORMATION

-------INDICATIONS AND USAGE-------
TECFIDERA is indicated for the treatment of patients with relapsing multiple sclerosis (b) (4) (1)

------- USE IN SPECIFIC POPULATIONS-------
Pregnancy: Based on animal data, may cause fetal harm (8.1)

8 USE IN SPECIFIC POPULATIONS

8.1 Pregnancy

Pregnancy Category C

There are no adequate and well-controlled studies in pregnant women with TECFIDERA. Oral studies of TECFIDERA in animals demonstrated evidence of embryolethality. TECFIDERA should be used during pregnancy only if the potential benefit justifies the potential risk to the fetus.

Administration of dimethyl fumarate to pregnant rats and rabbits resulted in adverse effects on offspring development, including embryolethality and developmental delays, at doses approximately 5 and 6 times the recommended human dose (RHD) of 240 mg BID.

When dimethyl fumarate was administered at oral doses of 25, 100 and 250 mg /kg (approximately 0.5, 2, and 5 times the RHD on a mg/m^2 basis) to pregnant rats during the period of organogenesis, maternal toxicity (i.e., reduced average body weights) and embryotoxic effects (including delayed development, as demonstrated by decreased fetal weights, increased overall alterations, and delayed ossification) occurred at 250 mg/kg/day. The no-effect dose for adverse effects on the embryo was approximately 2 times the RHD on a mg/m^2 basis). In pregnant rabbits treated during organogenesis with dimethyl fumarate at oral doses of 25, 75, and 150 mg/kg (approximately 1, 3, and 6 times the RHD on a mg/m^2 basis), maternal toxicity (body weight loss) and increased abortion occurred at 150 mg/kg. The no-effect dose for adverse effects on the embryo was approximately 3 times the RHD on a mg/m^2 basis. Administration of dimethyl fumarate to rats at oral doses of 25, 100, and 250 mg/kg (approximately 0.5, 2, and 5 times the RHD on a mg/m^2 basis) during the latter part of pregnancy and throughout lactation produced maternal toxicity (decreased average body weight and target organ

toxicity), decreased fetal viability, decreased fetal growth and delayed maturation, as well as effects on learning and memory, at 250 mg/kg; the no-effect dose is approximately 2 times the RHD on a mg/m^2 basis.

8.3 Nursing Mothers

There are no adequate and well-controlled studies in nursing women. It is not known whether TECFIDERA is excreted in human milk. The effect of dimethyl fumarate on the nursing infant is not known. Caution should be exercised when TECFIDERA is administered to a nursing mother.

12.1 Mechanism of Action

The mechanism by which TECFIDERA exerts therapeutic effects in multiple sclerosis is not fully understood. Dimethyl fumarate (DMF) has been shown to induce the nuclear factor (erythroid-derived 2)-like 2 (Nrf2) transcriptional pathway, which is the primary cellular defense system for responding to a variety of potentially toxic stimuli through up-regulation of antioxidant response genes, and inhibit nuclear factor kappa B (NF-κB)-dependent transcription. [***The following statement should be vetted by clinical pharmacology:*** TECFIDERA has also been shown to up regulate Nrf2-dependent antioxidant genes in patients, confirming clinical pharmacodynamic activity in humans.] Additionally, MMF (the primary metabolite of DMF) has been shown to be a nicotinic acid receptor agonist.

13 NONCLINICAL TOXICOLOGY

13.1 Carcinogenesis, Mutagenesis, Impairment of Fertility

Carcinogenesis: Carcinogenicity studies of TECFIDERA were conducted in mice and rats. In mice, dimethyl fumarate was administered at oral doses of 25, 75, 200, and 400 mg/kg/day for up to 2 years. The incidence of renal tubular adenomas and carcinomas, and nonglandular stomach leiomyosarcoma, papilloma, and squamous cell carcinoma were increased at less than the RHD, on a mg/m^2 basis. The relevance of these findings to human risk is unknown.

In rats, dimethyl fumarate was administered at oral doses of 25, 50, 100 and 150 mg/kg/day for up to 2 years. In males, an increased incidence of interstitial (Leydig) cell adenomas of the testes was observed at 2 times the RHD, on a mg/m^2 basis. The no-effect level was approximately equal to the RHD, on a mg/m^2 basis. The incidences of squamous cell papilloma and carcinoma of the nonglandular stomach was increased at less than the RHD, on a mg/m2 basis. The relevance of these findings to human risk is unknown.

Mutagenesis: DMF and MMF were negative in the *in vitro* bacterial reverse mutation assay and the *in vivo* rat micronucleus assay. DMF and MMF were positive only in *in vitro* chromosomal aberration assays in mammalian cells.

Impairment of fertility: Administration of dimethyl fumarate to male rats at daily oral doses of 75, 250, and 375 mg/kg prior to and during mating had few effects on male fertility up to the highest dose tested (8 times the RHD, based on mg/m^2). However, the number of nonmotile sperm was increased at 250 and 375 mg/kg (the no-effect level was approximately 1.5 times the RHD). Administration of dimethyl fumarate to female rats at daily oral doses of 25, 100, or 250 mg/kg/day prior to and during mating, and continuing to Day 7 of gestation, reduced the number of estrus cycles, increased the time spent in diestrus, and required slightly longer cohabitation times at 250 mg/kg. Post-implantation loss, nonviable embryo count, and the number of dams with any nonviable embryos were slightly increased, and viable embryo counts were slightly decreased, at 250 mg/kg. The no-effect level was approximately 2 times the RHD on a mg/m^2 basis.

13.2 Animal Toxicology and/or Pharmacology

Kidney toxicity was observed after repeated oral administration of dimethyl fumarate in mice, rats, dogs, and monkeys. Renal tubule toxicity epithelial regeneration, suggestive of tubule epithelial injury, was observed in all species. Treatment with DMF resulted in severe exacerbation of a common rodent renal disorder, and renal tubular hyperplasia and tumors were observed in rodents with lifetime dosing. Cortical atrophy was observed in dogs and monkeys and, in monkeys, single cell necrosis of the renal tubules and interstitial fibrosis were observed in animals that received daily oral doses of dimethyl fumarate for at least 11 months. These findings occurred at or below the RHD, on mg/m^2 basis.

GI toxicity was clearly observed in rodents. In rodents, extensive damage to the nonglandular stomach resulted in hyperplasia and tumor formation with as little as 3 months dosing. While humans do not have a direct anatomical correlate to the nonglandular stomach, some damage was observed in the glandular stomach.

In the testes, degeneration of the seminiferous epithelium was seen in rats and dogs, and interstitial hyperplasia and tumors were seen in rats. Findings were observed at

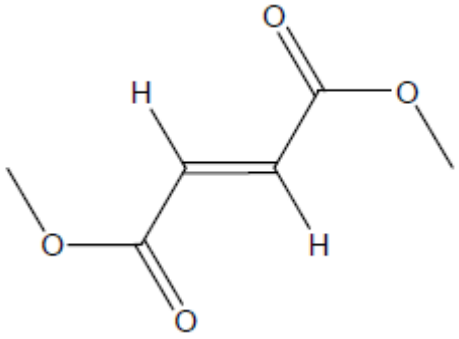
less than the RHD in rats, and approximately 3 times the RHD in dogs (on a mg/m^2 basis).

An increased incidence of retinal degeneration was observed in the 2-year mouse carcinogenicity study at 200 and 400 mg/kg. The no effect level is less than the RHD, on a mg/m^2 basis.

The relevance of these findings to humans is not known.

2 Drug Information

2.1 Drug

CAS Registry Number	624-49-7
Generic Name	dimethyl fumarate
Code Name	BG00012 BG-12 DiMF FAG-201 Fumarsäuredimethylester (German)
Chemical Name	dimethyl (E)-butenedioate (IUPAC name) dimethyl fumarate *trans*-1,2-Ethylenedicarboxylic acid dimethyl ester (E)-2-Butenedioic acid dimethyl ester fumaric acid, dimethyl ester 2-butenedioic acid, (2*E*)-, dimethyl ester dimethyl (2*E*)-but-2-enedioate
Molecular Formula/Molecular Weight	$C_6H_8O_4$; 144.13
Structure or Biochemical Description	
Pharmacologic Class	Immunomoduator (No identified EPC)

2.2 Relevant INDs, NDAs, BLAs and DMFs

IND 73,061 for Multiple Sclerosis, Relapsing-Remitting, Biogen Idec Inc. (Active)

(b) (4)

2.3 Drug Formulation

BG00012 drug product is formulated (b) (4) in gelatin capsules (120 and 240 mg) for oral administration.

2.4 Comments on Novel Excipients

The excipients used in the drug product formulation are commonly used in oral tablet formulations (b) (4). The excipients used (b) (4) are compliant with USP/NF and/or Ph. Eur. The amount of each excipient in the drug product is below the highest level published in the FDA Inactive Ingredient Search for Approved Drug Products.

2.5 Comments on Impurities/Degradants of Concern

No degradants or impurities were observed to be of concern. The potential formation of (b) (4) during the manufacturing process was discussed with the sponsor during development; monitoring for (b) (4) in the drug was performed on 17 commercial size batches, and (b) (4) was not found at the detection limit of (b) (4).

2.6 Proposed Clinical Population and Dosing Regimen

BG-12 is intended to be a first-line monotherapy for the treatment of relapsing forms of multiple sclerosis (b) (4). The starting dose is 120 mg twice a day orally; after 7 days, patients increase to the recommended dose of 240 mg twice a day orally.

2.7 Regulatory Background

(b) (4)

(b) (4) BG-12 was submitted under IND 73,061 for the treatment of multiple sclerosis on 7/12/06; this IND remains active and is the supporting IND for this NDA.

3 Studies Submitted

3.1 Studies Reviewed (excerpted from sponsor's tables)

			Overview			Test Article: BG00012	
Type of Study	**Species and Strain**	**Method of Administration**	**Duration of Dosing**	**Doses (mg/kg)**	**GLP Compliance**	**Testing Facility**	**Study Number**
Single-Dose Toxicity	NMRI/HAN Bö (SPF)Mice (3 M/3 F/group)	Oral Gavage	Single	0, 316, 681, 1000, 1210, 1470	Yes	(b) (4)	PD05-27
Single-Dose Toxicity	Sprague Dawley/Tif: RAI f (SPF) Crl:CD(SD)IGS BR Rats (3 M/3 F/group)	Oral Gavage	Single	681, 1470, 2150, 2610 (F only), 3160, 4640 (M only), 6810 (M only)	Yes		PD05-25
Genotoxicity	*Salmonella typhimurium* strains; TA 1535, TA 1537, TA 1538, TA 98, TA 100	*In vitro* ±Metabolic activation	*In vitro*	Initial and Confirmatory Assays: -S9: 0, 3.16, 10, 31.6, 100, 316, 1000, 3160 µg/plate +S9: 0, 3.16, 10, 31.6, 100, 316, 1000, 3160, 10000 µg/plate	Yes		5403/89
Genotoxicity[2]	*Salmonella* (TA98, TA100, TA1535, TA1537), *E. coli* strain (WP2*uvr*A)	*In vitro* ±Metabolic activation	*In vitro*	Initial Assay: ±S9: 0, 1.6, 5, 16, 50, 160, 500, 1600, 5000 µg/plate Confirmatory Assay: ±S9: 0, 50, 160, 500, 1600, 3330 5000 µg/plate	Yes		P00012-08-02
Genotoxicity	Chinese hamster cells (V79)	*In vitro* ±Metabolic activation	*In vitro*	Initial Assay: - S9: 0, 0.3, 1, 3, 10, 30 µg/ml +S9: 0, 312.5, 625, 1250, 2500, 5000 µg/ml Confirmatory Assay: -S9: 0, 0.3, 1, 3, 10, 30 µg/ml +S9: 0, 312.5, 625, 1250, 2500, 5000 µg/ml	Yes		5405/89
Genotoxicity	*In vitro* Human peripheral lymphocytes	*In vitro* ±Metabolic activation	*In vitro*	Initial Assay: -S9: 0, 1.56, 3.13, 6.25, 12.5, 25 µg/ml +S9: 0, 12.5, 25, 50, 100 µg/ml Confirmatory Assay: -S9: 0, 3.13, 6.25, 12.5, 25.0 µg/ml +S9: 0, 6.25, 12.5, 25, 50, 100, 150 µg/ml	Yes[3]		5407/89

Genotoxicity	*In vitro* Cultured Human Peripheral Blood Lymphocytes	*In vitro* ±Metabolic activation	*In vitro*	Initial Assay: ±S9: 0, 20.8, 29.7, 42.4, 60.5 µg/mL Confirmatory Assay: -S9: 0, 0.938, 1.88, 3.75, 7.5 µg/mL +S9: 0, 30, 40, 50, 60 µg/mL	Yes	(b) (4)	P00012-04-16
Genotoxicity[2]	*In vitro* Cultured Human Peripheral Blood Lymphocytes	*In vitro* ±Metabolic activation	3 hours 22 hours	Initial Assay -S9: 0, 153, 312, 446 µg/ml +S9: 0, 74.9, 153, 218 µg/ml Confirmatory Assay -S9: 0, 20.2, 28.8, 41.0 µg/mL +S9: 0, 100, 156, 196, 245 µg/mL	Yes		P00012-08-03
Genotoxicity	Male Sprague Dawley Crl:CD(SD)IGS BR Rats	Oral Gavage	Single	0, 250, 500, 1000	Yes		P00012-04-04
Carcinogenicity	CD-1 [Crl:CD-1®(ICR)Br] Mice	, 25, 75, 200, 600/400* Oral Gavage	2 years	0, 25, 75, 200, 600/400 *	Yes		P00012-05-03
Carcinogenicity	Sprague Dawley Crl:CD(SD)IGS BR Rats	Oral Gavage	2 Years	0, 25, 50 ,100, 150	Yes		P00012-04-11
Reproductive Fertility Segment I	Sprague Dawley Crl:CD(SD)IGS BR Rats (treated males and untreated females)	Oral Gavage	70 days + cohabitation	0, 75, 250, 375	Yes		P00012-04-03
Reproductive Fertility Segment I	Sprague Dawley Crl:CD(SD)IGS BR Rats (treated females and untreated males)	Oral Gavage	21 days prior to cohabitation up to DG 7	0, 25, 100, 250	Yes		P00012-10-01
Embryo-fetal Developmental Segment II	Sprague Dawley Crl:CD(SD)IGS BR Rats	Oral Gavage	DG7-DG17	0, 25, 100, 250	Yes		P00012-06-02
Embryo-fetal Developmental Toxicity Segment II	New Zealand White Rabbits	Oral Gavage	DG7-DG19	0, 25, 75, 150	Yes		P00012-06-01
Pre-and Post-natal Developmental Segment III	Sprague Dawley Crl:CD(SD)IGS BR Rats	Oral Gavage	DG7-DL20 or DG24	0, 25, 100, 250	Yes		P00012-09-02
Other Toxicity Studies Investigative	Sprague Dawley Crl:CD(SD)IGS BR Rats	Oral Gavage	14 Days	0, 83 TID, 250 QD, 50 Gentamicin (QD SC)	No		PD08-03
Other Toxicity Studies Investigative	Sprague Dawley Crl:CD(SD)IGS BR Rats	Oral Gavage	75 Days	0, 100	No		P00012-09-01

Other Toxicity Studies Investigative	Sprague Dawley Crl:CD(SD)IGS BR Rats	Oral Gavage	14 Weeks	0, 50 BID, 100 QD, 250 QD	Yes	(b) (4)	P00012-08-01

EXPERT REPORT ON HISTOPATHOLOGICAL RE-EVALUATION OF RAT AND MOUSE KIDNEY FROM CARCINOGENICITY STUDIES WITH ORALLY ADMINISTERED BG00012 (DIMETHYL FUMARATE) Non-GLP, report dated 5/11/12 (and requested individual and comparative data dated 10/20/12, submitted by request)

Studies briefly/previously reviewed (brief review undertaken and/or findings may be summarized in this review):

All pharmacology and ADME studies were briefly reviewed. Other studies were reviewed in brief to provide information on specific issues.

2.6.7.1. Toxicology

			Overview			Test Article: BG00012	
Type of Study	Species and Strain	Method of Administration	Duration of Dosing	Doses (mg/kg)	GLP Compliance	Testing Facility	Study Number
Other Toxicity Studies Investigative	Sprague Dawley Crl:CD(SD)IGS BR Rats	Oral Gavage	14 Weeks	0, 50 BID, 100 QD, 250 QD	Yes	(b) (4)	P00012-08-01

The following local tolerance study using Fumaderm® was also briefly reviewed, as it bears relevance to the irritancy potential of DMF, which has since been documented in scientific and regulatory literature:

Study 6053/90: Examination of Fumaderm in a Skin Sensitization Test in Guinea-Pigs According to Magnusson and Kligman (Maximisation Test)

These studies were briefly reviewed, as they were previously reviewed by (b) (4) Dr. T. Peters under IND 76031:

2.6.7.1. Toxicology

			Overview			Test Article: BG00012	
Type of Study	Species and Strain	Method of Administration	Duration of Dosing	Doses (mg/kg)	GLP Compliance	Testing Facility	Study Number
Repeat-Dose Toxicity	C57BL/6 Mice (6 M/6F/group)	Oral Gavage	28 Days	0, 50, 100, 250, 400	Yes	(b) (4)	P00012-04-02
Repeat-Dose Toxicity	CD-1 [Crl:CD-1®(ICR)Br] Mice (20 M/20 F/group main; 5 M/5 F/group recovery)	Oral Gavage	13 Week	0, 50, 200, 400	Yes		P00012-04-10
Repeat-Dose Toxicity	Sprague Dawley Crl:CD(SD)IGS BR Rats (10 M/10 F/group main; 5 M/5 F/group recovery)	Oral Gavage	90 Days	0, 50 (no recovery), 100, 250, 500	Yes		P00012-04-01

Repeat-Dose Toxicity	Cynomolgus Monkey (4 M/4 F/group main; 2 M/2 F/group recovery)	Oral Nasogastric Intubation	52 weeks	0, 5, 25,75	Yes	(b) (4)	P00012-05-08

3.2 Studies Not Reviewed

Most studies investigating the toxicity of Fumaderm® were not considered relevant, and were not reviewed here. Also, studies using different routes of administration or drug combinations were not reviewed here.

			Overview			Test Article: BG00012	
Type of Study	**Species and Strain**	**Method of Administration**	**Duration of Dosing**	**Doses (mg/kg)**	**GLP Compliance**	**Testing Facility**	**Study Number**
Single-Dose Toxicity	NMRI/HAN Bō (SPF) Mice (3 M/3 F/group)	Intraperitoneal	Single	0, 316, 464, 681, 825, 1000, 1470	Yes	(b) (4)	PD05-24
Single-Dose Toxicity	Sprague Dawley/Tif: RAI f (SPF) Crl:CD(SD)IGS BR Rats (3 M/3 F/group)	Intraperitoneal	Single	316, 681, 825 (F only), 1000, 1470 (M only)	Yes		PD05-26
Other Toxicity Studies Combination Toxicity	Sprague Dawley Crl:CD(SD)IGS BR Rats	Oral Gavage	90 Days	0, 100 BG00012, 0.5 methotrexate, 25 BG00012 + 0.5 methotrexate, 100 BG00012 + 0.5 methotrexate	Yes		P00012-07-02
Other Toxicity Studies Combination Toxicity	Cynomolgus monkeys	Nasogastric Intubation	90 Days	0, 25 BG00012, 0.5 methotrexate, 25 BG00012 + 0.5 methotrexate, 5 BG00012 + 0.5 methotrexate	Yes		P00012-07-01

3.3 Previous Reviews Referenced

Under IND 73,061 for the Treatment of Multiple Sclerosis;
Dr. Peter's review dated 10/1/07

(b) (4)

4 Pharmacology

4.1 Primary Pharmacology

A standard receptor binding assay was not submitted for BG-12. However, a number of immunomodulatory effects have been described in *in vitro* and *in vivo* studies. The exact mechanism by which DMF exerts its effect in the treatment of multiple sclerosis is unclear. Mechanism of action modeling studies indicated that BG-12 is likely to act through inhibition of nuclear factor kappa B (NF-κB) signaling and/or inhibition of cyclin-dependent kinase. *In vitro* studies have shown effects on the Nrf2 transcriptional pathway, the NF-κB-dependent transcription pathway, and kinase signaling pathways.

Effects demonstrated have included reduced cytokine secretion, reduced inflammation, and shifts in Th1 to Th2 T-helper cell responses.

The sponsor focused primarily on effects on the Nrf2 transcriptional pathway. DMF and MMF were shown to alkylate (although other research indicated that succination occurs; see Adam et al., 2011) a key cysteine residue(s) in KEAP 1 (an inhibitory regulator of Nrf2), which allowed Nrf2 to accumulate in the nucleus and go on to activate transcription from antioxidant response element (ARE)-driven promoters. In astrocytes and neuron cultures, DMF and MMF were shown to result in increased levels of active Nrf2, with subsequent upregulation of antioxidant target genes; concentration-dependent increases in cellular redox potential, glutathione, ATP levels, and mitochondrial membrane potential were observed. In studies of LPS-induced inflammation in macrophages and astrocytes, DMF was shown to suppress production of inflammatory cytokines; transcription factor Nrf2 contributed to, but was not required for, the demonstrated DMF-mediated effect on macrophages. *In vitro*, the pharmacodynamic responses of genes NQO1 and AKR1B8 (i.e., Nrf2 target genes) varied by tissue in rats and/or mice (NQO1 induced in lymphoid organs and AKR1B8 induced in GI tissue, but neither showing induction in CNS); these effects were found up to 24 hr post-DMF or MMF (mice only), at doses as low as 15 mg/kg.

Inhibition of NF-κB signaling has also been implicated as a mechanism by which fumaric esters inhibit expression of pro-inflammatory cytokines and adhesion molecules (see Vandermeeren et al., 1997; Loewe et al., 2001). *In vitro* studies in human endothelial cells have shown an inhibitory effect of DMF on NF-κB-dependent transcription of tumor necrosis factor-alpha (TNF-α) induced genes (Loewe et al., 2002). *In vitro* studies in human fibroblasts showed dramatically increased succinate dehydrogenase (SUDH) activity with DMF, and a lesser effect with MMF; it was proposed that the exogenous fumarates could stimulate SUDH-activity and cause accumulation of endogenous fumarate, leading to blockade of the citric acid cycle.

The sponsor briefly described *in vivo* experiments in several animal models; most submitted studies were not conducted in compliance with GLP regulations. In a Brown Norway rat MOG-induced experimental autoimmune encephalomyelitis (EAE) model of multiple sclerosis, DMF showed a dose-dependent reduction in symptoms. The sponsor indicated that the dose-response for efficacy corresponded with doses shown to induce Nrf2 responsive genes in liver and spleen. DMF appeared to decrease activation, as well as alter the morphology of, astrocytes and glia resulting from the administration of MOG. A MOG-induced EAE mouse model of central nervous system demyelination revealed preservation of myelin and axonal density in the plaque following DMF treatment; further mechanistic studies suggested that this resulted from an antioxidative mechanism of action via induction of the transcription factor Nrf2 (Linker et al, 2011).

4.2 Secondary Pharmacology

DMF is a lipophilic α,β-unsaturated ester that has been used both medically and commercially. Medically, DMF has been used to treat psoriasis (e.g., as part of the drug

Fumaderm®) and has also been proposed as a potential treatment for angiogenesis-related malignancies. DMF has been shown to inhibit angiogenesis *in vitro* and *in vivo*; it inhibits differentiation, proliferation and migration of endothelial cells (Garcia-Caballero et al., 2011). Commercially, DMF is also used to inhibit growth of mold, mostly for leather goods; this use has mostly been abandoned after it was found to be an allergic sensitizer at very low concentrations (as low as 1 ppm). Since this discovery, the EU has banned the use of DMF for consumer products (1998) and also the import of products containing DMF (2009) as a biocide.

DMF reacts rapidly with glutathione, by Michael addition. It depletes intracellular glutathione (GSH) by covalent bond formation in a reaction mediated by GSH-S-transferase (Held, 1991).

In vitro, DMF has shown several immunomodulatory properties. It has been proposed that DMF is the active component of fumaric acid ester (FAE) treatment (see Lehmann et al., 2007). DMF and diethylfumarate (DEF), but not fumaric acid, MMF, or ethylhydrogenfumarate, exhibited potent depression of inflammatory cytokine secretion (e.g., tumor necrosis factorα, IL-12, and IFNγ) in activated human peripheral blood mononuclear cells. Moreover, DMF and DEF inhibited T-cell proliferation in mixed leukocyte reaction. Interestingly, these immunosuppressive effects were accompanied by the strong induction of the anti-inflammatory stress protein heme oxygenase 1 (HO-1). Supplementation with exogenous glutathione (GSH), which is known to bind DMF, prevented both HO-1 induction as well as the anti-inflammatory effects of DMF. Inhibition of HO-1 activity restored the diminished IL-12 and IFNγ production after fumaric acid ester (FAE) treatment. These results suggest that DMF acts as an active compound within the FAE mixture, at least in part, by inducing the anti-inflammatory stress protein HO-1 through functional depletion of reduced GSH.

In vivo studies have demonstrated immunomodulatory effects, with some evidence of cytoprotection. In a Sprague-Dawley rat malonate-induced striatal damage model of cytoprotection, reductions in malonate lesion volume and concomitant apomorphine-induced behavioral manifestations (i.e., induced rotational behavior) were seen; neuron-specific staining suggested some evidence of neuronal protection. The sponsor also demonstrated anti-inflammatory effects (i.e., reduced paw swelling, reduced inflammatory infiltration of macrophages, decreased cytokine expression) of DMF in a collagen-induced rat arthritis model, and immunosuppressive effects (some prolongation of allograft survival [nss], compared to [ss] result with calcium MMF salt) in a rat kidney and heart transplant model.

DMF has a serum half-life of approximately 12 minutes, and is metabolized to MMF. MMF, but not DMF, is a potent nicotinic acid receptor agonist. In a transfected (i.e., GPR109, Gqi9 and aequorin) CHO cell assay, MMF induced a dose-dependent Ca^{2+} signal, with an EC_{50} of 9.4 μM. And, MMF completely inhibited forskolin-induced cAMP synthesis, with an IC_{50} of 70 nM. Nicotinic acid receptor GPR109A is expressed in immune cells (esp. neutrophils) and in human skin tissue (i.e., primary epidermal keratinocytes). Similar to nicotinic acid, MMF causes a skin flush, which the author

proposed similarly results from activation of GPR109A in epidermal Langerhans cells (Tang et al., 2008); notably, flushing was observed in a large proportion of subjects in the clinical trials.

4.3 Safety Pharmacology

The studies to address CNS safety pharmacology (i.e., hexobarbitone narcosis [sleeping time], spontaneous motility, nociceptive behavior [writhing test], and reserpine-reduced body temperature) were conducted using Fumaderm. DMF is one component of this drug; however, these studies were considered inadequate to support the safety of DMF (see pre-IND meeting minutes for the meeting held 9/1/05). Therefore, these studies will not be discussed here.

Using DMF and/or MMF, the sponsor conducted tests for cardiovascular safety (2 *in vitro* hERG assays, 2 *in vitro* cardiac action potential assays and an *in vivo* assessment in dogs) and respiratory safety (an *in vivo* assessment in dogs).

DMF

Study PD03-17: Effects of FAG201 on Cloned hERG Channels Expressed in Mammalian Cells ((b) (4) Study Number: 030811.CJP)

GLP, QA (b) (4) Initiated 9/2/03

The *in vitro* effects of FAG201 (i.e., DMF, lot F1177170, 99.6% pure, <0.1% MMF, <0.1% fumaric acid) on ionic currents in voltage-clamped HEK-293 stably expressing the hERG gene were determined (at near physiological temperature, 35 ±2° C). See the sponsor's summary Table 2, below, for concentrations tested and results. An IC_{50} could not be estimated for DMF. The vehicle control was 0.1% DMSO. Under identical experimental conditions, the positive control terfenadine (60 nM) inhibited hERG current by 92.9%. Analysis of the test samples at 60, 180, and 600 μM were outside of the acceptable limits of ±15% nominal; the actual concentrations were approximately 23, 135, and 510 μM. The highest tested concentration which did not significantly inhibit hERG current was found to be within the acceptable range (1500 μM ± 15%, approximately 1275 μM).

Table 2: Summary statistics for FAG201 inhibition of hERG current.
Mean percentage of current inhibited at each FAG201 concentration (Mean), standard deviation (SD), standard error of the mean (SEM), and number of cells (N).

FAG201 Concentration (μM)	Mean	SD	SEM	N
0	0.1%	0.1%	0.0%	4
60	0.3%	0.1%	0.1%	3
180	0.4%	0.2%	0.1%	3
600	0.3%	0.2%	0.1%	3
1500	3.6%	0.4%	0.2%	4

Study PD03-18 ((b) (4) Study 03082O.CJP): Effects of FAG201 on Action Potentials in Isolated Canine Cardiac Purkinje Fibers
GLP, QA (b) (4) Initiated 9/2/03

The *in vitro* effects of FAG201 (i.e., DMF, (b) (4) lot F1177170, 99.6% pure, < 0.1% MMF or fumaric acid) on cardiac action potentials in isolated canine Purkinje fibers (taken from 2 male beagle dogs aged 36 weeks; kept at 37 ± 1°C) were determined. The vehicle control was Tyrode's solution with 0.1% DMSO. Three concentrations of DMF (60, 600, and 1500 μM) were added sequentially to four fiber preparations at three stimulus intervals (basic cycle lengths of 2, 1, and 0.5 s). DMF did not induce a concentration-related, statistically significant prolongation of the action potential duration or the resting membrane potential (see sponsor's summary for DMF in Table 1 and for vehicle in Table 2, below). DMF significantly reduced action potential amplitude (less than 10%) and maximum rate of depolarization (~30%). By contrast, the positive control 100 μM sotalol produced significantly greater prolongation of the APD_{60} and APD_{90} at all stimulus intervals (approximately 20-40%). However, the formulation analysis indicated that nearly all of the DMF samples taken at the start of the study (with the exception of 3 of 4 samples of the 1500 μM reservoir and/or perfusate samples on the first day and the perfusate samples of 1500 μM on the second day) were outside the specification of ±15% of nominal (range -36% to -40%, -20% to -28%, and -7 to -19% at 60, 600, and 1500 μM, respectively). Sixty-day stability samples indicated that DMF formulations were highly unstable (concentrations were 50-60% less than nominal), with increases in MMF concentrations. Although a clear, concentration-dependent effect on action potential duration was not demonstrated for DMF, the results of the study are unreliable due to the formulation issues. Notably, DMF did appear to evoke a concentration-independent reduction of the action potential amplitude and rate of depolarization, which suggests an effect on sodium channels.

Table 1. Summary of the Effects of FAG201 on Action Potential Parameters at 2 s, 1s and 0.5s BCL's.

2 s BCL					
Concentration	APD_{60}, Δ%	APD_{90}, Δ%	RMP, ΔmV	APA, ΔmV	Vmax, Δ%
	Mean± S.E.M	Mean± S.E.M	Mean± S.E.M	Mean± S.E.M	Mean± S.E.M
60 μM	2.2 ± 2.0	2.0 ± 1.5	1.8 ± 2.8	-10.0 ± 4.4	-29.8* ± 10.1
600 μM	-0.9 ± 1.9	-0.6 ± 1.8	-2.6 ± 1.2	-7.8* ± 3.4	-29.2 ± 13.4
1500 μM	-2.1 ± 2.3	0.8 ± 2.3	0.7 ± 0.8	-9.6* ± 4.0	-35.1* ± 13.1

1s BCL					
Concentration	APD_{60}, Δ%	APD_{90}, Δ%	RMP, ΔmV	APA, ΔmV	Vmax, Δ%
	Mean± S.E.M	Mean± S.E.M	Mean± S.E.M	Mean± S.E.M	Mean± S.E.M
60 μM	2.1 ± 1.5	1.7 ± 1.5	0.6 ± 2.0	-6.5 ± 3.7	-24.7 ± 12.1
600 μM	0.6 ± 1.3	0.9 ± 1.4	-1.3 ± 0.7	-7.0 ± 3.3	-27.5* ± 13.4
1500 μM	-1.3 ± 2.5	1.4± 2.7	1.2 ± 1.0	-8.8 ± 3.9	-31.0 ± 14.1

0.5 s BCL					
Concentration	APD_{60}, Δ%	APD_{90}, Δ%	RMP, ΔmV	APA, ΔmV	Vmax, Δ%
	Mean± S.E.M	Mean± S.E.M	Mean± S.E.M	Mean± S.E.M	Mean±
60 μM	2.7 ± 1.1	2.7* ± 1.0	0.1 ± 1.5	-6.0 ± 2.9	-21.2 ± 10.8
600 μM	0.5 ± 0.7	1.0 ± 1.1	-0.9 ± 0.5	-7.4 ± 2.8	-29.2* ±
1500 μM	-1.7 ± 1.8	1.7 ± 2.3	1.6 ± 1.1	-8.9* ± 3.5	-30.6* ±

BCL, Basic Cycle Length; APD_{60} and APD_{90}, action potential duration measured at 60% and 90% repolarization; Δ%, Percent change from baseline values; ΔmV, absolute change from baseline in millivolts; RMP, resting membrane potential; APA, action potential amplitude; Vmax, maximum rate of depolarization. * Denotes statistical significance ($p<0.05$) when compared to time-matched vehicle control sequence.

Table 2. Summary of the Effects of Vehicle control on Action Potential Parameters at 2 s, 1s and 0.5s BCL's.

Vehicle Control Sequence	2 s BCL				
	APD_{60}, Δ%	APD_{90}, Δ%	RMP, ΔmV	APA, ΔmV	Vmax, Δ%
	Mean± S.E.M	Mean± S.E.M	Mean± S.E.M	Mean± S.E.M	Mean± S.E.M
1	-0.3 ± 1.4	-1.0± 1.1	-1.1 ± 0.4	1.1 ± 1.8	1.0 ± 5.6
2	0.5 ± 2.6	-0.3 ± 1.9	-0.6 ± 1.3	2.1 ± 1.2	4.6 ± 4.7
3	0.7 ± 2.4	0.9 ± 2.1	-0.5 ± 0.8	0.6 ± 0.4	-0.4 ± 4.1

Vehicle Control Sequence	1 s BCL				
	APD_{60}, Δ%	APD_{90}, Δ%	RMP, ΔmV	APA, ΔmV	Vmax, Δ%
	Mean± S.E.M	Mean± S.E.M	Mean± S.E.M	Mean± S.E.M	Mean± S.E.M
1	0.0 ± 1.3	-0.5 ± 1.0	-0.4 ± 0.8	1.1 ± 1.1	2.0 ± 5.2
2	0.4 ± 1.8	-0.4 ± 1.3	0.4 ± 0.7	1.0 ± 1.5	7.7 ± 3.7
3	-0.9 ± 1.5	-0.2 ± 1.5	-0.9 ± 0.6	0.4 ± 0.4	0.4 ± 4.8

Vehicle Control Sequence	0.5 s BCL				
	APD_{60}, Δ%	APD_{90}, Δ%	RMP, ΔmV	APA, ΔmV	Vmax, Δ%
	Mean± S.E.M	Mean± S.E.M	Mean± S.E.M	Mean± S.E.M	Mean± S.E.M
1	0.6 ± 0.9	-0.2 ± 0.5	-1.5 ± 1.6	1.0 ± 1.1	1.6 ± 3.6
2	0.5 ± 1.4	-0.2 ± 1.1	-0.1 ± 1.1	0.4 ± 1.7	6.6 ± 2.7
3	0.3 ± 1.4	0.0 ± 1.4	-0.8 ± 0.4	0.7 ± 0.7	2.0 ± 3.8

BCL, Basic Cycle Length; APD_{60} and APD_{90}, action potential duration measured at 60% and 90% repolarization; Δ%, Percent change from baseline values; ΔmV, absolute change from baseline in millivolts; RMP, resting membrane potential; APA, action potential amplitude; Vmax, maximum rate of depolarization.

Study PD03-19 ((b) (4) Study EBAW-0146): A Safety Pharmacology Study to Assess Potential Cardiovascular and Respiratory Effects of FAG201 Administered Orally to Beagle Dogs

GLP, QA (b) (4) Initiated 8/28/03

The effects of FAG201 (DMF, Lot F1177170, 99.6% pure, <0.1% MMF or fumaric acid) on hemodynamic parameters, respiratory parameters, and electrocardiographic activity were investigated in conscious telemetered Beagle dogs (4 male Beagle dogs, (b) (4)). DMF or vehicle (0.8% hydroxypropyl methylcellulose) was administered sequentially once per day (see sponsor's Text Table 1, below). After dosing, animals were monitored via radiotelemetry for 24 hours (see ECG tracing info below). Convenient summary data tables were not provided.

Text Table 1
Study Design

Session Number	Number of Animals	Test Article	Dosage Level (mg/kg)	Dosage Volume (mL/kg)	Dosing Regimen	Route	Last Day on Test
1	4	FAG201	0	10	Day 1	PO	Day 13
2			10		Day 6		
3			100		Day 8		
4			1000		Day 12		

Time Duration	Time Point Evaluated/Reported (Approximate)
Baseline = 24 hours	Every 4 hours (6 tracings)
Treatment to 6 hours posttreatment	Every 30 minutes (12 tracings)
12 hours posttreatment to 24 hours posttreatment	At 12 and 24 hours posttreatment (2 tracings)

DMF resulted in vomiting at 100 and 1000 mg/kg (at 8 min to ~2 hr postdose). Effects on heart rate and arterial blood pressure were observed from approximately 3 to 9 or 12 hours postdose. Compared to vehicle-treated, heart rates in the three DMF-treated groups were increased from approximately 3 hours post dose until approximately 9 to 12 hours post dose. Mean arterial blood pressure at 100 and 1000 mg/kg DMF was decreased approximately 12% at 3 hours postdose (and showed some reduction at 10 mg/kg at 6 hr postdose), and remained reduced 10-20% through 13 to 15 hours postdose. See sponsor's Figures 1 and 2, below. The timing of the heart rate and blood pressure effects was similar, although a clear dose relationship was not observed (the sponsor suggested that it may have been prevented by the vomiting observed at the two highest dose levels). Sinus tachycardia was noted in two animals, second degree AV block was noted in one animal, and ventricular premature complexes were noted in one animal; however, the sponsor did not consider the conduction disturbance and arrhythmias related to the administration of DMF. DMF did not cause QTc prolongation (QTc [Fridericia] values ranged from -3.1% to +5.8% of baseline). Effects on respiratory rate, peak thoracic pressure, body temperature, arterial blood gases, and electrocardiogram parameters were not observed. Dose-related increases in plasma DMF exposures were observed but were not dose-proportional (see sponsor's Table 1, below). Formulation analysis indicated that all doses were within ±15% of nominal.

Figure 1
Heart Rate (beats per minute) – Group means of actual values

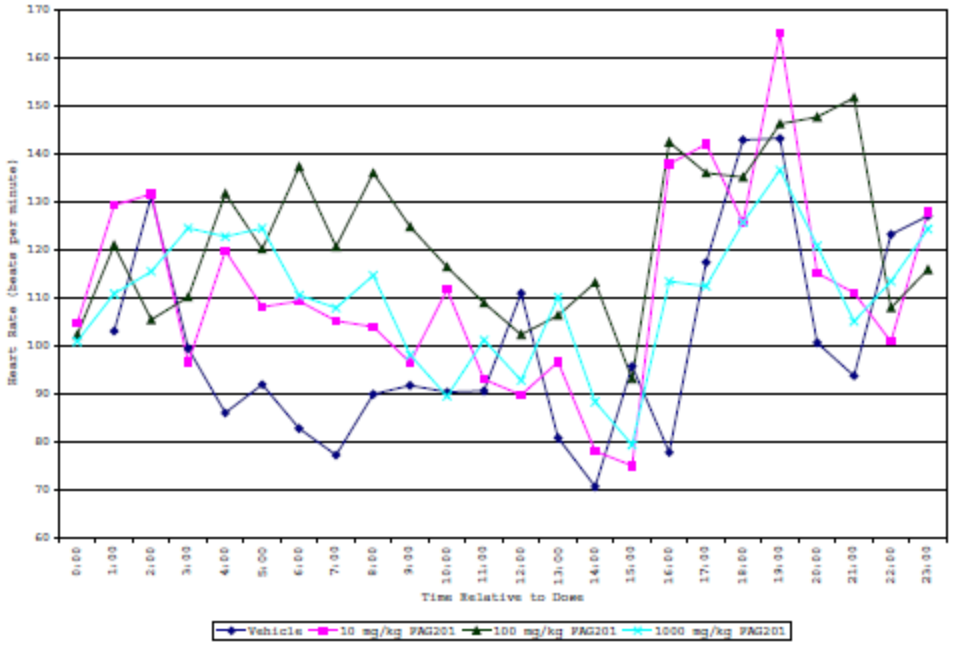


Figure 2
Mean Arterial Pressure (mmHg) – Group means of actual values

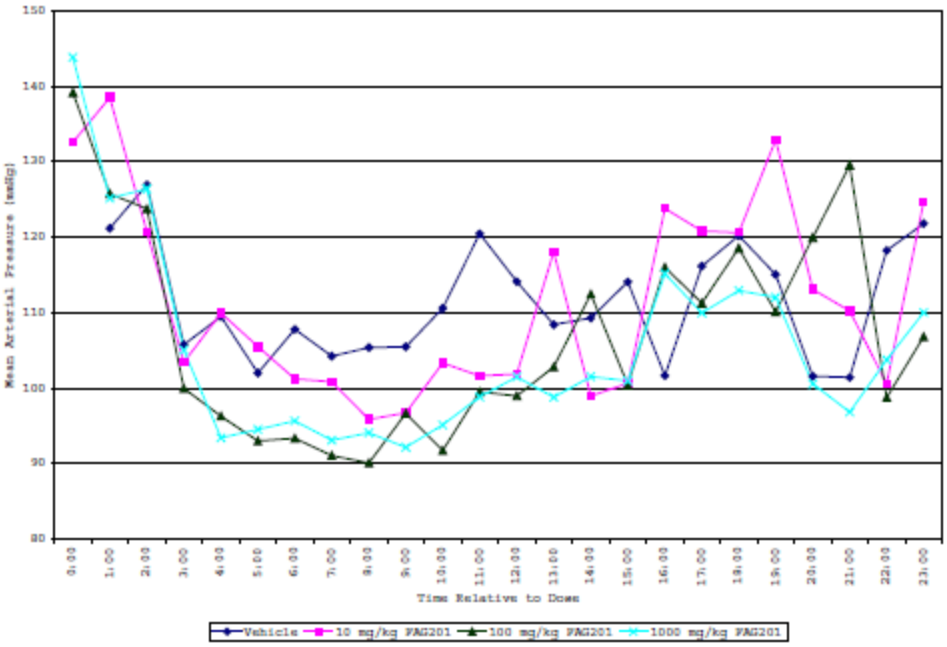


Table 1. Toxicokinetic parameters for FAG201 exposure in dogs after escalation of doses over 12 days.

Dose	10 mg/kg			100 mg/kg			1000 mg/kg		
Subject	AUClast	Cmax	Tmax	AUClast	Cmax	Tmax	AUClast	Cmax	Tmax
1001	4546	3060	1	52298	35300	0.33	53570	42600	1
1002	9127	5990	0.33	38244	26000	0.33	50094	45300	0.33
1003	6577	5480	0.33	47640	30400	0.33	56705	41700	0.33
1004	7930	5630	0.33	56061	36200	0.33	71916	61300	0.33
N	4	4	4	4	4	4	4	4	4
Mean	7045	5040	0.50	48561	31975	0.33	58071	47725	0.50
S.D.	1965	1337	0.34	7692	4729	0	9617	9178	0.34
% CV	27.9	26.5	67.3	15.8	14.8	0.0	16.6	19.2	67.3

MMF

Study PD03-21 ((b) (4) Study 030828.CJP): Effects of Methyl Hydrogen Fumarate on Cloned hERG Channels Expressed in Mammalian Cells

GLP, QA (b) (4) Initiated 9/12/03

The *in vitro* effects of methyl hydrogen fumarate (b) (4) lot EF386206, 100.1% pure) on ionic currents in voltage-clamped HEK-293 cells stably expressing the hERG gene were determined at near-physiological temperature (i.e., 35 ±2° C). The vehicle control was 0.1% DMSO. MHF (i.e., MMF) did not inhibit the hERG current at concentrations up to 1500 μM. Under identical experimental conditions, positive controls E-4031 (500 nM) and terfenadine (60 nM) inhibited hERG current by 100% and 86.1 ± 2.9%, respectively. Test concentration samples were within the acceptable limits of ±15% of nominal, with one exception at 1500 μM (-82%); however, only one of two samples of that concentration was outside of acceptable limits (see sponsor's table below.)

Table 2: Summary statistics for methyl hydrogen fumarate inhibition of hERG current.

Mean percentage of current inhibited at each methyl hydrogen fumarate concentration (Mean), standard deviation (SD), standard error of the mean (SEM), and number of cells (N).

Methyl hydrogen fumarate Concentration (μM)	Mean	SD	SEM	N
0	0.2%	2.2%	1.3%	3
60	1.0%	0.9%	0.5%	3
180	0.5%	0.6%	0.3%	3
600	0.1%	0.6%	0.4%	3
1500	1.2%	1.4%	0.8%	3

Study PD03-22 (b) (4) Study 030829.CJP): Effects of Methyl Hydrogen Fumarate on Action Potentials in Isolated Canine Cardiac Purkinje Fibers

GLP, QA (b) (4) Initiated 9/12/03

The *in vitro* effects of MMF [redacted] (b) (4) lot EF386206, 100% pure) on cardiac action potentials in isolated canine Purkinje fibers (from 2 male Beagle dogs, 37 weeks of age, [redacted] (b) (4)) were determined. The vehicle control was PFT with 0.1% DMSO. See sponsor's Figure 8.4.1 for the stimulus and solution application schedule. The effects of MMF on action potential parameters were compared to time-matched vehicle control sequences for statistical significance. MMF did not induce statistically significant prolongation of APD_{90} and APD_{60} at three stimulus intervals, or statistically significant changes in resting membrane potential, action potential amplitude, or the maximum rate of depolarization (V_{max}). By contrast, the positive control (sotalol, 100 pM) produced significantly greater prolongation of the APD_{60} and APD_{90} at all stimulus intervals. The formulation concentrations were within ±15% of nominal.

8.4.1 Stimulus and solution application schedule

Solution	Approximate Duration (minutes)	Measurement
Vehicle Control Solution	25	Stabilization
Vehicle Control Solution	20	APD rate dependence (BCL=2,1, 0.5 s)
60 µM Methyl Hydrogen Fumarate	20	Equilibration 60 µM Methyl Hydrogen Fumarate
60 µM Methyl Hydrogen Fumarate	3	APD rate dependence (BCL=2, 1, 0.5s)
600 µM Methyl Hydrogen Fumarate	20	Equilibration 600 µM Methyl Hydrogen Fumarate
600 µM Methyl Hydrogen Fumarate	3	APD rate dependence (BCL=2,1, 0.5 s)
1500 µM Methyl Hydrogen Fumarate	20	Equilibration with 1500 µM Methyl Hydrogen Fumarate
1500 µM Methyl Hydrogen Fumarate	3	APD rate dependence (BCL=2,1, 0.5 s)

5 Pharmacokinetics/ADME/Toxicokinetics

5.1 PK/ADME

The sponsor conducted a few studies of the ADME of DMF, MMF, and/or the Fumaderm-forte compound. For the purposes of this review, the data on Fumaderm-forte will not be considered.

Following ingestion, DMF is rapidly absorbed from the GI tract and believed to be pre-systemically hydrolyzed to MMF (its "primary metabolite"), which is further metabolized in the citric acid cycle. In actuality, MMF does not appear to circulate in amounts large enough to be considered a major human metabolite. In humans, the plasma circulating metabolites included glucose (~60%), fumaric and citric acids (~30%), unknowns (~7%) and MMF (5%). The sponsor stated that DMF has a short half-life, but the half-life of MMF is up to approximately 36 hours. The metabolism of DMF is mediated by high

capacity esterases and enzymes involved in the TCA cycle. DMF and MMF are rapidly metabolized and are primarily eliminated in expired air; generally, renal elimination plays a minor role.

Following a single oral dose administration of DMF (16.7 mg/kg) in SD rats, fumaric acid (the sponsor indicated no substance-related rise in physiological range could be detected) and MMF (T_{max} at 0.25 hr and $t_{1/2}$ ~10 min) but no DMF could be detected in plasma from 0.25- 4 hr postdose. In another study, a single oral administration of 100 mg/kg MMF (as MMF and MMF-calcium salt) administered to SD rats was well absorbed and yielded a C_{max} of ~70-80 μg/mL and $t_{1/2}$ of 0.25-1.3 hr; at 15 minutes postdose, skin showed a relatively high MMF concentration but MMF could not be detected in liver or kidney. An SD rat study using single oral dose 1000 mg/kg MMF-calcium salt found that bone marrow MMF exposure at 15 minutes postdose was similar to that of skin; bone marrow tissue half-life was ~60 minutes. Little unmetabolized MMF was excreted in urine (<15%), and no MMF was found in feces. Following a single oral dose of 16.7 mg/kg DMF in beagle dogs, fumaric acid (the sponsor indicated no substance-related rise in physiological range could be detected) and MMF (T_{max} at 2 hr and $t_{1/2}$ ~85 min), but no DMF, could be detected in plasma from 0.25 to 96 hr postdose.

The sponsor provided two radiolabeled-DMF ADME studies in Long Evans rats (one dated 1987, the other 2008). The radiolabel was placed on the two central carbon atoms for both studies. Following a single oral dose of 10.3 mg/kg, systemic bioavailability was reported as ~120%, radioactivity peaked at ~0.5 hr (1^{st} sampling interval) and rapidly declined over 2 hr; widespread distribution was observed by 96 hr postdose. The terminal elimination half-life was approximately 40 hr. The highest concentrations of radioactivity were observed in liver, kidney, and adrenal gland. The recovery of radioactivity was greatest in the residual carcass and liver. The majority of the radioactivity (~80%) had been eliminated within 24 hr, with almost 50% being eliminated in expired air within 4 hr. Within the 96 hr period, ~98% was recovered, including: expired air (60-70%), urine (~20%), tissues (~5%), and feces (<5%). In the more recent study, a single oral dose of 10 mg/kg was administered and the findings were generally similar. The radioactivity peaked at the first sampling time (0.5 hr), and the half-lives in blood and plasma were ~110 and ~40 hr, respectively; retention of radioactivity in the cellular fraction of the blood was observed. Although the tubes contained sodium fluoride to inhibit esterases, MMF was BLOQ at all but the first timepoint. Radiolabeled-DMF was readily absorbed and the radioactivity was widely distributed within 72 hr, with concentrations highest in the kidney, stomach, liver, pancreas, brain, small intestine, and salivary glands. Tissue concentrations were maximal at the first sampling time, except for reproductive fat. Selective melanin binding was not observed. Over the 168-hr study period, ~60% was recovered in expired air (~30% within the first 2 hr), ~20% was recovered in urine, ~10% was left in the carcass, and <5% was recovered from feces. Labeled-DMF was extensively metabolized. In rat plasma, the circulating metabolites included: glucose (~50%), fumaric acid (~30%), citric acid (35%), and MMF (<1%). Cysteine or N-acetylcysteine conjugates of monomethyl- and dimethyl- succinate were the major radioactive components in urine (representing ~10% of the administered dose).

The sponsor also provided some information on [redacted] formulations of DMF in dogs. Oral administration of DMF to dogs was complicated by frequent emesis. Generally, DMF concentrations were reported to be BLOQ, so PK analysis was based on MMF. Plasma analyses were conducted by Biogen Idec labs. One study examined the effects of administering a single 5 mg/kg DMF dose to specific regions of the GI tract (i.e., duodenum, jejunum, ileum, or colon). Observed clinical signs included redness of the ears, neck, and eyes for approximately 2 hr. Generally, the absorption of MMF was relatively consistent across the intestinal regions, although there was a trend towards higher exposures (1.3-1.5x) in the duodenum and jejunum. Another study examined the PK profiles of 6 different formulations, with and without [redacted], of DMF in dogs; an [redacted] formulation was necessary to reduce GI side effects. Finally, the sponsor conducted a study to determine the PK and tolerability of the [redacted] commercial formulation of BG-12 in dogs; the sponsor's summary Table 1, below, gives the summary PK data following a single administration. Interpretation of the data was again limited by emesis observed in dogs; itching and redness of the skin (e.g., ears, neck; generally lasting ~2 hr) were also observed.

Table 1: Individual and Mean (± standard deviation) BG12 Pharmacokinetic Parameter Estimates Following a Single Oral Dose to Male Beagle Dogs.

Dose (mg/kg)	AUC_{last} (hr*µg/mL)	Cl/F (mL/hr)	$t_{½}$ (hr)	C_{max} (µg)	T_{max} (hr)	V_z/F (mL/kg)
5	4.86	1030	0.639	2.79	0.5	947
5	6.53	764	0.601	2.42	2.5	663
5	6.54	764	0.73	5.81	1	805
5	5.74	868	0.734	2.85	2.5	921
Average	**5.92 ± 0.798**	**856 ± 124**	**0.676 ± 0.067**	**3.47 ± 1.57**	**1.63 ± 1.03**	**834 ± 129**
50	68.0	734	0.690	33.3	1.5	730
50	42.2	1170	0.590	12.6	1	995
50	43.9	886	2.73	14.4	2.5	3490
50	32.0	1490	0.805	17.9	1	1730
Average	**46.5 ± 15.3**	**1070 ± 334**	**1.20 ± 1.02**	**19.6 ± 9.43**	**1.50 ± 0.707**	**1740 ± 1250**
75	54.7	1370	1.17	41.1	1	2310
75	46.2	1620	0.753	27600	1	1760
Average	**50.5 ± 6.01**	**1490 ± 179**	**0.962 ± 0.295**	**34.4 ± 9.56**	**1.00 ± 0.00**	**2040 ± 389**
100*	73.3	1360	0.990	32.2	1	1930
100*	118	839	0.680	45.4	3.5	824
Average	**95.7 ± 31.6**	**1100 ± 365**	**0.834 ± 0.217**	**38.8 ± 9.33**	**2.25 ± 1.77**	**1380 ± 782**

Averages represent mean ± standard deviation

*In the 100 mg/kg dose groups, one dog vomited [redacted] (645 mg) at approximately 45 min post dose, and the other dog vomited [redacted] (114 mg) followed by a second episode [redacted] 90 mg) at approximately 3 hrs post dose.

Generally, the toxicokinetic analyses for MMF in the pivotal chronic toxicity and carcinogenicity studies were conducted under GLP by validated methods (usually protein precipitation extraction, HPLC-MS). One exception to this is the 11-month study

in dog (not a validated method). A validated method was also used for plasma MMF levels in the rabbit reproductive (embryofetal) study. In most cases, the calibration range for MMF was 50- 5000 ng/mL. When measured, methods utilized for the detection of formic acid and/or methanol in plasma were non-GLP. Even under circumstances of validated methodology, there appeared to be issues (e.g., repeated assays, failed runs) with the plasma sample analyses.

6 General Toxicology

6.1 Single-Dose Toxicity

The sponsor conducted GLP acute oral toxicity studies via PO and IP routes in rats and mice (the IP studies will not be discussed here).

In rats (SD; mostly 3/sex/group), the maximum nonlethal dose was 2150 mg/kg (minimal lethal dose was 2610 mg/kg and 3160 mg/kg in females and males, respectively). Mortalities occurred within 24-48 hr (these animals showed reddened gastric mucosa); surviving animals recovered within 24 hrs after dosing (no pathological findings). At 1470 mg/kg, reduced food consumption was observed. At 2150 mg/kg, weight gain was decreased. At 2610 mg/kg (F only), ataxia, muscular hypotonia, and reduced mobility and respiratory rate were observed. The histopathology observed included hemorrhagic erosions in the stomach, engorgement of the mucosa in the forestomach, hemorrhages into the mucosa, and epithelial desquamation with edema at 3160 mg/kg, and minimal congestion of the forestomach at 6810 mg/kg.

In mice (NMRI; 3/sex/group), the LD_{50} (24 hr/14 d) was 1200 mg/kg in males and 1340 mg/kg in females. Death occurred within 90 min to 24 hr; surviving animals recovered within 2 days after dosing (without pathological findings). The lowest toxic dose was 681 mg/kg in males and females, based on clinical signs of reduced mobility, ataxia, dyspnea, muscular hypotonia, and cyanosis. At 1210 (and/or 1000, not clear from report) mg/kg, tremor and abdominal position were also observed. At 1470 mg/kg, Straub's phenomenon (seizure-related behavior) was also observed. Partially pale liver, pale kidneys, fine-grain structured surface of the kidney, thin-walled stomach with hemorrhagic foci or reddened/ severely reddened gastric mucosa, and bloody intestinal contents were detected in animals that died. At 1470 mg/kg, minimal congestion in the stomach was observed histologically.

6.2 Repeat-Dose Toxicity

The sponsor conducted repeated dose toxicity studies in mice, rats, dogs, and monkeys. The longest duration toxicity studies will be reviewed and/or summarized here. Shorter duration studies are summarized to support the maximum dose used in the longer studies (as necessary) and/or to describe findings of importance observed at doses not tested in the longer duration studies.

Rodent

Although the sponsor conducted two 3-month studies in rats, one study (19416-005, in CD rats) was not listed in the toxicology tabulated summary and was not discussed further by the sponsor. Upon brief review, the primary purpose of this study appeared to be testing 2 different vehicles (3% cornstarch suspension and 0.8% aqueous HPMC). It tested DMF oral doses of 50 and 250 mg/kg in male and female Sprague Dawley rats (10/sex/gp, 5/sex/gp recovery), and showed generally similar toxicities as the pivotal 3-month study. A transient (week 1 only) reduction in food consumption was observed at 250 mg/kg. Decreased body weights were observed at 250 mg/kg in males (up to 13%, compared to controls), reticulocytes (up to 80%) and leukocytes were increased (HDM), and creatinine was mildly decreased (6-16%) in males and females; generally, these changes were not fully recovered after four weeks. Heart (7-15%, M), kidney (~10-50%), liver (~10-30%), and stomach (20-160%) weights were increased in treated males and/or females; increased stomach weights did not fully recover. Clear dose-related toxicity was observed in the nonglandular stomach (hyperplasia, hyperkeratosis, ulcer, inflammation, and subepithelial granulation) stomach, but glandular stomach alterations (minimal-moderate glandular ectasia, as well as low incidence ulceration and erosion) also occurred. The pathologist described nonglandular stomach "epithelial hypertrophy … of a papilloma-like structure..." Inflammation was observed in several organs, and was associated with minimal-mild lymphoid hyperplasia in the spleen, lymph nodes and GALT of the intestines. In the kidney, minimal fatty infiltration of the tubular epithelial cells, hydronephrosis, basophilic tubular cells and hyaline tubular casts were described and discounted as incidental findings.

The pivotal 3-month toxicity study (Study P00012-04-01; EBAW-0154) in Sprague-Dawley rats with a 4-week recovery period was reviewed by Dr. See (see review dated 10/15/04). DMF oral doses of 0, 50, 100 and 250 mg/kg (an initial high dose group of 500 mg/kg was terminated on D8) in male and female Sprague Dawley rats (10/sex/gp; 5/sex control and 250 mg/kg recovery). No drug-related mortalities were reported (note that the original HD was terminated early). Salivation was observed. Average mean body weight reductions of >20% were observed at HD. RBC parameters appeared to show slight reductions at HD. Stomach (up to ~2x), kidney (up to ~50%) and liver (up to ~30%) weights were increased at MD and HD. Target organs included stomach (nonglandular and glandular), pancreas, kidney, thymus and lymph nodes. The eye and skin were potential targets. Clearly dose-related alterations were observed in the nonglandular stomach (e.g., hyperkeratosis, inflammation, squamous hyperplasia, and squamous carcinoma) in all treated groups. Notably, squamous cell carcinoma of the nonglandular stomach was observed in 2 HD animals (1/sex; 6.7%); the pathologist described these as, "focal areas of squamous cell carcinoma, which infiltrated the submucosa and was typically associated with a scirrhous response." Additionally, rats that received 500 mg/kg for 8 days showed necrotizing, ulcerative gastritis of the nonglandular mucosa, "frequently" with similar findings present in the glandular mucosa near the junction of the nonglandular and glandular portions. The pathologist described these findings as, "gastritis consisted of areas of mucosal necrosis with abundant infiltrates of neutrophils with variable proliferation of reactive fibrovascular tissue within the submucosa (i.e., granulation tissue). In the pancreas, a dose-related increased

incidence and/or severity of acinar epithelial cell apoptosis and vacuolization and/or pancreatic heterophagia was observed in treated groups. In the kidney, dose-related (all treated groups) minimal-mild tubular basophilia and dilatation was observed in males, as was mineralization in females and proteinosis in males and females. Transcript profiling of kidney samples was also performed. Genes upregulated in association with DMF treatment in both genders of rats included the glutathione-S-transferase mu 1 Yc subunit, glutathione peroxidase, and NADPH dehydrogenase. These are consistent with the expected pharmacology of DMF to induce Phase II metabolic enzymes. Alterations in expression of genes related to kidney injury and tubular regeneration were also identified: Kim-1 was upregulated in both males and females, while clusterin was induced only in male rats. Decreased expression of cytochrome P450 2C and 2B was observed in males and females; since testosterone levels affect the expression of clusterin and cytochrome P450 2C, the sponsor believed that alterations in their expression may reflect changes in testosterone levels. Minimal-mild thymic atrophy was observed in many rats but was clearly increased in HD animals. Lymphoid hyperplasia was observed at the HD, and plasmacytosis was observed in a few HDF. Although of unclear importance, retinal atrophy was observed in 1 HDM and retinal dysplasia in 1 MDF. An undifferentiated sarcoma was observed in 1 HDM.

Study title: A 6-Month Oral (Gavage) Toxicity Study of Dimethyl Fumarate in CD®IGS Rats

Study no.:	P00012-04-06, CRO Study EBA00016
Study report location:	EDR
Conducting laboratory and location:	(b) (4)
Date of study initiation:	8/16/04
GLP compliance:	Yes, pg. 2 (except TK and drug characterization)
QA statement:	Yes, pgs. 3-4
Drug, lot #, and % purity:	DMF (BG00012), Lot Nos. F1177170 and Lot No. 1102642 33004998, 99.6% and 100.2% pure respectively (MMF < 0.1% or 0.02%, respectively)

KEY FINDINGS

- No NOAEL in males or females due to changes in the forestomach and kidney
- Target organs included: stomach, kidney, and liver in males and females

Methods (see the sponsor's summary table, below)

Frequency of dosing: QD for 26 weeks, then no dosing for a-4 week recovery period

Route of administration: PO, gavage

Species/Strain: Sprague Dawley Crl:CD®(SD)IGSBR rats [redacted] (b) (4)

Age: Approximately 8 weeks

Weight: 266-344 g (M); 157-235 g (F)

Deviation from study protocol: Dosing was not performed on 2 days due to inclement weather (12/23/04 and 1/6/05).

Group	No. of Animals (Recovery) [Satellite TK] Male	Female	Test Material	Dose Level (mg/kg/day)	Concentration of Dosing Solution (mg/mL)	Dosing Volume (mL/kg)
1	15 (5) [8]	15 (5) [8]	Vehicle	0	0	10
2	15 (5) [8]	15 (5) [8]	BG00012	25	2.5	10
3	15 (5) [8]	15 (5) [8]	BG00012	100	10	10
4	15 (5) [8]	15 (5) [8]	BG00012	200	20	10
Note: The vehicle utilized was 0.8% HPMC in RODI water.						

Observations and Results

Mortality [2x/day]

There were no drug-related deaths. One MDM died on D91; the sponsor attributed the death to an interim bleeding for evaluation of clinical pathology parameters. Also, one recovery HDF was found dead on D197; the sponsor attributed this death to a urinary tract disorder.

Clinical Signs [daily, detailed exams weekly]

Drug-related clinical signs included salivation (prior to dosing and post-dose), hairloss, scab(s), tail enlargement, ocular discharge, and red material around the mouth or eyes. Salivation was the only frequent finding. Salivation prior to dosing was observed at the HD; struggling was sometimes observed. Dose-related increases in the incidence and frequency of post-dose salivation were observed in MD and HD animals. Few signs were still evident during the recovery period. See excerpts from the sponsor's summary tables for details.

MALES SUMMARY OF SURVIVAL AND CLINICAL OBSERVATIONS (FREQUENCY/ANIMALS)

GROUP:	1	2	3	4
LEVEL: MG/KG/DAY	0	25	100	200
DAY 0 to 183				
ACCIDENTAL DEATH	0/ 0	0/ 0	1/ 1	0/ 0
BODY				
HAIRLOSS	15/ 2	23/ 2	1/ 1	71/ 5
SCAB(S)	4/ 2	17/ 2	26/ 8	5/ 4
OTHER				
SALIVATION PRIOR TO DOSING	0/ 0	0/ 0	0/ 0	13/10
STRUGGLED DURING DOSING	0/ 0	1/ 1	1/ 1	1/ 1

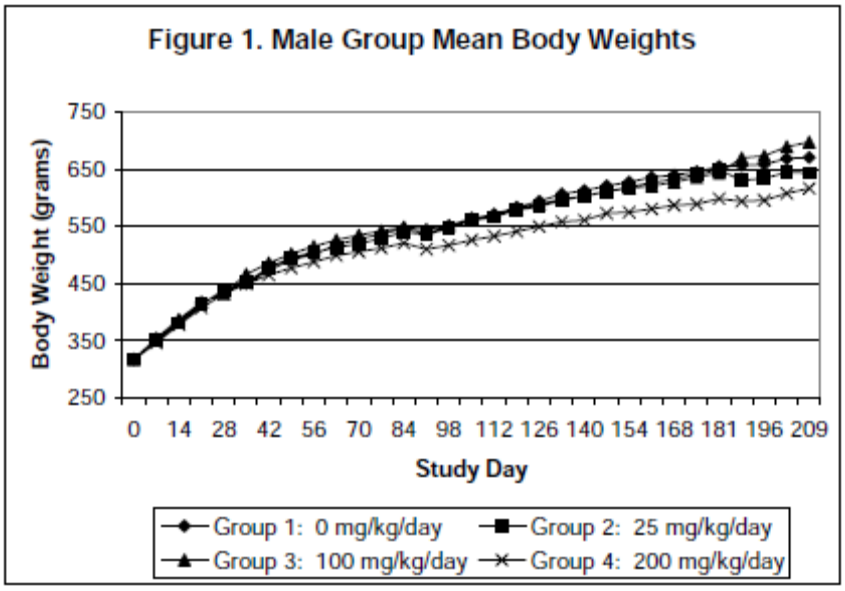
POST-DOSE OBS				
SALIVATION	0/ 0	0/ 0	63/11	137/15
RED MATERIAL AROUND MOUTH	0/ 0	0/ 0	1/ 1	2/ 1
DAY 184 to 210				
BODY				
HAIRLOSS	0/ 0	0/ 0	0/ 0	5/ 1
SCAB(S)	0/ 0	1/ 1	5/ 2	2/ 1

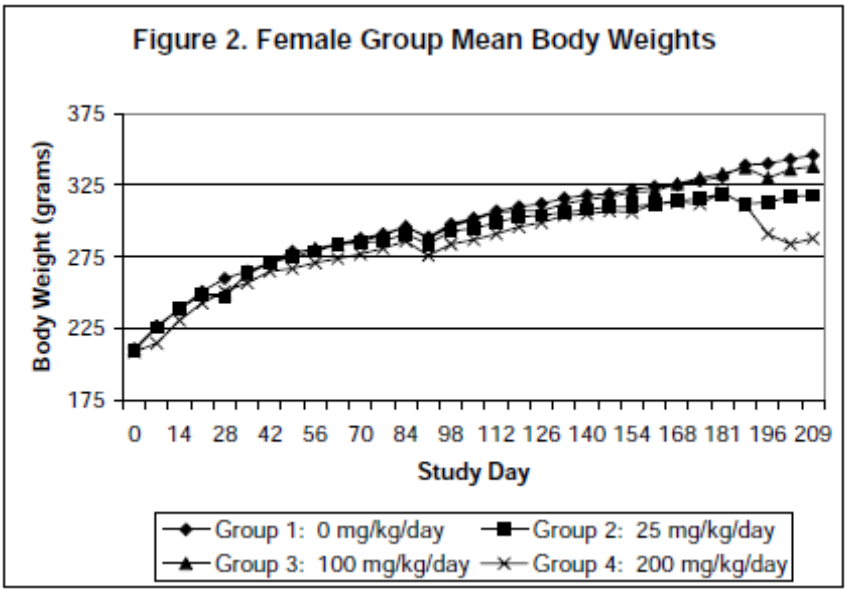
FEMALES SUMMARY OF SURVIVAL AND CLINICAL OBSERVATIONS (FREQUENCY/ANIMALS)

GROUP:	1	2	3	4
LEVEL: MG/KG/DAY	0	25	100	200
DAY 0 to 183				
BODY				
HAIRLOSS	44/ 6	117/ 8	81/ 5	6/ 2
TAIL ENLARGEMENT	4/ 1	0/ 0	0/ 0	14/ 2
EYE(S)				
OCULAR DISCHARGE	1/ 1	0/ 0	8/ 2	4/ 2
DARK MATERIAL AROUND EYE(S)	1/ 1	1/ 1	15/ 4	15/ 5
OTHER				
SALIVATION PRIOR TO DOSING	0/ 0	0/ 0	0/ 0	10/ 5
STRUGGLED DURING DOSING	0/ 0	1/ 1	0/ 0	4/ 4
POST-DOSE OBS				
SALIVATION	0/ 0	0/ 0	7/ 4	131/15
RED MATERIAL AROUND MOUTH	0/ 0	0/ 0	0/ 0	1/ 1
DAY 184 to 210				
FOUND DEAD	0/ 0	0/ 0	0/ 0	1/ 1
EYE(S)				
OCULAR DISCHARGE	0/ 0	0/ 0	3/ 1	0/ 0
DARK MATERIAL AROUND EYE(S)	0/ 0	0/ 0	5/ 1	2/ 1

Body Weights

Reduced average body weights were observed in HDM from D7 on, reaching statistical significance between D112 and D181 (differences of 6.8% to 9.6%); during the recovery period, MDM and HDM tended to gain more weight than controls. While HDF showed reduced average weight gain during the first week (-63%), treated females generally had average body weights that were within 5% of those of controls. The sponsor reported that apparent decreases in average body weights observed in LDF and HDF during the recovery period were artifactual. HDF lost weight (20 g) over days 189-196; the reason for this substantial change was not clear. See the sponsor's summary figures for males and females, below.





Food Consumption

Despite some statistically significant differences in food consumption among groups, the sponsor reported that food consumption was generally comparable among all groups. However, during the first 2 weeks, HD animals consumed less than controls (8 to 7% in HDM, and 20 to 5% in HDF). MDM and HDM tended to eat slightly more than controls and LDM during the recovery period.

Ophthalmoscopy

Corneal crystals were observed in the eyes of several animals among all groups, including controls; the veterinary ophthalmologists (2) indicated that these were

compatible with the species and age of the animals, and reported no drug-related ocular findings. A moderate cortical cataract of the lens was observed in 1 HDM. No other ocular abnormalities were reported.

Hematology

The sponsor reported no toxicologically meaningful drug-related differences in hematological or coagulation parameters in males or females; however, there were some clear dose-related differences in RBC and WBC parameters. On D91/92, treated males showed a 7-8% reduction in RBC parameters (erythrocyte count, hemoglobin, and hematocrit); generally, these parameters were similar to controls on D182/183 and D210. Generally, these parameters were within 5% of controls in females. In males, platelets were increased on D91/92 (19% at HD), D182/183 (25-32% at MD and HD), and D210 (21% at HD). In HDF, platelets were increased 19% on D91/92 and 30% on D182/183. Platelet clumps were observed in 1 MDF and 1 HDF. Although variability was notably large in all groups (including controls), activated PTT time was decreased up to 27% on D182/183 and 15% on D210 in treated females.

WBC counts showed dose-related increases in treated males and HDF. On D182/183, increases were observed in leukocytes (1.2-1.6x), lymphocytes (1.2-1.4x), segmented neutrophils (1.1-2x; 21% at HD on D91), eosinophils (1.6-3.6x), and basophils (HD only, 4x) in males. In HDF, leukocytes (21% on D182/182, 39% on D210), lymphocytes (23% on D182/183), segmented neutrophils (27% on D182/183, 24% on D210 excluding 1 recHDF at ~3x), eosinophils (2x on D182/183, 4x on D210), and basophils (on D182/183 and D210) were increased.

Clinical Chemistry

The sponsor reported no toxicologically meaningful treatment-related differences in clinical chemistry parameters in males or females but noted there were a number of statistically significant differences in clinical chemistry parameters observed throughout the course of the study. According to the sponsor, notable changes included very slight decreases (<5%) in sodium and chloride in MD and HD animals on D91/92 and D182/183, and small elevations (up to 11%) in potassium levels in HD animals D182/183.

Other notable changes were observed. Effects on liver enzymes showed a sex-related difference (this resulted primarily from 3 HDF, the increased values correlating with findings of minimal focal-multifocal liver necrosis and minimal bile duct hyperplasia). In HDF, AST was increased 36% on D91/92 (9% in MDF), 29% on D182/183 (although this primarily due to high values for 2 individuals, #3606 and 3614), and 58% on D210 (although this was due to 1 individual with 3.5x, #3618). For ALT, increases were also observed (87% on D91/92, 14% on D182/183- due to the same 2 animals, and 2.4x on D210 due to the same animal). GGT was also increased by 20-30% in HDF at D182/183 and D210, due to the same animals. On D210, drug-related decreases in AST and ALT were observed in treated males (24-45% for AST and ~50% for ALT).

Cholesterol was increased 10-26% in MDF and HDF on D91/92, 27-21% in MDF and HDF on D182/183, and 8-45% in treated females on D210; cholesterol was increased 1.5-1.7x in MDM and HDM on D182/183. Total bilirubin was increased 9-16% in MDF and HDF on D91/21, 7% in HDF on D182/183, and 10-24% in treated females on D210; bilirubin was only increased 6-8% in HDM across all days. Changes that would indicate renal toxicity were not clearly observed, except in a few individuals. In HDM, BUN was increased 14% on D182/183 (although this was mostly due to ≤2x increases in 2 animals, correlating with moderate nephropathy), but BUN was decreased (up to 18%) in MDF and HDF on all days (except for HDF #3617 on D210, with a 2.5x increase, correlating with mild nephropathy). Creatinine was decreased 11-17% in MDM and/or HDM on all days, and decreased 9-18% in MDF and HDF on all days (also decreased in LDF on D91/92). Increased phosphorus was observed on D91/92 (9-12%) in MD and HD animals, and on D182/182 (13-35%) in LDF and MD and/or HD animals. Creatine kinase was decreased (~50%) in MDF, HDF and HDM on D210. In MDM and HDM, small increases in total protein (~7% on D182/183 and 4% at HD on D210) and globulin (10-11% on D182/183, 9% at HD on D210) resulting in a slightly reduced A/G ratio (6-7% on D182/183, 6-10% on D210); very slightly increased total protein and globulin (8-10%, respectively) were noted in HDF on D210.

Urinalysis

The sponsor reported no toxicologically meaningful treatment-related differences in urinalysis parameters in males or females. There were only a few clear changes. The presence of ketones, protein and leukocytes was observed. The incidence of increased urinary ketones was increased in MD and HD animals (acetest confirmed in MDM and HDM). Protein in the urine was clearly increased in incidence and severity in treated males, and slightly increased in MDF and HDF. And, although resolved in most by D210, small-large numbers of leukocytes were observed in the urine of treated males and females. Other changes were less obvious. Although the amounts were clearly variable, total urine volume was increased 1.7x in HDM on D182/183, and 1.4-3x in MDF and/or HDF across days. The pH was slightly decreased in treated males on D91/92 and D182/183. Hazy/cloudy urine was observed more frequently in HD animals on D91/92. One HDF was noted to show red urine on D210. A large amount of hemolyzed blood was observed on D91/92 in 3 HDF, and a small amount of bilirubin was observed in the urine of 1 HDF on D182/183. Nitrite was observed in the urine of treated females on D91/92. A few animals at HD were noted to show urinary RBCs and/or epithelials, and increased casts and bacteria were noted in treated groups.

Gross Pathology

Gross necropsy observations indicated clear drug-related effects on the kidney, stomach, and liver. Enlarged kidneys were observed at the end of the dosing period in both males and females at MD and HD; the finding persisted in MDM and HDM at recovery. At the end of the dosing period, gross necropsy findings of prominent epithelials in the stomach were observed primarily in MD and HD animals; this correlated with increased stomach weights. This finding persisted in HDF at recovery. Liver showed foci and/or dark red color in MDF and HDF at the end of dosing; this was

not observed in recovery females. Adrenal, lymph nodes, skin, thymus and urinary bladder showed a few changes.

MAIN/RECOVERY	MALES				FEMALES			
N: 15/5	Con	LD	MD	HD	Con	LD	MD	HD
KIDNEY								
Enlarged	-	1/0	7/1	12/1	-	-	-	1/0
Pitted	1/0	0/1	0/1	-	-	-	1/0	2/0
Dilated pelvis	-	0/2	1/0	1/0	1/0	-	-	0/2
Depressed area(s)	-	-	-	-	-	-	-	0/2
STOMACH								
Prominent epithelials	-	1/0	15/0	15/0	-	-	14/0	15/2
Foci	2/0	-	1/0	1/0	-	1/0	3/0	1/1
Thickened	-	-	-	0/2	-	-	0/1	-
LIVER								
Foci	-	-	-	-	-	-	4/0	3/0
Dark red	-	-	-	-	-	-	-	1/0
ADRENAL								
Foci	-	-	-	1/0	-	-	-	1/0
LYMPH NODES, ALL								
Enlarged	-	0/1	-	5/0	-	-	-	2/0
SKIN								
Scabbing	-	0/1	1/3	3/1	1/0	-	-	0/2
THYMUS								
Foci	7/0	6/4	6/2	4/1	1/1	4/0	6/2	1/0
URINARY BLADDER								
Calculi	-	--	-	-	-	-	-	0/1
Thickened	-	-	-	-	-	-	-	0/1

Organ Weights

Drug-related changes in organ weights were observed for kidney, stomach, and liver. Dose-related increases in relative (to body) kidney weights were observed at the end of the dosing period (day 182/183) in treated males and females, compared to controls (1.1x, 1.5x, and 1.8x in males, respectively; 16% and 34% in MDF and HDF); at recovery, weights remained 1.3x and 1.5x controls in MDM and HDM, and 1.1x and 1.4x in MDF and HDF. At the end of the dosing period, dose-related increases in relative (to body) stomach weights were observed in treated males and females (1.1x, 1.8x, and 2.4x in males; 1.5x and 2.2x in MDF and HDF). At recovery, increased relative (to body) stomach weights persisted in treated animals (1.2x, 1.2x, and 1.3x in males, respectively; 1.2x and 1.3x in MDF and HDF). Dose-related increases in relative (to body) liver weights were observed on D182/183 in both treated males and females

(1.1x, 1.3x and 1.4x in males, respectively; 13% and 23% in MDF and HDF); liver weights were still increased 10-20% in MDF, HDM, and HDF at recovery.

Changes were also suggested for brain, lung, spleen, heart, and epididymides. Absolute brain weight was reduced (8%) in recHDF. Relative (to body) lung weight was increased 9% [ss] in HDM; this increase remained (11%) at recovery in HDM. Relative (to body) spleen weights were increased 10% in HDM; recovery spleen weights in males and females were increased 10-20%. At recovery, relative (to body) heart weight was increased 14% in HDF. At recovery, relative (to body) epididymides weight was increased 30%.

Histopathology

Adequate Battery **Yes (bone marrow smears were taken but not examined)**
Peer Review **Yes, by Karrie Brenneman, DVM, PhD, DACVP of Biogen Idec**

Histological Findings

Clear drug-related histopathological findings were observed in the kidney, stomach, and liver.

Drug-related findings in the kidneys were often of greater incidence and severity in treated males. An increased incidence and severity of nephropathy was clearly observed in males, and suggested in MD and HD females. Additionally, according to the pathologist, "other renal findings not classically associated with nephropathy, including cortical tubular changes (diffuse dilation, hyaline droplet accumulation, nuclear/cellular hypertrophy of epithelial cells, segmental epithelial regeneration) and hypertrophy of the parietal epithelium of Bowman's capsule" were observed in a dose-related fashion in males. In females, hypertrophy, hyaline droplet accumulation, and segmental regeneration in the tubule epithelium occurred at MD and HD. At recovery, the pathologist indicated that the "character and severity of test article-related findings in the kidney were generally similar to those at the main study time point," but noted that the incidence was decreased in some dose groups. Tubular dilation was also observed in HDF at recovery. See the sponsor's tables, below.

Table 3 – Intergroup Comparison of Test Article-Related Kidney Findings Main Study

Sex:	Male				Female			
Group[a]:	1	2	3	4	1	2	3	4
Kidney #Ex:	**15**	**15**	**14**	**15**	**15**	**15**	**15**	**15**
Nephropathy								
minimal	7	9	5	5	7	6	7	8
mild	1	1	8	5	0	0	1	1
moderate	0	0	1	5	0	0	0	0
Dilation, tubular								
minimal	4	7	7	6	4	3	4	3
mild	0	1	5	6	0	0	0	0
Hyaline droplet, tubule, epithelium								
minimal	0	4	6	7	0	0	3	3
mild	0	0	1	3	0	0	0	1
Hypertrophy, Bowman's capsule, epithelium								
minimal	1	7	6	3	0	0	1	0
mild	0	0	0	7	0	0	0	0
Hypertrophy, tubule, epithelium								
minimal	0	2	11	13	0	1	12	15
Regeneration, segmental, tubule, epithelium								
minimal	1	11	6	9	0	0	2	1
mild	0	1	7	6	0	0	0	0

[a] Test material and dosage for each groups are defined in Table 1

Table 4 – Intergroup Comparison of Test Article-Related Kidney Findings Recovery

Sex:	Male				Female			
Group[a]:	1	2	3	4	1	2	3	4
Kidney #Ex:	**5**	**5**	**5**	**5**	**5**	**5**	**5**	**4**
Nephropathy								
minimal	5	5	2	0	1	1	2	2
mild	0	0	3	4	0	0	0	1
moderate	0	0	0	1	0	0	0	0
Dilation, tubular								
minimal	1	2	5	2	0	0	0	2
mild	0	0	0	3	0	0	0	0
Hyaline droplet, tubule, epithelium								
minimal	1	0	2	4	0	0	0	0
mild	0	0	0	0	0	0	0	1
Hypertrophy, Bowman's capsule, epithelium								
minimal	1	3	5	3	0	0	0	0
mild	0	0	0	1	0	0	0	0
Hypertrophy, tubule, epithelium								
minimal	2	1	5	5	0	2	5	4
Regeneration, segmental, tubule, epithelium								
minimal	1	3	3	2	0	0	0	1
mild	0	0	1	3	0	0	0	0

[a] Test material and dosage for each groups are defined in Table 1

At the end of the dosing period, most drug-related findings occurred in the nonglandular portion of the stomach. These included increased incidence and severity of squamous epithelial hyperplasia and hyperkeratosis (all treated, dose-related), minimal-mild subacute inflammation (treated, dose-related), squamous cell carcinoma (1 HDM), and squamous papilloma (1 MDM). In the glandular stomach, minimal-mild subacute inflammation was observed (treated, roughly dose-related). At recovery, drug-related findings continued to be observed in the stomach, generally occurring at much lower incidence and severity than at the end of the dosing period. Nonglandular stomach changes included minimal hyperkeratosis (MD and/or HD), minimal squamous epithelial hyperplasia (treated, dose-related), keratinized cysts (HD), and subacute inflammation (low incidence, roughly dose-related). Subacute inflammation also continued to be observed in the glandular stomach (low incidence, roughly dose-related). See the sponsor's summary tables, below.

Table 5 – Intergroup Comparison of Test Article-Related Stomach Findings Main Study

Sex:	Male				Female			
Group[a]:	1	2	3	4	1	2	3	4
Nonglandular Stomach #Ex:	**15**	**15**	**14**	**15**	**15**	**15**	**15**	**15**
Hyperkeratosis								
minimal	0	8	0	0	0	9	3	1
mild	0	0	8	6	0	0	12	7
moderate	0	0	6	9	0	0	0	7
Hyperplasia, squamous epithelium								
minimal	0	9	1	0	0	5	8	0
mild	0	0	10	10	0	0	7	15
moderate	0	0	3	5	0	0	0	0
Inflammation, subacute								
minimal	0	3	8	8	0	0	8	9
mild	0	0	1	3	0	0	2	0
Squamous cell carcinoma	0	0	0	1	0	0	0	0
Squamous papilloma, benign	0	0	1	0	0	0	0	0
Glandular Stomach								
Inflammation, subacute								
minimal	2	0	7	10	0	3	6	4
mild	0	0	1	2	0	0	1	2

[a] Test material and dosage for each groups are defined in Table 1

Table 6 – Intergroup Comparison of Test Article-Related Stomach Findings Recovery

Sex:	Male				Female			
Group[a]:	1	2	3	4	1	2	3	4
Nonglandular Stomach #Ex:	5	5	5	5	5	5	5	4
Hyperkeratosis								
minimal	0	0	2	3	0	0	1	0
Hyperplasia, squamous epithelium								
minimal	0	1	5	4	0	0	1	3
Cyst, keratinized	0	0	0	2	0	0	0	2

[a] Test material and dosage for each groups are defined in Table 1

Inflammation, Subacute; glandular	(0)	(1)	(2)	(1)	(0)	(0)	(1)	(2)
minimal	0	1	2	1	0	0	1	2
Inflammation, Subacute; nonglandular	(0)	(1)	(0)	(2)	(1)	(1)	(1)	(2)
minimal	0	1	0	2	1	0	1	2
mild	0	0	0	0	0	1	0	0

At the end of the dosing period, lymphoid hyperplasia of pancreatic or abdominal lymph nodes was seen in a few HD animals (see the sponsor's summary table, below); the pathologist indicated that this finding may have been secondary to stomach inflammation. Plasmacytosis in these lymph nodes was also observed at HD.

Table 9 – Intergroup Comparison of Test Article-Related Lymph Node Findings Main Study

Sex:	Male				Female			
Group[a]:	1	2	3	4	1	2	3	4
#Ex:	0	0	0	4	0	0	0	2
Lymph node, pancreatic/ abdominal: Hyperplasia, lymphoid								
minimal	0	0	0	1	0	0	0	0
mild	0	0	0	2	0	0	0	2
Plasmacytosis								
minimal	0	0	0	1	0	0	0	0
mild	0	0	0	3	0	0	0	2

[a] Test material and dosage for each groups are defined in Table 1

Drug-related findings in the liver occurred primarily in females. At MD and HD, histopathological findings at the end of the dosing period included minimal focal-multifocal hepatic necrosis and minimal bile duct hyperplasia (also in 1 LDF). At the end of the recovery period, minimal hepatic necrosis continued to be observed in MD and HD females (with low incidence). The low incidence of bile duct hyperplasia was not drug-related. See the sponsor's summary tables, below.

Table 7 – Intergroup Comparison of Test Article-Related Liver Findings
Main Study

Sex:	Male				Female			
Group[a]:	1	2	3	4	1	2	3	4
Liver #Ex:	**15**	**15**	**14**	**15**	**15**	**15**	**15**	**15**
Necrosis, focal/multifocal								
minimal	0	0	1	1	0	0	4	5
Hyperplasia, bile duct								
minimal	0	0	0	1	0	1	3	8

[a] Test material and dosage for each groups are defined in Table 1

Table 8 – Intergroup Comparison of Test Article-Related Liver Findings
Recovery

Sex:	Male				Female			
Group[a]:	1	2	3	4	1	2	3	4
Liver #Ex:	**5**	**5**	**5**	**5**	**5**	**5**	**5**	**4**
Necrosis, focal/multifocal								
minimal	0	0	0	0	0	0	1	1
Hyperplasia, bile duct								
minimal	0	0	0	0	1	0	0	1

[a] Test material and dosage for each groups are defined in Table 1

Changes in a few other organs were observed, generally at lower incidence. In HD animals, changes were observed in the adrenal gland, mammary gland, testis, and parathyroid gland at the end of the dosing period. Increased alterations were also observed in treated animals in spleen at the end of the dosing period. At the end of the recovery period, these changes were either not noted or not dose-related. In treated females, lung alterations were observed at low incidence. See excerpts from the sponsor's summary tables, below, for details.

Observations: Neo-Plastic and Non Neo-Plastic
Removal Reason: Terminal Euthanasia

	MALES				FEMALES			
	0 mg/kg	25 mg/kg	100 mg/kg	200 mg/kg	0 mg/kg	25 mg/kg	100 mg/kg	200 mg/kg
Number of Animals on Study :	15	15	14	15	15	15	15	15
Number of Animals Completed:	(15)	(15)	(14)	(15)	(15)	(15)	(15)	(15)
ADRENAL GLAND;								
Examined	(15)	(15)	(14)	(15)	(15)	(15)	(15)	(15)
Degeneration; cystic; Cortex	(1)	(0)	(1)	(0)	(1)	(0)	(0)	(3)
minimal	1	0	1	0	1	0	0	3
MAMMARY GLAND;								
Examined	(15)	(15)	(14)	(15)	(15)	(15)	(15)	(15)
Within Normal Limits	14	15	13	14	15	15	13	14
Hyperplasia; focal	(0)	(0)	(1)	(1)	(0)	(0)	(1)	(1)
minimal	0	0	1	1	0	0	1	1
TESTIS;								
Examined	(15)	(15)	(14)	(15)	(-)	(-)	(-)	(-)
Within Normal Limits	14	14	14	13	-	-	-	-
Hyperplasia; interstitial	(1)	(0)	(0)	(2)	(-)	(-)	(-)	(-)
minimal	1	0	0	2	-	-	-	-
PARATHYRCID GLAND;								
Examined	(12)	(14)	(10)	(11)	(12)	(13)	(11)	(12)
Within Normal Limits	12	14	10	11	12	13	11	11
Not Examined: NOT PRESENT	3	1	4	4	3	2	4	3
Hyperplasia	(0)	(0)	(0)	(0)	(0)	(0)	(0)	(1)
minimal	0	0	0	0	0	0	0	1
SPLEEN;								
Examined	(15)	(15)	(14)	(15)	(14)	(15)	(15)	(15)
Withir Normal Limits	5	2	1	4	9	10	7	7
Not Examined: NOT PRESENT	0	0	0	0	1	0	0	0
Hematcpoiesis; extramedullary	(10)	(13)	(13)	(11)	(5)	(4)	(8)	(8)
minimal	9	13	13	11	5	4	8	8
moderate	1	0	0	0	0	0	0	0

Observations: Neo-Plastic and Non Neo-Plastic

Removal Reason: Recovery Euthanasia

	MALES				FEMALES			
	0 mg/kg	25 mg/kg	100 mg/kg	200 mg/kg	0 mg/kg	25 mg/kg	100 mg/kg	200 mg/kg
Number of Animals on Study :	5	5	5	5	5	5	5	4
Number of Animals Completed:	(5)	(5)	(5)	(5)	(5)	(5)	(5)	(4)
LUNG WITH BRONCHI;								
Examined	(5)	(5)	(5)	(5)	(5)	(5)	(5)	(4)
Histocytosis	(0)	(0)	(0)	(0)	(0)	(1)	(2)	(1)
mininal	0	0	0	0	0	1	2	1

Toxicokinetics

DMF showed rapid and dose-independent absorption, as indicated by MMF exposures. Control group and pre-dose MMF concentrations were BLOQ (i.e., no dosing of the control group or MMF accumulation was observed). T_{max} ranged from 15 to 30 minutes. The AUC increased with dose in an approximately dose-proportional manner; however, C_{max} did not show a consistent dose-proportionality. Consistent sex differences in exposure were indicated by AUC values at the end of the dosing period (D181), i.e., exposure in females were 1.2-2x those in males. The elimination half-life of MMF was estimated to be 20-60 minutes. See the sponsor's summary table, below.

Table 3. Toxicokinetic parameter estimates of MMF on Days 0 and 181 after daily oral administration of BG00012 to Sprague Dawley rats for 6-month at 25, 100 or 100 mg/kg/day.

Dose group (mg/kg)	Study Day	Gender	$t_{1/2}$ (min)	T_{max} (min)	C_{max} (μg/mL)	AUC_{last} (hr*μg/mL)	AUC_{inf} (hr*μg/mL)
25	Day 0	Female	24	15	3.7	2.7	2.9
		Male	41	15	3.2	2.6	3.0
	Day 181	Female	48	15	7.0	4.1	4.6
		Male	51	15	3.6	2.4	2.7
100	Day 0	Female	30	30	16	14	16
		Male	42	30	10	11	13
	Day 181	Female	50	15	41	24	27
		Male	40	15	12	10	13
200	Day 0	Female	26	15	34	26	28
		Male	49	15	26	22	29
	Day 181	Female	60	15	48	36	46
		Male	57	15	56	30	37

Dosing Solution Analysis

The stability of BG00012 in 0.8% HPMC was established from 5 to 50 mg/mL when stored at room temperature for 24 hours at 2 to 8°C for 11 days; the 2.5 mg/mL concentration was found to be stable for 24 hours at room temperature and for ten days when stored at 2 to 8°C. Formulations were prepared weekly. The homogeneity results for week 0 were all within the acceptable range of ≤5% RSD for Groups 2 and 4. Formulations for weeks 0, 1, 2, 3, 7, 11, 19, and 23 were within the acceptable limits of ±15% error (except for week 7 Group 3 samples, which were -17.9% of the expected concentration).

Non-Rodent

Monkey

To support the doses used in the pivotal chronic toxicity study in monkey, the sponsor conducted a non-GLP 14-day dose-ranging study (Study P00012-05-07 [EBA00174]: "BG00012: A 14 Day Dose Range Finding Study of Dimethyl Fumarate Suspension Administered by Nasogastric Intubation to Cynomolgus Monkeys", conducted (b) (4)). DMF (Lot No. 1102643) in 0.8 % hydroxypropylmethylcellulose was orally administered once daily to 16 non-naive female cynomolgus monkeys (4 F/group, 4-11 years old) at 0, 5, and 25 for 14 days and at 75 mg/kg for 8 days, followed by a 1-day washout period, 100 mg/kg on the 10th day, and 125 mg/kg from D11 until D15. Only in-life examinations were performed. DMF was generally well-tolerated at 0, 5, and 25 mg/kg for 14 days, and at 75 mg/kg for 8 days; a minimal average body weight loss was observed at 75 mg/kg (<1%), and low food consumption and/or emesis were sporadically observed. Beginning on D10 at HD, drug-related reductions in body weight (<5% BW loss), episodes of low food consumption and/or emesis (1 to 3 of 4), and emaciation (2 of 4) were observed. On D14, the HD animals showed minimally decreased serum albumin concentrations. Monomethylfumarate (MMF) exposures were confirmed for 2 hours at LD, for 2 to 6 hours at MD, and for 6 hours at HD; AUC and C_{max} exposures were dose-dependent and approximately dose-proportional. There was no apparent accumulation of MMF in the systemic circulation for any DMF-treated group during the dosing period. $T_{1/2}$ was in the range of 0.6 to 1.6 hours. The systemic levels of methanol (MtOH) and FA were low, within two-fold of levels in the control group. The sponsor considered the MTD to be at or near 75 mg/kg/day based on the body weight and food consumption effects; however, it is unclear whether an MTD was approximated.

To support registration, the sponsor conducted a 12-month chronic toxicity study in monkeys (Study P00012-05-08 [EBA00176], entitled "BG00012: A 1 Year Chronic Toxicity Study of Dimethyl Fumarate Suspension Administered by Nasogastric Intubation to Cynomolgus Monkeys"; see review by Dr. Peters, dated 10/1/07). Cynomolgus monkeys (*Macaca mulatta*; N=4/sex/gp main, 2/sex Con and HD satellite TK) were administered 0, 5, 25, or 75 mg/kg/day b.i.d. DMF (lot 1102643 33004999) in 0.8% hydroxypropylmethylcellulose by oral gavage via nasogastric tube. The male monkeys were noted (at histologic exam) to be sexually immature. Histologic peer review was performed by Dr. Brenneman of Biogen Idec, Inc.

No drug-related deaths were reported. Decreased food consumption and body weight gain were noted early (week one) in the study for HD males and females but normalized for the remainder of the study. At MD and HD, decreased BUN and creatinine (females) were observed in most animals, with concurrent decreases in phosphorus levels in some HD animals. Mean kidney weight was increased in HDF (and in HDM after recovery). Changes were observed in the kidneys at MD and HD. Grossly, kidneys were described as enlarged and "pale" (4/4 males, 2/4 females); "watery" was also used as a descriptor in 2 HDF. The renal lesions, including interstitial fibrosis with tubular atrophy and/or regeneration in the HDM, were not consistent with the serum chemistry

findings. Tubular regeneration and/or single cell necrosis of the epithelial cells were reported at MD and HD (see table from review below), with increased incidence and severity at HD. According to the pathology report, the observed tubular regeneration "affected entire tubular profiles in some cases, but only segments in others, and consisted of scattered individual or clumps of cortical tubules in which tubular epithelial cells had one or more of the following changes: increased or decreased size, cytoplasmic basophilia, cytoplasmic vacuolation, increased or decreased size of the nucleus, irregular shape of nucleus, mitotic figures. The nuclei within affected tubules were often irregularly distributed, being sometimes clumped together and sometimes sparse. The lumen of some affected tubules appeared large while others were small due to decreased or increased size of affected epithelial cells." The observed single cell necrosis consisted of "individual tubular epithelial cells in the cortex that were eosinophilic, shrunken, and had pyknotic or karyorrhexic nuclei. Some affected cells remained adjacent to the tubular basement membrane, while others had become detached and were in the tubular lumen. The necrotic cells were in some cases widely scattered in the renal cortex, although there were occasionally more than one affected cell in a single tubular profile." The report also indicated that both single cell necrosis of tubular epithelium and regeneration of tubular epithelium were sometimes concentrated in the medullary rays. Focal or multifocal cortical scars (described as "small cortical scars characterized by small areas of a few tubules that were severely atrophic and had peritubular interstitial fibrosis with a clear loss of tissue, also with sclerotic adjacent glomeruli in some") were observed in two MDF and one HDM; according to Dr. Peters, these are found not infrequently in control kidneys in monkey studies. Also, a slight increase in the severity score (mild) for multifocal mononuclear cell infiltrates in the cortical interstitium of 2 of 4 HDF was observed.

Incidence of Treatment-Related Renal Histologic Lesions at Terminal Necropsy

	Males				Females			
Dose/ Lesion	**0**	**5**	**25**	**75**	**0**	**5**	**25**	**75**
Kidney- Tubular necrosis	N= 4	N= 4	N= 4	N= 4	N= 4	N= 4	N= 5	N= 4
Minimal	0	0	1	3	0	0	2	0
Mild	0	0	0	0	0	0	0	4
Tubular regeneration								
Minimal	0	0	0	1	0	0	2	1
Mild	0	0	0	1	0	0	0	0
Moderate	0	0	0	1	0	0	0	3

After 4 weeks recovery, the incidence and severity of the renal lesions were decreased, although not completely recovered, at MD and HD (see table from review, below). Mild to moderate tubular atrophy was observed in some recovery animals. According to Dr. Peters, it is unclear whether these findings indicated "repair or simply discontinuation of the insult." The two recovery HDM showed mild to moderate, multifocal to diffuse, interstitial fibrosis with tubular atrophy (one of which also showed increased BUN [~39%] and creatinine at weeks 38, 52 and 56); this is a sign of irreversible loss of

tissue and function. Minimal to mild fibrosis of Bowman's capsule and mild multifocal mononuclear infiltrates were reported as secondary changes associated with the interstitial fibrosis.

Incidence of Treatment-Related Renal Histologic Lesions at Recovery Necropsy

	Males				Females			
Dose/ Lesion	**0**	**5**	**25**	**75**	**0**	**5**	**25**	**75**
Kidney- Tubular necrosis	N= 2	N= 2	N= 2	N= 2	N=2	N= 2	N= 1	N= 2
Minimal	0	0	0	1	0	0	1	0
Tubular regeneration								
Minimal	0	0	0	0	0	0	1	1
Mild	0	0	0	2	0	0	0	1
Interstitial fibrosis								
Mild	0	0	0	1	0	0	0	0
Moderate	0	0	0	1	0	0	0	0

The following TK data were provided (see tables from review and the sponsor, below) for MMF, methanol, and formic acid. Dose-proportional increases in MMF C_{max} were observed, but MMF $AUC_{0\text{-}24hr}$ increased greater than dose-proportionally between MD and HD. The terminal half-life was also prolonged at MD and HD. However, accumulation was not observed. There were no statistically significant drug-related changes in plasma methanol or formic acid. Plasma methanol exposures were increased ~40% in MDM, HDF, and HDM at 26 weeks, and were again increased at HD in week 52. Formic acid plasma exposures were similar among all groups, except for a ~30% decrease in HD animals at week 52.

Table 2. Toxicokinetic parameter estimates of **MMF** on Day 1, Weeks 4, 26 and 52 after once daily nasogastric intubation of BG00012 to cynos at 5 mg/kg (Table 2A), 25 mg/kg (Table 2B) and 75 mg/kg (Table 2C). (N=6 per gender in each dose group)

Table 2A

Dose	Study Day	Gender		C_{max} (µg/mL)	$AUC_{0\text{-}24hr}$ (µg*hr/mL)	T_{max} (hr)	Vz/F (L/kg)	CL/F (L/hr/kg)	$T_{1/2}$ (hr)
5 mg/kg	Day 1	Female	**Mean**	**1.65**	**2.83**	**0.5**	4.48	2.58	1.17
			SD	0.95	1.38	0	2.50	0.60	0.51
			CV%	57.9	48.9	0	55.8	23.1	43.5
		Male	**Mean**	**2.03**	**2.82**	**0.52**	-	-	-
			SD	0.47	0.59	0.04	-	-	-
			CV%	23.1	20.8	7.9	-	-	-
	Week 4	Female	**Mean**	**1.83**	**2.23**	**0.37**	1.95	2.32	0.58
			SD	0.33	0.25	0.1	0.37	0.24	0.08
			CV%	18.1	11.3	27.3	18.7	10.5	13.6
		Male	**Mean**	**1.88**	**1.99**	**0.33**	1.74	2.70	0.46
			SD	0.35	0.44	0.15	0.63	0.77	0.19
			CV%	18.8	22.3	45.5	36.5	28.7	41.5
	Week 26	Female	**Mean**	**2.46**	**2.51**	**0.33**	1.65	2.09	0.53
			SD	0.85	0.4	0.13	0.83	0.34	0.17
			CV%	34.5	15.9	38.7	50.2	16.1	33.0
		Male	**Mean**	**1.99**	**2.05**	**0.36**	1.93	2.68	0.52
			SD	0.44	0.56	0.13	0.59	0.86	0.16
			CV%	22.2	27.4	34.8	30.8	32.0	30.1
	Week 52	Female	**Mean**	**2.06**	**2.73**	**0.3**	1.98	1.88	0.73
			SD	0.39	0.34	0.09	0.41	0.25	0.13
			CV%	19.1	12.5	28.5	20.8	13.4	18.0
		Male	**Mean**	**2.03**	**2.32**	**0.35**	1.95	2.29	0.60
			SD	0.99	0.53	0.14	0.66	0.52	0.20
			CV%	48.8	22.9	39.1	33.8	22.7	33.2

Table 2B

Dose	Study Day	Gender		C_{max} (µg/mL)	$AUC_{0\text{-}24hr}$ (µg*hr/mL)	T_{max} (hr)	Vz/F (L/kg)	CL/F (L/hr/kg)	$T_{1/2}$ (hr)
25 mg/kg	Day 1	Female	**Mean**	**7.03**	**11.6**	**0.5**	2.91	2.50	0.84
			SD	1.83	3.21	0	0.63	0.94	0.14
			CV%	26.1	27.7	0	21.8	37.7	16.4
		Male	**Mean**	**9.89**	**14.43**	**0.5**	2.37	1.88	0.92
			SD	1.76	3.24	0	0.82	0.50	0.41
			CV%	17.8	22.4	0	34.8	26.5	44.5
	Week 4	Female	**Mean**	**9.71**	**12.9**	**0.54**	1.71	2.03	0.58
			SD	2.66	3.02	0.19	0.74	0.45	0.18
			CV%	27.4	23.4	34.7	43.1	22.2	31.6
		Male	**Mean**	**10.98**	**12.74**	**0.52**	1.44	2.51	0.42
			SD	3.41	4.51	0.26	0.83	1.81	0.10
			CV%	31	35.4	49.2	57.5	72.2	23.7
	Week 26	Female	**Mean**	**11.62**	**15.26**	**0.51**	1.55	1.72	0.62
			SD	3.1	2.56	0.14	0.76	0.30	0.30
			CV%	26.7	16.8	28.1	49.1	17.2	47.6
		Male	**Mean**	**10.92**	**14.57**	**0.54**	1.95	2.01	0.80
			SD	3.55	4.93	0.1	1.11	0.92	0.71
			CV%	32.5	33.8	18.8	56.7	45.9	88.1
	Week 52	Female	**Mean**	**11.79**	**15.58**	**0.58**	2.14	1.69	0.88
			SD	4.85	2.22	0.26	0.97	0.26	0.39
			CV%	41.1	14.2	44.3	45.5	15.1	44.3
		Male	**Mean**	**8.82**	**12.21**	**0.43**	2.68	2.17	0.89
			SD	2.39	2.31	0.19	0.80	0.40	0.38
			CV%	27.1	18.9	45.4	29.8	18.3	42.4

Table 2C

Dose	Study Day	Gender		C_{max} (µg/mL)	AUC_{0-24hr} (µg*hr/mL)	T_{max} (hr)	Vz/F (L/kg)	CL/F (L/hr/kg)	$T_{1/2}$ (hr)
75 mg/kg	Day 1	Female	Mean	27.78	39.46	0.67	1.96	1.96	0.74
			SD	12.35	19.15	0.26	0.60	0.66	0.28
			CV%	44.5	48.5	38.7	30.7	33.4	37.8
		Male	Mean	30.33	40.71	0.53	2.33	2.14	0.73
			SD	10.57	10.76	0.06	1.21	0.74	0.31
			CV%	34.8	26.4	11.7	52.1	34.5	42.3
	Week 4	Female	Mean	26.97	50.39	0.62	1.87	1.56	0.87
			SD	8.12	8.15	0.14	0.62	0.20	0.40
			CV%	30.1	16.2	21.9	33.1	12.9	46.2
		Male [a]	Mean	31.95	58.41	0.86	1.71	1.32	0.90
			SD	6.99	7	0.13	0.38	0.14	0.21
			CV%	21.9	12	15.2	22.2	11.0	23.3
	Week 26	Female	Mean	35.22	53.21	0.69	1.81	1.48	0.84
			SD	14.6	9.26	0.27	0.64	0.26	0.24
			CV%	41.4	17.4	39.5	35.2	17.6	28.0
		Male	Mean	29	49.63	0.79	1.88	1.61	0.81
			SD	9.14	12.09	0.19	0.50	0.37	0.11
			CV%	31.5	24.4	23.8	26.3	22.8	13.5
	Week 52	Female	Mean	31.47	43.63	0.54	2.53	1.87	0.91
			SD	12.3	11.41	0.19	1.14	0.47	0.23
			CV%	39.1	26.2	34.7	44.9	25.1	25.5
		Male	Mean	23.92	47.42	1	2.60	1.73	1.04
			SD	6.8	11.27	0.52	0.91	0.50	0.15
			CV%	28.4	23.8	52.4	35.1	28.9	14.1

[a] N=4

Mean Plasma MMF Concentrations on Weeks 13 and 39

Plasma MMF (µg/mL)	Time point (hr)	Dose (mg/kg) 0	5 mg/kg F	M	25 mg/kg F	M	75 mg/kg F	M
Week 13	0	0	0	0	0	0	0.02 (0.04)	0.03 (0.08)
	1	0	0.94 (0.22)	0.76 (0.17)	5.29 (1.51)	4.97 (1.26)	20.33 (3.27)	21.88 (4.7)
39	0	0	0	0	0	0	0.01 (0.03)	0
	1	0	0.98 (0.23)	0.82 (0.22)	5.94 (0.96)	5.33 (1.37)	16.61 (4.22)	23.17 (6.72)

Table 3. The $AUC_{0\text{-}24hr}$ of **methanol** on Weeks 26 and 52 after once daily administration of BG00012 at 0 (Control, Table 3A), 5 mg/kg (Table 3B), 25 mg/kg (Table 3C), and 75 mg/kg (Table 3D). (N=6 per gender in each dose group)

Table 3A

BG-12 Dose	Study Day	Gender		$AUC_{0\text{-}24hr}$ (µg*hr/mL)
Control	Week 26	Female	**Mean**	**198.61**
			SD	12.73
			CV%	6.4
		Male	**Mean**	**206.27**
			SD	22.84
			CV%	11.1
	Week 52	Female	**Mean**	**341.81**
			SD	136.11
			CV%	39.8
		Male	**Mean**	**337.64**
			SD	115.6
			CV%	34.2

Table 3B

BG-12 Dose	Study Day	Gender		$AUC_{0\text{-}24hr}$ (µg*hr/mL)
5 mg/kg	Week 26	Female	**Mean**	**237.54**
			SD	37.98
			CV%	16
		Male	**Mean**	**206.29**
			SD	29.94
			CV%	14.5
	Week 52	Female	**Mean**	**313.72**
			SD	83
			CV%	26.5
		Male [a]	**Mean**	**312.12**
			SD	101.88
			CV%	32.6

Table 3C

BG-12 Dose	Study Day	Gender		$AUC_{0\text{-}24hr}$ (µg*hr/mL)
25 mg/kg	Week 26	Female	**Mean**	**232.69**
			SD	64.34
			CV%	27.6
		Male	**Mean**	**284.2**
			SD	88.3
			CV%	31.1
	Week 52	Female	**Mean**	**310.32**
			SD	90.52
			CV%	29.2
		Male	**Mean**	**319.88**
			SD	75.47
			CV%	23.6

Table 3D

BG-12 Dose	Study Day	Gender		$AUC_{0\text{-}24hr}$ (µg*hr/mL)
75 mg/kg	Week 26	Female	**Mean**	**283.54**
			SD	37.93
			CV%	13.4
		Male	**Mean**	**296.47**
			SD	84.6
			CV%	28.5
	Week 52	Female	**Mean**	**349.05**
			SD	51.5
			CV%	14.8
		Male	**Mean**	**383.39**
			SD	88.03
			CV%	23

[a] N=5

Table 4. The $AUC_{0\text{-}24hr}$ of **formic acid** on Weeks 26 and 52 after once daily administration of BG00012 at 0 (Control, Table 4A), 5 mg/kg (Table 4B), 25 mg/kg (Table 4C), and 75 mg/kg (Table 4D).

Table 4A

BG-12 Dose	Study Day	Gender		$AUC_{0\text{-}24hr}$ (µg*hr/mL)
Control	Week 26	Female	**Mean**	**66.42**
			SD	17.6
			CV%	26.5
		Male	**Mean**	**73.33**
			SD	22.27
			CV%	30.4
	Week 52	Female	**Mean**	**62.6**
			SD	17.58
			CV%	28.1
		Male	**Mean**	**58.39**
			SD	13.38
			CV%	22.9

Table 4B

BG-12 Dose	Study Day	Gender		$AUC_{0\text{-}24hr}$ (µg*hr/mL)
5 mg/kg	Week 26	Female	**Mean**	**55.31**
			SD	12.82
			CV%	23.2
		Male	**Mean**	**55**
			SD	13.17
			CV%	23.9
	Week 52	Female	**Mean**	**55.19**
			SD	4.72
			CV%	8.5
		Male[a]	**Mean**	**57.58**
			SD	24.84
			CV%	43.1

Table 4C

BG-12 Dose	Study Day	Gender		$AUC_{0\text{-}24hr}$ (µg*hr/mL)
25 mg/kg	Week 26	Female	**Mean**	**60.13**
			SD	15
			CV%	24.9
		Male	**Mean**	**58.41**
			SD	6.58
			CV%	11.3
	Week 52	Female	**Mean**	**48.64**
			SD	13.46
			CV%	27.7
		Male	**Mean**	**57.3**
			SD	8.98
			CV%	15.7

Table 4D

BG-12 Dose	Study Day	Gender		$AUC_{0\text{-}24hr}$ (µg*hr/mL)
75 mg/kg	Week 26	Female	**Mean**	**44.93**
			SD	4.17
			CV%	9.3
		Male	**Mean**	**51.6**
			SD	4.8
			CV%	9.3
	Week 52	Female	**Mean**	**43.84**
			SD	9.13
			CV%	20.8
		Male	**Mean**	**58.16**
			SD	13.91
			CV%	23.9

[a] N=5

The NOEL for the study was 5 mg/kg/day, and the NOAEL was 25 mg/kg/day.

Dog

Subchronic toxicity in dogs was assessed in a 3-week dose-escalation toxicity study (Study P00012-05-04; EBA00132) and a 4-week (with 14-day recovery) oral gavage study (Study P00012-04-05; EBA00014). Chronic toxicity was assessed in a 11-month toxicity study of BG-12 using oral capsules (Study P00012-05-05).

In the 3-week dose-escalation study, oral doses (25-100 mg/kg, QD for one day, and 75-125 [37.5-62.5 mg/kg given twice], BID for 1-7 days) were administered by capsule once daily from D1 to D4 (followed by a three-day recovery period) and twice daily (approximately four hours apart) from D8 to D18 in male and female dogs (2/sex). Dose-related emesis (≥ 50 mg/kg), inappetance (food and Science Diet®), and reduced body weights were observed. All animals were "euthanized moribund"; average body weight losses of 12% in males and 19% in females were observed at sacrifice. Macroscopic findings were observed (see sponsor's summary Text Table 7, below). The sponsor defined doses ≥ 50 mg/kg as exceeding an MTD.

Text Table 7
Presence of Necropsy Findings

Necropsy Finding	Presence of Finding			
	D347M	D348M	D349F	D350F
Abnormal stomach content; one (b) (4) present	+			
Abnormal jejunum content; two (b) (4) present	+			
Abnormal colon content; yellow mucoid material mixed with fecal material	+			
Discoloration of duodenum (entire length), reddened mucosal surface		+	+	
Dehydration of carcass			+	
Small thymus			+	+
Abnormal gall bladder content			+	
Gall bladder foci			+	

In the 4-week oral gavage study, doses of 0, 50, 100, and 250 mg/kg were administered once daily to male and female beagle dogs (4/sex/gp main; 3/sex/gp recovery). (A high-dose Fumaderm comparator dose of 180/60 mg/kg was used but will not be discussed here). One animal, a MDF, was found dead on D29; the death was attributed to "confounding nutritional factors secondary to persistent postdose emesis related to test article administration." Dose-related persistent emesis and "related" clinical observations (e.g., thin, vomitus, loose skin, skin discolored, salivation, and decreased activity) occurred, the severity increasing with dose. Due to the severity of the clinical signs, the HD main study animals were sacrificed early (D13-15); the recHD animals were sacrificed on D29, following a 14-day recovery period. Dose-related body weight losses (5-18% in treated animals at week 1 and >20% in MD animals on D28) and decreases in food consumption were observed; veterinary treatments (e.g., soft feed and electrolytes) were provided. (Body weights increased during recovery.) MD and HD animals were allowed dosing holidays on D11-12 and D17-19 (in males) or D10-11

and D16-18 (in females). Decreased heart rate was observed, with concomitant increases in RR, PR, and QT intervals. QTc prolongation reached a maximum of ~10% (compared to controls; 20 and 18 ms in LDM and MDM) in treated males in week 1, and remained for >1 week. Several pulse rate, blood pressure, hematological, and clinical chemistry alterations were observed in the treated groups; the sponsor indicated that these changes might have resulted from poor nutritional status. Macroscopic findings were observed in the stomach (red foci, corresponding to mucosal hemorrhage in some) and thymus (small, corresponding to lymphoid atrophy). Decreased thymus and spleen weights, as well as increased adrenal weights, were observed at the MD and HD. In HD animals, stomach erosion, mononuclear cells and hemorrhage, thymic lymphoid atrophy (also MDM), and bone marrow hypocellularity (also MDM) were observed. Vacuolation in the tubular epithelium of the kidney ("characterized by round, clear cytoplasmic vacuoles in the tubules of the medullary rays in the deep cortex"; 3/4 HDM and 4/4 HDF at interim sacrifice, and greater severity in treated groups) was observed; the sponsor (not the pathologist) considered this finding incidental because: it was seen in several control animals, it was common in recovery females, and there was little dose proportionality. The findings did not fully recover. No NOEL could be defined. The histopathology NOAEL was 50 mg/kg.

Table 3 – Incidence of Selected Histopathology Findings

Interim Sacrifice										
Organ and Finding	**Males**					**Females**				
Group*	**1**	**2**	**3**	**4**	**5**	**1**	**2**	**3**	**4**	**5**
No. Examined				4					4	
Thymus										
Lymphoid atrophy				4					4	
Bone marrow, sternum										
Hypocellular				3					2	
Bone marrow, femur										
Hypocellular				3					2	
Kidneys										
Vacuolation				3					4	
Stomach										
Hemorrhage				3					1	
Mononuclear cells				3					4	
Erosion				3					0	
Main Study Sacrifice										
Organ and Finding	**Males**					**Females**				
Group*	**1**	**2**	**3**	**4**	**5**	**1**	**2**	**3**	**4**	**5**
No. Examined	4	4	4	3	4	4	4	3	3	4
Thymus										
Lymphoid atrophy	0	0	4	3	2	0	1	2	0	2
Bone marrow, sternum										
Hypocellular	0	0	3	0	1	0	0	1	0	1
Bone marrow, femur										
Hypocellular	0	0	1	0	1	0	0	1	0	2
Kidneys										
Vacuolation	1	2	4	2	3	3	4	3	3	4
Stomach										
Hemorrhage	2	0	0	1	0	2	0	0	0	0
Mononuclear cells	4	0	0	1	3	0	1	1	3	1
Recovery Sacrifice										
Organ and Finding	**Males**					**Females**				
Group*	**1**	**2**	**3**	**4**	**5**	**1**	**2**	**3**	**4**	**5**
No. Examined	3	3	3		3	3	3	3		3
Thymus										
Lymphoid atrophy	0	0	0		0	0	0	2		0
Bone marrow, sternum										
Hypocellular	0	0	1		2	0	0	1		0
Bone marrow, femur										
Hypocellular	0	0	1		2	0	0	1		0
Kidneys										
Vacuolation	0	0	1		1	3	3	2		3
Stomach										
Hemorrhage	1	0	0		0	0	1	0		0
Mononuclear cells	3	0	1		0	0	0	0		1

* Group 1 = vehicle control
Group 2-4 = 50, 100, and 250 mg/kg BG00012, respectively
Group 5 = 180 mg/kg Fumaderm

Study title: An 11-Month Toxicity Study of BG00012 Administered by the Oral (Capsule) Route to Dogs with a 1-Month Recovery Period

Study no.: P00012-05-05, EBA00066
Study report location: EDR,
Conducting laboratory and location: (b) (4)
Date of study initiation: 10/4/05
GLP compliance: Yes, pg. 9 except Test article and TK
QA statement: Yes, pg. 10-12
Drug, lot #, and % purity: BG12 (b) (4), Lot 25700, 118 mg/capsule

Methods (see design table from the sponsor's submission, below)

Frequency of dosing: BID, 4 hr apart

Route of administration: PO, tablets

Formulation/Vehicle: Torpac "Lock Ring" Size 13 capsules (porcine gelatin, methyl paraben, propyl paraben & SDS), [redacted] (b) (4)

Species/Strain: Beagle dog, [redacted] (b) (4)

Number/Sex/Group: 4/sex/gp main, 2/sex/gp recovery

Age: (not provided)

Weight: M: 7759-9852 g; F: 6454-8203 g

Significant Protocol Amendments and Deviations: There were a number of amendments and identified deviations, including:
HD dogs received supplemental feeding (1 can Science Diet per day) starting day 15, to overcome inappetance.

A number of HD animals underwent dosing holidays (based on a 20% body weight loss); animals were not dosed until they regained to within 10% of their day 1 weight.

The dosing period was shortened from 12 to 11 months (with other measures adjusted accordingly).

A number of dogs, esp. HD, received veterinary treatments for a few conditions (e.g., interdigital cyst or mass, conjunctivitis, nailbed infection).

Group No.	No. of Main Study (Recovery) Animals		Test Material	Dose Level (mg/kg)	Necropsy Day (Recovery)
	Males	Females			
1	4 (2)	4 (2)	BG12 placebo [redacted] (b) (4)	75/50[a] (0 mg/kg test article)	332/333 (365)
2	4 (2)	4 (2)	BG12 [redacted] (b) (4)	5	332/333 (365)
3	4 (2)	4 (2)	BG12 [redacted]	25	332/333 (365)
4	4 (2)	4 (2)	BG12 [redacted]	75/50[a]	332/333 (365)

[a]Beginning on Day 7, due to adverse signs noted in Group 4 animals, the dose level for Groups 1 and 4 was decreased to 50 mg/kg.

Dosing Holiday

Group No.	Animal No./Gender	Days of Dosing Holiday	Day Dosing was Resumed
4	D458/M	Days 26-28	Day 29
4	D462/M	Days 32-34	Day 35
4	D458/M	Days 35-62	Day 63
4	D459/M	Days 38-48	Day 49
4	D460/M	Days 38-55	Day 56
4	D462/M	Days 38-48	Day 49
4	D480/F, D486/F, D493/F	Days 38-41	Day 42
4	D458/M, D460/M	Days 77-90	Day 91
4	D486/F[a]	Days 82-91	Day 92
4	D458/M	Days 98-118	Day 119
4	D458/M	Days 126-139	Day 140
4	D458/M	Days 147-167	Day 168
4	D458/M	Days 175-181	Day 182
4	D458/M	Days 189-202	Day 203
4	D458/M	Days 210-216	Day 217

Note: Prior to Study Day 35, animals were placed on dosing holidays upon the veterinarian's recommendation based on the animal's food consumption and clinical signs. In order to provide more objective criteria for placing animals on dosing holidays, effective Study Day 35, any animal with a 30% body weight loss was placed on a dosing holiday until the animal achieved a body weight within 10% of its Day 1 body weight. Group 4 females #D480, #D486, and #D493 received the first dose of the day on Study Day 38 before being placed on dosing holiday.

[a]Group 4 female #D486 was placed on a dosing holiday since the animal was treated with antibiotics due to an infection.

Observations and Results

Mortality [2x/day]

There were no drug-related deaths.

Clinical Signs [daily, 30-120 min postdose; detailed exams 1x/week]

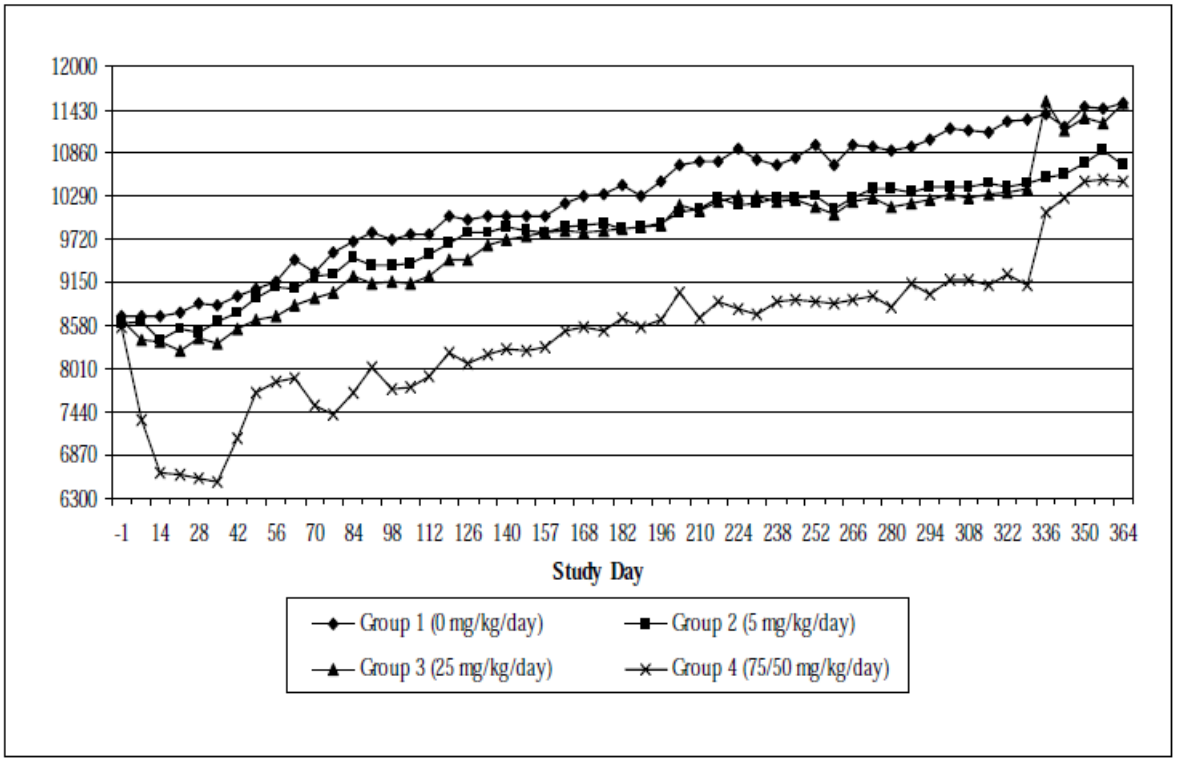
Drug-related clinical signs included: soft stools (HD), mucoid stools (MD and HD), no/few feces (HDM), mucoid material in the tray (MD and HD), emesis (mostly HD), thin appearance and/or dehydration (HD), and ocular discharge (HD). Soft stools, mucoid stools, and no/few feces were observed throughout the dosing phase of the study but were rarely observed during the recovery phase. Mucoid material in the tray also occurred at the MD and HD. Post-dose diarrhea was occasionally observed at the HD. According to the sponsor, emesis occurred primarily during the first 60 days of the study, with the greatest frequency observed during the first 30 days. Science diet food was offered to all HD animals throughout the dosing period; it was seldom given during the recovery period. Ocular discharge was observed sporadically in 2 HDM and 3 HDF during the dosing phase. Scabs occurred with greater frequency in the treated animals. Raised and/or reddened area(s) occurred with greater frequency in MDF and HDF.

Body Weights [weekly]

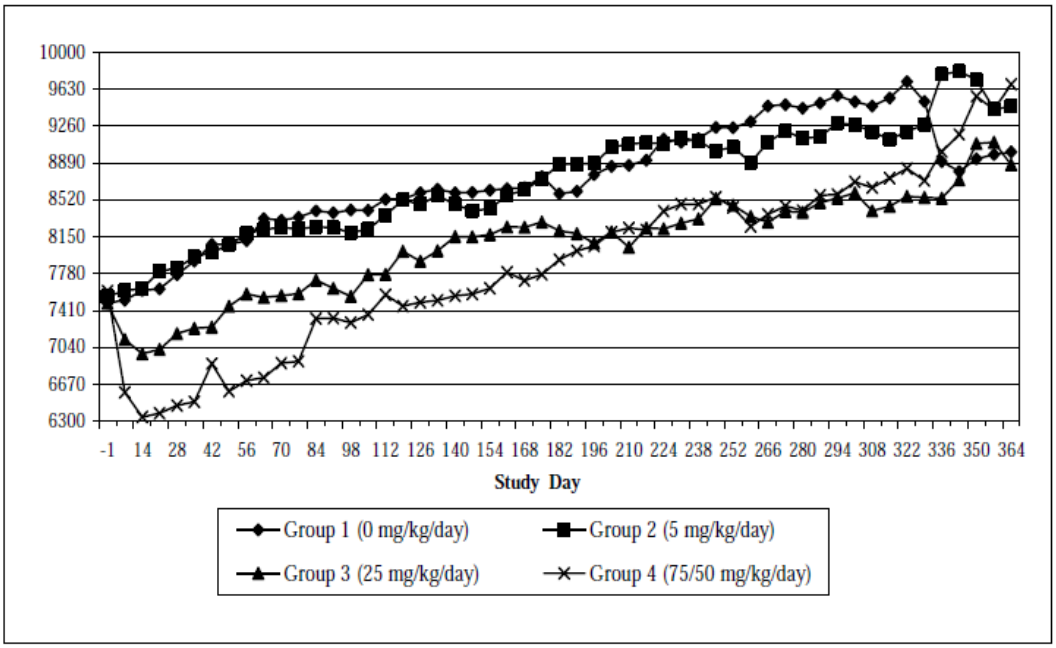
Clear drug-related decreased average body weights and average body weight gains were observed in HD animals during the dosing phase. HDM lost 24% of their initial body weight from D7 to D35 but regained some body weight starting D42. Dosing holidays were given to a number of HDM due to weight decrements. HDF lost up to 17% of their initial body weight from D7 to D14 but regained some body weight starting D70. Average body weight gains were variable in HD animals throughout the dosing period. At the end of the dosing period, average body weight reductions in HDM, MDM, and LDM were 19.4%, 7.5% and 8.2%, respectively, compared to controls. Average body weight reductions in HDF, MDF, and LDF were 8.3%, 10.1%, and 2.4%, respectively, compared to controls. See the sponsor's figures, below.

HD animals continued to gain weight during the recovery period. The average body weights of the HDM, MDM, and LDM were 8.9% less, similar to, and 7.1% less than controls, respectively, at the end of the recovery period. Average body weights of HDF, MDF, and LDF were +5%, -1.4%, and +7.5% of controls, respectively, at the end of the recovery period.

Male Group Mean Body Weights (g)



Female Group Mean Body Weights (g)



Food Consumption [daily]

Drug-related inappetance was observed in HD animals beginning the first week, resulting in substantially lower food consumption, early average body weight losses, and later reductions in body weight gains. Decreased food consumption persisted until the end of the dosing phase in HD animals; due to this effect, dietary supplements were offered to HD animals (and to some MD animals). Beginning on D8, Nutrical was offered to animals when dry food consumption intakes were ≤ 150 grams. Beginning on D15, all HD animals were offered one can of Science Diet® per day; qualitative documentation of intake was provided in the clinical signs but is not reflected in the food consumption calculations. Food consumption was similar to control for MD and LD animals. On D338, all Nutrical and Science Diet® supplements were discontinued. During the recovery period, food consumption showed a roughly dose-related increase in treated animals.

Ophthalmoscopy [pre-dosing, end of treatment, end of recovery]

The sponsor reported no drug-related ophthalmic findings in the study. No ocular abnormalities were observed pretest. A slight corneal scar on the left eye of 1 HDM was observed at the end of the dosing period. No significant ocular lesions were found at recovery.

ECG [2x pre-dosing, month 3, 6, and 9, end of dosing, end of recovery]

The sponsor reported no drug-related effects on ECG parameters or blood pressure. All dogs maintained normal sinus rhythms throughout the study. A few deviations were

noted during the study (e.g., atrial premature depolarization in a LDM on a single day and left axis deviation across groups sporadically). No statistically significant abnormalities were detected in heart rate or interval duration, with one exception. On D178, the heart rate in HDF was increased compared to control females (mean increase of 19 bpm, 27%), resulting in a reduced RR interval. Although these changes were statistically significant, they were not considered toxicologically relevant due to the relatively small magnitude of the difference and the isolated instances. Recovery traces were reported as within normal limits.

Although blood pressure was assessed, the pretest data were extremely variable (differing from controls by up to 23% in males and up to 55% in females). On D178, diastolic pressures appeared decreased in MDM and HDM (39% and 47%, respectively, less than controls). On D361 during recovery, decreased systolic pressure was detected in HD (32%, compared to controls).

Hematology [pre-dosing, days 31, 88, 179, 270, and 332/333 or 365]

The sponsor reported no "remarkable" drug-related findings, although there were several statistically significant changes (see the sponsor's summary table, below). The sponsor did not consider any of the noted differences toxicologically meaningful because: the differences were small in magnitude, the statistically significant values were within the "normalized" range of historical control values for each parameter, and/or there were no consistent differences. Notably, the data were extremely variable and interpretation was difficult. Slightly reduced (up to ~15% during dosing period, up to ~20% during recovery) erythrocytes, hemoglobin, and hematocrit were observed in HD animals throughout the study, including pretest (up to ~5%). In treated males, reticulocytes were decreased early in the dosing period and increased late in the dosing period (differences pretest were reductions of up to 20%, and the control value at recovery was nearly double other times); in HDF, reticulocytes were 2.3x controls on D270, which resolved at recovery. Increases in platelets (up to ~40% in males and ~20% in females) were observed at HD from D88 to D365 and D88 to D270, respectively, compared to controls. Monocytes were increased in MD and HD males (20-30%) during D179-365. The observed decreases in eosinophils and increases in monocytes in HDF, although statistically significant, did not appreciably differ from the absolute pretest values. Segmented neutrophils were increased 15-50% in HDF from D88 onward (absolute values decreased some during recovery).

Statistically Significant Differences in Hematology Parameters

Gender	Day	Parameter	Group	Statistically Significant Value	Concurrent Control Value	Historical Control Normalized Range
Males	31	MCHC	Group 4 ↑	36.4	35.3	31.6 – 37.3
		Mean Corpus Volume	Group 4 ↓	62.6	65.3	60.30 – 72.20
		Reticulocytes	Group 2 ↓	0.45	0.97	0.07 – 0.85
			Group 4 ↓	0.33		
	88	Mean Corpus Volume	Group 3 ↓	64.7	67.1	60.30 – 72.20
		Reticulocytes	Group 2 ↓	0.57	1.13	0.07 – 0.85
		Platelets	Group 4 ↑	422	322	254.0 – 562.0
	179	Erythrocytes	Group 4 ↓	6.78	7.89	6.270 – 7.690
		Hemoglobin	Group 4 ↓	15.6	18.4	14.80 – 18.30
		Hematocrit	Group 4 ↓	44.1	52.2	41.80 – 51.20
		MCH	Group 3 ↓	22.6	23.3	22.70 – 24.60
		Mean Corpus Volume	Group 3 ↓	64.1	66.1	63.50 – 69.00
		Reticulocytes	Group 2 ↓	0.30	0.72	0.20 – 0.50
		Platelets	Group 4 ↑	364	284	226.0 – 372.0
	270	Platelets	Group 4 ↑	374	288	226.0 – 394.0
	332/333	Platelets	Group 4 ↑	370	268	226.0 – 394.0
Females	31	Erythrocytes	Group 4 ↓	5.91	6.79	5.220 – 7.200
		Hemoglobin	Group 4 ↓	13.6	15.5	11.90 – 16.40
		Hematocrit	Group 4 ↓	38.4	43.9	34.20 – 48.50
		Eosinophils	Group 2 ↓	0.14	0.29	0.0 – 4.1
			Group 3 ↓	0.16		
			Group 4 ↓	0.05		
	88	MCHC	Group 3 ↑	35.3	34.8	31.70 – 37.10
	179	MCHC	Group 3 ↑	35.8	34.9	34.80 – 35.60
	270	Reticulocytes	Group 4 ↑	1.03	0.44	0.10 – 0.70
		Monocytes	Group 4 ↑	0.67	0.37	0.25 – 0.87
	332/333	Erythrocytes	Group 4 ↓	6.27	6.93	5.710 – 7.730
		Hemoglobin	Group 4 ↓	14.7	16.2	13.30 – 18.00
		Hematocrit	Group 4 ↓	41.5	46.5	37.80 – 50.80
		Monocytes	Group 4 ↑	0.67	0.37	0.22 – 0.87

Group 2 (5 mg/kg/day) Group 3 (25 mg/kg/day) Group 4 (75/50 mg/kg/day)

Clinical Chemistry [pre-dosing, days 31, 88, 179, 270, and 332/333 or 365]

The sponsor reported several statistically significant changes (see the sponsor's summary tables for males and females, below). The most notable changes in drug-treated animals for most of the dosing period were decreased creatinine and BUN; the sponsor considered all other changes incidental because they were minimal in magnitude, did not exhibit a dose-response relationship, or were generally within the "normalized" historical control ranges. Dose-related decreases in creatinine (~20-40%) and blood urea nitrogen (20-60%) were observed at MD and HD in males and females. After a 1-month recovery period, however, blood urea nitrogen and serum creatinine levels had returned to control levels and, according to the sponsor, were consistent with absolute pretest values for each individual animal. There were a few other changes that reflected changes seen in studies in other species. Albumin was slightly increased in

HDM during the dosing period (up to ~10%); this appeared to resolve. Globulin was also slightly increased (~10-15%) in MDM and HDM at the end of the dosing period. These alterations were reflected in a slightly decreased A/G ratio in HDM from D88 through recovery. ALT was increased in HDM but decreased in HDF (~20%), at the end of the dosing period and during recovery (>20%; MDM increased 8% on D365). GGT was increased (~10-40%) at the MD and/or HD throughout the dosing period but not at the end of recovery. Cholesterol was increased in HDM throughout the dosing period and recovery (~10-20%), and at the end of recovery only in HDF (50-70%). Increased glucose (up to ~20%) was observed in MD and HD animals at the end of recovery. Serum potassium was decreased in HD animals (up to ~10%) at the end of the dosing period; this appeared to resolve at the end of recovery. Serum calcium was slightly decreased in HDF (up to 7%) throughout the dosing period; this appeared to resolve.

Statistically Significant Differences in Male Clinical Chemistry Parameters

Gender	Parameter	Day	Group	Statistically Significant Value	Concurrent Control Value	Historical Control Normalized Range
Males	Total Protein	31	Group 3 ↑	6.35	5.73	4.814 – 6.103
			Group 4 ↓	5.19		
		270	Group 3 ↑	6.51	5.99	4.940 – 5.918
	Albumin	31	Group 4 ↓	2.79	3.11	2.341 – 3.123
		88	Group 4 ↓	2.88	3.21	
		179	Group 4 ↓	3.04	3.36	2.772 – 3.506
	Globulin	31	Group 3 ↑	3.05	2.62	2.276 – 3.260
		270	Group 3 ↑	3.17	2.76	2.082 – 2.674
	A/G Ratio	88	Group 4 ↓	1.02	1.16	0.795 – 1.245
		270	Group 4 ↓	0.99	1.17	1.154 – 1.513
	Urea Nitrogen	31	Group 4 ↓	10	17	9.2 – 23.3
		88	Group 3 ↓	13	17	
			Group 4 ↓	12		
		179	Group 3 ↓	15	19	13.8 – 19.4
			Group 4 ↓	11		
		270	Group 4 ↓	10	18	
		332/333	Group 3 ↓	14	20	
			Group 4 ↓	9		
	Creatinine	31	Group 4 ↓	0.66	0.80	0.54 – 0.897
		88	Group 2 ↓	0.73	0.81	
			Group 3 ↓	0.65		
			Group 4 ↓	0.66		
		179	Group 3 ↓	0.69	0.85	0.689 – 1.026
			Group 4 ↓	0.60		
		270	Group 3 ↓	0.66	0.82	
			Group 4 ↓	0.58		
		332/333	Group 3 ↓	0.65	0.79	
			Group 4 ↓	0.62		
	Sodium	31	Group 3 ↑	149	144	140.9 – 148.4
	Potassium	179	Group 3 ↓	4.38	4.71	4.064 – 4.558
			Group 4 ↓	4.05		
		270	Group 4 ↓	3.97	4.37	
	Glucose	270	Group 2 ↓	92	103	85.4 – 106.8
	Triglyceride	88	Group 2 ↓	32	43	14.9 – 57.5

Statistically Significant Differences in Female Clinical Chemistry Parameters

Gender	Parameter	Day	Group	Statistically Significant Value	Concurrent Control Value	Historical Control Normalized Range
Females	Total Protein	88	Group 4 ↓	5.27	5.78	4.750 – 5.957
	Albumin	88	Group 4 ↓	2.82	3.20	2.372 – 3.133
		179	Group 4 ↓	2.93	3.22	2.732 – 3.361
		270	Group 3 ↑	3.33	3.10	
	Urea Nitrogen	31	Group 3 ↓	12	16	9.2 – 22.7
			Group 4 ↓	11		
		88	Group 4 ↓	10	15	
		179	Group 4 ↓	8	17	12.6 – 21.4
		270	Group 4 ↓	7	17	
		332/333	Group 4 ↓	7	15	
	Creatinine	31	Group 3 ↓	0.65	0.78	0.542 – 0.893
			Group 4 ↓	0.57		
		88	Group 3 ↓	0.63	0.77	
			Group 4 ↓	0.49		
		179	Group 3 ↓	0.61	0.76	0.665 – 0.863
			Group 4 ↓	0.50		
		270	Group 3 ↓	0.56	0.73	
			Group 4 ↓	0.50		
		332/333	Group 3 ↓	0.59	0.76	
			Group 4 ↓	0.50		
	Calcium	31	Group 4 ↓	10.98	11.47	9.901 – 11.660
		88	Group 2 ↓	10.22	10.62	
			Group 3 ↓	10.18		
			Group 4 ↓	9.86		
		179	Group 4 ↓	10.00	10.68	10.255 – 11.105
		270	Group 4 ↓	9.89	10.52	
		332/333	Group 4 ↓	10.02	10.50	
	Chloride	31	Group 4 ↑	114	112	108.7 – 117.9
		270	Group 4 ↑	114	112	113.6 – 118.1
	ALT	179	Group 3 ↓	20	29	20.3 – 36.4
	GGT	179	Group 2 ↑	4.27	3.24	2.586 – 5.588
	Glucose	179	Group 2 ↓	84	97	90.6 – 107.8
	Triglyceride	270	Group 3 ↑	44	28	15.9 – 62.7
	Potassium	332/333	Group 4 ↓	3.99	4.43	4.005 – 4.584

Group 2 (5 mg/kg/day) Group 3 (25 mg/kg/day) Group 4 (75/50 mg/kg/day)

Urinalysis [days 332/333 or 365]

The sponsor reported no drug-related, toxicologically meaningful differences in macroscopic or microscopic urinalysis parameters. However, urine volumes at terminal necropsy were increased in HD animals (6-7x; 2.5x in MDF), as compared to controls. Decreased specific gravity (~3%, [ss]) was noted in HD animals on D332/333 only. At the end of recovery, no differences in urine volume or specific gravity were observed. Urine pH was slightly decreased in treated males. Small to moderate amounts of bilirubin, ICTOTEST-confirmed, were observed in 3 LD and 3 MD animals (2M, 1F) at the end of the dosing period (results in 1MDM and 1 HDM were not confirmed). Slightly increased leukocytes were observed in HDM. Macroscopically, hemolyzed blood was observed at low incidence in treated females. Microscopically, slightly increased RBCs, WBCs, epithelials, bacteria, and/or amorphous crystals (F) were observed at low incidence in treated animals.

Gross Pathology

Drug-related findings at terminal necropsy were observed in the kidney (MDM and HDM) and the adrenal gland (HDM). Enlargement of the kidneys correlated with increased kidney weight and hypertrophy of the tubular epithelium and diffuse cortical tubular dilation. Adrenal enlargement correlated with enlargement of the zona fasciculata.

Test Article-Related Gross Necropsy Findings for Main Study Animals

Gender	Day(s)	Finding	Group 1	Group 2	Group 3	Group 4
Males	332/333	Adrenal – enlarged	--	--	--	2/4
		Kidney – enlarged	--	--	1/4	2/4
Group 1 (0 mg/kg/day) Group 2 (5 mg/kg/day) Group 3 (25 mg/kg/day) Group 4 (75/50 mg/kg/day)						

In addition to the sponsor-noted findings above, the following were also observed at terminal necropsy: small prostate (1/4 MDM, 2/4 HDM) and discoloration and/or adhesions of the spleen (1/4 HDF). Enlarged lymph nodes were observed in a few treated animals. At recovery, a few findings were observed, including: hematocyst of the heart (1/2 HDM), discoloration of the spleen (1/2 HDM), and swollen reproductive tissues (2/2 LDF, 1/2 MDF, 2/2 HDF).

Organ Weights

Relative (to body) weight organ weights were not provided.

Clear drug-related changes in organ weight were observed for kidney (dose-related, treated males and females), testis (HDM), and epididymides (HDM). Increased kidney weights correlated with enlargement and microscopic findings of hypertrophy of the tubular epithelium (males) and diffuse cortical tubular dilation (males and females). Decrease testes weight correlated with degeneration of the seminiferous tubules, and decreased epididymides weight correlated with hypospermia. The sponsor considered other differences to not be toxicologically meaningful because the magnitude of the differences was small, microscopic correlates were not present, and/or dose-related trends were absent.

In addition to the findings above, a few other alterations were observed in absolute organ weights (complicated by the lack of body weight-corrected data, in the presence of clear body weight loss/reduction in MD and HD animals). In HDM, absolute brain weight was decreased 8%. Prostate weight was decreased at HD. Ovaries and uterus weights were decreased in treated females. Absolute adrenal (treated females, HDM), thyroid (treated males, HDF), liver (MD and HD animals), spleen (treated females) weights suggest an increased weight. Absolute thymus weight was increased in HDM but decreased in treated females. At recovery, testes (HDM) and epididymides (MDM and HDM) weights were still decreased. Ovaries and uterus weights were increased in treated females. Spleen weight was decreased in treated males. Thymus (MDM, HDM,

and treated females), thyroid (treated females), and liver (treated females) weights were increased.

Histopathology

Adequate Battery **Yes**
Peer Review **Yes, Karrie Brenneman, DM, PhD, DACVP of Biogen Idec, Inc.**

Histological Findings
Drug-related findings were observed at terminal necropsy in the kidney, testis, epididymis, and adrenal gland. The pathologist stated that the incidence and/or severity of the findings were generally greater in males than females. The sponsor considered the effects in kidney and testis primary treatment-related effects but considered the hypospermia observed in the epididymis secondary to the degeneration of seminiferous tubules observed in the testis. Additionally, the sponsor considered the adrenal effects (i.e., hypertrophy of the zona fasciculata) to be related to chronic stress.

Findings in the kidney occurred in both sexes (see the sponsor's summary table, below); the pathologist provided the following descriptions of the findings.

Kidney:

Hypertrophy of tubular epithelium in the cortex was characterized by enlarged, cuboidal epithelial cells with abundant eosinophilic cytoplasm lining cortical tubules. Some of the cells also had enlarged nuclei. The altered cells appeared to involve primarily convoluted tubules in the deep cortex.

Dilation of cortical tubules was characterized by diffuse expansion of tubular lumens.

Regeneration of tubular epithelium in the cortex was characterized by short segments of columnar to cuboidal tubular epithelial cells with increased basophilia and nuclear crowding sometimes overlying a thickened basement membrane. Regeneration, epithelial hypertrophy and tubular dilation were often seen concurrently.

Atrophy of the cortical parenchyma was characterized by atrophic tubules with or without sclerotic/degenerate glomeruli usually with interstitial fibrosis and mononuclear infiltrates. This change sometimes had a linear distribution radiating outward from the inner cortex towards the capsular surface. Other minimal foci near the periphery of the cortex were difficult to distinguish from common background.

Infiltration of mixed inflammatory cells in the renal papillae was characterized by numerous mononuclear cells, especially macrophages, and fewer neutrophils within the intertubular interstitium at the tip of the distal papillae. Although this finding was observed in only one male and one female from the high-dose group (75/50 mg/kg), it was considered to be treatment-related because it has not been seen as an incidental finding.

Hyperplasia of papillary urothelial cells lining the renal pelvis was characterized by an increase in the number of cell layers and cell size. Distribution of this change was either locally extensive or multifocal involving one or both kidneys.

Generally, the incidences were dose-related. Findings only in male dogs included hypertrophy of the cortical tubular epithelium and regeneration of the cortical tubular

epithelium; cortical thickening of Bowman's capsule basement membrane was observed in 2HDM. Dilation of cortical tubules and hyperplasia of papillary urothelial cells were observed in dogs of both sexes. Atrophy of the cortical parenchyma and infiltration of mixed inflammatory cells in the renal papillae were predominantly observed in HD male and females. Increased incidence and/or severity of tubular epithelium pigmentation were observed in MD and HD animals. Following a 1-month recovery, the incidence and/or severity of most renal findings were reduced or not observed suggesting a trend towards recovery; however, papillary hyperplasia had not decreased in incidence. Tubular epithelium pigmentation was also still observed at the end of recovery.

Incidence and Severity of Treatment-related Histopathology Findings at Terminal Euthanasia (Days 332/333)

	Males				Females			
Group	1	2	3	4	1	2	3	4
Dose of BG12 (mg/kg)	0	5	25	75/50	0	5	25	75/50
No. animals examined	4	4	4	4	4	4	4	4
Kidney								
Hypertrophy, tubular epithelium	(0)[a]	(2)	(3)	(4)	(0)	(0)	(0)	(0)
Minimal	0	1	2	2	0	0	0	0
Mild	0	1	1	2	0	0	0	0
Dilation, cortical tubules	(0)	(1)	(1)	(4)	(0)	(1)	(0)	(3)
Minimal	0	1	0	3	0	1	0	3
Mild	0	0	1	1	0	0	0	0
Regeneration, tubular epithelium	(0)	(0)	(1)	(2)	(0)	(0)	(0)	(0)
Minimal	0	0	0	1	0	0	0	0
Mild	0	0	1	1	0	0	0	0
Atrophy, cortical parenchyma	(0)	(1)	(1)	(3)	(1)	(0)	(1)	(4)
Minimal	0	1	0	1	1	0	1	4
Mild	0	0	1	2	0	0	0	0
Infiltration, mixed cell, papilla	(0)	(0)	(0)	(1)	(0)	(0)	(0)	(1)
Mild	0	0	0	0	0	0	0	1
Moderate	0	0	0	1	0	0	0	0
Hyperplasia, papillary urothelium	(0)	(1)	(2)	(4)	(0)	(0)	(3)	(4)
Minimal	0	1	2	2	0	0	1	1
Mild	0	0	0	2	0	0	2	3

[a] Total incidence of a finding is in parentheses ( )

Observations: Neo-Plastic and Non Neo-Plastic	MALES				FEMALES			
Removal Reason: TERMINAL EUTHANASIA	1	2	3	4	1	2	3	4
Number of Animals on Study :	4	4	4	4	4	4	4	4
Number of Animals Completed:	(4)	(4)	(4)	(4)	(4)	(4)	(4)	(4)
KIDNEY;								
Examined	(4)	(4)	(4)	(4)	(4)	(4)	(4)	(4)
Pigmentation; tubular; Epithelium; bilateral; multifocal	(2)	(2)	(3)	(3)	(0)	(0)	(1)	(3)
minimal	2	2	2	3	0	0	1	1
mild	0	0	1	0	0	0	0	2
Thickening; cortical; Bowmans Capsule; Basement Membrane; hyaline; bilateral; multifocal	(0)	(0)	(0)	(2)	(0)	(0)	(0)	(0)
minimal	0	0	0	1	0	0	0	0
mild	0	0	0	1	0	0	0	0

Incidence and Severity of Treatment-related and Adrenal Histopathology Findings at Recovery Euthanasia (Day 365)

	Males				Females			
Group	1	2	3	4	1	2	3	4
Dose of BG12 (mg/kg)	0	5	25	75/50	0	5	25	75/50
No. animals examined	2	2	2	2	2	2	2	2
Kidney								
Hypertrophy, tubular epithelium	(0)[a]	(2)	(0)	(1)	(0)	(0)	(0)	(0)
Minimal	0	2	0	0	0	0	0	0
Mild	0	0	0	1	0	0	0	0
Dilation, cortical tubules	(0)	(0)	(1)	(1)	(0)	(0)	(0)	(0)
Minimal	0	0	1	1	0	0	0	0
Atrophy, cortical parenchyma	(1)	(1)	(2)	(1)	(0)	(0)	(0)	(0)
Minimal	1	1	0	1	0	0	0	0
Mild	0	0	2	0	0	0	0	0
Infiltration, mixed cell, papilla	(0)	(0)	(0)	(0)	(0)	(0)	(0)	(1)
Minimal	0	0	0	0	0	0	0	1
Hyperplasia, papillary urothelium	(0)	(0)	(2)	(1)	(0)	(0)	(1)	(1)
Minimal	0	0	1	1	0	0	1	0
Mild	0	0	1	0	0	0	0	1

[a] Total incidence of a finding is in parentheses ( )

Observations: Neo-Plastic and Non Neo-Plastic	MALES				FEMALES			
Removal Reason: RECOVERY EUTHANASIA	1	2	3	4	1	2	3	4
Number of Animals on Study :	2	2	2	2	2	2	2	2
Number of Animals Completed:	(2)	(2)	(2)	(2)	(2)	(2)	(2)	(2)
KIDNEY;								
Examined	(2)	(2)	(2)	(2)	(2)	(2)	(2)	(2)
Pigmentation; tubular; Epithelium; bilateral; multifocal	(1)	(0)	(0)	(2)	(0)	(0)	(0)	(2)
minimal	1	0	0	1	0	0	0	1
mild	0	0	0	1	0	0	0	1

Findings in the testis included degeneration of the seminiferous tubular epithelium and the presence of spermatid giant cells in the lumen of the seminiferous tubules in MDM and HDM at the end of the dosing period and at the end of the recovery period. The pathologist provided the following descriptions of the testis findings (see excerpt below). However, the incidence and/or severity of seminiferous tubular degeneration were generally reduced at the end of recovery. The only microscopic finding in the epididymis was hypospermia (described by the pathologist as "characterized by a decreased number of spermatozoa in the tubules") in HDM; hypospermia was not observed at the end of the recovery period. 1 MDM showed unilateral moderate spermatid giant cells in the epididymides at the end of recovery.

Testis:

Degeneration of seminiferous epithelium was characterized by a decrease in all stages of germinal epithelial cells with retention of Sertoli cells. Degeneration of epithelium was sometimes accompanied by increased numbers of spermatid giant cells in the affected tubules.

Spermatid giant cells was a finding characterized by large, multinucleated cells in the lumen of seminiferous tubules. This finding was frequently associated with degeneration of seminiferous tubules.

Incidence and Severity of Treatment-related Histopathology Findings at Terminal Euthanasia (Days 332/333)

	Males				Females			
Group	1	2	3	4	1	2	3	4
Dose of BG12 (mg/kg)	0	5	25	75/50	0	5	25	75/50
No. animals examined	4	4	4	4	4	4	4	4
Testis								
Degeneration, epithelium	(1)	(0)	(1)	(3)	-	-	-	-
Minimal	1	0	0	0	-	-	-	-
Mild	0	0	1	2	-	-	-	-
Moderate	0	0	0	1	-	-	-	-
Spermatid giant cells	(0)	(0)	(0)	(2)	-	-	-	-
Minimal	0	0	0	2	-	-	-	-
Epididymis								
Hypospermia	(0)	(0)	(0)	(3)	-	-	-	-
Moderate	0	0	0	3	-	-	-	-

[a] Total incidence of a finding is in parentheses ( )

Incidence and Severity of Treatment-related and Adrenal Histopathology Findings at Recovery Euthanasia (Day 365)

	Males				Females			
Group	1	2	3	4	1	2	3	4
Dose of BG12 (mg/kg)	0	5	25	75/50	0	5	25	75/50
No. animals examined	2	2	2	2	2	2	2	2
Testis								
Degeneration, epithelium	(0)	(0)	(1)	(0)	-	-	-	-
Minimal	0	0	1	0	-	-	-	-
Spermatid giant cells	(0)	(0)	(0)	(2)	-	-	-	-
Minimal	0	0	0	2	-	-	-	-

[a] Total incidence of a finding is in parentheses ( )

Adrenal gland showed minimal to moderate hypertrophy of the zona fasciculata in MD and HD animals. The pathologist described the adrenal hypertrophy as, "characterized by expansion of the zona fasciculata by large, finely vacuolated cells." At the end of recovery, minimal hypertrophy of the zona fasciculata was still observed in HD dogs but was not considered drug-related by the sponsor because the incidence was similar in control dogs.

Incidence and Severity of Treatment-related Histopathology Findings at Terminal Euthanasia (Days 332/333)

	Males				Females			
Group	1	2	3	4	1	2	3	4
Dose of BG12 (mg/kg)	0	5	25	75/50	0	5	25	75/50
No. animals examined	4	4	4	4	4	4	4	4
Adrenal Gland								
Hypertrophy, zona fasciculata	(0)	(0)	(2)	(1)	(0)	(0)	(2)	(2)
Minimal	0	0	1	0	0	0	2	2
Mild	0	0	0	1	0	0	0	0
Moderate	0	0	1	0	0	0	0	0

[a] Total incidence of a finding is in parentheses ( )

Incidence and Severity of Treatment-related and Adrenal Histopathology Findings at Recovery Euthanasia (Day 365)

	Males				Females			
Group	1	2	3	4	1	2	3	4
Dose of BG12 (mg/kg)	0	5	25	75/50	0	5	25	75/50
No. animals examined	2	2	2	2	2	2	2	2
Adrenal Gland								
Hypertrophy, zona fasciculata	(1)	(0)	(0)	(1)	(0)	(0)	(0)	(1)
Minimal	1	0	0	1	0	0	0	1

[a] Total incidence of a finding is in parentheses ( )

A few other findings were observed at low or increased incidence. Changes were observed in brain, heart, mammary gland, prostate, spleen, stomach, thyroid, urinary bladder, and uterus. Lymph node findings of lymphoid hyperplasia, hemorrhage, and/or hemosiderin pigmentation were observed sporadically.

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Observations: Neo-Plastic and Non Neo-Plastic	MALES				FEMALES			
Removal Reason: TERMINAL EUTHANASIA	1	2	3	4	1	2	3	4
Number of Animals on Study :	4	4	4	4	4	4	4	4
Number of Animals Completed:	(4)	(4)	(4)	(4)	(4)	(4)	(4)	(4)
BRAIN;								
Examined	(4)	(4)	(4)	(4)	(4)	(4)	(4)	(4)
Within Normal Limits	3	3	3	3	3	4	4	4
Infiltration, Mononuclear Cell; meningeal; focal	(0)	(0)	(1)	(1)	(0)	(0)	(0)	(0)
minimal	0	0	1	1	0	0	0	0
HEART;								
Examined	(4)	(4)	(4)	(4)	(4)	(4)	(4)	(4)
Within Normal Limits	4	4	4	3	4	4	4	4
Increased Thickness; Atrioventricular Valve	(0)	(0)	(0)	(1)	(0)	(0)	(0)	(0)
mild	0	0	0	1	0	0	0	0
MAMMARY GLAND;								
Examined	(2)	(3)	(4)	(4)	(4)	(4)	(4)	(4)
Galactocele; multifocal	(0)	(0)	(0)	(0)	(1)	(2)	(3)	(2)
minimal	0	0	0	0	0	1	0	0
mild	0	0	0	0	0	1	2	1
moderate	0	0	0	0	1	0	1	1
PROSTATE GLAND;								
Examined	(4)	(4)	(4)	(4)	(-)	(-)	(-)	(-)
Within Normal Limits	4	4	0	3	-	-	-	-
Immature	0	0	1	0	-	-	-	-
Dilation; glandular; Acinus; focal	(0)	(0)	(2)	(0)	(-)	(-)	(-)	(-)
minimal	0	0	1	0	-	-	-	-
mild	0	0	1	0	-	-	-	-
Dilation; glandular; Acinus; multifocal	(0)	(0)	(1)	(1)	(-)	(-)	(-)	(-)
minimal	0	0	1	1	-	-	-	-
Infiltration, Mononuclear Cell; interstitial; multifocal	(0)	(0)	(1)	(0)	(-)	(-)	(-)	(-)
mild	0	0	1	0	-	-	-	-
SPLEEN;								
Examined	(4)	(4)	(4)	(4)	(4)	(4)	(4)	(4)
Within Normal Limits	0	0	0	0	2	0	0	0
Congestion; vascular; Red Pulp	(4)	(4)	(4)	(4)	(2)	(4)	(4)	(3)
minimal	3	2	3	1	2	4	1	2
mild	1	2	1	3	0	0	3	1
Adhesion; focal	(0)	(0)	(0)	(0)	(0)	(0)	(0)	(1)
mild	0	0	0	0	0	0	0	1
STOMACH;								
Examined	(4)	(4)	(4)	(4)	(4)	(4)	(4)	(4)
Within Normal Limits	4	4	4	4	4	4	4	3
Hyperplasia; Fundus; Parietal Cell; diffuse	(0)	(0)	(0)	(0)	(0)	(0)	(0)	(1)
mild	0	0	0	0	0	0	0	1
THYROID GLAND;								
Examined	(4)	(4)	(4)	(4)	(4)	(4)	(4)	(4)
Metaplasia; squamous cell; unilateral; focal	(0)	(0)	(1)	(0)	(0)	(0)	(0)	(1)
mild	0	0	1	0	0	0	0	1
Dilation; follicular; bilateral; multifocal	(0)	(0)	(0)	(1)	(0)	(0)	(0)	(0)
minimal	0	0	0	1	0	0	0	0
Dilation; follicular; unilateral; focal	(0)	(0)	(0)	(1)	(0)	(0)	(0)	(0)
minimal	0	0	0	1	0	0	0	0

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Observations: Neo-Plastic and Non Neo-Plastic	MALES				FEMALES			
Removal Reason: RECOVERY EUTHANASIA	1	2	3	4	1	2	3	4
Number of Animals on Study :	2	2	2	2	2	2	2	2
Number of Animals Completed:	(2)	(2)	(2)	(2)	(2)	(2)	(2)	(2)
MAMMARY GLAND;								
Examined	(2)	(2)	(1)	(2)	(2)	(2)	(2)	(2)
Hyperplasia; alveolar; epithelial; diffuse	(0)	(0)	(0)	(0)	(0)	(0)	(1)	(2)
mild	0	0	0	0	0	0	0	1
moderate	0	0	0	0	0	0	1	1
OVARY;								
Examined	(-)	(-)	(-)	(-)	(2)	(2)	(2)	(2)
Within Normal Limits	-	-	-	-	2	0	0	0
Corpus Luteum; bilateral; multiple	-	-	-	-	0	2	2	2
PROSTATE GLAND;								
Examined	(2)	(2)	(2)	(2)	(-)	(-)	(-)	(-)
Within Normal Limits	1	1	2	1	-	-	-	-
Dilation; glandular; Acinus; focal	(0)	(0)	(0)	(1)	(-)	(-)	(-)	(-)
moderate	0	0	0	1	-	-	-	-
URINARY BLADDER;								
Examined	(2)	(2)	(2)	(2)	(2)	(2)	(2)	(2)
Within Normal Limits	2	2	2	2	2	2	2	1
Mineralization; Periarterial; focal	(0)	(0)	(0)	(0)	(0)	(0)	(0)	(1)
mild	0	0	0	0	0	0	0	1
UTERUS;								
Examined	(-)	(-)	(-)	(-)	(2)	(2)	(2)	(2)
Within Normal Limits	-	-	-	-	2	1	1	0
Dilation; Lumen; bilateral	(-)	(-)	(-)	(-)	(0)	(0)	(1)	(2)
minimal	-	-	-	-	0	0	1	0
mild	-	-	-	-	0	0	0	1
moderate	-	-	-	-	0	0	0	1
Retained Placental Tissue; unilateral	-	-	-	-	0	1	0	0

Toxicokinetics [days 1, 91, 182, 273 and 330]

The plasma analysis assay was not reliable. While all analytical results were within acceptable limits, the study overall had a high number of failed batches (especially during the month of November 2005). It was later determined that the analysis of human plasma (from (b) (4) Project Number EBA00182MX) interfered with the analysis of dog plasma when analyzed in that order. Subsequently, analyses of MMF in human and dog plasma samples were performed on different LC/MS/MS instruments. The assay's LLOQ was 50 ng/mL.

The TK portion of the study was not conducted under GLP. On days 1, 182, and 330, samples were taken pre-dose and at 0.5, 1, 2, 4 (prior to second dose), 4.5, 5, 6, 8, and 24 hours post-dose (after first dose). On days 91 and 273, samples were taken pre-dose (prior to first and second doses), and at 1 and 5 hours post-dose (after first dose). TK samples were analyzed for MMF concentration.

MMF concentrations were BLOQ in controls at all timepoints, and pre-dose exposures in treated animals were BLOQ, with one exception (Animal 462 on D330 with 133 ng/mL MMF). Exposure was confirmed in all treated animals throughout the 11-month study interval. Plasma T_{max} was 0.5-4 hours postdose. AUC and C_{max} increased with dose. In most animals, AUC and C_{max} decreased between D1 and D182; exposures then remained level until D330. The reason for the decrease in exposures between D1 and D182 is unknown. Exposures were not sex-dependent. See the sponsor's summary TK table, below.

Table 2. Toxicokinetic parameter estimates of MMF on Days 1, 182 and 330 after twice-daily oral administration of BG00012 to dogs for 11 months at 5, 25, 50 or 75 mg/kg/day.

Dose per day	Gender	Study_Day	PK Paramter	AUC(0-24hr) hr*ng/mL	Cmax ng/mL	Tmax hr
5 mg/kg	Female	Day 1	**Mean**	**9837**	**2892**	**2.5**
			SD	4992	726	2.4
			CV%	51	25	94
		Day 182	**Mean**	**5697**	**2252**	**2.3**
			SD	1811	593	2.1
			CV%	32	26	95
		Day 330	**Mean**	**6969**	**1687**	**3.0**
			SD	4084	385	2.9
			CV%	59	23	97
	Male	Day 1	**Mean**	**8544**	**2610**	**4.3**
			SD	2339	1335	2.8
			CV%	27	51	65
		Day 182	**Mean**	**5805**	**2406**	**1.7**
			SD	1292	910	1.6
			CV%	22	38	98
		Day 330	**Mean**	**5684**	**2072**	**3.3**
			SD	1768	837	2.7
			CV%	31	40	82
25 mg/kg	Female	Day 1	**Mean**	**49076**	**11388**	**3.7**
			SD	12691	4686	2.3
			CV%	26	41	61
		Day 182	**Mean**	**22413**	**8033**	**3.8**
			SD	3406	4162	2.6
			CV%	15	52	69
		Day 330	**Mean**	**27336**	**9105**	**3.2**
			SD	7499	1566	2.4
			CV%	27	17	76
	Male	Day 1	**Mean**	**40250**	**11230**	**2.8**
			SD	11605	3495	2.1
			CV%	29	31	75
		Day 182	**Mean**	**21037**	**8613**	**3.1**
			SD	6358	2465	2.5
			CV%	30	29	81
		Day 330	**Mean**	**23998**	**10962**	**2.5**
			SD	8800	4526	2.2
			CV%	37	41	89
50 mg/kg	Female	Day 182	**Mean**	**34996**	**13563**	**1.4**
			SD	11076	5540	0.7
			CV%	32	41	47
		Day 330	**Mean**	**44785**	**14548**	**1.1**
			SD	12233	4476	0.5
			CV%	27	31	45
	Male	Day 182	**Mean**	**42250**	**12380**	**2.5**
			SD	27715	5482	2.1
			CV%	66	44	83
		Day 330	**Mean**	**52045**	**9662**	**1.9**
			SD	30758	4080	1.2
			CV%	59	42	63
75 mg/kg	Female	Day 1	**Mean**	**91086**	**24850**	**3.4**
			SD	10718	5355	2.2
			CV%	12	22	64
	Male	Day 1	**Mean**	**74632**	**22933**	**2.7**
			SD	9371	8869	2.3
			CV%	13	39	87

Dosing Solution Analysis

Dose sample analysis was not performed for this study.

Histopathology inventory

Study	04-06, 6 mo	05-08, 12 mo	05-05, 11 mo
Species	Rat	Monkey	Dog
Adrenals	X*	X*	X*
Aorta	X	X	X
Bone Marrow smear	X, femur (not assessed)	X	X, from 7th rib
Bone (femur)	X, w/stifle	X, 7th rib	X
Brain	X*	X*	X*
Cecum	X	X	X
Cervix	X, w/uterus	X	X
Colon	X	X	X
Duodenum	X	X	X
Epididymis	X*	X*	X*
Esophagus	X	X	X
Eye	X, w/optic nerve	X	X
Fallopian tube			
Gall bladder	N/A	X	X
Gross lesions	X	X	X
Harderian gland	X		
Heart	X*	X*	X*
Ileum	X	X	X, with Peyer's Patch
Injection site			
Jejunum	X	X	X
Kidneys	X*	X*	X*
Lachrymal gland	X, exorbital		X
Larynx	X		
Liver	X*	X*	X*
Lungs	X*	X*	X*
Lymph nodes, cervical			
Lymph nodes mandibular	X	X	X
Lymph nodes, mesenteric	X	X	X
Mammary Gland	X, w/skin	X	X
Nasal cavity			
Optic nerves	X	X	X
Ovaries	X*, w/oviducts	X*	X*
Pancreas	X	X	X
Parathyroid	X, w/thyroid	X	X
Peripheral nerve			
Pharynx			
Pituitary	X*	X*	X*
Prostate	X	X	X*
Rectum	X	X	X

Salivary gland	X, submandibular	X	X*, mandibular
Sciatic nerve	X	X	X
Seminal vesicles	X	X	
Skeletal muscle	X, thigh	X, psoas & diaphragm	X, thigh
Skin	X, w/mammary	X	X, mammary
Spinal cord	X, cervical, midthoracic, lumbar	X, cervical, thoracic, lumbar	X, cervical, thoracic, lumbar
Spleen	X*	X*	X*
Sternum	X, w/marrow		X
Stomach	X*	X	X, cardiac, fundic, pyloric
Testes	X*	X*	X*
Thymus	X*	X*	X*
Thyroid	X*	X* (wt with parathyroids)	X* (wt with parathyroids)
Tongue	X	X	X
Trachea	X	X	X
Urinary bladder	X	X	X
Uterus	X, w/cervix	X	X*
Vagina	X	X	X
Zymbal gland			

X, histopathology performed
*, organ weight obtained

Also: RAT: popliteal lymph nodes
MONKEY: femoral-tibial joint (gross, no histology), ureter
DOG: femoral-tibial joint

7 Genetic Toxicology

7.1 *In Vitro* Reverse Mutation Assay in Bacterial Cells (Ames)

Study title: MUTAGENICITY STUDY OF DIMETHYLFUMARATE IN THE AMES SALMONELLA/MICROSOME PLATE TEST (*IN VITRO*)

Study no.:	5403/89
Study report location:	EDR
Conducting laboratory and location:	(b) (4)
Date of study initiation:	9/8/89, protocol dated 4/21/89
GLP compliance:	Yes, FDA, page 3
QA statement:	Yes, page 4
Drug, lot #, and % purity:	Dimethylfumarate, batches 8660686 and L980737, 100.6% (0.26% fumaric acid)

Methods

Strains:	*Salmonella typhimurium* strains TA 98, TA 100, TA 1535, TA 1537, and TA 1538
Concentrations in definitive study:	-S9: 0, 3.16, 10, 31.6, 100, 316, 1000 & 3160 μg/plate Repeat: 10, 31.6, 100, 316, 1000, 3160 & 10000 μg/plate +S9: 0, 3.16, 10, 31.6, 100, 316, 1000 & 3160 μg/plate Repeat: 10, 31.6, 100, 316, 1000, 3160 & 10000 μg/plate
Basis of concentration selection:	Cytotoxicity at 1000 μg/plate without metabolic activation (Little if any cytotoxicity with metabolic activation)
Negative control:	DMF, 100 μl
Positive control:	+S9: 2-aminoanthracene in DMSO (2 μg/plate) -S9: TA 100 & 1535- sodium azide (10 μg/plate) TA 98 & 1538- 2NF (10 μg/plate) TA 1537- 9-aminoacridine (50 μg/plate)
Formulation/Vehicle:	DMF
Incubation & sampling time:	48 hr at 37°C

Study Validity

The study is lacking a strain for the detection of cross-linking mutagens, S. typhimurium TA 102 or a repair-proficient E. coli strain. 2-aminoanthracene was the only positive control used in the presence of metabolic activation; that is, was the sole indicator of efficacy of the S9 mix. Two separate experiments were conducted using the plate incorporation method (triplicate platings), with and without metabolic activation (Aroclor 1254-induced rat liver S9 fraction). Eight concentrations (ranging from 3.16- 10000 μg) of dimethylfumarate/plate were tested. Cytotoxicity was observed at 1000 μg/plate without metabolic activation, but very little cytotoxicity was observed with metabolic activation (up to a concentration of 10000 μg/plate). A positive result was reported when: 1) the number of revertants was significantly increased (Mann-Whitney) compared to control by 2-3 fold, 2) a significant dose-related effect was demonstrated, and 3) the result was reproducible. The formulations were 99-102.1% of nominal. Historical controls were not provided.

Results: Negative, but inadequate

(Lacks a cross-link detector strain and 2-AA was the only positive control with S9.)

In the first experiment without S9, DMF showed at least double the number of revertants at 3.16 - 316 mg/plate (the highest value was at the lowest concentration, and was due to 1 of 3 cultures); however, this was not seen in the repeat experiment. Cytotoxicity was seen at 3160 &/or 10000 μg/plate. No clearly mutagenic effect was observed for dimethylfumarate tested up to 10000 μg/plate in any of the tester strains in two independent experiments with and without activation.

TABLE 2 Mutagenicity test with Dimethylfumarate in the AMES Salmonella/microsome plate test (in vitro)

Substance (µg/plate)		Summarized data without metabolic activation revertants per plate TA 1535	TA 1537	TA 1538	TA 98	TA 100
1 s t e x p e r i m e n t						
Dimethylfumarate:						
3160.0	M	0.0	7.0	1.3	2.7	14.3
	±SD	0.0	3.6	1.5	1.2	4.6
1000.0	M	4.0	5.7	23.3	23.0	70.0
	±SD	1.0	1.5	4.0	5.3	4.0
316.0	M	7.7	7.7	27.7	34.0	106.0
	±SD	0.6	0.6	6.0	8.2	5.3
100.0	M	9.0	6.3	19.3	37.7	122.3
	±SD	1.0	1.5	3.1	4.5	1.2
31.6	M	7.7	6.3	25.0	33.3	122.7
	±SD	1.5	1.5	5.3	2.5	12.9
10.0	M	8.3	5.0	22.0	30.7	109.0
	±SD	2.1	2.0	7.5	7.6	7.8
3.16	M	11.7	6.0	22.3	38.0	112.7
	±SD	8.3	2.0	5.9	7.9	12.1
Solvent control: 100 µl DMF/ plate	M	4.0	4.7	23.7	32.3	120.0
	±SD	1.7	1.5	8.0	10.1	3.5
Positive control: substance		Sodium-azide	9-Amino acridine	2-Nitro-9H-fluorene	2-Nitro-9H-fluorene	Sodium-azide
Concentration (µg/plate)		10	50	10	10	10
	M	4132.7	879.7	1634.7	2099.3	1765.7
	±SD	952.7	122.2	152.1	247.6	118.4

M = mean number of revertants
SD = standard deviation

TABLE 2 Mutagenicity test with Dimethylfumarate in the AMES Salmonella/microsome plate test (in vitro)

Substance (µg/plate)		Summarized data without metabolic activation revertants per plate TA 1535	TA 1537	TA 1538	TA 98	TA 100
2 n d e x p e r i m e n t						
Dimethylfumarate:						
10000.0	M	0.3	0.0	0.0	0.0	0.0
	±SD	0.6	0.0	0.0	0.0	0.0
3160.0	M	0.0	0.0	0.7	0.3	2.7
	±SD	0.0	0.0	0.6	0.6	1.5
1000.0	M	2.0	6.7	15.3	14.3	61.0
	±SD	1.0	2.3	1.2	5.1	5.2
316.0	M	7.0	3.7	20.7	14.3	64.0
	±SD	1.0	2.3	4.0	0.6	4.6
100.0	M	7.0	3.3	23.0	20.7	90.0
	±SD	4.6	1.5	4.4	6.7	14.8
31.6	M	9.3	6.0	22.3	23.7	104.7
	±SD	2.1	2.0	8.1	4.0	7.6
10.0	M	8.3	3.3	22.7	28.0	122.0
	±SD	2.1	0.6	5.8	6.1	11.5
Solvent control: 100 µl DMF/ plate	M	8.7	4.7	22.0	21.0	80.0
	±SD	4.7	1.2	3.6	5.6	4.4
Positive control: substance		Sodium-azide	9-Amino acridine	2-Nitro-9H-fluorene	2-Nitro-9H-fluorene	Sodium-azide
Concentration (µg/plate)		10	50	10	10	10
	M	1995.3	946.0	1251.7	1279.3	1267.3
	±SD	25.1	58.8	73.7	238.4	57.4

M = mean number of revertants
SD = standard deviation

TABLE 2 Mutagenicity test with Dimethylfumarate in the AMES Salmonella/microsome plate test (in vitro)

Substance (µg/plate)		Summarized data with metabolic activation revertants per plate TA 1535	TA 1537	TA 1538	TA 98	TA 100
1 s t e x p e r i m e n t						
Dimethylfumarate:						
3160.0	M	11.3	6.7	35.0	44.3	82.3
	±SD	0.6	3.8	7.0	0.6	10.1
1000.0	M	12.7	4.3	34.7	39.7	96.3
	±SD	5.1	2.3	5.9	5.0	6.4
316.0	M	8.7	12.0	31.7	34.7	103.3
	±SD	2.1	2.0	2.5	7.4	11.8
100.0	M	8.3	9.0	31.0	42.7	104.0
	±SD	5.7	3.6	3.0	4.0	7.9
31.6	M	10.0	11.0	31.3	40.3	113.7
	±SD	6.1	3.6	7.4	2.5	8.1
10.0	M	8.0	8.7	39.0	45.3	107.0
	±SD	1.7	3.2	3.6	6.8	9.5
3.16	M	8.3	8.3	31.0	38.3	104.7
	±SD	6.7	1.5	4.0	5.7	6.0
Solvent control: 100 µl DMF/ plate	M	11.0	8.0	33.7	36.7	110.3
	±SD	6.1	2.6	3.5	8.6	11.4
Positive control: substance		2-Aninoanthracene				
Concentration (µg/plate)		2	2	2	2	2
	M	302.3	190.0	426.3	1137.0	1110.0
	±SD	56.4	19.7	114.9	275.0	147.8

M = mean number of revertants
SD = standard deviation

TABLE 2 Mutagenicity test with Dimethylfumarate in the AMES Salmonella/microsome plate test (in vitro)

Substance (µg/plate)		Summarized data with metabolic activation revertants per plate TA 1535	TA 1537	TA 1538	TA 98	TA 100
2 n d e x p e r i m e n t						
Dimethylfumarate:						
10000.0	M	5.3	7.0	34.0	33.0	111.3
	±SD	3.2	1.7	7.2	8.2	7.0
3160.0	M	6.3	5.7	28.3	34.3	134.0
	±SD	1.2	0.6	5.0	3.8	2.0
1000.0	M	13.7	9.3	33.3	42.7	124.3
	±SD	4.0	3.2	5.5	7.4	10.1
316.0	M	13.7	7.0	45.7	45.0	137.0
	±SD	1.2	3.0	4.7	7.9	13.0
100.0	M	16.0	10.3	36.0	48.0	140.7
	±SD	1.7	2.5	3.5	7.8	11.1
31.6	M	6.7	8.3	37.0	53.7	142.0
	±SD	2.3	1.2	5.3	4.9	13.9
10.0	M	10.3	6.3	41.0	48.0	129.3
	±SD	3.8	2.5	5.6	5.3	15.6
Solvent control: 100 µl DMF/ plate	M	8.7	9.7	40.0	45.0	134.0
	±SD	2.1	2.1	6.6	8.5	6.6
Positive control: substance		2-Aminoanthracene				
Concentration (µg/plate)		2	2	2	2	2
	M	261.3	226.7	453.7	1254.7	1044.3
	±SD	17.9	3.8	49.9	313.0	148.7

M = mean number of revertants
SD = standard deviation

In Vitro Reverse Mutation Assay in Bacterial Cells (Ames)

Study title: Bacterial Reverse Mutation Assay with a Confirmatory Assay

Study no.:	P00012-08-02 ; (b) (4) 6538525
Study report location:	EDR
Conducting laboratory and location:	(b) (4)
Date of study initiation:	2/9/10
GLP compliance:	Yes, page 2, except GMP of test article
QA statement:	Yes, Page 3
Drug, lot #, and % purity:	Mono-methyl fumarate (MMF) 97%; CAS 2756-87-8; lot 75798MJ; 97.5% pure

Key Study Findings

Methods

Strains:	*S. typhimurium* TA98, TA100, TA1535, and TA1537, and *E. coli* WP2*uvr*A
Concentrations in definitive study:	Initial +S9: 1.60, 5.00, 16.0, 50.0, 160, 500, 1600, and 5000 µg/plate -S9: 1.60, 5.00, 16.0, 50.0, 160, 500, 1600, and 5000 µg/plate Confirmatory ±S9: 50.0, 160, 500, 1600, 3330, and 5000 µg/plate
Basis of concentration selection:	Limit dose
Negative control:	Dimethylsulfoxide (DMSO); CAS 67-68-5; lots 07796KK & 35596LK >99% pure
Positive control:	See Table I from sponsor, below
Formulation/Vehicle:	DMSO
Incubation & sampling time:	incubated for 52 ± 4 hours at 37 ± 2°C

Table I
Positive Control Articles

Tester Strain(s)	S9	Positive Control	Dose (µg/plate)	CAS No.	Lot No.
TA98	–	2-nitrofluorene	1.0	607-57-8	01508BE
TA100, TA1535	–	sodium azide	2.0	26628-22-8	017K0136
TA1537	–	ICR-191	2.0	17070-45-0	116K1026
WP2*uvr*A	–	4-nitroquinoline-N-oxide	1.0	56-57-5	117K1485
TA98	+	benzo[a]pyrene	2.5	50-32-8	087K0733
TA100, TA1535, TA1537	+	2-aminoanthracene	2.5	613-13-8	12317CE
WP2*uvr*A	+	2-aminoanthracene	25.0	613-13-8	12317CE

Study Validity

Duplicate plates using the plate incorporation method were used in the initial assay, but the confirmatory assay (also plate incorporation) was plated in triplicate. Test article was freely soluble at all doses with and without S9. Male rat liver homogenate (S9) was purchased [redacted] (b) (4) (Lot No. 2538, containing 34.2 mg/mL protein). Growth inhibition was observed at 5000 and/or 1600 μg/plate (and ≥3330 μg/plate in the confirmatory assay). A positive response was defined as: 1) a dose-dependent increase in revertant frequency that is ≥2.0-fold vehicle control values for tester strains TA98, TA100, and WP2*uvr*A, or ≥3.0-fold vehicle control values for tester strains TA1535 and TA1537, and 2) reproducible. Tested formulations were 96.3 to 103% of nominal.

Results- Negative

MMF did not cause an increase in revertant frequency in any of the strains tested (see sponsor's summary, below). The sponsor also provided historical data (see the sponsor's table, below).

Test Article	Dose (μg/plate)	Revertant Colony Counts (Mean ± SD) Strain TA98	TA100	TA1535	TA1537	WP2*uvr*A
Without Metabolic Activation						
DMSO	50μl/plate	13 ± 1	100 ± 3	19 ± 4	5 ± 1	13 ± 0
Mono-methyl fumarate	1.60	11 ± 1	80 ± 6	23 ± 4	9 ± 5	17 ± 1
	5.00	14 ± 2	90 ± 6	26 ± 3	6 ± 2	15 ± 1
	16.0	12 ± 3	89 ± 8	23 ± 4	3 ± 0	15 ± 2
	50.0	15 ± 5	96 ± 5	14 ± 3	3 ± 0	13 ± 0
	160	16 ± 6	102 ± 0	17 ± 7	4 ± 0	12 ± 7
	500	14 ± 2	109 ± 13	15 ± 4	4 ± 1	15 ± 1
	1600	10 ± 6	88 ± 6	15 ± 4	5 ± 1 [R]	15 ± 6
	5000	0 ± 0 [R]	34 ± 9 [R]	4 ± 1 [R]	2 ± 2 [R]	13 ± 3 [R]
2-nitrofluorene	1.0	287 ± 18				
sodium azide	2.0		947 ± 49	733 ± 27		
ICR-191	2.0				199 ± 21	
4-nitroquinoline-N-oxide	1.0					227 ± 54

GLP = Good Laboratory Practice
Positive response (increase over corresponding control): 2-fold for TA98, TA100, and WP2*uvr*A; 3-fold for TA1535 and TA1537
[R] Reduced background bacterial lawn

With Metabolic Activation						
DMSO	50μl/plate	18 ± 2	119 ± 11	14 ± 4	10 ± 2	19 ± 6
Mono-methyl fumarate	1.60	21 ± 1	91 ± 4	14 ± 2	8 ± 4	17 ± 1
	5.00	21 ± 5	105 ± 30	8 ± 6	9 ± 1	19 ± 6
	16.0	24 ± 16	106 ± 3	11 ± 0	6 ± 0	18 ± 6
	50.0	22 ± 1	114 ± 6	12 ± 4	6 ± 1	20 ± 1
	160	17 ± 3	101 ± 16	15 ± 1	10 ± 4	15 ± 3
	500	26 ± 4	103 ± 4	12 ± 1	8 ± 1	17 ± 5
	1600	17 ± 4	100 ± 9	10 ± 7	7 ± 2	16 ± 1
	5000	24 ± 0 [R]	70 ± 1 [R]	11 ± 2 [R]	2 ± 1 [R]	11 ± 3 [R]
benzo[a]pyrene	2.5	330 ± 101				
2-aminoanthracene	2.5		953 ± 57	231 ± 49 [N]	89 ± 3	
2-aminoanthracene	25.0					595 ± 16

GLP = Good Laboratory Practice
Positive response (increase over corresponding control): 2-fold for TA98, TA100, and WP2*uvr*A; 3-fold for TA1535 and TA1537
[R] Reduced background bacterial lawn

Test Article	Dose (µg/plate)	Revertant Colony Counts (Mean ± SD) Strain TA98	TA100	TA1535	TA1537	WP2*uvr*A
Without Metabolic Activation						
DMSO	50µl/plate	16 ± 2	91 ± 5	15 ± 5	7 ± 2	15 ± 5
Mono-methyl fumarate	50.0	11 ± 2	88 ± 17	13 ± 4	4 ± 3	9 ± 2
	160	10 ± 1	81 ± 2	13 ± 5	9 ± 2	14 ± 3
	500	14 ± 4	83 ± 6	12 ± 2	7 ± 3	16 ± 2
	1600	12 ± 1	73 ± 10	12 ± 3	8 ± 1	17 ± 10
	3330	6 ± 3 [R]	60 ± 10 [R]	6 ± 3 [R]	4 ± 1 [R]	10 ± 1 [R]
	5000	3 ± 2 [R]	42 ± 1 [R]	7 ± 2 [R]	1 ± 1 [R]	12 ± 1 [R]
2-nitrofluorene	1.0	186 ± 12				
sodium azide	2.0		993 ± 78	689 ± 54		
ICR-191	2.0				110 ± 8	
4-nitroquinoline-N-oxide	1.0					123 ± 34

GLP = Good Laboratory Practice
Positive response (increase over corresponding control): 2-fold for TA98, TA100, and WP2*uvr*A; 3-fold for TA1535 and TA1537
[R] Reduced background bacterial lawn

With Metabolic Activation						
DMSO	50µl/plate	27 ± 4	104 ± 5	13 ± 3	8 ± 3	17 ± 4
Mono-methyl fumarate	50.0	23 ± 2	100 ± 11	11 ± 3	8 ± 1	20 ± 1
	160	22 ± 4	115 ± 5	9 ± 2	6 ± 3	15 ± 4
	500	20 ± 5	102 ± 10	10 ± 3	8 ± 2	12 ± 3
	1600	22 ± 1	78 ± 8	11 ± 1	7 ± 2	11 ± 2
	3330	20 ± 4	74 ± 8	5 ± 3	6 ± 3	13 ± 2
	5000	15 ± 4	64 ± 5	5 ± 2	6 ± 2	11 ± 1
benzo[a]pyrene	2.5	337 ± 112				
2-aminoanthracene	2.5		993 ± 9	145 ± 17	91 ± 7	
2-aminoanthracene	25.0					504 ± 74

GLP = Good Laboratory Practice
Positive response (increase over corresponding control): 2-fold for TA98, TA100, and WP2*uvr*A; 3-fold for TA1535 and TA1537

Historical Control Data for Bacterial Mutagenicity Studies

Plate Incorporation Method - Report Period 01/2007 through 12/2007

Vehicle Controls with S9 Mix					
Strain	TA98	TA100	TA1535	TA1537	WP2*uvrA*
Mean Revertants per Plate	22.2	106.4	12.6	8.0	17.2
Standard Deviation	6.9	21.6	5.5	4.0	5.9
Maximum	56	184	41	35	42
Minimum	8	50	2	0	5
Count	725	723	712	714	696
Vehicle Controls without S9 Mix					
Strain	TA98	TA100	TA1535	TA1537	WP2*uvrA*
Mean Revertants per Plate	14.7	88.0	11.9	6.5	14.8
Standard Deviation	5.5	23.6	5.4	3.8	5.0
Maximum	42	274	47	28	33
Minimum	2	47	1	0	2
Count	733	722	711	711	696
Positive Controls with S9 Mix[a]					
Strain	TA98	TA100	TA1535	TA1537	WP2*uvrA*
Mean Revertants per Plate	393.1	854.5	127.2	94.9	417.9
Standard Deviation	109.8	323.7	46.1	67.1	126.1
Maximum	765	2926	693	971	849
Minimum	102	108	44	10	89
Count	721	721	707	710	695
Positive Controls without S9 Mix[b]					
Strain	TA98	TA100	TA1535	TA1537	WP2*uvrA*
Mean Revertants per Plate	443.1	1121.6	826.1	431.4	285.4
Standard Deviation	449.9	207.7	163.4	246.6	126.3
Maximum	3446	2247	1459	1447	1283
Minimum	68	117	278	43	34
Count	732	721	699	701	695

a TA98 benzo[a]pyrene 2.5 µg/plate
TA100 2-aminoanthracene 2.5 µg/plate
TA1535 2-aminoanthracene 2.5 µg/plate
TA1537 2-aminoanthracene 2.5 µg/plate
WP2*uvrA* 2-aminoanthracene 25.0 µg/plate

b TA98 2-nitrofluorene 1.0 µg/plate
TA100 sodium azide 2.0 µg/plate
TA1535 sodium azide 2.0 µg/plate
TA1537 ICR-191 2.0 µg/plate
WP2*uvrA* 4-nitroquinoline-N-oxide 1.0 µg/plate

7.2 *In Vitro* Assays in Mammalian Cells

Study title: *IN VITRO* MUTATION ASSAY OF DIMETHYLFUMARATE IN CHINESE HAMSTER CELLS (V79)

Study no.:	5405-89
Study report location:	EDR
Conducting laboratory and location:	(b) (4)
Date of study initiation:	9/4/89, protocol dated 4/21/89
GLP compliance:	Yes, Page 3, FDA
QA statement:	Yes, Page 4
Drug, lot #, and % purity:	DMF, lots 8660686 & L980737, 100.6% pure (0.26% fumaric acid)

Methods

Cell line:	V79 Chinese Hamster lung cells
Concentrations in definitive study:	+S9: 312.5, 625, 1250, 2500 & 5000 μg/mL -S9: 0.3, 1, 3, 10 and 30 μg/mL
Basis of concentration selection:	Cytotoxicity
Negative control:	DMSO, Lot 025350
Positive control:	-S9: Ethyl methanesulfonate (EMS) +S9: 9,10-dimethyl-1,2-benzanthracene (DMBA)
Formulation/Vehicle:	DMSO
Incubation & sampling time:	+S9 exposed for 2 hr -S9 exposed for 24 hr Plates are incubated for 8 days (evaluate plating efficiency) or 12 days (selection mutants).

Study Validity

Triplicate plates were used in 2 independent experiments in a gene mutation assay in cultured mammalian cells (V79, genetic marker HGPRT) both in the presence and absence of metabolic activation (rat liver Aroclor-1254-induced S9 mix). The top concentrations exerted cytotoxic effects (≥ 5 μg/mL for +S9 and ≥1000 μg/mL for -S9). A positive result was defined as a dose-dependent increase in the mutation frequency in both independent experiments (at similar concentrations) to at least 2-fold solvent control and a count of at least 20 x 10^{-6} both in the presence and/or absence of S9 mix. Formulations were 98.5-103% of nominal.

Results: Negative, but inadequate (inadequate cytotoxicity)

According to the sponsor, DMF tested up to cytotoxic concentrations, in the absence and presence of metabolic activation, in two independent experiments was negative in the V79 cell mutagenicity test under conditions where the positive controls exhibited potent mutagenic effects. However, although increases in revertant frequencies were not seen, the tests did not evaluate cultures with adequate cytotoxicity (RTGs ~20%); the report only provided PEs (see the sponsor's summary data tables below).

ABLE 2 Results of the in vitro V79 HGPRT point mutation assay of Dimethylfumarate (1st experiment) without S9 mix

ompound µg/ml)	Solvent (µl/plate)	Plating Efficiencies PE_1 (a)	PE_2 (b)	Thioguanine-resistant colonies (c)					Total number of viable cells exposed to thioguanine x 10^6 (d)	Mutation frequency x 10^{-6} (e)
imethyl-umarate	DMSO									
0	300	0.82	0.84	6	11	14	13	10	4.20	12.9
0.3	300	1.07	0.73	3	4	6	0	3	3.65	4.4
1	300	0.69	0.86	8	4	5	1	8	4.30	6.1
3	300	0.89	0.77	9	12	13	10	13	3.85	14.8
10	300	0.00	#			#			#	#
30	300	0.00	#			#			#	#
:MS	DMSO									
;00	100	0.40	0.39	539	589	523	570	428	1.95	1358.5
'00	100	0.31	0.45	520	579	589	608	606	2.25	1289.8

for a, b, c, d, e please see page 15

) concentration 100% cytotoxic, not carried through beyond the initial treatment

TABLE 3 Results of the in vitro V79 HGPRT point mutation assay of Dimethylfumarate (1st experiment) without S9 mix

Compound (µg/ml)	Solvent (µl/plate)	Plating Efficiencies PE_1 (a)	PE_2 (b)	Thioguanine-resistant colonies (c)					Total number of viable cells exposed to thioguanine x 10^6 (d)	Mutation frequency x 10^{-6} (e)
Dimethyl-fumarate	DMSO									
0	300	0.82	1.03	8	7	2	12	5	5.20	6.5
0.3	300	0.73	0.91	14	12	6	17	12	4.55	13.4
1	300	0.89	1.08	13	7	6	8	18	5.40	9.6
3	300	0.96	0.92	15	17	13	12	13	4.60	15.2
10	300	0.00	0.71	10	13	10	8	16	3.55	16.1
30	300	0.00	#			#			#	#
EMS	DMSO									
600	100	0.55	0.45	352	337	332	331	345	2.25	754.2
700	100	0.31	0.25	222	322	313	346	357	1.25	1248.0

for a, b, c, d, e please see page 15

concentration 100% cytotoxic, not carried through beyond the initial treatment

TABLE 4 Results of the in vitro V79 HGPRT point mutation assay of Dimethylfumarate (1st experiment) with S9 mix

Compound (µg/ml)	Solvent (µl/plate)	Plating Efficiencies PE_1 (a)	Plating Efficiencies PE_2 (b)	Thioguanine-resistant colonies (c)					Total number of viable cells exposed to thioguanine x 10^6 (d)	Mutation frequency x 10^{-6} (e)
Dimethyl-fumarate	DMSO									
0	180	0.98	0.82	5	4	6	4	5	4.10	5.9
312.5	180	0.67	0.75	4	10	8	15	10	3.75	12.5
625	180	0.95	0.86	10	11	12	12	9	4.30	12.6
1250	180	0.40	0.66	6	8	10	4	6	3.30	10.3
2500	180	0.09	0.78	8	8	16	11	9	3.90	13.3
5000	180	0.00	#			#			#	#
DMBA	DMSO									
20	180	0.99	0.59	259	232	270	248	#	2.95	427.6
30	180	0.82	0.73	345	348	384	335	328	3.65	476.7

for a, b, c, d, e please see page

concentration 100% cytotoxic, not carried through beyond the initial treatment

TABLE 5 Results of the in vitro V79 HGPRT point mutation assay of Dimethylfumarate (2nd experiment) with S9 mix

Compound (µg/ml)	Solvent (µl/plate)	Plating Efficiencies PE_1 (a)	Plating Efficiencies PE_2 (b)	Thioguanine-resistant colonies (c)					Total number of viable cells exposed to thioguanine x 10^6 (d)	Mutation frequency x 10^{-6} (e)
Dimethyl-fumarate	DMSO									
0	180	0.74	0.84	7	6	9	11	8	4.20	9.8
312.5	180	0.93	0.90	24	10	9	15	16	4.50	16.4
625	180	0.72	0.75	10	9	8	12	6	3.75	12.0
1250	180	0.33	1.00	8	11	10	8	14	5.00	10.2
2500	180	0.36	0.64	12	7	13	7	8	3.20	14.7
5000	180	0.02	#			#			#	#
DMBA	DMSO									
20	180	0.58	0.76	495	520	429	428	462	3.80	614.2
30	180	0.45	0.64	430	465	393	408	459	3.20	673.4

for a, b, c, d, e please see page 15

concentration 100% cytotoxic, not carried through beyond the initial treatment

Study title: *IN VITRO* ASSESSMENT OF THE CLASTOGENIC ACTIVITY OF DIMETHYLFUMARATE IN CULTURED HUMAN PERIPHERAL LYMPHOCYTES

Study no.:	5407-89
Study report location:	EDR
Conducting laboratory and location:	(b) (4)
Date of study initiation:	9/25/89, protocol dated 4/21/89
GLP compliance:	Yes, page 3, FDA
QA statement:	Yes, page 4
Drug, lot #, and % purity:	DMF, lot 8660686 L980737, 100.6% pure

Methods

Cell line:	Cultured human peripheral blood lymphocytes
Concentrations in definitive study:	**-S9**: 1.56. 3.13. 6.25, 125, 25.0 & 50.0 μg/mL **+S9:** 6.25, 125, 25.0, 50.0, 100.0 & 150.0 μg/mL
Basis of concentration selection:	Preliminary cytotoxicity assay showed toxicity at 50 μg/mL in -S9 and >5 μg/mL in +S9 (assay was not reliable).
Negative control:	DMSO, lot 025350
Positive control:	**-S9:** mitomycin C **+S9:** cyclophosphamide
Formulation/Vehicle:	
Incubation & sampling time:	**-S9:** 24 hours at 37°C **+S9:** 2 hours at 37°C

Study Validity

Human peripheral blood was obtained by venipuncture from 2 healthy donors that were medication-free and collected in heparinized tubes. In this study, DMF was tested up to cytotoxic concentrations both in the absence and presence of metabolic activation (rat liver Aroclor-1254 induced S9). One thousand lymphocytes per culture were examined to determine cytotoxicity. Two hours before termination, cell division was arrested with colcemid. Two slides were made from each culture. For each treatment and culture, 100 metaphases were counted. In the positive control groups treated with mitomycin C or cyclophosphamide, a significant ($p \leq 0.05$) increase in aberrant metaphases occurred. The formulations were 97.7 - 101% of nominal.

Results: Positive, but inadequate assay

The incidence of chromosomal aberrations was increased at cytotoxic concentrations in the absence of metabolic activation. At 12.5 μg DMF/mL, there was a concentration-

related significant (p≤ 0.05; by Chi-Square) increase in the frequency of aberrations (excluding gaps), compared to the solvent control cultures for both independent tests. Excessive cytotoxicity was observed in both assays at the highest concentration tested (25 μg DMF/mL). The mean incidence of chromosomal aberrations (excluding gaps) for DMF in the presence of metabolic activation ranged from 0.0% to 3.0% in the two independent tests. These results are within the normal range of the solvent control. For details, see the sponsor's summary tables, below.

Dimethylfumarate
Chromosome analysis in human peripheral lymphocytes in vitro
Table 2 c 1st experiment without metabolic activation (S9 mix)

Treatment (μg/ml)	Mitotic index#	Number of metaphases scored	% of cells with gaps	% of cells with aberrations including gaps	% of cells with aberrations excluding gaps	Significance chi² - test (aberrations excluding gaps)
Dimethyl-fumarate						
0	1.00	100	4.0	5.0	1.0	-
1.56	1.33	100	3.0	5.0	2.0	n.s.
3.13	0.75	100	3.0	6.0	3.0	n.s.
6.25	0.67	100	5.0	8.0	3.0	n.s.
12.5	0.29	100	12.0	27.0	21.0	s.
25.0	0.33	100	99 aut of 100 metaphases pulverised			s.
Mitomycin C						
0.1	0.50	100	20.0	31.0	18.0	s.

n.s. not significant at p ≤ 0.05 s. significant at p ≤ 0.05 # solvent control = 1.00

Dimethylfumarate
Chromosome analysis in human peripheral lymphocytes in vitro
2nd experiment without metabolic activation (S9 mix)

Table 3 c

Treatment (µg/ml)	Mitotic index#	Number of metaphases scored	% of cells with gaps	% of cells with aberrations including gaps	% of cells with aberrations excluding gaps	Significance chi^2 - test (aberrations excluding gaps)
Dimethyl-fumarate						
0	1.00	100	4.0	4.0	0.0	-
3.13	0.85	100	1.0	2.0	1.0	n.s.
6.25	1.00	100	1.0	3.0	2.0	n.s.
12.5	0.31	69##	4.4	11.6	7.3	s.
25.0	0.23	4##	-	-	-	-
Mitomycin C						
0.1	0.62	100	21.0	36.0	21.0	s.

n.s. not significant at $p \leq 0.05$ s. significant at $p \leq 0.05$
solvent control = 1.00
no more metaphases of sufficient quality for evaluation due to cytotoxicity of Dimethylfumarate

Dimethylfumarate
Chromosome analysis in human peripheral lymphocytes in vitro
1st experiment with metabolic activation (S9 mix)

Table 4 c

Treatment (µg/ml)	Mitotic index#	Number of metaphases scored	% of cells with gaps	% of cells with aberrations including gaps	% of cells with aberrations excluding gaps	Significance chi^2 - test (aberrations excluding gaps)
Dimethyl-fumarate						
0	1.00	100	3.0	4.0	1.0	-
12.5	1.23	100	3.0	3.0	0.0	n.s.
25.0	1.08	100	3.0	3.0	0.0	n.s.
50.0	1.23	100	5.0	5.0	0.0	n.s.
100.0	0.69	100	5.0	7.0	2.0	n.s.
Cyclophos-phamide						
10.0	1.69	100	9.0	25.0	18.0	s.

n.s. not significant at $p \leq 0.05$ s. significant at $p \leq 0.05$
solvent control = 1.00

Dimethylfumarate
Chromosome analysis in human peripheral lymphocytes in vitro
Table 5 c 2nd experiment with metabolic activation (S9 mix)

Treatment (µg/ml)	Mitotic index#	Number of metaphases scored	% of cells with gaps	% of cells with aberrations including gaps	% of cells with aberrations excluding gaps	Significance chi^2 - test (aberrations excluding gaps)
Dimethyl-fumarate						
0	1.00	100	4.0	4.0	0.0	-
6.25	0.91	100	9.0	12.0	3.0	n.s.
12.5	1.25	100	8.0	9.0	1.0	n.s.
25.0	1.00	100	5.0	6.0	1.0	n.s.
50.0	0.83	100	4.0	6.0	2.0	n.s.
100.0	0.17	100	2.0	4.0	2.0	n.s.
150.0	0.25	23##	8.7	13.0	4.3	n.s.
Cyclophos-phamide						
10.0	0.83	100	12.0	28.0	22.0	s.

n.s. not significant at $p \leq 0.05$ s. significant at $p \leq 0.05$ # solvent control = 1.00
no more metaphases of sufficient quality for evaluation due to cytotoxicity of Dimethylfumarate

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Study title: Chromosomal Aberrations in Cultured Human Peripheral Blood Lymphocytes

Study no.:	P00012-04-16, (b) (4) 6538-357
Study report location:	EDR
Conducting laboratory and location:	(b) (4)
Date of study initiation:	11/29/04
GLP compliance:	Yes, page 3, FDA
QA statement:	Yes, page 2
Drug, lot #, and % purity:	DMF (dimethyl fumarate, BG00012), Lot 1102643 33004999, 99.8% (0.03% methylhydrogen fumarate)

Methods

Cell line:	Cultured human peripheral blood lymphocytes
Concentrations in definitive study:	**Initial Assay (3 hours, ± S9):** Tested 10.2, 14.5, 20.8, 29.7, 42.4, 60.5, 86.5, 124, 176, 252, 360, 515, 735, 1050, and 1500 µg/mL 20.8, 29.7, 42.4, and 60.5 µg/mL analyzed

	Confirmatory Assay (3 hr +S9, 22 hr -S9)
	+S9: 30, 40, 50 and 60 μg/mL -S9: 0.938, 1.88, 3.75 and 7.50 μg/mL
Basis of concentration selection:	Cytotoxicity in initial assay The highest dose used in the study, 1500 μg/mL DMF, was slightly >10 mM DMF (molecular weight 144.13, 10 mM=1441 μg/mL DMF), the high dose recommended by the OECD Testing Guidelines at the time the study was conducted.
Negative control:	DMSO, (b) (4), Lots A019540701 and A019779001
Positive control:	+S9: cyclophosphamide -S9: mitomycin C
Formulation/Vehicle:	DMSO
Incubation & sampling time:	3 hr ± S9, 22 hr -S9 at 37 □2□C
Deviations	Samples were not saved for homogeneity from the lowest dose formulation; the sponsor indicated that this had no impact on the study.

Study Validity

Human venous blood from a healthy, adult donor (nonsmoker without a history of radiotherapy, chemotherapy, or drug usage, and lacking current viral infections) was collected; whole blood cultures were initiated. The S9 fraction was purchased ((b) (4) Lot No. 1722). To arrest the cell cycles, 0.1 μg/mL Colcemid was present during the last 2 ± 0.5 hours of incubation. Replicate cultures were used at each concentration. One hundred cells, if possible, from each duplicate culture from four concentrations of the test article, the negative and vehicle controls, and one dose level from the positive control cultures were analyzed for the different types of chromosomal aberrations. Mitotic index was evaluated from the negative control, vehicle control and a range of test article concentrations by analyzing the number of mitotic cells in at least 1000 cells per culture, if available, and the ratio expressed as a percentage of mitotic cells. Percent polyploidy and endoreduplication were also analyzed by evaluating 100 metaphases per culture, if available, and tabulated. A test article was considered positive for inducing chromosomal aberrations if a significant increase (the difference was considered significant when $p < 0.01$) in the number of cells with chromosomal aberrations was observed at one or more dose levels. The linear trend test evaluated the dose-responsiveness. If a significant increase was seen at one or more dose levels, a dose-response should be observed. Precipitate was observed at ≥ 735 μg/mL DMF, with and without metabolic activation.

Results: Positive without metabolic activation

The results of the initial assay without metabolic activation are presented in sponsor's Table 2, below. A significant increase in cells with chromosomal aberrations was observed in the cultures treated with 42.4, and 60.5 μg/mL DMF. No significant increase in polyploidy or endoreduplication was observed at the concentrations analyzed.

Table 2: Chromosomal Aberrations in Human Lymphocytes - Without Metabolic Activation - 3-Hour Treatment, ~22-Hour Harvest

Assay No.: 26208-0-449OECD Trial No.: B1 Date: 12/15/04 Lab No.: CY121604 Test Article: DMF

				# Cells Scored for Aberrations	% Mitotic Index Reduction[a]	# Cells Scored for pp and er	# of pp Cells	# of er Cells	Judge-ment (+/-)[b]	Numbers and Percentages of Cells Showing Structural Chromosome Aberrations							Judge-ment (+/-)[d]
										gaps	simple breaks	chte	chre	mab	Totals[c] g-	Totals[c] g+	
Controls																	
Negative:	RPMI 1640		A	100		100	0	0		1	1	3			4	5	
			B	100		100	0	0				1	2		3	3	
			Total	**200**		**200**				1	1	4	2		7	8	
			Average	%	--		**0.0**	**0.0**		**0.5**	**0.5**	**2.0**	**1.0**		**3.5**	**4.0**	
Vehicle:	DMSO	10.0 μL/mL	A	100		100	0	0		2			1		1	3	
			B	100		100	0	0		1					0	1	
			Total	**200**		**200**				3			1		1	4	
			Average	%	**0**		**0.0**	**0.0**		**1.5**			**0.5**		**0.5**	**2.0**	
Positive:	MMC	1.00 μg/mL	A	25		100	0	0		1	7	3			9	9	
			B	25		100	0	0		2	5	3	1		8	10	
			Total	**50**		**200**				3	12	6	1		17	19	
			Average	%	--		**0.0**	**0.0**	-	**6.0**	**24.0**	**12.0**	**2.0**		**34.0**	**38.0**	+
Test Article		20.8 μg/mL	A	100		100	0	0		3					0	3	
			B	100		100	0	0		1	1				1	2	
			Total	**200**		**200**				4	1				1	5	
			Average	%	--		**0.0**	**0.0**	-	**2.0**	**0.5**				**0.5**	**2.5**	-
		29.7 μg/mL	A	100		100	0	0		1	5				5	6	
			B	100		100	0	0		1	2	2			4	5	
			Total	**200**		**200**				2	7	2			9	11	
			Average	%	**1**		**0.0**	**0.0**	-	**1.0**	**3.5**	**1.0**			**4.5**	**5.5**	-
		42.4 μg/mL	A	25		100	0	0			8	1			8	8	
			B	100		100	0	0		5	15	1		3	19	23	
			Total	**125**		**200**				5	23	2		3	27	31	
			Average	%	**41**		**0.0**	**0.0**	-	**4.0**	**18.4**	**1.6**		**2.4**	**22.4**	**25.6**	+
		60.5 μg/mL	A	25		100	0	0			7			8	15	15	
			B	25		100	0	0		1	9	2		3	13	13	
			Total	**50**		**200**				1	16	2		11	28	28	
			Average	%	**56**		**0.0**	**0.0**	-	**2.0**	**32.0**	**4.0**		**22.0**	**56.0**	**56.0**	+

chte: chromatid exchange chre: chromosome exchange mab: multiple aberrations, greater than 4 aberrations pp: polyploidy er: endoreduplication

[a] % Mitotic index reduction as compared to the vehicle control.

[b] Significantly greater in % polyploidy and % endoreduplication than the vehicle control, $p \leq 0.01$.

[c] -g = # or % of cells with chromosome aberrations; +g = # or % of cells with chromosome aberrations + # or % of cells with gaps.

[d] Significantly greater in -g than the vehicle control, $p \leq 0.01$. RPMI 1640 = culture medium DMSO = dimethylsulfoxide MMC = Mitomycin C

The results of the initial assay with metabolic activation are presented in sponsor's Table 4, below. No significant increase in chromosomal aberrations, polyploidy, or endoreduplication was observed at the concentrations analyzed.

Table 4: Chromosomal Aberrations in Human Lymphocytes - With Metabolic Activation - 3-Hour Treatment, ~22-Hour Harvest

Assay No.: 26208-0-449OECD Trial No.: B1 Date: 12/15/04 Lab No.: CY121604 Test Article: DMF

				# Cells Scored for Aberrations	% Mitotic Index Reduction[a]	# Cells Scored for pp and er	# of pp Cells	# of er Cells	Judge-ment (+/-)[b]	Numbers and Percentages of Cells Showing Structural Chromosome Aberrations							Judge-ment (+/-)[d]
											simple breaks	chte	chre	mab	Totals[c] g-	Totals[c] g+	
Controls																	
Negative:	RPMI 1640		A	100		100	0	0		2	2				2	4	
			B	100		100	0	0		2			2		2	4	
			Total	200		200				4	2		2		4	8	
			Average	%	--		0.0	0.0		2.0	1.0		1.0		2.0	4.0	
Vehicle:	DMSO	10.0 µL/mL	A	100		100	0	0							0	0	
			B	100		100	0	0		1					0	1	
			Total	200		200				1					0	1	
			Average	%	0		0.0	0.0		0.5					0.0	0.5	
Positive:	CP	25.0 µg/mL	A	50		100	0	0			15	4			19	19	
			B	50		100	0	0		1	14	3			17	18	
			Total	100		200				1	29	7			36	37	
			Average	%	--		0.0	0.0	-	1.0	29.0	7.0			36.0	37.0	+
Test Article		20.8 µg/mL	A	100		100	1	0		2					0	2	
			B	100		100	0	0		1	1				1	2	
			Total	200		200				3	1				1	4	
			Average	%	5		0.5	0.0	-	1.5	0.5				0.5	2.0	-
		29.7 µg/mL	A	100		100	0	0		1					0	1	
			B	100		100	0	0							0	0	
			Total	200		200				1					0	1	
			Average	%	12		0.0	0.0	-	0.5					0.0	0.5	-
		42.4 µg/mL	A	100		100	0	0		1					0	1	
			B	100		100	0	0		2					0	2	
			Total	200		200				3					0	3	
			Average	%	48		0.0	0.0	-	1.5					0.0	1.5	-
		60.5 µg/mL	A	100		100	0	0		3	1				1	4	
			B	100		100	0	0		1					0	1	
			Total	200		200				4	1				1	5	
			Average	%	56		0.0	0.0	-	2.0	0.5				0.5	2.5	-

chte: chromatid exchange chre: chromosome exchange mab: multiple aberrations, greater than 4 aberrations pp: polyploidy er: endoreduplication

[a] % Mitotic index reduction as compared to the vehicle control.

[b] Significantly greater in % polyploidy and % endoreduplication than the vehicle control, $p \leq 0.01$.

[c] -g = # or % of cells with chromosome aberrations; +g = # or % of cells with chromosome aberrations + # or % of cells with gaps.

[d] Significantly greater in -g than the vehicle control, $p \leq 0.01$. RPMI 1640 = culture medium DMSO = dimethylsulfoxide CP = Cyclophosphamide

The results of the confirmatory assay without metabolic activation are presented in sponsor's Table 6, below. No significant increase in cells with chromosomal aberrations, polyploidy, or endoreduplication was observed in the cultures analyzed.

Table 6: Chromosomal Aberrations in Human Lymphocytes - Without Metabolic Activation - ~22-Hour Treatment, ~22-Hour Harvest

Assay No.: 26208-0-449OECD Trial No.: C1 Date: 02/09/05 Lab No.: CY020705A Test Article: DMF

				# Cells Scored for Aberrations	% Mitotic Index Reduction[a]	# Cells Scored for pp and er	# of pp Cells	# of er Cells	Judge-ment (+/-)[b]	Numbers and Percentages of Cells Showing Structural Chromosome Aberrations							Judge-ment (+/-)[d]
											simple				Totals[c]		
										gaps	breaks	chte	chre	mab	g-	g+	
Controls																	
Negative:	RPMI 1640		A	100		100	0	0		4					0	4	
			B	100		100	0	0		1					0	1	
			Total	**200**		**200**				5					0	5	
			Average	%	--		**0.0**	**0.0**		**2.5**					**0.0**	**2.5**	
Vehicle:	DMSO	10.0 µL/mL	A	100		100	0	0		1					0	1	
			B	100		100	0	0			1				1	1	
			Total	**200**		**200**				1	1				1	2	
			Average	%	**0**		**0.0**	**0.0**		**0.5**	**0.5**				**0.5**	**1.0**	
Positive:	MMC	0.300 µg/mL	A	50		100	0	0		5	21	5			25	29	
			B	50		100	0	0		3	17	3			19	21	
			Total	**100**		**200**				8	38	8			44	50	
			Average	%	--		**0.0**	**0.0**	-	**8.0**	**38.0**	**8.0**			**44.0**	**50.0**	+
Test Article		0.938 µg/mL	A	100		100	0	0		1	2				2	3	
			B	100		100	0	0		2	2				2	4	
			Total	**200**		**200**				3	4				4	7	
			Average	%	**21**		**0.0**	**0.0**	-	**1.5**	**2.0**				**2.0**	**3.5**	-
		1.88 µg/mL	A	100		100	0	0		2					0	2	
			B	100		100	0	0		1					0	1	
			Total	**200**		**200**				3					0	3	
			Average	%	**25**		**0.0**	**0.0**	-	**1.5**					**0.0**	**1.5**	-
		3.75 µg/mL	A	100		100	0	0		2					0	2	
			B	100		100	0	0		1					0	1	
			Total	**200**		**200**				3					0	3	
			Average	%	**39**		**0.0**	**0.0**	-	**1.5**					**0.0**	**1.5**	-
		7.50 µg/mL	A	100		100	0	0		1	3				3	4	
			B	100		100	0	0			2				2	2	
			Total	**200**		**200**				1	5				5	6	
			Average	%	**58**		**0.0**	**0.0**	-	**0.5**	**2.5**				**2.5**	**3.0**	-

chte: chromatid exchange chre: chromosome exchange mab: multiple aberrations, greater than 4 aberrations pp: polyploidy er: endoreduplication

[a] % Mitotic index reduction as compared to the vehicle control.

[b] Significantly greater in % polyploidy and % endoreduplication than the vehicle control, $p \leq 0.01$.

[c] -g = # or % of cells with chromosome aberrations; +g = # or % of cells with chromosome aberrations + # or % of cells with gaps.

[d] Significantly greater in -g than the vehicle control, $p \leq 0.01$. RPMI 1640 = culture medium DMSO = dimethylsulfoxide MMC = Mitomycin C

The results of the confirmatory assay with metabolic activation are presented in sponsor's Table 8, below. Due to toxicity, only 87 metaphases were available from one of the cultures treated with 60.0 µg/mL DMF and 113 metaphases were evaluated from the duplicate culture. Given this issue, the cultures at 75 mg/mL with a similar mitotic index reduction should have been analyzed. No significant increase in cells with chromosomal aberrations, polyploidy, or endoreduplication was observed in the cultures analyzed.

Table 8: Chromosomal Aberrations in Human Lymphocytes - With Metabolic Activation - 3-Hour Treatment, ~22-Hour Harvest

Assay No.: 26208-0-449OECD Trial No.: C1 Date: 02/09/05 Lab No.: CY020705A Test Article: DMF

				# Cells Scored for Aberrations	% Mitotic Index Reduction[a]	# Cells Scored for pp and er	# of pp Cells	# of er Cells	Judge-ment (+/-)[b]	Numbers and Percentages of Cells Showing Structural Chromosome Aberrations							Judge-ment (+/-)[d]
											simple				Totals[c]		
										gaps	breaks	chte	chre	mab	g-	g+	
Controls																	
Negative:	RPMI 1640		A	100		100	0	0							0	0	
			B	100		100	0	0		1					0	1	
			Total	200		200				1					0	1	
			Average	%	--		0.0	0.0		0.5					0.0	0.5	
Vehicle:	DMSO	10.0 µL/mL	A	100		100	0	0							0	0	
			B	100		100	0	0		2					0	2	
			Total	200		200				2					0	2	
			Average	%	0		0.0	0.0		1.0					0.0	1.0	
Positive:	CP	25.0 µg/mL	A	50		100	0	0		3	23	1			24	26	
			B	50		100	0	0		1	18	1			18	19	
			Total	100		200				4	41	2			42	45	
			Average	%	--		0.0	0.0	-	4.0	41.0	2.0			42.0	45.0	+
Test Article		30.0 µg/mL	A	100		100	0	0		1	1				1	2	
			B	100		100	0	0			1				1	1	
			Total	200		200				1	2				2	3	
			Average	%	--		0.0	0.0	-	0.5	1.0				1.0	1.5	-
		40.0 µg/mL	A	100		100	0	0			4				4	4	
			B	100		100	0	0		3	2				2	5	
			Total	200		200				3	6				6	9	
			Average	%	25		0.0	0.0	-	1.5	3.0				3.0	4.5	-
		50.0 µg/mL	A	100		100	0	0		7	4				4	10	
			B	100		100	0	0		6	2				2	7	
			Total	200		200				13	6				6	17	
			Average	%	37		0.0	0.0	-	6.5	3.0				3.0	8.5	-
		60.0 µg/mL	A	87		100	0	0		1	4				4	5	
			B	113		81	0	0		4					0	4	
			Total	200		181				5	4				4	9	
			Average	%	54		0.0	0.0	-	2.5	2.0				2.0	4.5	-

chte: chromatid exchange chre: chromosome exchange mab: multiple aberrations, greater than 4 aberrations pp: polyploidy er: endoreduplication

[a] % Mitotic index reduction as compared to the vehicle control.

[b] Significantly greater in % polyploidy and % endoreduplication than the vehicle control, $p \leq 0.01$.

[c] -g= # or % of cells with chromosome aberrations; +g= # or % of cells with chromosome aberrations + # or % of cells with gaps.

[d] Significantly greater in -g than the vehicle control, $p \leq 0.01$. RPMI 1640 = culture medium DMSO = dimethylsulfoxide CP = Cyclophosphamide

DMF is considered positive for inducing chromosomal aberrations with a 3-hour treatment under non-activation conditions, but negative with a prolonged treatment (when greater cytotoxicity occurred at lower concentrations) and under conditions with metabolic activation. DMF is considered negative for inducing polyploidy or endoreduplication under non-activation conditions. The negative and vehicle control cultures had ≤3.5% cells with aberrations, which is within the range of the historical control data (sponsor's table included below). The positive control results were statistically significant ($p \leq 0.01$) compared to vehicle controls.

HISTORICAL CONTROL DATA

Chromosome Aberrations In Human Lymphocytes
~22 Hour Harvest - 7/2003 through 12/2003

	Activation		% -g	% +g	% Polyploid Cells	%Endore-duplicated Cells
Negative Control 3 Hour Treatment	Without	MIN	0.0	0.0	0.0	0.0
		MAX	4.5	8.0	0.0	0.0
		AVG	0.7	3.1	0.0	0.0
		SD (±)	1.03	2.50	0.00	0.00
		N	20	20	20	20
Vehicle Control (Pooled) 3 Hour Treatment	Without	MIN	0.0	0.0	0.0	0.0
		MAX	3.0	11.5	0.5	0.0
		AVG	0.6	2.5	0.0	0.0
		SD (±)	0.76	2.35	0.10	0.00
		N	23	23	23	23
Positive Control - MMC 3 Hour Treatment	Without	MIN	21.3	30.7	0.0	0.0
		MAX	63.0	66.0	0.5	0.0
		AVG	41.1	47.6	0.0	0.0
		SD (±)	9.74	10.10	0.10	0.00
		N	23	23	23	23
Negative Control Continuous Treatment	Without	MIN	0.0	0.0	0.0	0.0
		MAX	3.0	9.0	0.5	0.0
		AVG	0.7	2.5	0.0	0.0
		SD (±)	0.87	2.14	0.13	0.00
		N	14	14	14	14
Vehicle Control (Pooled) Continuous Treatment	Without	MIN	0.0	0.0	0.0	0.0
		MAX	3.0	9.0	1.0	0.0
		AVG	1.1	3.1	0.1	0.0
		SD (±)	0.92	2.46	0.24	0.00
		N	20	20	20	20
Positive Control - MMC Continuous Treatment	Without	MIN	18.7	24.2	0.0	0.0
		MAX	56.0	56.0	0.0	0.0
		AVG	34.5	40.6	0.0	0.0
		SD (±)	10.45	9.78	0.00	0.00
		N	20	20	20	20
Negative Control 3 Hour Treatment	With	MIN	0.0	0.0	0.0	0.0
		MAX	1.5	9.0	0.5	0.0
		AVG	0.5	2.4	0.0	0.0
		SD (±)	0.49	1.95	0.09	0.00
		N	34	34	34	34
Vehicle Control (Pooled) 3 Hour Treatment	With	MIN	0.0	0.0	0.0	0.0
		MAX	3.5	7.5	0.5	0.0
		AVG	0.6	2.6	0.0	0.0
		SD (±)	0.90	2.11	0.11	0.00
		N	42	42	42	42
Positive Control - CP 3 Hour Treatment	With	MIN	18.9	28.7	0.0	0.0
		MAX	49.0	62.0	1.0	0.0
		AVG	36.8	44.4	0.0	0.0
		SD (±)	7.81	7.66	0.17	0.00
		N	42	42	42	42

N = Number of trials
MMC = Mitomycin C
CP = Cyclophosphamide
-g = % of cells with chromosome aberrations
+g = % of cells with chromosome aberrations + % of cells with gaps

Study title: Chromosomal Aberrations in Cultured Human Peripheral Blood Lymphocytes

Study no.:	P00012-08-03, (b) (4) 6538526
Study report location:	EDR
Conducting laboratory and location:	(b) (4)
Date of study initiation:	2/9/10
GLP compliance:	Yes, page 2, FDA
QA statement:	Yes, page 3
Drug, lot #, and % purity:	mono-methyl fumarate (MMF), 97%, CAS 2756-87-8, lot 75798MJ, 97.5% pure

Methods

Cell line:	Cultured HPBL
Concentrations in definitive study:	**Initial assay (3 hrs ± S9)** -S9: 153, 312, and 446 µg/mL +S9: 74.9, 153, and 218 µg/mL **Confirmatory assay (22 hr -S9, 3 hr +S9)** -S9: 2.50, 5.00, 10.0, 20.2, 28.8, 41.0, 58.0, 80.0, 100, 125, 156, 196, 245, 350, and 500 µg/mL +S9: 64.0, 80.0, 100, 125, 156, 196, 245, and 350 µg/mL **2nd Confirmatory assay** -S9: 20.2, 28.8, and 41.0 µg/mL +S9: 100, 156, and 245 µg/mL
Basis of concentration selection:	The highest concentration tested in the assay was 1300 µg/mL, which was approximately 10 mM of MMF (molecular weight 130.10).
Negative control:	DMSO, CAS 67-68-5, lots 35596LK & 02896BM, 99.92% & 99.97% pure, respectively
Positive control:	-S9: mitomycin C +S9: cyclophosphamide
Formulation/Vehicle:	DMSO
Incubation & sampling time:	3 hrs and/or 22 hrs, incubated at 37 ± 2°C
Deviations	Documentation that the test article was characterized under GLP or GMP conditions is not available. The sponsor indicated that the test article was provided by a reputable, high quality commercial vendor; therefore, is not likely to have an adverse impact upon the integrity of the study or the conclusions derived from it.

Study Validity

Human venous blood samples were collected from healthy, adult donors (nonsmokers without a history of radiotherapy, chemotherapy, or drug usage, and lacking current viral infections). The vehicle control cultures were in the historical control range for cells with chromosomal aberrations and the positive control cultures had significant increases in cells with chromosomal aberrations as compared with the vehicle control cultures. The high doses selected for analysis in the assay had a ≥50% reduction in mitotic index, as recommended for this assay by the OECD Testing Guidelines. The formulations in the 2nd confirmatory assay were 92.8-103% of nominal.

Results: Positive without metabolic activation

The initial chromosomal aberrations assay treated the cultures for 3 hr, without and with metabolic activation. Concentrations of 8.82, 12.6, 18.0, 25.7, 36.7, 52.5, 74.9, 107, 153, 218, 312, 446, 637, 910, and 1300 µg/mL were tested without and with metabolic activation. Of those concentrations, 153, 312, and 446 µg/mL without metabolic activation and 74.9, 153, and 218 µg/mL with metabolic activation were analyzed for chromosomal aberrations. Without metabolic activation, statistically significant increases in cells with chromosomal aberrations were observed at 312 and 446 µg/mL (at 39% and 56% reductions in the mitotic index, respectively). With metabolic activation, no significant increases in cells with chromosomal aberrations, polyploidy or endoreduplication were observed. However, the formulation analyses showed that the formulations used were only 60.7-98.9% of nominal (most between 60-76%). See the sponsor's summary table, below.

Table 11 Genotoxicity: In Vitro **Report Title: Chromosomal Aberrations in Cultured Human Peripheral Blood Lymphocytes** **Test Article:** mono-methyl fumarate

Test for Induction of: Chromosome aberrations **Number of Independent Assays:** 3 **Study Number:** 6538526
Strains: Primary human lymphocytes **Number of Replicate Cultures:** 2 **Sponsor Reference Number:** P00012-08-03
Metabolizing System: Aroclor™-induced rat liver S9 **Number of Cells Analyzed/Culture:** 100 (or ≥25 if >25% cells with aberrations) **Location in CTD:** Vol. Page
Vehicles: Test Article: Dimethylsulfoxide (DMSO) Positive Controls: Water
Treatment: Pulse treatment 3 hr and recovery time 19 hr with and without S9. **GLP Compliance:** Yes
Cytotoxic Effects: Dose-related decreases in mitotic indices. **Date of Treatment:** 24-Feb-2010
Genotoxic Effects: Statistically significant increase in the percent aberrant cells without activation only.

Test Article	Dose (µg/mL)	Total Cells Analyzed	Mitotic Suppression[a] Mean %	Aberrant Cells Mean% -g	Aberrant Cells Mean% +g	Polyploidy Mean %	Endoreduplicate Mean %
3-Hour Treatment Without Metabolic Activation							
Vehicle DMSO	10.0 µL/mL	200	100	0.5	5.0	1.0	0.0
mono-methyl fumarate	153	200	100	1.0	4.0	0.0	0.0
	312	200	61	6.0*	12.0	2.0	0.0
	446	200	44	8.5*	15.0	5.0	1.5
Mitomycin C	1.00	75	--	37.3*	45.3	0.0	0.0
3-Hour Treatment With Metabolic Activation							
Vehicle DMSO	10.0 µL/mL	200	100	0.5	2.0	0.0	0.0
mono-methyl fumarate	74.9	200	100	0.0	1.5	0.0	0.0
	153	200	58	0.0	2.0	0.0	0.0
	218	200	41	4.0	6.5	3.0	0.0
Cyclophosphamide	25.0	100	--	44.0*	49.0	0.0	0.0

GLP = Good Laboratory Practice; -g = % of aberrant cells excluding those with gaps only; +g = % of aberrant cells plus % of cells with gaps only.
* Significantly greater in -g than the vehicle control, $p \leq 0.01$
[a] Mitotic suppression = [One minus the quotient (mean test article mitotic index/mean vehicle control mitotic index)] x 100.

In the first confirmatory chromosomal aberrations assay, the treatment period was for 22 hours without metabolic activation (2.50, 5.00, 10.0, 20.2, 28.8, 41.0, 58.0, 80.0, 100, 125, 156, 196, 245, 350, and 500 µg/mL) and 3 hours with metabolic activation (64.0, 80.0, 100, 125, 156, 196, 245, and 350 µg/mL). The sponsor indicated that this assay was discontinued due to low metaphase frequency.

A repeat confirmatory chromosomal aberrations assay was conducted under identical conditions. Concentrations of 20.2, 28.8, and 41.0 µg/mL without metabolic activation and 100, 156, and 245 µg/mL with metabolic activation were analyzed for chromosomal aberrations. Statistically significant increases in cells with chromosomal aberrations were observed at 41.0 µg/mL without metabolic activation (at 54% reduction in mitotic index; though the sponsor indicated that this may be a statistical anomaly due to 0 aberrant cells in the concurrent vehicle control) and at 245 µg/mL with metabolic activation (at 56% reduction in the mitotic index). No significant increase in cells with polyploid or endoreduplication was observed in the cultures analyzed. The sponsor believes that the increases observed at the single high doses are probably induced as effects secondary to cytotoxicity rather than a true clastogenic potential; the small increases (almost all of which were simple breaks) were at relatively cytotoxic dose levels (~50% reduction in mitotic index), with no increase observed at the less cytotoxic dose levels. See the sponsor's summary table below.

Table 12 Genotoxicity: In Vitro **Report Title: Chromosomal Aberrations in Cultured Human Peripheral Blood Lymphocytes** **Test Article:** mono-methyl fumarate

Test for Induction of: Chromosome aberrations **Number of Independent Assays:** 3 **Study Number:** 6538526
Strains: Primary human lymphocytes **Number of Replicate Cultures:** 2 **Sponsor Reference Number:** P00012-08-03
Metabolizing System: Aroclor™-induced rat liver S9 **Number of Cells Analyzed/Culture:** 100 (or ≥25 if >25% cells with aberrations) **Location in CTD:** Vol. Page
Vehicles: Test Article: Dimethylsulfoxide (DMSO) Positive Controls: Water
Treatment: Continuous 22 hr without S9; pulse treatment 3 hr and recovery time 19 hr with S9. **GLP Compliance:** Yes
Cytotoxic Effects: Dose-related decreases in mitotic indices. **Date of Treatment:** 07-Apr-2010
Genotoxic Effects: Without activation statistically significant due to lack of aberration in concurrent vehicle control, however not significant from the historical control viewpoint and also biologically insignificant. With activation, weak positive at cytotoxic dose levels only.

Test Article	Dose (µg/mL)	Total Cells Analyzed	Mitotic Suppression[a] Mean %	Aberrant Cells Mean% -g	Aberrant Cells Mean% +g	Polyploidy Mean %	Endore-duplicate Mean %
22-Hour Treatment Without Metabolic Activation							
Vehicle DMSO	10.0 µL/mL	200	100	0.0	1.0	0.0	0.0
mono-methyl fumarate	20.2	200	93	1.5	5.0	0.0	0.0
	28.8	200	68	3.0	9.0	0.0	0.0
	41.0	200	46	4.0*	11.5	0.0	0.0
Mitomycin C	0.300	100	--	46.0*	58.0	0.0	0.0
3-Hour Treatment With Metabolic Activation							
Vehicle DMSO	10.0 µL/mL	200	100	0.0	0.5	0.0	0.0
mono-methyl fumarate	100	200	98	0.0	2.0	0.5	0.0
	156	200	56	0.5	3.5	0.0	0.0
	245	200	44	6.0*	11.0	2.5	0.5
Cyclophosphamide	25.0	100	--	46.0*	61.0	0.5	0.0

GLP = Good Laboratory Practice; -g = % of aberrant cells excluding those with gaps only; +g = % of aberrant cells plus % of cells with gaps only.
* Significantly greater in -g than the vehicle control, $p \leq 0.01$
[a] Mitotic suppression = [One minus the quotient (mean test article mitotic index/mean vehicle control mitotic index)] x 100.

Mono-methyl fumarate was positive in the initial (B1) assay in the absence of metabolic activation, yielding positive results at concentrations of 312 and 446 µg/mL. In the confirmatory assay, either in the presence or absence of metabolic activation, positive results were at the maximum concentration used (>50% cytotoxicity).

7.3 *In Vivo* Clastogenicity Assay in Rodent (Micronucleus Assay)

Study title: *In Vivo* Rat Micronucleus Assay of DMF

Study no:	P00012-04-04; (b) (4) #6538-337
Study report location:	EDR
Conducting laboratory and location:	(b) (4)
Date of study initiation:	6/9/04
GLP compliance:	Yes, page 2
QA statement:	Yes, Page 3
Drug, lot #, and % purity:	BG00012, Lot F1177170, 99.6% pure

Methods- See Sponsor's table, below.

Frequency of dosing:	1x
Route of administration:	PO via gavage
Dose volume:	10 ml/kg
Formulation/Vehicle:	0.8% hydroxypropylmethylcellulose in reverse osmosis deionized water
Species/Strain:	Male Crl:CD (SD)IGS BR rats 8 weeks of age, 245-277 g
Basis of dose selection:	The maximum dose used (1000 mg/kg) depressed the PCE/NCE ratio (caused bone marrow toxicity).

Target Dose Level (mg/kg)	Animals/Harvest Timepoint 24 Hour Male	48 Hour Male
Positive Control[a]	6	-
Vehicle Control	6	6
250	6	-
500	6	-
1000	6	6

[a] Positive Control = Cyclophosphamide (60mg/kg)

Study Validity: questionable maximum dose

The formulation analyses indicated that the test doses were within ±15% of nominal (85.8 - 93%). DMF did not induce signs of clinical toxicity or mortality in the animals at any dose tested; however, the sponsor indicated that bone marrow toxicity was detected at 1000 mg/kg (see sponsor's Tables 4 & 5, below). The evidence of cytotoxicity demonstrated was weak; in fact, individual animals in the vehicle group demonstrated lower PCE/NCE ratios than animals in the HD group at the 24 hr timepoint, and the effect was not clearly dose-related. Evidence of cytotoxicity at the 48-hr timepoint was a little more convincing, but not strong. Bone marrow was extracted and at least 2000 PCEs per animal were analyzed for the frequency of micronuclei. Cytotoxicity was assessed by scoring the number of PCEs and NCEs in at least 500 total erythrocytes for each animal. The historical background frequency of micronuclei in the rat strains at this laboratory is 0.0 - 0.4%.

Table 4: Micronucleus Assay – 24-Hour Male Individual Animal Data

Assay No.: 26208-0-454OECD
Test Article: DMF
Initiation of Dosing: 21 June 2004

Treatment	Dose	Animal Number	# MN PCE/ 2000 PCE	Ratio PCE:NCE
Vehicle Control (0.8% HPMC)	0 mg/kg	5807	1	0.79
		5812	0	1.00
		5817	1	0.91
		5818	2	0.97
		5837	0	0.67
Positive Control (CP)	60 mg/kg	5808	73	0.78
		5825	77	0.76
		5832	22	0.84
		5833	57	0.95
		5835	67	0.76
Test Article	250 mg/kg	5802	3	0.97
		5806	2	0.78
		5809	1	0.67
		5814	2	0.89
		5821	1	0.81
	500 mg/kg	5820	0	1.20
		5822	1	1.05
		5827	1	1.07
		5828	2	0.78
		5839	0	0.74
	1000 mg/kg	5803	0	0.81
		5805	0	1.07
		5811	1	0.80
		5823	2	0.70
		5834	1	0.77

HPMC = Hydroxypropylmethlycellulose
CP = Cyclophosphamide
PCE = Polychromatic erythrocyte
MN PCE = Micronucleated PCE
NCE = Normochromatic erythrocyte

Table 5: Micronucleus Assay – 48-Hour Male Individual Animal Data

Assay No.: 26208-0-454OECD
Test Article: DMF
Initiation of Dosing: 21 June 2004

Treatment	Dose	Animal Number	# MN PCE/ 2000 PCE	Ratio PCE:NCE
Vehicle Control (0.8% HPMC)	0 mg/kg	5801	0	1.17
		5804	1	1.15
		5810	1	1.04
		5815	0	0.97
		5816	0	0.95
Test Article	1000 mg/kg	5813	0	0.92
		5819	2	0.97
		5824	1	0.74
		5830	2	0.75
		5831	1	0.84

HPMC = Hydroxypropylmethlycellulose
PCE = Polychromatic erythrocyte
MN PCE = Micronucleated PCE
NCE = Normochromatic erythrocyte

Results: negative, but maximum dose defined by questionable cytotoxicity

DMF did not induce statistically significant increases in micronucleated PCEs. The sponsor indicated that DMF was cytotoxic to the bone marrow, as evidenced by a statistically significant decrease in the PCE:NCE ratio at the HD at the 48 hr timepoint. This evidence is not convincing; it appears that a higher dose could have been used. (In an acute oral toxicity study in rat, a single dose up to 2150 mg/kg was achieved without mortality in males [the only clinical sign was inhibition of body weight gain].)

Table 3: Micronucleus Assay – Summary Table

Assay No.: 26208-0-454OECD
Test Article: DMF
Initiation of Dosing: 21 June 2004

Treatment	Dose	Harvest Time	% Micronucleated PCEs Mean of 2000 per Animal ± S.E. Males	Ratio PCE:NCE Mean ± S.E. Males
Controls				
Vehicle (0.8%HPMC)	0 mg/kg	24 hr	0.04 ± 0.02	0.87 ± 0.06
		48 hr	0.02 ± 0.01	1.06 ± 0.04
Positive (CP)	60 mg/kg	24 hr	2.96 ± 0.49*	0.82 ± 0.04
Test Article	250 mg/kg	24 hr	0.09 ± 0.02	0.82 ± 0.05
	500 mg/kg	24 hr	0.04 ± 0.02	0.97 ± 0.09
	1000 mg/kg	24 hr	0.04 ± 0.02	0.83 ± 0.06
		48 hr	0.06 ± 0.02	0.84 ± 0.04**

* Significantly greater than the corresponding vehicle control, $p \leq 0.01$.
** Significantly less than the corresponding vehicle control, $p \leq 0.05$.
HPMC = Hydroxypropylmethlycellulose
CP = Cyclophosphamide
PCE = Polychromatic erythrocyte
NCE = Normochromatic erythrocyte

Appendix 4
Historical Control Data

Rat Micronucleus - 1/2003 through 12/2003

		% Micronucleated PCEs From 2000 PCEs per Animal Mean ± S.E. Males	PCE:NCE Ratio Mean ± S.E. Males
Pooled Vehicle Controls			
24 hour harvest	Minimum	0.00	0.49
	Maximum	0.35	1.91
	Average	0.085 ± 0.007	0.916 ± 0.023
	N	105	105
48 hour harvest	Minimum	0.00	0.45
	Maximum	0.30	2.42
	Average	0.073 ± 0.007	0.909 ± 0.035
	N	75	75
Positive Controls – Cyclophosphamide, 60 mg/kg			
24 hour harvest	Minimum	0.15	0.33
	Maximum	6.55	2.81
	Average	2.891 ± 0.127	0.840 ± 0.038
	N	102	102

PCE = Polychromatic erythrocyte
NCE = Normochromatic erythrocyte
N = Number of animals

In Vivo Clastogenicity Assay in Rodent (Chromosome Aberration)

Study title: *IN VIVO* BONE MARROW CYTOGENETIC TEST OF METHYLHYDROGEN FUMARATE AS CALCIUM SALT BY ORAL ADMINISTRATION IN SPRAGUE-DAWLEY RATS (Chromosomal Analysis)

Study no:	9337/1/95
Study report location:	EDR
Conducting laboratory and location:	(b) (4)
Date of study initiation:	8/14/95, 8/21/95 (main); protocol 7/17/95
GLP compliance:	Yes, pg 3, FDA
QA statement:	Yes, pg 4
Drug, lot #, and % purity:	MMF as calcium salt, lot 2305681, 99.7-99.9 % pure

Methods

Doses in definitive study:	0 and 1000 mg/kg
Frequency of dosing:	One dose; assessments at 6, 24 and 48 hours after administration
Route of administration:	PO
Dose volume:	20 ml/kg
Formulation/Vehicle:	0.8% aqueous hydroxypropyl- methylcellulose gel
Species/Strain:	Rat, Sprague-Dawley/Crl: CD®BR
Number/Sex/Group:	5/sex/gp
Basis of dose selection:	Preliminary assay: Doses of 400 and 800 mg/kg MMF Calcium salt PO caused slight toxicity, while 1600 mg/kg resulted in moderate toxicity lasting for 3 hours.
Negative control:	0.8% aqueous hydroxypropyl- methylcellulose gel
Positive control:	Cyclophosphamide (27 mg/kg IP), dissolved in 0.9% NaCl solution

Study Validity

This is a nonstandard study. Only one dose-level was used; this dose resulted in clinical signs (i.e., reduced mobility, ataxia and dyspnea) at 1 - 3 hr postdose. From the preliminary assay, it appears that a higher dose could have been used. Two hours prior to the sampling time, the animals received Colcemid (IP). The mitotic index was determined by counting the number of metaphases per 1000 cells in each cell preparation. The mean mitotic index of 10 animals (both sexes combined) was compared with the mean mitotic index of the negative control (mitotic index: 1.0). The analysis for structural aberrations (chromosome- and chromatid type) was carried out in 50 cells per animal; the OECD guideline indicates that 100 cells/animal should be analyzed. Cells with an incomplete number of centromeres or insufficient spreading were not used for analysis.

Results: inadequate, negative

The mitotic index in bone marrow was not affected. The mean incidence of chromosomal aberrations (excluding gaps) of the cells treated with MMF calcium salt was not increased above the negative control range (0.4 - 1.2% at all three sampling time-points, compared to the normal range of the negative control [0.6%]). The number of cells with gaps was also within the range of the negative controls (treated groups: 2.8% to 4.8%; controls: 2.8%). Cyclophosphamide, the positive control, induced significant levels of chromosomal aberrations.

Table 1 Methylhydrogen fumarate as Calcium salt
Rat bone marrow cytogenetic test, chromosomal analysis
Summary

Compound mg/kg b.w. p.o.	Sampling time (h)	Number of metaphases analysed	Mitotic index*	% of cells with gaps	% of cells with aberrations including gaps	% of cells with aberrations excluding gaps	Significance chi^2-test (aberrations excluding gaps)
0.8% aqueous hydroxypropyl-methylcellulose gel							
(20 ml/kg b.w. p.o.)	24	500	1.00	2.8	3.0	0.6	-
Methylhydrogen fumarate as Calcium salt							
1000	6	500	1.12	2.8	3.8	1.2	n.s.
1000	24	500	0.93	3.6	3.6	0.4	n.s.
1000	48	500	0.86	4.8	5.2	0.6	n.s.
Cyclophosphamide							
27 mg/kg b.w. i.p.	24	500	0.42	22.2	40.0	28.0	s.

n.s. not significant
s. significant at $p \leq 0.05$
* negative control = 1.00

7.4 Other Genetic Toxicology Studies

Study 5409-89: MICRONUCLEUS TEST OF FUMADERM (FD) ACTIVE AGENTS IN BONE MARROW CELLS OF TREATED NMRI MICE

Conducted [redacted] (b) (4)
GLP, QA, protocol signed 4/21/89

Fumaderm, lot 905412, analysis from sponsor (below)

Tests	Requirements	Results
Contents		
Fumaric acid free	1.1 % maximum	0.98 %
Ca/Mg/Zn monoethylfumarate	39.8-48.6 %	43.1 %
Dimethylfumarate	50.2-61.3 %	55.5 %
Calcium monoethylfumarate	36.5-44.5 %	39.8 %
Magnesium monoethylfumarate	2.1-2.5 %	2.4 %
Zinc monoethylfumarate	1.26-1.54 %	1.49 %

Formulations were 94.8-97.5% of nominal.

Fumaderm® was orally administered once at 300, 600 and 1200 mg/kg (40 ml/kg in 0.8% aqueous hydroxypropyl-methylcellulose gel). Three sampling times were used: 16, 48 and 72 hours after administration of Fumaderm. MMS (130 mg/kg) served as a positive control. The MMS group was assessed at 48 hours postdose. One thousand

PCE per animal were scored for the incidence of micronuclei. The ratio of PCE to NCE was determined for each animal by counting a total of 1000 erythrocytes.

The highest tested dose (1200 mg/kg) was, according to the sponsor, "in the range of the MTD" by the sponsor; all animals showed reduced mobility, ataxia and dyspnea. (In a preliminary study in 2 female mice, animals showed reduced mobility, ataxia, and dyspnea at 1000 mg/kg [reported as "slight" symptoms], decreased muscle tone and tremor at 2000 mg/kg ["moderate"], and abdominal position and clonic convulsion at 4000 mg/kg ["severe"].) Clinical signs were not reported at the low dose levels (300 and 600 mg/kg). No substance-related increase in micronucleated PCE was observed in the treated groups compared to controls. The positive control group exhibited a significant increase in the number of micronucleated PCE.

As conducted, Fumaderm® was negative in the mouse micronucleus assay. Methyl methanesulfonate was clearly clastogenic.

8 Carcinogenicity

Study title: A Two Year Oral (Gavage) Carcinogenicity Study in Rats with BG00012

Study no.:	P00012-04-11; (b) (4) Study EBA00009
Study report location:	EDR, 4.2.3.4.1
Conducting laboratory and location:	(b) (4)
Date of study initiation:	10/12/04
GLP compliance:	Yes, pg. 9; except TK and test article characterization
QA statement:	Yes, pgs. 10-13
Drug, lot #, and % purity:	BG00012 (2 lots) Lot 1102642 33004998, 100.2% pure, with 0.02% MMF Lot 1102643 33004999, 99.8% pure, with 0.03% MMF
CAC concurrence:	Yes, see FAX dated 10/6/04

Key Study Findings

- Dosing was discontinued early for HDM (week 80) and HMDM (week 82).
- Early termination occurred for HDM (week 86) and HMDM (week 88).
- Dose-related reduction in survival was observed in males (not F).
- Dose-related reductions in body weight were observed (M & F).
- Dose-related exacerbation of chronic progressive nephropathy was observed in males and females; this was a common cause of death, especially in males.

Adequacy of Carcinogenicity Study

The study is acceptable, based on MTD; an MTD was exceeded in males, based on decreased survival at 100 and 150 mg/kg. The sponsor received FDA ExecCAC concurrence on the doses of 0, 25, 50, 100, and 150 mg/kg BG-12. The ExecCAC noted that concurrence was contingent upon "there being no substantive differences between the draft report and the final report of the 90-day study" (Study EBAW-0154).

Appropriateness of Test Models

The Sprague-Dawley rat is an acceptable rodent species for nonclinical toxicity testing. Oral route of exposure was selected since it is the intended clinical route of administration.

Evaluation of Tumor Findings

Tumors were observed in:

- Nonglandular stomach- SC carcinoma & papilloma (treated M & F)
- Kidney- renal adenoma (M & F) and carcinoma (F)
- Testes- interstitial cell adenoma (HMDM, HDM)
- Parathyroid- adenoma (HDM)
- Brain- granular cell tumor (HDM)
- Mammary gland- carcinoma (HDF)

Methods (see details in sponsor's table below)

Frequency of dosing:	QD
Route of administration:	PO, oral intubation
Basis of dose selection:	Study EBAW-0154, 90 day study in rats
Species/Strain:	Sprague Dawley rat, Crl:CD(SD)IGS BR
Age and weight:	Main: ~6 weeks of age at the time of randomization; 154-202 g (M) and 126-163 g (F) TK: 7 weeks of age at the time of randomization, 180-214 g (M) and 157-186 g (F)
Animal housing:	Animals were individually housed.
Paradigm for dietary restriction:	Food and water were provided *ad libitum* throughout the study
Deviation from study protocol:	There were 14 protocol amendments. One notable deviation from the original protocol was the use of 5 sentinel animals/sex at 26, 52, 78 and 105 weeks. Pinworms (*Syphacia muris*) were detected in the sentinel and study animals. According to the sponsor, no definitive lesions attributable to the worms were recorded; therefore, the presence of pinworms was not considered to have had a significant negative impact on either the animals or the study.

Experimental Design for the Carcinogenicity and Toxicokinetic Phases

Group No.	No. of Animals (TK Satellite Animals)[a]		Dosage Material	Dosage Level (mg/kg/day)	Dosage Conc. (mg/mL)	Dosage Volume (mL/kg)
	Male	Female				
1	75 (15)	75 (15)	HPMC[b]	0	0	10
2	75 (15)	75 (15)	BG00012	25	2.5	10
3	75 (15)	75 (15)	BG00012	50	5	10
4	75 (15)	75 (15)	BG00012	100	10	10
5	75 (15)	75 (15)	BG00012	150	15	10

[a]Toxicokinetic phase animals began dosing during Week 24 of the carcinogenicity phase. The first day of dosing was designated Day 1. The duration of the toxicokinetic phase was a minimum of 180 days.
[b]Hydroxypropylmethylcellulose or Hypromellose (3,500-5,600 cps), 0.8% w/v in reverse osmosis deionized water.

Observations and Results

Mortality [2x daily]

A dose-related reduction in survival was observed for males (31%, 27%, 17%, 13%, and 13%, respectively; see sponsor's summary table and Figure 1, below). Dosing was suspended early in HDM (D547, week 80) and HMDM (D564, week 82) in an attempt to prolong survival; however, this was unsuccessful, and these groups were terminated early (HDM on week 86 and HMHDM on week 88). Although the LMDM group was terminated at scheduled euthanasia, this group also demonstrated reduced survival compared to controls. Survival was not significantly affected in LDM or in females (33%, 37%, 27%, 31% and 31%, respectively; see sponsor's summary table and Figure 2, below).

Summary of Male Mortality during Weeks 1-105

Males	Group 1	Group 2	Group 3	Group 4	Group 5
No. Found Dead	31	22	35	43	40
No. Euthanized Moribund	21	32	26	22	25
No. Accidental Death	0	1	1	0	0
No. Surviving[a]	23	20	13	10	10

Group 1 (0 mg/kg/day) Group 2 (25 mg/kg/day) Group 3 (50 mg/kg/day) Group 4 (100 mg/kg/day) Group 5 (150 mg/kg/day)
[a]Surviving males in Group 5 were euthanized during Week 86 and surviving males in Group 4 were euthanized during Week 88.

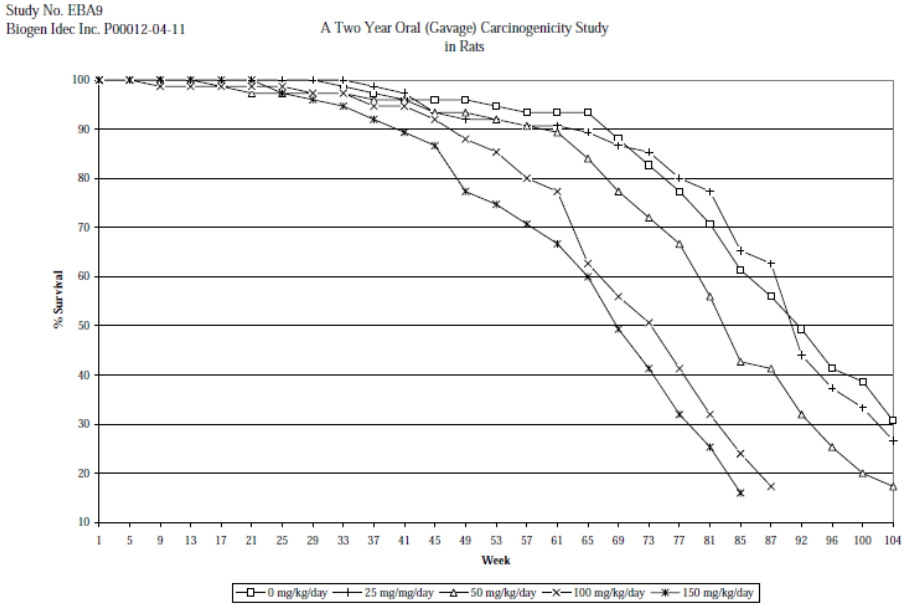

Figure 1. Summary of Male Mortality

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Summary of Female Mortality during Weeks 1-105

Females	Group 1	Group 2	Group 3	Group 4	Group 5
No. Found Dead	21	21	25	28	21
No. Euthanized Moribund	29	26	30	25	31
No. Surviving	25	28	20	22	23
Group 1 (0 mg/kg/day) Group 2 (25 mg/kg/day) Group 3 (50 mg/kg/day) Group 4 (100 mg/kg/day) Group 5 (150 mg/kg/day)					

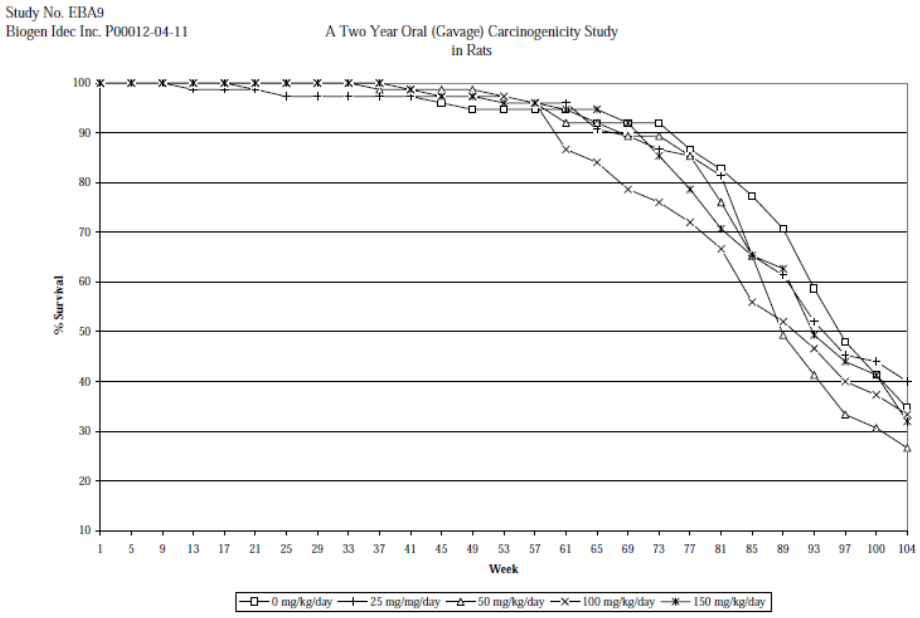

Figure 2. Summary of Female Mortality

Clinical Signs [Cage-side, 1x/daily at 2-3 hr postdose; Detailed 1x/week]

Few treatment-related clinical signs were reported, and mostly at the HMD and HD; these included thin appearance, "pale skin," and "pale eyes." HMD and HD animals were reported to struggle during dosing. Dose-related increases in "Cool to touch" were observed in treated males and females. Hunched posture and decreased activity were also observed in treated females. There was no apparent difference between groups for palpable masses. A few notable signs were reported at low incidences. An increased incidence in abnormal colored urine was observed in HDF (16/3 vs. 6/2 ConF). Convulsions were reported in ConM, ConF, LMDM, HMDM, and HMDF (observations/animals; 1/1, 1/1, 1/1, 2/2, and 9/2, respectively); one HMDM showed convulsions prior to dosing. Head tilt was observed in 2 LMDM, 3 HMDM, and 1 HDM but there was no clear dose-related effect in females.

Body Weights [Day 1, Weekly for 13 wks, then 1x/4 wks and Week 104]

Dose-related body weight reductions (often [ss]) were observed in treated males and females (see sponsor's Figures 3 and 4, below), with larger effects in males. Generally, LDM and LMDM showed average body weights similar to controls (difference < 10%, except 10-11% at weeks 97 & 101 in LMDM). At week 104, average body weight

reductions of 4% and 8% were observed in LDM and LMDM. Average body weights of HMDM began to significantly differ from those of controls by week 57 (>10% by week 61), while those of HDM differed by week 2 (>10% by week 37). By week 85 (termination), average body weights of HMDM and HDM were reduced 16-17%.

Effects on body weight were less pronounced in treated females. Average body weights of LDF and LMDF were similar to controls; average body weights of HMDF were similar to controls through week 69, but were reduced 3-8% in weeks 73-104. Average body weights of HDF differed from controls by <10% through week 65 but were reduced 12-17% during weeks 69-104. At week 104, average body weights were +1%, +1.5%, -7.7%, and -12.4% those of controls for LDF, LMDF, HMDF, and HDF.

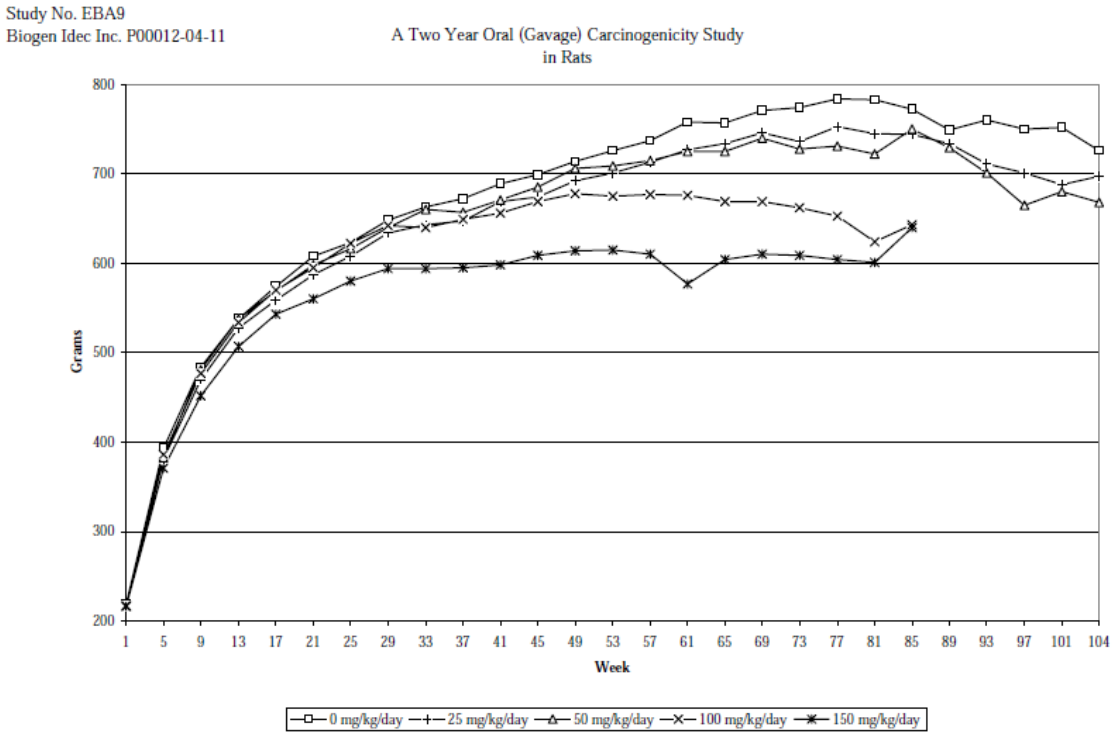

Figure 3. Summary of Male Body Weight Data

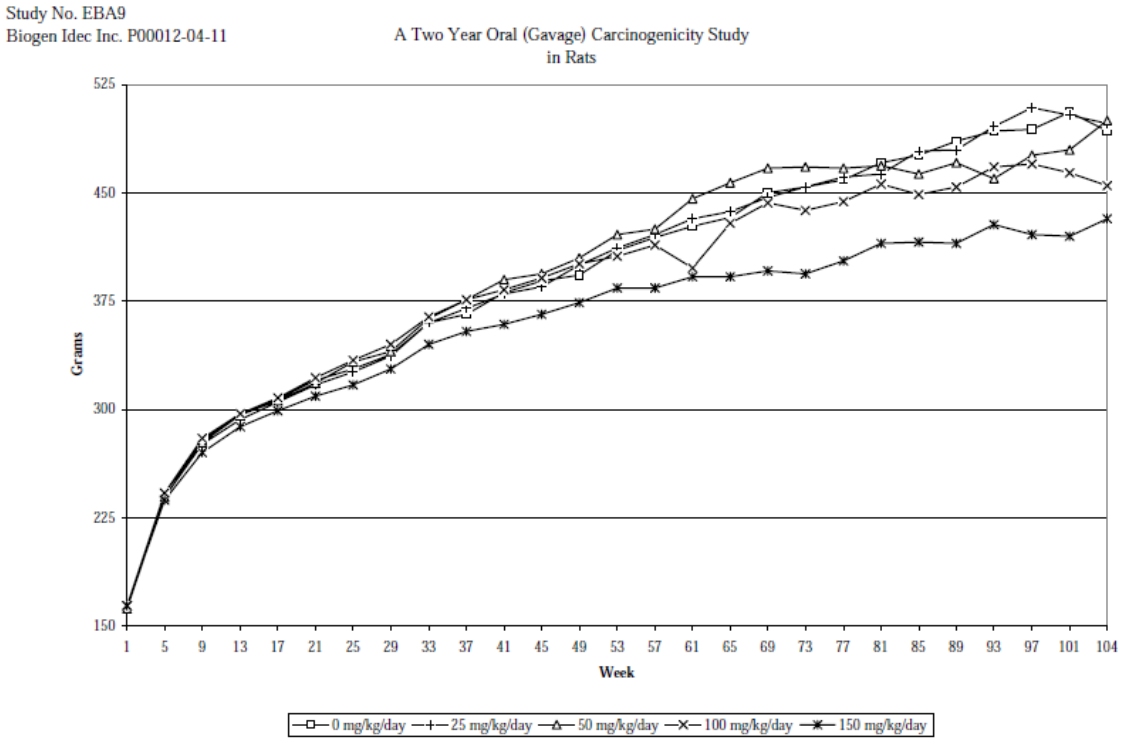

Figure 4. Summary of Female Body Weight Data

Food Consumption [Weekly for 13 wks, then 1x/4 wks and Week 104]

Food consumption was comparable across groups; differences between controls and treated animals were generally ± 10%. See sponsor's Figures 5 and 6, below.

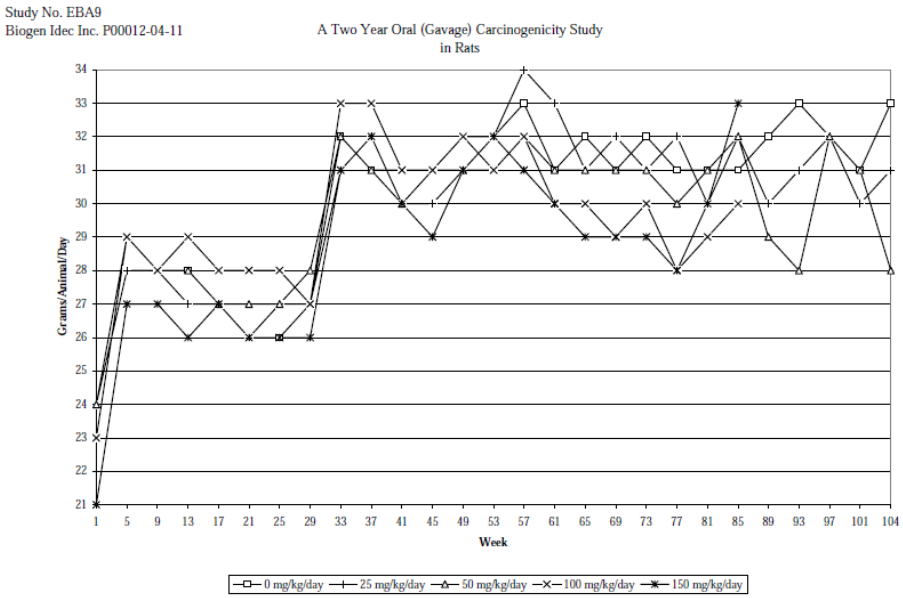

Figure 5. Summary of Male Food Consumption Data

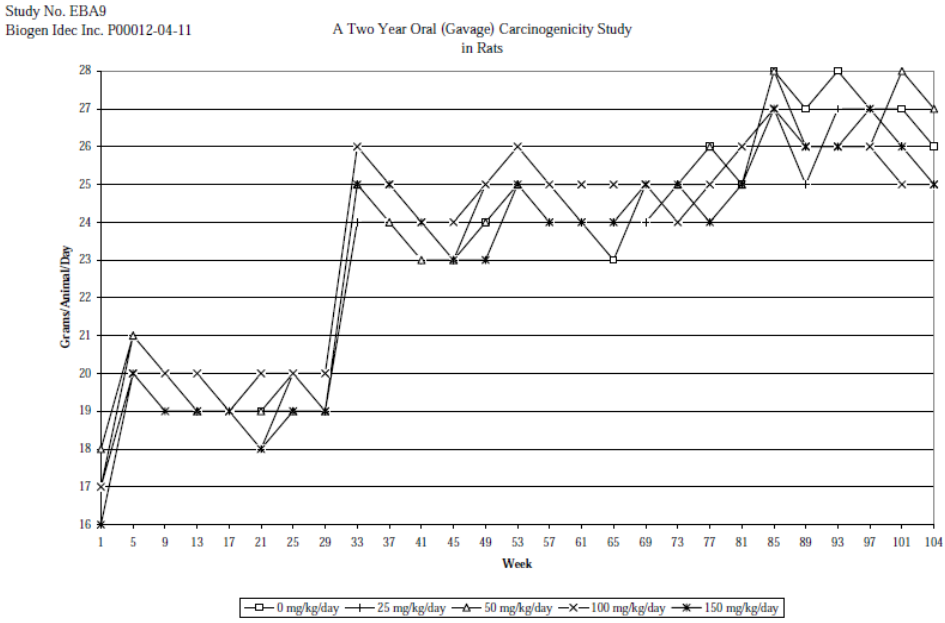

Figure 6. Summary of Female Food Consumption Data

Clinical Pathology [at necropsy]

Only hematological parameters were assessed. Clear drug-related effects on red cell parameters were not observed in males; however, reductions in reticulocytes were observed in LMDM, HMDM, and HDM (30%, 38% and 43%, respectively at weeks 105, 88 and 86, compared to controls at week 105). Treated females showed reduced red cell parameters (8-15% RBC, 7-12% hemoglobin, 6-10% hematocrit, 1-3% MCHC) and increased reticulocytes (28-49%), MCV (4-9%), and platelets (14-18%, not clearly dose-related). See the sponsor's summary table for details, below. One to three females in each treated group exhibited slight to moderate macrocytosis, polychromasia, and anisocytosis. Also, nucleated RBC counts were increased in HDF (17 vs. 0 in all other groups).

HDM showed increased mean leukocytes (1.7x), lymphocytes (1.5x), and segmented neutrophils (2x) at week 86, compared to controls (week 105); these WBC elevations were outside of the historical control ranges for the majority of the HDM. Although not noted by the sponsor, increases were also observed for average eosinophil (2.4x) and basophil (2x) counts; decreased LUC counts were observed (~60% reduction). HDF demonstrated significant increases in total leukocytes (45%), lymphocytes (16%), monocytes (62%), segmented neutrophils (71%), and LUCs (55%), as compared to controls (for details, see sponsor's summary table, below).

Statistically Significant Differences in Hematology Parameters

Sex	Week	Parameter	Group	Statistically Significant Value	Concurrent Control Value
Females	105	Erythrocytes	Group 5 ↓	6.15	7.24
		MCHC	Group 5 ↓	36.3	37.5
		Mean Corpus Volume	Group 5 ↑	58.6	53.8
		Reticulocytes	Group 4 ↑	235.69	169.96
			Group 5 ↑	253.73	
		Leukocytes	Group 5 ↑	14.00	9.69
		Monocytes	Group 5 ↑	0.94	0.58
		Segmented Neutrophils	Group 5 ↑	7.95	4.63
Group 4 (100 mg/kg/day) Group 5 (150 mg/kg/day)					

Gross Pathology [at necropsy]

According to the sponsor, dose-related findings were observed in the stomach, kidney, and testes. See the sponsor's summary table, below.

Test Article-Related Gross Pathology Findings

Gross Finding	Sex	No. of Animals with Findings[a]				
		Group 1	Group 2	Group 3	Group 4	Group 5
Enlarged kidney	Male	1	11	20	35	48
	Female	0	3	3	13	21
Roughened surface, kidney	Male	10	13	30	49	54
	Female	1	6	7	23	37
Discoloration, kidney	Male	7	9	8	12	14
	Female	4	4	4	8	18
Prominent epithelial surface, stomach	Male	0	32	64	74	73
	Female	1	11	58	73	73
Small testes	Male	6	9	11	18	28
Soft testes	Male	8	11	14	19	24
Group 1 (0 mg/kg/day) Group 2 (25 mg/kg/day) Group 3 (50 mg/kg/day) Group 4 (100 mg/kg/day) Group 5 (150 mg/kg/day) [a]Includes all deaths.						

Other organs were noted to show dose-related effects. Additional selected gross findings are provided in the following table:

	MALE					FEMALE				
	Con	LD	LMD	HMD	HD	Con	LD	LMD	HMD	HD
ABDOMINAL CAVITY										
Adhesion(s)	0	0	0	0	4	0	0	0	0	5
BLOOD										
Thin and watery	2	2	2	9	11	1	1	5	6	10
BLOOD VESSEL										
Enlarged	1	5	11	11	6	2	0	0	1	4
BONE										
Brittle	-	-	-	-	-	0	0	1	1	1
CARCASS/GROSS										
Body fat depletion	2	1	2	8	11	4	5	1	5	15
Discoloration	5	2	3	6	5	1	2	2	2	12
EPIDIDYMIS										
Small	0	5	1	10	15	-	-	-	-	-
Soft	0	0	0	1	5	-	-	-	-	-
HEART										
Enlarged	2	5	6	6	6	1	0	0	1	3
Foci	1	1	0	2	3	0	0	0	0	1
KIDNEY										
Cyst	3	6	5	5	11	0	2	4	7	6
Dilated pelvis	1	3	2	5	4	0	5	1	2	4
Pitted	5	5	5	6	5	0	4	8	6	3
LIVER										
Enlarged	1	3	3	5	1	1	1	1	0	4
Discoloration	4	5	7	11	2	3	6	7	11	10
Nodules	0	2	0	0	0	0	1	0	1	2
LYMPH NODES (ALL)										
Enlarged	23	27	39	39	37	15	16	15	25	44
PARATHYROID										
Enlarged	9	8	29	31	23	2	3	2	8	11
PITUITARY										
Enlarged	4	0	1	3	0	11	10	13	11	19
Discoloration	2	0	0	1	1	0	4	4	2	6
Focus/Foci	3	3	2	0	0	13	10	13	12	14
PROSTATE										
Firm	3	7	4	2	9	-	-	-	-	-
Discoloration	5	12	12	6	18	-	-	-	-	-
THYMUS										
Small	1	2	3	6	14	1	3	2	3	2

Histopathology [at necropsy]

Peer Review Yes, all neoplasms and all tissues from 10% of Con & HD animals

Neoplastic

Dose-related increases in tumors were observed in the stomach (nonglandular; treated M and F), kidney (HDF and M, trend), parathyroid, (HDM), brain (HDM), and testes (HMDM and HDM). The pathologist's report stated that the "treatment-related early morbidity and mortality were associated with earlier onset and/or progression of treatment-related tumors and related toxicities." The sponsor's pathologist reported the tumorigenic potential as follows:

> BG00012 had tumorigenic potential in Sprague Dawley rats, inducing squamous epithelial hyperplasia, squamous cell papillomas and squamous cell carcinomas in the nonglandular mucosa of the stomach, renal tubular hyperplasia, adenoma, and carcinoma in the kidneys and testicular interstitial (Leydig) cell hyperplasia and adenoma. Secondary renal hyperparathyroidism resulted in parathyroid adenoma. The tumorigenic effects of BG00012 on Sprague Dawley rats were dose-related and increased in incidence and/or severity with increasing dose and duration of treatment.

The nonglandular stomach was a target organ at all doses; both toxicity and proliferative changes were clearly observed. Statistically significant increases were observed in papillomas and carcinomas (see the sponsor's summary table, below). By the pathologist's descriptions, the benign papillomas were "seen as exophytic growths into the lumen" and the malignant carcinomas were "usually invasive neoplasms associated with chronic active inflammation and degenerative changes." Although the sponsor did not mention proliferative changes in the glandular stomach, these also occurred with low incidence (see excerpts from the sponsor's summary data, below).

Incidence of Proliferative Changes in Nonglandular Stomach

Sex	Males					Females				
Group	1	2	3	4	5	1	2	3	4	5
Dose (mg/kg/day)	0	25	50	100	150	0	25	50	100	150
Hyperplasia, squamous epithelium	3 (0.05)	71 (2.32)	75 (2.99)	75 (3.57)	75 (3.88)	7 (0.23)	73 (1.81)	74 (2.75)	75 (3.37)	75 (3.76)
Squamous Cell Papilloma (including multiple)	0	22	24	46	49	0	11	21	31	24
Squamous Cell Carcinoma	0	5	18	51	58	0	1	4	30	48

Note: The average severity of grade (score: 0-4, ranging from absent to marked) is shown in parentheses.

GLANDULAR STOMACH

	MALE					FEMALE				
	Con	LD	LMD	HMD	HD	Con	LD	LMD	HMD	HD
Hyperplasia	0	0	0	2	1	0	0	1	1	1
Adenoma	0	0	1	0	0	-	-	-	-	-

Although a dose-related exacerbation of rodent-specific chronic progressive nephropathy (CPN) was clearly observed in males and females (generally, CPN is of greater incidence and severity in males), a number of other changes, not typical of CPN, also occurred in the kidney. A low incidence of proliferative changes in the kidney

was also observed (see the sponsor's summary table, below). The pathologist noted that the incidence of hyperplasia/neoplasia was greatest in animals that did not have the more severe exacerbations of CPN, and therefore were able to survive for longer durations, allowing time for the neoplasia to present. Regardless, the exacerbated CPN resulted in renal failure in a number of animals, and was believed to have led to secondary hyperparathyroidism (and its related effects); a low incidence of parathyroid neoplasia was also noted (see the sponsor's summary table, below). Although low, these incidences exceeded the concurrent control, as well as historical control incidences.

Incidence of Proliferative Changes in Renal Tubule

Sex	Males					Females				
Group	1	2	3	4	5	1	2	3	4	5
Dose (mg/kg/day)	0	25	50	100	150	0	25	50	100	150
Hyperplasia	0 (0.00)	5 (0.11)	5 (0.13)	15 (0.44)	11 (0.35)	0 (0.00)	0 (0.00)	4 (0.11)	4 (0.05)	9 (0.21)
Adenoma	0	0	1	1	4	1	0	0	0	2*
Carcinoma	0	0	0	0	0	0	0	0	2	4*

Note: The average severity of grade (score: 0-4, ranging from absent to marked) is shown in parentheses.
* = Animal E700 (Group 5 female) exhibited both Renal Tubule Adenoma and Renal Tubule Carcinoma.

Incidence of Treatment-Related Parathyroid Findings

Sex	Males					Females				
Group	1	2	3	4	5	1	2	3	4	5
Dose (mg/kg/day)	0	25	50	100	150	0	25	50	100	150
Hyperplasia	9 (0.25)	16 (0.48)	32 (1.01)	42 (1.31)	48 (1.61)	0 (0.00)	4 (0.09)	5 (0.14)	11 (0.33)	22 (0.68)
Adenoma (including bilaterally)	0	0	1	0	3	0	0	0	0	0

Note: The average severity of grade (score: 0-4, ranging from absent to marked) is shown in parentheses.

Drug-related, dose-dependent toxicity (i.e., atrophic and degenerative lesions) and proliferative changes were also observed in the testis. Interstitial cell (Leydig cell) hyperplasia and adenomas were observed. For interstitial cell adenoma, there was a statistically significant increase for the trend and pairwise comparisons for HMDM and HDM.

Incidence of Proliferative Changes in Testes

Sex	Males				
Group	1	2	3	4	5
Dose (mg/kg/day)	0	25	50	100	150
Interstitial Cell -Hyperplasia	0 (0.00)	2 (0.05)	2 (0.07)	6 (0.17)	10 (0.28)
Interstitial Cell –Adenoma (including bilaterally)	3	3	2	9	19

Note: The average severity of grade (score: 0-4, ranging from absent to marked) is shown in parentheses.

Granular cell tumors were observed in the brains of 2 HDM (compared with no occurrences in any other group), which yielded a statistically significant trend. The sponsor did not consider these tumors treatment-related because the incidence was within the historical control incidence (0-4%) for this tumor in Sprague-Dawley rats; however, it should be noted that these tumors are very rare.

The sponsor reported all other observed tumors as "spontaneous, incidental tumors, common … in aging Sprague Dawley rats of this stock." Increased tumor incidences were suggested for mammary gland carcinoma (19 in HDF vs. 10 in ConF) and vaginal endometrial stromal polyps (1 HMDF, 2 HDF); these were not statistically significant.

Non Neoplastic

There were numerous non-neoplastic changes in several organ systems; the most common cause of death or euthanasia in the study was a dose-related exacerbation of chronic progressive nephropathy (CPN), especially in males (see the sponsor's summary table, below). BG-12 induced a dose-related exacerbation of the incidence and/or severity of changes (described as "characteristic of CPN"), including: glomerular hypertrophy, glomerular atrophy and sclerosis, Bowman's capsule hyperplasia, adhesions, basophilic tubules, thickened basement membranes, interstitial inflammation and fibrosis, tubular cysts, and tubular casts. According to the pathologist, these changes were not individually diagnosed, but were given the summary diagnosis of CPN and an overall grade. The pathologist stated that the severe CPN led to uremia with secondary hyperparathyroidism, and then yielded many secondary systemic effects in numerous organs as expected in chronic renal failure (such as parathyroid hyperplasia and adenomas, fibrous osteodystrophy of bones, mineralization of numerous vessels and soft tissues, and degeneration, erosions and mineralization of the glandular mucosa of the stomach).

However, in addition to the dose-related exacerbation of CPN, some dose groups developed renal tubule proliferative lesions including tubular hyperplasia, adenomas and/or carcinomas. According to the pathologist, these proliferative changes were seen in both sexes, but were "most evident in the females that had not developed CPN as severely as the males and thus had better longevity and more time to develop renal tubular hyperplasia and tumors." In addition to the hyperplasia observed in the renal tubules, hyperplasia was also noted to occur with increased incidence in the pelvis in HMDM and HDM (see excerpt from the sponsor's summary data, below). The pathologist also noted that "another treatment-related renal change, not directly related to CPN, was the increased incidence and severity of venous thrombosis and mineralization." See the sponsor's summary table, below. Other important findings were observed in the kidney, although at lower incidence; see the excerpts from the sponsor's summary data, below.

Incidence of Treatment-Related Kidney Findings

Sex	Males					Females				
Group	1	2	3	4	5	1	2	3	4	5
Dose (mg/kg/day)	0	25	50	100	150	0	25	50	100	150
Nephropathy, Chronic Progressive	68 (2.04)	75 (2.71)	75 (3.16)	75 (3.52)	75 (3.53)	49 (0.95)	55 (1.32)	68 (1.84)	69 (2.44)	73 (3.24)
Vein - Thrombosis	2 (0.07)	6 (0.23)	14 (0.55)	16 (0.55)	24 (0.91)	1 (0.05)	2 (0.09)	1 (0.04)	8 (0.33)	21 (0.85)
Vein - Mineralization	2 (0.05)	4 (0.11)	9 (0.32)	9 (0.21)	11 (0.31)	0 (0.00)	2 (0.05)	1 (0.03)	7 (0.23)	13 (0.32)
Note: The average severity grade (score: 0-4) is shown in parentheses.										

WEEKS ON TEST: ALL SEX: MALE

INCIDENCE OF NEOPLASTIC and NON-NEOPLASTIC MICROSCOPIC FINDINGS

GROUP:		1	2	3	4	5
		(1)	(2)	(3)	(4)	(5)
NUMBER OF ANIMALS:		75	75	75	75	75
KIDNEYS	# EX	75	75	75	75	75
PELVIS- TRANSITIONAL EPITHELIUM- HYPERPLASIA		27	19	23	31	32
CYST(S)		27	34	44	54	58
MINERALIZATION		13	8	19	19	22

WEEKS ON TEST: ALL SEX: FEMALE

INCIDENCE OF NEOPLASTIC and NON-NEOPLASTIC MICROSCOPIC FINDINGS

GROUP:		1	2	3	4	5
		(1)	(2)	(3)	(4)	(5)
NUMBER OF ANIMALS:		75	75	75	75	75
KIDNEYS	# EX	75	75	75	75	75
CYST(S)		6	9	13	33	55
MINERALIZATION		6	4	5	6	8
PELVIS- DILATATION		14	21	24	24	35
RENAL TUBULE- HYALINE DROPLETS		1	2	1	2	2
ARTERY- MINERALIZATION		0	2	0	5	6

The stomach, both nonglandular and glandular, was identified as a target organ. The nonglandular mucosa of the stomach was a treatment-related target tissue in all treated groups. A dose-related progression of lesion severity and tumor development was observed. Proliferative lesions, squamous cysts, and hyperkeratosis were observed, frequently associated with chronic active inflammation, ulceration, erosions, mineralization and inflammation of the submucosal tissues and vessels through the serosa. Similar changes were also observed in the glandular stomach (e.g., proliferative lesions, degeneration, edema, inflammation, mineralization, ulceration), although with lower incidence. Generally, the sponsor interpreted these changes as secondary effects resulting from the hyperparathyroidism resulting from exacerbated

CPN. Given the effects on the nonglandular stomach, it is not clear whether the toxicities seen in the glandular stomach are entirely secondary effects.

WEEKS ON TEST: ALL SEX: MALE

INCIDENCE OF NEOPLASTIC and NON-NEOPLASTIC MICROSCOPIC FINDINGS

GROUP:		1	2	3	4	5
		(1)	(2)	(3)	(4)	(5)
NUMBER OF ANIMALS:		75	75	75	75	75
STOMACH	# EX	75	75	75	75	75
SEROSA- INFLAMMATION, CHRONIC ACTIVE		1	1	3	10	18
GLANDULAR- DEGENERATION		3	8	20	18	21
GLANDULAR- EDEMA		1	1	0	0	4
GLANDULAR- INFLAMMATION, CHRONIC ACTIVE		1	2	5	18	13
GLANDULAR- MINERALIZATION		5	4	15	15	24
GLANDULAR- ULCER		0	1	0	1	1
NONGLANDULAR- ARTERY- INFLAMMATION, CHRONIC ACT		2	2	11	12	19
NONGLANDULAR- EDEMA		4	1	3	2	1
NONGLANDULAR- EPITHELIUM- EROSION		1	1	3	12	14
NONGLANDULAR- HYPERPLASIA, SQUAMOUS EPITHELIUM		3	71	75	75	75
NONGLANDULAR- HYPERKERATOSIS		2	69	75	75	75
NONGLANDULAR- SQUAMOUS CYST(S)		2	9	28	53	64
NONGLANDULAR- INFLAMMATION, CHRONIC ACTIVE		3	11	24	48	51
NONGLANDULAR- MINERALIZATION		1	1	9	9	17
NONGLANDULAR- SQUAMOUS CELL CARCINOMA		0	5	18	51	58
NONGLANDULAR- SQUAMOUS CELL PAPILLOMA		0	22	23	46	49
NONGLANDULAR- SQUAMOUS CELL PAPILLOMA, MULTIPLE		0	0	1	0	0
NONGLANDULAR- ULCER		0	1	0	4	15
ARTERY- MINERALIZATION		0	0	4	3	1

WEEKS ON TEST: ALL SEX: FEMALE

INCIDENCE OF NEOPLASTIC and NON-NEOPLASTIC MICROSCOPIC FINDINGS

GROUP:		1	2	3	4	5
		(1)	(2)	(3)	(4)	(5)
NUMBER OF ANIMALS:		75	75	75	75	75
STOMACH	# EX	75	75	75	75	75
SEROSA- INFLAMMATION, CHRONIC ACTIVE		0	0	2	6	23
GLANDULAR- DEGENERATION		0	4	1	8	14
GLANDULAR- EROSION		1	3	3	6	5
GLANDULAR- GLAND- CYST(S)		54	65	66	66	73
GLANDULAR- INFLAMMATION, CHRONIC ACTIVE		0	0	0	0	2
GLANDULAR- MINERALIZATION		0	3	2	6	11
GLANDULAR- ULCER		0	1	0	0	2
NONGLANDULAR- ARTERY- INFLAMMATION, CHRONIC		0	0	0	1	2
NONGLANDULAR- ARTERY- INFLAMMATION, CHRONIC ACT		0	0	2	0	1
NONGLANDULAR- EPITHELIUM- ULCER		4	1	0	6	16
NONGLANDULAR- EPITHELIUM- EROSION		0	4	8	9	20
NONGLANDULAR- HYPERKERATOSIS		6	63	74	75	75
NONGLANDULAR- SQUAMOUS CYST(S)		1	0	11	45	62
ARTERY-MINERALIZATION		0	1	1	3	6
NONGLANDULAR- INFLAMMATION, CHRONIC ACTIVE		4	8	13	40	55
NONGLANDULAR- MINERALIZATION		0	0	1	1	8
NONGLANDULAR- SQUAMOUS CELL CARCINOMA		0	1	4	30	48

Other GI organs were also affected. The esophagus was noted to show hyperkeratosis, particularly in HDM. Although the sponsor stated that their presence did not affect the study, observations in the intestines (such as inflammation) may have been confounded by the presence of pinworms in the colony (recorded as oxyuriasis). Effects such as edema, inflammation, erosions, ulcers, and necrosis were observed with relatively low incidence in HMDM and/or HDM.

WEEKS ON TEST: ALL SEX: MALE

INCIDENCE OF NEOPLASTIC and NON-NEOPLASTIC MICROSCOPIC FINDINGS

GROUP:		1	2	3	4	5
		(1)	(2)	(3)	(4)	(5)
NUMBER OF ANIMALS:		75	75	75	75	75
ESOPHAGUS	# EX	75	75	75	75	75
HYPERKERATOSIS		0	1	2	0	5
RECTUM	# EX	74	75	75	74	74
OXYURIASIS		5	5	7	6	5
INFLAMMATION, CHRONIC ACTIVE		0	0	0	1	0
COLON	# EX	74	75	75	70	74
OXYURIASIS		7	6	5	3	11
EDEMA		0	0	0	0	2
INFLAMMATION, CHRONIC ACTIVE		0	0	0	2	3
EROSION		0	0	0	0	2
ULCER		0	0	0	0	1
CECUM	# EX	73	68	68	65	70
OXYURIASIS		4	1	5	4	5
DILATATION		0	0	1	0	2
EDEMA		1	0	3	0	4
EROSION		1	0	0	1	2
INFLAMMATION, CHRONIC ACTIVE		0	0	0	4	5
MINERALIZATION		0	0	0	2	1
ULCER		0	0	1	0	1
NECROSIS		0	0	1	0	1
INFARCTION		0	0	0	2	0

WEEKS ON TEST: ALL SEX: FEMALE

INCIDENCE OF NEOPLASTIC and NON-NEOPLASTIC MICROSCOPIC FINDINGS

GROUP:		1	2	3	4	5
		(1)	(2)	(3)	(4)	(5)
NUMBER OF ANIMALS:		75	75	75	75	75
COLON	# EX	75	75	75	75	75
OXYURIASIS		7	4	4	7	14
CECUM	# EX	75	75	75	75	75
OXYURIASIS		1	4	2	5	4

Findings were also observed in the heart and the cardiovascular system. A dose-related increased severity of cardiomyopathy and an increased incidence and severity of thrombosis of the atrium were observed (see summary table from the sponsor, below). The sponsor interpreted a number of these findings as secondary effects (due to the previously discussed effects leading to morbidity/mortality). In the aorta and

heart, arteriosclerosis, dilatation, mineralization, and/or necrosis were observed (see excerpts from the sponsor's summary table, below). In addition to these findings, dose-related increases in the incidence and severity of chronic active inflammation of the arteries throughout numerous organs (to include the pancreas, thymus, spleen, kidneys, intestine, and lymph nodes; stomach and epididymides should have been included) were observed; see the sponsor's summary table, below. This was often accompanied by mineralization. In some animals with chronic blood loss and/or severe inflammatory lesions, an increased incidence of extramedullary hematopoiesis in the spleen and erythroid and/or myeloid hyperplasia of the bone marrow were noted. Other arterial effects were also noted in a few organs, with low incidence, including: hypertrophy, medial hypertrophy, mineralization, and/or thrombosis.

Incidence of Treatment-Related Heart Findings

Sex	Males					Females				
Group	1	2	3	4	5	1	2	3	4	5
Dose (mg/kg/day)	0	25	50	100	150	0	25	50	100	150
Atrium - Thrombosis	1 (0.04)	2 (0.09)	3 (0.15)	5 (0.21)	6 (0.21)	0 (0.00)	2 (0.08)	1 (0.04)	2 (0.08)	9 (0.35)
Cardiomyopathy	70 (2.04)	68 (2.05)	73 (2.28)	74 (2.49)	71 (2.59)	63 (1.52)	63 (1.57)	68 (1.51)	64 (1.65)	66 (1.87)

Note: The average severity grade (score: 0-4, ranging from absent to marked) is shown in parentheses.

WEEKS ON TEST: ALL SEX: MALE

INCIDENCE OF NEOPLASTIC and NON-NEOPLASTIC MICROSCOPIC FINDINGS

GROUP:		1	2	3	4	5
		(1)	(2)	(3)	(4)	(5)
NUMBER OF ANIMALS:		75	75	75	75	75
AORTA	# EX	75	75	75	75	75
ARTERIOSCLEROSIS		2	3	8	7	6
DILATATION		3	4	7	6	8
MINERALIZATION		4	4	12	8	15
HEART	# EX	75	75	75	75	75
ARTERY- ARTERIOSCLEROSIS		2	3	9	5	8
ARTERY- MINERALIZATION		6	3	12	9	14
MINERALIZATION		5	3	10	12	8
VALVE- MINERALIZATION		1	0	0	1	2
MYOCARDIUM- NECROSIS		2	1	1	1	1
NECROSIS		2	2	5	8	3
BONE MARROW (FEMUR)	# EX	75	74	75	74	74
DEPLETION, CELLULAR		2	2	6	11	5
HYPERPLASIA, MYELOID		11	10	12	11	9
HYPERPLASIA, ERYTHROID		2	6	8	6	19
BONE MARROW (STERNUM)	# EX	75	74	75	74	74
HYPERPLASIA, MYELOID		11	8	12	11	10
HYPERPLASIA, ERYTHROID		2	7	7	4	18
DEPLETION, HEMATOPOIETIC CELLS		1	2	4	11	6

WEEKS ON TEST: ALL SEX: FEMALE

INCIDENCE OF NEOPLASTIC and NON-NEOPLASTIC MICROSCOPIC FINDINGS

GROUP:		1	2	3	4	5
		(1)	(2)	(3)	(4)	(5)
NUMBER OF ANIMALS:		75	75	75	75	75
AORTA	# EX	75	75	75	75	75
ARTERIOSCLEROSIS		0	4	0	6	6
DILATATION		0	0	0	5	5
MINERALIZATION		0	3	1	6	6
HEART	# EX	75	75	75	75	75
ARTERY- ARTERIOSCLEROSIS		0	4	1	6	6
ARTERY- INFLAMMATION, CHRONIC ACTIVE		0	1	0	1	1
ARTERY- MINERALIZATION		0	3	1	6	6
PERICARDIUM- INFLAMMATION, CHRONIC ACTIVE		1	0	1	3	2
NECROSIS		0	1	0	1	0
VENTRICLE- THROMBOSIS		0	1	0	1	1
BONE MARROW (FEMUR)	# EX	75	75	75	75	75
HYPERPLASIA, MYELOID		6	8	10	19	27
HYPERPLASIA, ERYTHROID		4	7	4	6	10
BONE MARROW (STERNUM)	# EX	74	75	75	75	75
HYPERPLASIA, MYELOID		6	7	6	16	15
HYPERPLASIA, ERYTHROID		5	5	3	5	10

Sponsor's Table of Sites of Chronic/Active Inflammation of the Arteries

	Male					Female				
	Dose level (mg/kg)					Dose level (mg/kg)				
Organ/tissue	0	25	50	100	150	0	25	50	100	150
Thymus	1	0	3	5	5	ND	ND	ND	ND	ND
Pancreas	6	10	13	20	22	4	4	5	11	10
Spleen	1	1	1	2	5	0	0	0	1	0
Kidney	2	1	1	4	5	1	0	0	1	0
Intestine-jejunum	2	1	1	1	4	1	0	0	1	1
Intestine-duodenum	4	5	7	9	13	1	1	1	5	2
Intestine-cecum	1	0	2	2	11	1	0	0	1	0
Lymph node-mesenteric	1	5	9	16	21	2	2	1	3	7
Lymph node-pancreatic	2	3	3	8	7	0	0	0	1	1
Testis	10	17	17	26	31	NA	NA	NA	NA	NA
ND - not described. NA - not applicable.										

Male reproductive organs were also identified as target organs. In addition to the proliferative lesions of the testes previously discussed, an increased incidence and severity of inflammation of the testicular and epididymal arteries, atrophy of the epididymides, and degeneration or atrophy of the testis were observed. Specifically, BG-12 induced dose-related degeneration and atrophy of the testicular tubular epithelium. When testicular atrophy was observed, it was usually associated with atrophy of the epididymides. In prostate and seminal vesicles, atrophy, abscess, and/or inflammation were observed at LMD, HMD, and/or HD.

WEEKS ON TEST: ALL SEX: MALE

INCIDENCE OF NEOPLASTIC and NON-NEOPLASTIC MICROSCOPIC FINDINGS

GROUP:		1	2	3	4	5
		(1)	(2)	(3)	(4)	(5)
NUMBER OF ANIMALS:		75	75	75	75	75
TESTES	# EX	75	75	75	75	75
ARTERY- INFLAMMATION, CHRONIC ACTIVE		10	17	17	26	31
ATROPHY		12	16	15	17	29
GERMINAL EPITHELIUM- DEGENERATION		7	8	5	19	15
EPIDIDYMIDES	# EX	75	75	75	75	75
ARTERY- INFLAMMATION, CHRONIC ACTIVE		4	5	9	9	15
ATROPHY		13	16	17	29	42
PROSTATE	# EX	73	74	75	75	75
ABSCESS(ES)		0	0	1	1	2
ATROPHY		2	2	5	14	8
SEMINAL VESICLES	# EX	74	75	75	75	75
ATROPHY		8	10	10	30	28
INFLAMMATION, CHRONIC ACTIVE		10	16	8	9	17

In addition to the observed brain neoplasia in HDM, there was a low incidence of necrosis and gliosis in males and females. See excerpts from the sponsor's summary data, below.

WEEKS ON TEST: ALL SEX: MALE

INCIDENCE OF NEOPLASTIC and NON-NEOPLASTIC MICROSCOPIC FINDINGS

GROUP:		1	2	3	4	5
		(1)	(2)	(3)	(4)	(5)
NUMBER OF ANIMALS:		75	75	75	75	75
		#	#	#	#	#
BRAIN	# EX	73	75	75	75	74
CEREBELLUM- DEGENERATION		1	0	0	0	0
CEREBELLUM- GLIOSIS		0	0	0	2	0
CEREBELLUM- NECROSIS		0	0	0	2	0
CEREBRUM- DEGENERATION		0	0	0	1	0
CEREBRUM- GRANULAR CELL TUMOR, BENIGN		0	0	0	0	2
CEREBRUM- GLIOSIS		0	0	2	1	2
CEREBRUM- NECROSIS		1	0	4	1	3

WEEKS ON TEST: ALL SEX: FEMALE

INCIDENCE OF NEOPLASTIC and NON-NEOPLASTIC MICROSCOPIC FINDINGS

GROUP:		1	2	3	4	5
		(1)	(2)	(3)	(4)	(5)
NUMBER OF ANIMALS:		75	75	75	75	75
BRAIN	# EX	75	75	75	75	75
CEREBELLUM- GLIOSIS		0	1	0	0	0
CEREBELLUM- NECROSIS		0	1	0	0	1
CEREBRUM- GLIOSIS		0	0	0	1	2
CEREBRUM- NECROSIS		1	0	0	1	1

A few other findings of relatively low incidence, but appearing potentially treatment-related, were observed. In addition to the renal findings, there was an increased incidence in urinary bladder findings. In addition to the hyperplasia noted by the sponsor, necrosis was also observed in the parathyroid in HDM. Related to the hyperplasia of the parathyroid, and discussed by the sponsor as a marker of secondary hyperparathyroidism, bone was noted to show fibrous osteodystrophy. As a general body condition finding, fat atrophy and/or necrosis was observed to occur across sites. Skeletal muscle (thigh) also showed atrophy and damage. Changes were also observed in the liver of MD, MHD and HD animals (often showing more in females). Thymus and skin were altered in both sexes. In males, changes were also observed in Harderian gland, lymph nodes, and spleen. In females, changes were observed in pituitary, thyroid (LD and LMD females, but these animals were on drug for a longer duration than HMDF and HDF), and female reproductive organs.

WEEKS ON TEST: ALL SEX: MALE

INCIDENCE OF NEOPLASTIC and NON-NEOPLASTIC MICROSCOPIC FINDINGS

GROUP:		1	2	3	4	5
		(1)	(2)	(3)	(4)	(5)
NUMBER OF ANIMALS:		75	75	75	75	75
URINARY BLADDER	# EX	74	75	75	75	74
INFLAMMATION, CHRONIC ACTIVE		9	8	7	8	11
MINERALIZATION		0	0	0	2	1
TRANSITIONAL EPITHELIUM- CARCINOMA		0	1	0	0	0
TRANSITIONAL EPITHELIUM- HYPERPLASIA		9	7	8	8	14
PARATHYROID	# EX	68	64	70	71	70
NECROSIS		0	0	0	0	3
FEMUR WITH JOINT	# EX	75	74	75	75	74
FIBROUS OSTEODYSTROPHY		6	8	20	29	36
STERNUM	# EX	75	74	75	74	74
FIBROUS OSTEODYSTROPHY		5	5	14	23	24
BONE	# EX	2	1	1	0	0
FIBROUS OSTEODYSTROPHY		0	0	1	0	0
HYPEROSTOSIS		0	0	1	0	0
HEAD	# EX	0	0	0	1	1
NASOTURBINATE- FIBROUS OSTEODYSTROPHY		0	0	0	1	0
CAVITY, ABDOMINAL	# EX	4	4	4	5	8
FAT- NECROSIS		0	2	1	0	5
FAT-ATROPHY		0	1	1	1	1
CAVITY, THORACIC	# EX	1	3	1	0	0
FAT- ATROPHY		1	1	0	0	0
FAT	# EX	1	1	2	0	3
ATROPHY		0	0	2	0	3
SKELETAL MUSCLE (THIGH)	# EX	75	75	75	75	75
ATROPHY		13	18	17	19	31
DEGENERATION		2	3	3	8	3
MINERALIZATION		1	0	5	6	3
NECROSIS		0	0	0	1	1

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HARDERIAN GLAND	# EX	75	75	75	74	75
INFILTRATING CELL, LYMPHOCYTE		3	3	6	3	5
INFILTRATING CELL, MIXED		1	0	5	0	1
THYMUS	# EX	75	75	74	73	75
EPITHELIUM- HYPERPLASIA		6	9	9	5	13
HEMORRHAGE		0	0	4	4	0
LYMPH NODE. MANDIBULAR	# EX	75	75	75	74	75
DEPLETION, LYMPHOID		2	1	1	4	5
ERYTHROPHAGOCYTOSIS		2	3	7	5	6
HYPERPLASIA, LYMPHOID		15	14	19	23	16
LYMPH NODE, MESENTERIC	# EX	74	74	75	74	74
LYMPHANGIOECTASIA		5	6	9	11	13
HYPERPLASIA, LYMPHOID		3	5	8	10	13
ERYTHROPHAGOCYTOSIS		5	3	6	9	6
DEPLETION, LYMPHOID		4	3	12	15	16
LYMPH NODE, MEDIASTINAL	# EX	7	10	8	7	5
HYPERPLASIA, LYMPHOID		3	4	3	1	3
DEPLETION, LYMPHOID		0	0	0	1	2
LYMPH NODE, PANCREATIC	# EX	3	5	9	23	20
HYPERPLASIA, LYMPHOID		0	2	4	11	10
LYMPHANGIOECTASIA		0	2	5	18	19
ERYTHROPHAGOCYTOSIS		0	1	5	14	15
LYMPH NODE, RENAL	# EX	4	4	3	11	4
HYPERPLASIA, LYMPHOID		3	4	2	5	3
DEPLETION, DEPLETION		0	0	0	3	0
ERYTHROPHAGOCYTOSIS		3	3	1	8	3
LYMPHANGIOECTASIA		3	4	1	10	3
LYMPH NODE, ILIAC	# EX	7	10	11	3	2
LYMPHANGIOECTASIA		5	10	11	3	2
HYPERPLASIA, LYMPHOID		7	10	11	1	2
LYMPH NODE, AXILLARY	# EX	0	2	2	0	0
HYPERPLASIA, LYMPHOID		0	1	2	0	0
LYMPHANGIOECTASIA		0	0	2	0	0
SPLEEN	# EX	75	75	75	75	75
CONGESTION		4	4	7	9	7
DEPLETION, LYMPHOID		10	7	12	22	27
FIBROSIS		0	0	1	1	2
LIVER	# EX	75	74	75	75	75
CONGESTION, CENTRILOBULAR		14	11	20	13	9
VACUOLIZATION, CENTRILOBULAR		7	10	15	16	11
VACUOLIZATION, PERIPORTAL		0	4	3	2	1
CAPSULE- FIBROSIS		1	1	0	3	3
SKIN	# EX	75	74	75	74	75
HAIR FOLLICLE- ATROPHY		0	3	3	10	10

WEEKS ON TEST: ALL SEX: FEMALE

INCIDENCE OF NEOPLASTIC and NON-NEOPLASTIC MICROSCOPIC FINDINGS

GROUP:		1 (1)	2 (2)	3 (3)	4 (4)	5 (5)
NUMBER OF ANIMALS:		75	75	75	75	75
URINARY BLADDER	# EX	75	75	75	75	75
INFLAMMATION, CHRONIC ACTIVE		0	2	3	6	5
TRANSITIONAL EPITHELIUM- HYPERPLASIA		1	2	3	6	6
FEMUR WITH JOINT	# EX	75	74	75	74	75
FIBROUS OSTEODYSTROPHY		0	2	2	8	15
STERNUM	# EX	74	75	75	75	75
FIBROUS OSTEODYSTROPHY		0	2	0	5	8
SKELETAL MUSCLE (THIGH)	# EX	75	75	74	74	75
ATROPHY		7	9	6	9	17
THYMUS	# EX	75	75	74	75	75
EPITHELIUM- HYPERPLASIA		35	34	45	50	43
PITUITARY	# EX	75	75	74	73	75
PARS DISTALIS- ANGIECTASIS		8	7	18	12	16
PARS INTERMEDIA- CYST(S)		1	2	3	3	2
PARS DISTALIS- HYPERPLASIA		11	11	12	16	16
LYMPH NODE, MESENTERIC	# EX	75	75	75	75	75
DEPLETION, LYMPHOID		6	3	1	2	11
LYMPH NODE, PANCREATIC	# EX	2	2	3	10	24
HYPERPLASIA, LYMPHOID		0	0	2	10	23
LYMPHANGIOECTASIA		0	0	1	8	21
ERYTHROPHAGOCYTOSIS		0	0	1	2	7
LYMPH NODE, RENAL	# EX	2	2	0	5	8
LYMPHANGIOECTASIA		0	1	0	4	8
ERYTHROPHAGOCYTOSIS		0	1	0	5	6
HYPERPLASIA, LYMPHOID		0	0	0	5	8
SPLEEN	# EX	75	75	75	75	75
EXTRAMEDULLARY HEMATOPOIESIS, INCREASED		24	30	21	39	52
NECROSIS		0	0	1	0	2
PIGMENT		0	0	1	0	1
THYROID	# EX	75	75	75	75	75
C CELL- HYPERPLASIA		8	12	17	9	6
LIVER	# EX	75	75	75	75	75
EXTRAMEDULLARY HEMATOPOIESIS		11	12	10	15	13
BILE DUCT- CYST(S)		0	3	4	6	7
BILE DUCT- HYPERPLASIA		38	43	50	46	43
CAPSULE- FIBROSIS		1	1	1	1	9
VACUOLIZATION, CENTRILOBULAR		3	6	12	10	25
CONGESTION, CENTRILOBULAR		2	1	4	4	10
NECROSIS, CENTRILOBULAR		0	1	3	3	5
OVARIES	# EX	75	75	75	75	75
FOLLICLE- CYST(S)		15	17	16	17	26
PERIOVARIAN TISSUE- CYST		8	11	11	13	7

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GROUP:		1 (1)	2 (2)	3 (3)	4 (4)	5 (5)
NUMBER OF ANIMALS:		75	75	75	75	75
VAGINA	# EX	75	75	75	75	75
INFLAMMATION, CHRONIC ACTIVE		4	5	1	1	12
SKIN	# EX	75	75	75	75	75
HAIR FOLLICLE- ATROPHY		3	1	0	3	8

Toxicokinetics

The sponsor stated that DMF undergoes rapid and extensive metabolism by non-specified esterase and was not detectable in the majority of *in vivo* and *in vitro* ADME studies; therefore, MMF (the primary, active, metabolite) was measured in these studies. Exposure to BG00012 was confirmed through plasma concentrations of MMF. MMF exposures demonstrated a rapid peak (T_{max} = 15-30 minutes) and elimination ($t_{1/2}$ = 26-60 minutes). C_{max} and AUC_{∞} values were highly variable across study days in each group. No consistent trend in dose proportionality was seen either over sex or dose; only the C_{max} values of both males and females on Day 1 and AUC_{∞} values for females exhibited dose proportionality. In general, higher exposures were observed in females than in males across all groups. Trough (pre-dose) concentrations of MMF on D90 and D180 were BLOQ, consistent with no evidence of accumulation of MMF with once daily administration. See sponsor's summary Table 4, below, for details.

Table 4. Toxicokinetic parameter estimates of MMF on Days 1, 90, and 180 after once-daily oral administration of BG00012 to Sprague Dawley rats for 6 months at 25, 50, 100 or 150 mg/kg/day.

Dose (mg/kg)	Gender	Study Day	$T_{1/2}$ (min)	T_{max} (min)	C_{max} (µg/mL)	AUC_{last} (hr*µg/mL)	AUC_{inf} (hr*µg/mL)	Cl/F (mL/hr/kg)	Vz/F (mL/kg)
25	Female	1	52	15	5.5	3.3	3.4	7392	9187
		90	26	15	5.6	3.1	3.3	7537	4777
		180	34	15	11.3	7.4	7.5	3356	2759
	Male	1	31	15	4.5	3.2	3.2	7822	5754
		90	47	15	3.1	1.8	2.2	11347	12689
		180	47	15	3.3	4.0	4.2	5998	6860
50	Female	1	31	15	10.0	8.6	8.6	5818	4303
		90	29	30	8.3	7.5	7.5	6643	4675
		180	28	15	18.2	16.6	16.6	3009	2027
	Male	1	47	15	9.2	7.5	7.5	6649	7587
		90	54	15	3.4	4.3	4.5	11062	14293
		180	49	15	8.2	8.7	9.1	5502	6491
100	Female	1	35	15	21.4	22.1	22.3	4477	3837
		90	41	15	11.2	14.7	14.7	6787	6712
		180	47	30	24.2	22.9	23.0	4352	4919
	Male	1	42	30	20.1	20.1	20.2	4962	5007
		90	53	30	7.3	7.5	7.8	12752	16391
		180	56	30	10.6	11.9	12.1	8299	11244
150	Female	1	62	15	31.0	33.7	34.3	4370	6481
		90	37	30	17.5	23.1	23.1	6488	5815
		180	55	15	37.8	37.6	38.4	3911	5140
	Male	1	52	30	29.3	33.0	33.2	4517	5626
		90	53	15	25.6	19.1	19.5	7680	9857
		180	52	15	51.2	30.8	30.9	4857	6072

Dosing Solution Analysis

Samples for analysis were prepared from dosing formulations weekly until week 4, then every 4 weeks until week 20, weekly until week 24, and every 4 weeks until week 104. Generally, the samples were within acceptable limits of ±15% error. Homogeneity was determined for all dose formulation concentration levels during Weeks 1 and 2; all results were within the acceptable range of ≤5% RSD.

EXPERT REPORT ON HISTOPATHOLOGICAL RE-EVALUATION OF RAT AND MOUSE KIDNEY FROM CARCINOGENICITY STUDIES WITH ORALLY ADMINISTERED BG00012 (DIMETHYL FUMARATE)

Non-GLP, Performed by (b) (4) on March 16-29, 2012, report dated 5/11/12.
Individual and comparative data tables dated 10/20/12, were provided upon Agency request

(b) (4) generated new data based on the re-examination of the renal histopathology occurring in the rat (and mouse; see review for mouse following the review of the mouse 2-yr bioassay) carcinogenicity study of DMF. The sponsor requested that (b) (4) "identify the nature of the lesions reported, and to attempt to determine key histopathological events that would support mode(s) of action underlying the development of renal tubule tumors in… [the] carcinogenicity studies." The observed renal tumors were assessed to determine whether any exhibited an amphophilic-vacuolar (A-V) morphology, which has since been determined to be of spontaneous origin (Hard et al., 2008). And for the hyperplasia and tumors observed in the study, the hyperplasia was evaluated for the atypical tubule hyperplasia (ATH) form, which is accepted as being on the continuum with renal tubule adenoma and carcinoma (Hard, 1987, Lipsky and Trump, 1988; Dietrich and Swenberg, 1991; Nogueira et al., 1993).

The previously prepared histology slides of rat kidney stained with hematoxylin and eosin (H&E) were examined. (b) (4) used the criteria recommended by the Society of Toxicologic Pathology (Hard et al., 1999) to diagnose rat kidney lesions. The criteria of Hard et al. (2005, 2006) were used for identifying "non-biologically significant, proliferative lesions occurring in chronic progressive nephropathy (CPN) and distinguishing them from ATH and adenoma." (b) (4) graded CPN on a 0 to 8 scale, based on the progressive development of the disease (Hard et al., 2012). He stated that " …for expediency, the numbers of foci in the lower grades were not counted, and grades 1-4 were combined as they were not significant to the outcome of the review." See the sponsor's summary tables of the original pathologist's findings and (b) (4) re-evaluation, below.

Incidence (and Severity) of Nephropathy, Renal Hyperplasia, Adenoma and Carcinoma in Rats of 2-Year Carcinogenicity Study – Results from the Final Study Report

Gender	Male					Female				
Dosage (mg/kg/day)	0	25	50	100	150	0	25	50	100	150
Nephropathy, Incidence	68	75	75	75	75	49	55	68	69	73
Average severity score [a]	2.04	2.71	3.16	3.52	3.53	0.95	1.32	1.84	2.44	3.24
Hyperplasia, tubule	0	5	5	15	11	0	0	4	4	9
Adenoma, tubule	0	0	1	1	4	1	0	0	0	2
Carcinoma, tubule	0	0	0	0	0	0	0	0	2	4

a: Average severity score based on following grades: 0 = absent, 1= minimal, 2 = mild, 3 = moderate, 4 = marked

Incidence (and Severity) of Nephropathy, Renal Hyperplasia, Adenoma and Carcinoma in Rats of 2-Year Carcinogenicity Study – Results from [redacted] (b) (4) Evaluation

Gender	Male					Female				
Dosage (mg/kg/day)	0	25	50	100	150	0	25	50	100	150
CPN, Incidence	73	75	75	75	75	67	70	74	70	74
Average severity score [a]	4.5	5.8	6.5	6.9	7.0	2.9	3.5	4.1	5.5	6.6
Hyperplasia (ATH), tubule [c]	0	0	1	1	1	0	0	1	0	2
Adenoma, tubule	0	0	1	1	0	1 [b]	0	1	0	2
Carcinoma, tubule	0	0	0	0	0	0	0	0	0	1
A-V Hyperplasia, tubule [d]	0	1	0	1	1	0	0	1	0	2
A-V Adenoma, tubule	0	0	0	0	2	0	0	0	0	0
A-V Carcinoma, tubule	0	0	0	0	0	0	0	0	1	1

a: The severity grades for CPN (chronic progressive nephropathy) were averaged based on 0-8 for the rat where Grades 1 to 4 represented increasing numbers of countable foci of CPN; Grade 5 represented focal CPN in which the foci were too numerous to quantify; Grades 6 to 8 represented the progressive coalescence of CPN foci into a continuous network of affected tissue, culminating in end-stage kidney disease (Grade 8).
b: Slide missing (result maintained from the original evaluation).
c: Hyperplasia noted were described as atypical tubule hyperplasia (ATH).
d: A-V = amphophilic-vacuolar proliferation (spontaneous entity).

Renal tubule cells with no or low grade CPN did not show evidence of cytotoxicity. [redacted] (b) (4) concluded that there was clear evidence of exacerbation of CPN by BG-12. The advanced severity of the CPN was a risk factor for a low incidence of low-grade renal tubule tumor development (cf. Hard et al., 2012). CPN has no counterpart in humans and, therefore, chemical exacerbation of CPN is likely to have no relevance for humans (Hard et al., 2004, 2009). After exclusion of A-V ATH/tumors and other differences from the original study pathologist's diagnoses (e.g., non-renal secondary growths from the mammary gland), the "ATH and tumor incidence in the rat carcinogenicity study of BG00012 was not significantly increased, but the majority of lesions were closely associated with CPN of advanced grade and linked to the CPN exacerbation associated with BG00012 administration." Only 1 HDF (No. 700) was observed to show both an adenoma and an early carcinoma that were not associated with CPN.

Study title: Two-Year Oral (Gavage) Carcinogenicity Study in Mice with BG00012

Study no.:	P00012-05-03; CRL No. P00012-05-03
Study report location:	Electronic,
Conducting laboratory and location:	(b) (4)
Date of study initiation:	6/16/05
GLP compliance:	Yes, pg. 9, except: • Animals were received prior to protocol signing • Characterization and stability were conducted with GMP compliance • "Toxicokinetic interpretation was not conducted in compliance with the GLP regulations."
QA statement:	Yes, pg. 10-13
Drug, lot #, and % purity:	BG00012 (dimethyl fumarate), lot 1102642 33004998, 100.2% pure (MMF 0.02%)
CAC concurrence:	No, concurrence was not obtained

Key Study Findings

- The HD was reduced from 600 mg/kg (D1-D5) to 400 mg/kg on D9 (dosing holiday D6-D8), due to deaths on D5-D8 (15 HDM and 13 HDF).
- Dosing was suspended in HDM in week 72 (day 503) and in HDF in week 82 (day 571).
- Survival was reduced at HMD and HD; the HD groups were terminated during week 101.
- Average body weight was reduced at HD before the dose reduction (D9); afterward, average body weight was similar to controls.
- Target organs were kidney, stomach, and eye:
  - Kidney: dose-dependent increase in nephropathy (esp. males), other changes not typically noted in nephropathy (i.e., changes in the outer stripe and medullary rays), and increased adenomas and/or carcinomas in both sexes (HMDM, HDM, & HDF)
  - Stomach, nonglandular: dose-dependent increase in squamous cell papillomas and carcinomas and leiomyosarcomas in males and females, and a number of lesions such as squamous cysts, squamous hyperplasia, hyperkeratosis, ulcers, erosions, and inflammation that extended into the submucosa and serosa (HMD & HD)
  - Eye: dose-related increase in the severity and/or incidence of retinal degeneration (HMD & HD)

Adequacy of Carcinogenicity Study

The study is acceptable based on MTD; the mortality at 600 mg/kg/day, necessitating the reduction in the dose to 400 mg/kg/day (which still resulted in increased mortality in males), demonstrated that a maximally tolerated dose was achieved/exceeded.

The sponsor did not receive FDA ExecCAC concurrence (communications dated 6/15/05 and 8/9/05) on their proposed 2-year bioassay protocol because the submitted 13-week study in mice (Study EBA00044) was not adequate to define an MTD. Furthermore, the Agency had the following comments on the sponsor's proposal (from communication dated 8/9/05):

> The Agency cannot comment upon the suitability of an exposure of 600 mg/kg/day in the proposed bioassay, as adequate data have not been submitted to support selection of that exposure level. It is, again, recommended that the sponsor obtain fully adequate data concerning exposure of mice for at least 13 weeks to the test material at levels of exposure that induce dose-limiting toxicity (or adequately support dosage selection through means other than the MTD, as discussed in the ICH S1C document, "Dose Selection for Carcinogenicity Studies of Pharmaceuticals", and related documents) if concurrence of the exec-CAC is desired. Those data should be obtained under conditions identical to those proposed for use in the two-year study, including use of the same strain of mouse. The sponsor is, of course, free to conduct a study without having received concurrence from the committee. In that case, the study would be evaluated upon submission as if the protocol had not been submitted for concurrence.

Appropriateness of Test Models

According to the sponsor, the CD-1 mouse was chosen as the animal model for this study as it is a preferred rodent species for oral toxicity studies. The mouse is a standard model used in 2-year bioassay carcinogenicity testing. The oral route of exposure was selected since this is the intended clinical route of administration.

Evaluation of Tumor Findings

- Kidney: adenomas and/or carcinomas in both sexes (HMDM, HDM, & HDF)
- Stomach, nonglandular: squamous cell papillomas and carcinomas (MHD & HD) and leiomyosarcomas (HD) in both sexes

Methods (see details in sponsor's summary table, below)

Frequency of dosing:	QD
Route of administration:	PO, gavage
Basis of dose selection:	According to the sponsor, the doses were "selected by the Sponsor according to FDA-CDER recommendations as a June, 2005 Executive CAC response…" The selected doses were based on the marked dose-dependent increase in stomach weights and gross and histological changes in the forestomach in treated animals, compared to control animals, in a 90-day dose range-finding study in CD-1 mice. The sponsor estimated that MHD of 200 mg/kg would be the approximate maximum tolerated dose (MTD), based on the dose range-finding study. The HD of 600 mg/kg/day was added based on "the FDA CDER CAC response to evaluate higher doses than 200 mg/kg in order to determine an MTD within the mouse study (June 2005)."
Species/Strain:	CD-1 [Crl:CD-1R(ICR)Br] mice, (b) (4)
Age:	8 weeks at randomization, 27-35 g (M) and 22-29 g (F)
Animal housing:	individually in suspended stainless steel cages
Satellite groups:	TK Sentinel animals (20/sex) were also maintained. Many sentinel, main study and TK animals were affected by and treated for Staph. aureus infections. The number of treatment days varied, but began on D64 and continued for up to 104 weeks. Although the sponsor indicated that this had no bearing on the study, there is some question as to the general health and well-being of the animals on study.
Deviation from study protocol:	A few animals were replaced; for main study animals, the following replacements were made: HDF 937 with 1055 on D3, gavage error LDM 104 with 1060 on D14, found dead ConM 17 with 1064 on D21, found dead

Experimental Design for the Carcinogenicity and Toxicokinetic Phases

Group No.	No. of Animals (Toxicokinetic Phase)		Dosage Material	BG00012 Dose Level (mg/kg/day)	BG00012 Dose Volume (mL/kg)	BG00012 Dose Conc. (mg/mL)
	Males	Females				
1	75 (12)	75 (12)	HPMC[a]	0	10	0
2	75 (30)	75 (30)	BG00012	25	10	2.5
3	75 (30)	75 (30)	BG00012	75	10	7.5
4	75 (30)	75 (30)	BG00012	200	10	20
5	75 (30)	75 (30)	BG00012	600/400[b]	10	60/40[b]

[a]Hydroxypropylmethylcellulose or Hypromellose (3,500-5,600 cps), 0.8% w/v in reverse osmosis deionized water.
[b]Due to mortality observed in the high-dose group, the dose level for Group 5 was decreased to 400 mg/kg/day (40 mg/mL) beginning on Day 9.

Observations and Results

Mortality [2x daily]

Survival was reduced at the HMD and HD in both sexes, in a dose-dependent fashion. See sponsor's Figures 1 and 2, and the sponsor's summary survival tables, below. Initial decreases in survival at the HD (15 HDM and 13 HDF were found dead during D5-8) were not resolved by the reduction in dose after the first week (HD was reduced from 600 mg/kg to 400 mg/kg on D9, after a dosing holiday D5-8); increased mortality was clearly observed between approximately week 40 (M) or 60 (F) and week 70. Final survival rates in males were 47%, 35%, 25% and 13% at LD, LMD, HMD, and HD, compared to 32% in controls; final survival rates in females were 36%, 38%, 32% and 13% at LD, LMD, HMD, and HD, compared to 45% in controls.

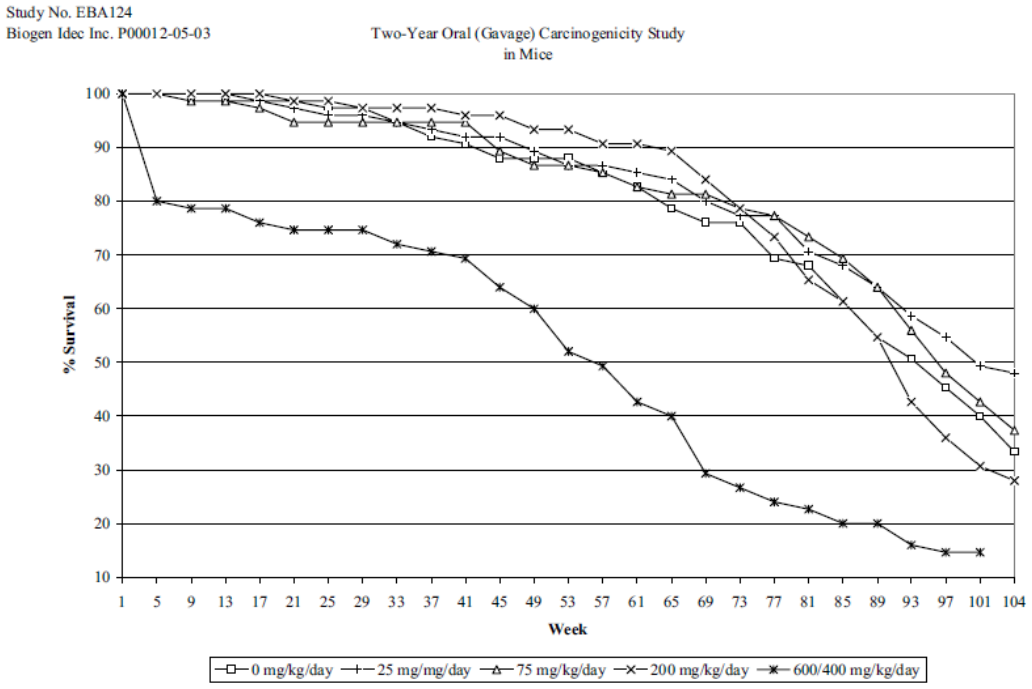


Figure 1. Summary of Male Mortality

Summary of Male Mortality during Weeks 1-107

Males	Group 1	Group 2	Group 3	Group 4	Group 5
No. Found Dead	39	30	33	46	49
No. Euthanized Moribund	12	10	16	10	16
No. Surviving[a]	24	35	26	19	10

Group 1 (0 mg/kg/day) Group 2 (25 mg/kg/day) Group 3 (75 mg/kg/day) Group 4 (200 mg/kg/day) Group 5 (600/400 mg/kg/day)
[a]Surviving males in Group 5 were euthanized during Week 101.

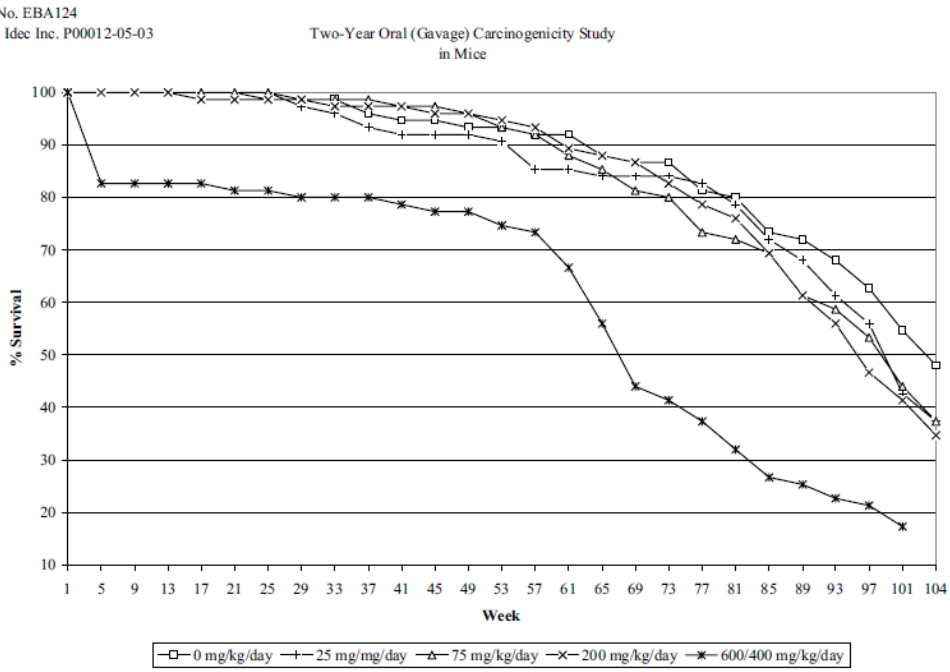


Figure 2. Summary of Female Mortality

Summary of Female Mortality during Weeks 1-107

Females	Group 1	Group 2	Group 3	Group 4	Group 5
No. Found Dead	30	31	35	28	38
No. Euthanized Moribund	11	17	10	22	27
No. Accidental Death	1	0	2	1	0
No. Surviving[a]	33	27	28	24	10

Group 1 (0 mg/kg/day) Group 2 (25 mg/kg/day) Group 3 (75 mg/kg/day) Group 4 (200 mg/kg/day) Group 5 (600/400 mg/kg/day)
[a]Surviving females in Group 5 were euthanized during Week 101.

Clinical Signs [2x daily; detailed obs pre- and weekly thereafter, with mass check]

Although the sponsor stated that there were no clearly dose-related (d-r) clinical signs, a few findings were slightly increased, mostly at the HD. The incidence and number of animals affected were slightly increased for distended abdomen (d-r M, HDF), rough coat (d-r M, HMDF, and HDF), few feces (d-r M), cool to touch (HMDF and HDF), tail enlargement (HMDF and HDF), decreased activity (HD), thin appearance (HDF), and wobbly gait (HD).

Body Weights [pre-, weekly for 13 weeks, and 1x/4 weeks until week 104]

The HD groups demonstrated average body weight reductions, compared to controls, during the first two weeks, prior to the dose reduction (5.0 - 7.3% in HDF and HDM, respectively, [ss], compared to controls); in HDM, this reflected a 3% average body weight loss between weeks 1 and 2. Following a brief dosing holiday (i.e., D6-8) and a

reduction in dose to 400 mg/kg/day, the average body weights of the HD animals were comparable to those of controls. See the sponsor's Figures 3 and 4, below. The HDF actually weighed approximately 4 - 9% more than controls during Weeks 3-49; otherwise, average body weight gains were fairly similar across groups.

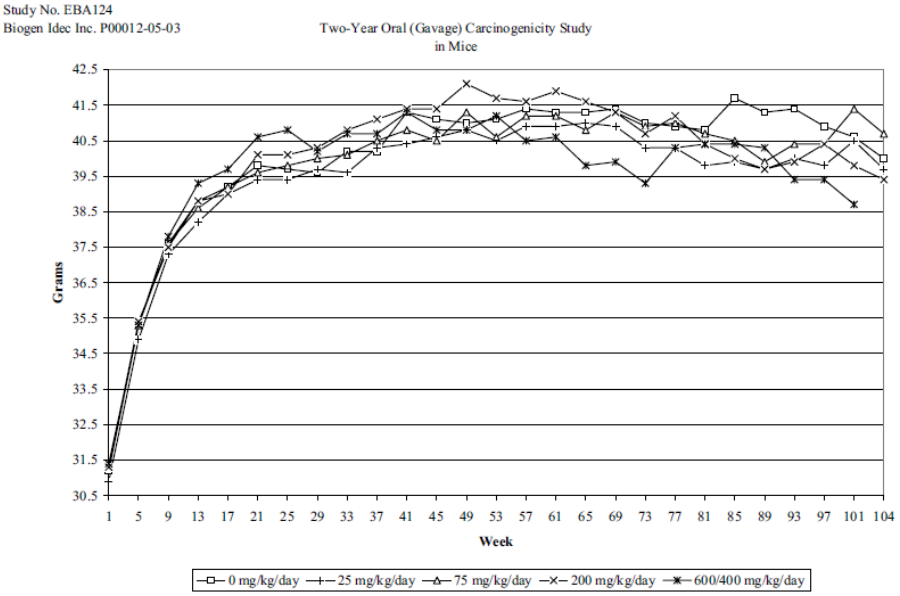


Figure 3. Summary of Male Body Weight Data

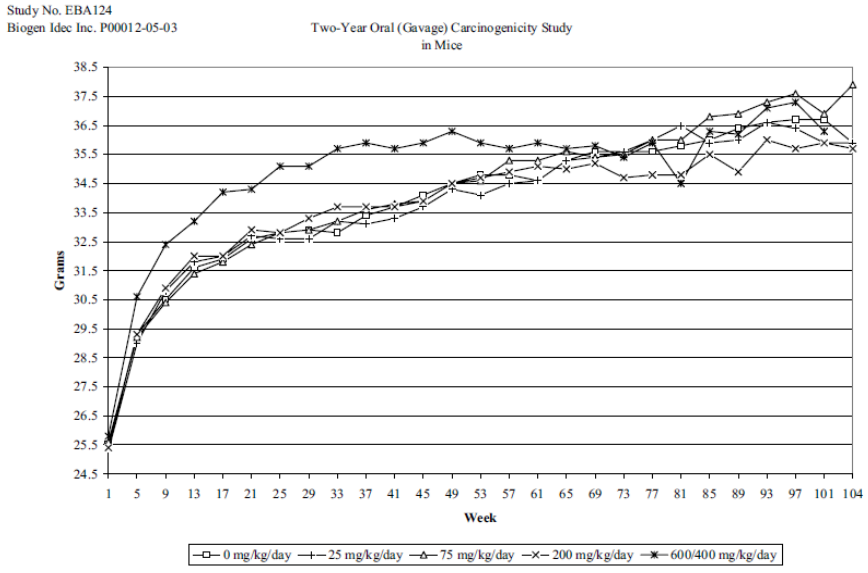


Figure 4. Summary of Female Body Weight Data

Food Consumption [weekly for 13 weeks, and 1x/4 weeks until week 104]

Food consumption was reduced in HD animals prior to the dose reduction; afterwards, food consumption was slightly greater at HD. Overall, food consumption data were fairly similar among groups. See the sponsor's summary Figures 5 and 6, below.

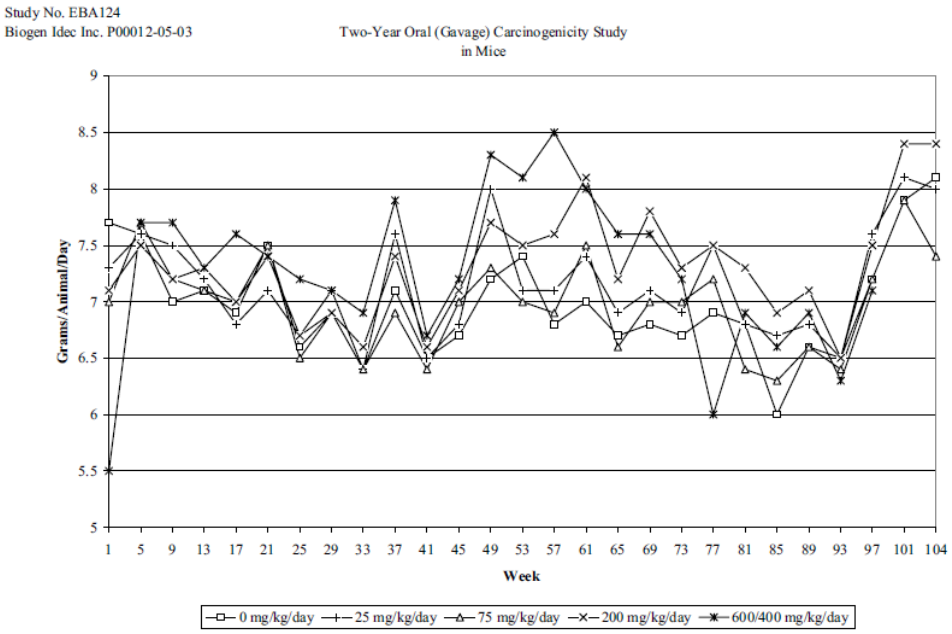


Figure 5. Summary of Male Food Consumption Data

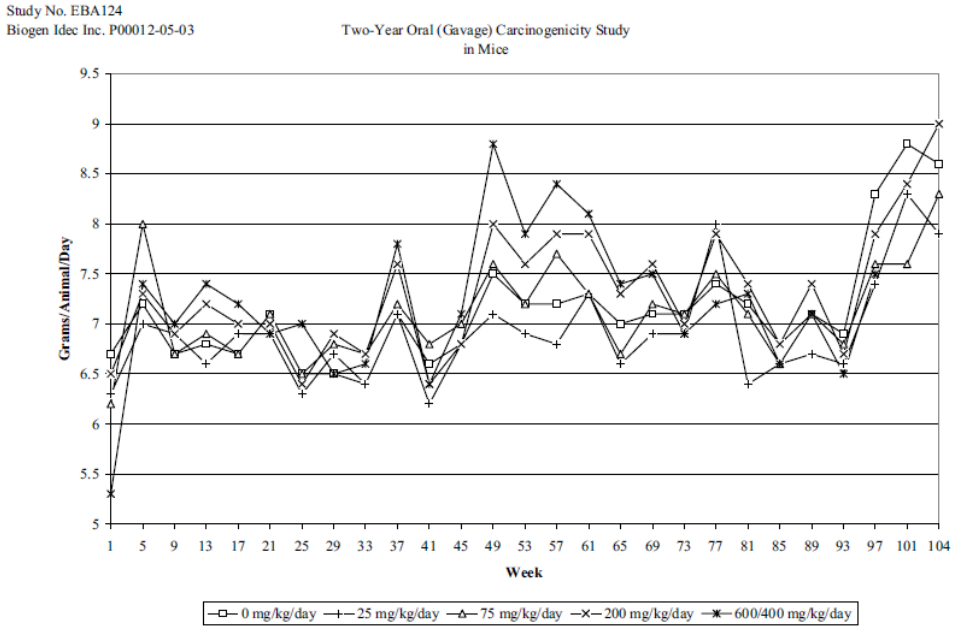


Figure 6. Summary of Female Food Consumption Data

Clinical Pathology- Hematology [at necropsy]

There were few significant hematological findings; the largest changes occurred in WBC parameters. HMDM and HDM showed reduced erythrocytes counts, hemoglobin, and hematocrit (~9-10%) compared to controls. Treated males also had reduced

reticulocyte counts (~10-20%). HMDM showed increased platelets (~30%). HDF showed increased reticulocytes (~20%). HDM and HDF had increased total leukocytes (~1.5-1.7x), lymphocytes (~1.5x), segmented neutrophils (1.4-1.8x), monocytes (~1.5-2x), and eosinophils (~2-3x) compared to controls (statistical significance was not assessed due to the difference in time of necropsy). However, the sponsor indicated that none of the observed increases appeared to deviate from the historical control range for CD-1 mice at this facility. There were no clear drug-related differences in red cell morphology.

Gross Pathology [at necropsy]

Clearly dose-related gross observations were found in the stomach and kidney (see the sponsor's summary table, below). The sponsor noted that most gross observations were correlated with histological findings. Prominent epithelial surface of the stomach was observed in LMD, HMD, and HD animals of both sexes; the sponsor indicated that this correlated with microscopic findings of squamous epithelial hyperplasia, squamous cell papilloma, and squamous cell carcinoma of the nonglandular forestomach. Kidney findings were prevalent in LMDM, HMDM, and HDM, whereas the kidney was less affected in females.

There were also some findings that suggested a dose relationship that the sponsor did not note (see reviewer's table, below). Additional findings in stomach at HD included: reddened, distended, thickened and/or rupture. In kidney, cysts and foci were also noted. General body fat depletion and abdominal cavity adhesions were observed at HD. Scabbing of the skin was noted at HD. Alterations of male and female reproductive organs were also observed.

Test Article-Related Gross Pathology Findings

Gross Finding	Sex	No. of Animals with Findings[a]				
		Group 1	Group 2	Group 3	Group 4	Group 5
Discoloration, kidney	Male	5	5	11	15	14
	Female	11	8	0	6	6
Enlarged kidney	Male	6	6	16	19	23
	Female	2	0	1	1	3
Roughened surface, kidney	Male	6	1	13	10	6
	Female	5	1	3	6	8
Prominent epithelial surface, stomach	Male	3	2	15	49	50
	Female	1	6	21	51	47

Group 1 (0 mg/kg/day) Group 2 (25 mg/kg/day) Group 3 (75 mg/kg/day) Group 4 (200 mg/kg/day) Group 5 (600/400 mg/kg/day)
[a]Includes all deaths.

	MALES					FEMALES				
	Con	LD	LMD	HMD	HD	Con	LD	LMD	HMD	HD
CARCASS										
Body fat depletion	10	10	12	6	14	9	13	10	14	18
ABDOMINAL CAVITY										
Adhesion	6	1	3	3	36	2	3	4	3	39
KIDNEY										
Cysts	20	22	36	43	29	8	3	3	6	7
Focus/Foci	0	0	2	1	3	0	0	0	1	0
SEMINAL VESICLE										
Enlarged	14	17	20	23	3					
SKIN										
Scabbing	12	6	6	12	19	4	9	5	3	16
STOMACH										
Reddened	1	0	0	0	14	0	0	0	0	12
Distended	1	0	0	0	2	1	0	0	0	8
Thickened	2	0	5	4	4	0	0	1	2	3
Rupture	0	0	0	0	1	0	0	0	0	0
TESTES										
Small	4	1	4	8	4					
UTERUS										
Polyp(s)						6	7	16	11	8

Histopathology [at necropsy]

Peer Review- Yes, for all target organ tissues, all neoplasms, and all tissues from 10% of the control and HD groups.

Neoplastic

Dose-related neoplastic findings were observed in the nonglandular stomach and kidney; the pathologist stated that "these .. tumorigenic changes increased in incidence and/or severity with increasing dose and duration of exposure to the test compound."

The nonglandular mucosa of the stomach was a target tissue in both sexes and in all DMF-treated groups. A dose-related increase in epithelial lesion incidence and severity and progression to tumor development was observed in all treated groups. The observed chronic-active inflammation and epithelial ulceration was associated with squamous epithelial hyperplasias, hyperkeratosis, squamous papillomas, and squamous cell carcinomas. The pathologist described the benign squamous papillomas as exophytic growths into the gastric lumen and the malignant squamous cell carcinomas as "usually invasive neoplasms associated with degenerative changes and chronic-active inflammation that frequently extended into the submucosa and serosa of the stomach." In addition to these neoplastic lesions, fibrosarcomas and leiomyosarcomas were also observed in the nonglandular submucosa at the HD.

Incidence of Proliferative Changes in Nonglandular Stomach

Sex	Males (N=75/Group)					Females (N=75/Group)				
Group	1	2	3	4	5	1	2	3	4	5
Dose (mg/kg/day)	0	25	75	200	600/400	0	25	75	200	600/400
Hyperplasia	2	12	49	68	60	0	33	56	70	58
Papilloma	0	1	3	12	14	1	0	3	6	16
Squamous Cell Carcinoma	0	1	0	2	6	0	1	1	5	12
Leiomyosarcoma	0	0	0	0	3	0	0	0	0	3
Fibrosarcoma	0	0	0	0	1	0	0	0	0	2

Kidney was also a target tissue for toxicity and tumors. Dose-related increases in the incidence and severity of "age-related" nephropathy (particularly in males), as well as proliferative changes that included renal tubular hyperplasias, renal tubular adenomas, and renal tubular carcinomas, were observed. Renal tumors were observed in all treated male groups, HMDF, and HDF. The sponsor stated that the "renal injury eventually leading to increased renal tumor incidence in this study may have been due to BG00012 exacerbation of nephropathy, BG00012 specific renal injury or a combination of the two factors."

Incidence of Proliferative Changes in Renal Tubule

Sex	Males					Females				
Group	1	2	3	4	5	1	2	3	4	5
Dose (mg/kg/day)	0	25	75	200	400/600	0	25	75	200	400/600
Hyperplasia	1	7	16	40	15	0	7	8	13	13
Adenoma	1	2	0	5	3	0	0	0	2	4
Carcinoma	0	0	2	4	3	0	0	0	0	1

Increased tumor incidences were suggested in a few other organs but did not reach statistical significance. Increased lung tumors were suggested for HMDM and HMDF (possibly relevant, taking into account the mortality at the HD). Also, uterine endometrial stromal polyp appeared increased at LMD and HMD. See excerpts from the statistical review below).

organ	Incidence					Significance Levels				
tumor	Veh	Low	Med	MedHi	High	Trend	Hi vs Veh	MedHi vs Veh	Med vs Veh	Low vs Veh
MALE										
LUNG WITH BRONCHI										
# Evaluated	75	75	75	75	75					
Adjusted # at risk	50.0	53.9	52.5	51.4	23.2					
Bronchiolo-Alveolar Adenocarcinoma	6	6	5	11	5	.0411	.2292	.1541	.7598	.6607
FEMALE										
LUNG WITH BRONCHI										
# Evaluated	75	75	75	75	75					
Adjusted # at risk	58.6	56.9	54.6	55.8	31.8					
Bronchiolo-Alv. Adenoma	12	14	9	20	10	.0372	.1714	.0503	.7839	.3725
UTERUS										
# Evaluated	75	75	75	75	75					
Adjusted # at risk	57.4	56.3	55.1	54.4	30.3					
Endometrial Stromal Polyp	6	9	14	11	8	.0597	.0529	.1197	.0340	.2776

Non Neoplastic

According to the pathologist, dose-related early gastric nonglandular epithelial necrosis (and the later development of tumors in the nonglandular stomach), as well as nephropathy (and renal tumors), were associated with the observed drug-related mortality.

Drug-related non-neoplastic lesions were observed in the nonglandular stomach mucosa, the serosa of the nonglandular and/or glandular stomach, and the glandular stomach mucosa. Non-neoplastic lesions observed in the nonglandular stomach included squamous cysts, hyperkeratosis, acute and/or chronic-active inflammation, ulceration, erosions, necrosis, and hemorrhage. Ulceration and inflammation often extended into the submucosa and the serosa. Necrosis of the epithelium of the nonglandular stomach was associated with severe inflammation of the submucosa and mortality; the pathologist stated that fibrosarcomas and leiomyosarcomas were later observed in the submucosa. Both acute and chronic-active inflammation, as well as adhesions in HDF, was observed in the serosa of the stomach (these observations were of mixed location- nonglandular and/or glandular stomach). In the glandular stomach, cystic hyperplasia was clearly observed; acute inflammation, erosion, ulceration, and/or necrosis were observed with lower incidence.

Nonglandular Stomach

	MALES					FEMALES				
	Con	**LD**	**LMD**	**HMD**	**HD**	**Con**	**LD**	**LMD**	**HMD**	**HD**
Inflammation, acute, mucosa, multifocal	**-**	**-**	**-**	**-**	**-**	**0**	**0**	**0**	**0**	**8**
Mild	-	-	-	-	-	0	0	0	0	1
Marked	-	-	-	-	-	0	0	0	0	7
Inflammation, chronic-active, mucosa, mild	**-**	**-**	**-**	**-**	**-**	**0**	**0**	**0**	**0**	**1**
Hemorrhage, mucosa, moderate	**-**	**-**	**-**	**-**	**-**	**0**	**0**	**0**	**0**	**2**
Cyst, squamous, mucosa	**-**	**-**	**-**	**-**	**-**	**1**	**0**	**1**	**1**	**5**
Mild	-	-	-	-	-	1	0	1	0	2
Moderate	-	-	-	-	-	0	0	0	1	3
Erosion, mucosa	**0**	**0**	**0**	**0**	**1**	**1**	**0**	**0**	**1**	**1**
Mild	0	0	0	0	1	0	0	0	1	1
Marked	-	-	-	-	-	1	0	0	0	0
Ulceration	**0**	**0**	**1**	**1**	**14**	**0**	**0**	**0**	**3**	**8**
Mild	0	0	0	1	5	0	0	0	1	2
Moderate	0	0	0	0	1	0	0	0	0	1
Marked	0	0	1	0	8	0	0	0	2	5
Necrosis, marked	**0**	**0**	**0**	**0**	**13**	**0**	**0**	**0**	**0**	**13**
Hyperkeratosis	**6**	**15**	**64**	**70**	**64**	**5**	**39**	**63**	**75**	**62**
Minimal	1	4	6	2	3	1	17	11	8	0
Mild	4	9	26	22	11	2	20	22	17	9
Moderate	1	2	31	45	49	2	2	29	48	48
Marked	0	0	1	1	1	0	0	1	2	5
Hyperplasia	**2**	**12**	**49**	**68**	**60**	**0**	**33**	**56**	**70**	**58**
Minimal	0	6	7	3	4	0	23	13	8	1
Mild	1	5	31	23	16	0	8	29	24	8
Moderate	1	1	11	42	40	0	2	13	36	44
Marked						0	0	1	2	5

Stomach Serosa (includes both nonglandular and/or glandular locations)

	MALES					FEMALES				
	Con	**LD**	**MD**	**MHD**	**HD**	**Con**	**LD**	**MD**	**MHD**	**HD**
Inflammation, acute, multifocal	**0**	**0**	**1**	**0**	**13**	**0**	**0**	**0**	**0**	**11**
Moderate	0	0	1	0	5	0	0	0	0	3
Marked	0	0	0	0	8	0	0	0	0	8
Inflammation, chronic-active, focal	**0**	**0**	**0**	**0**	**14**	**0**	**0**	**0**	**0**	**11**
Mild	-	-	-	-	-	0	0	0	0	3
Moderate	0	0	0	0	8	0	0	0	0	4
Marked	0	0	0	0	6	0	0	0	0	4
Adhesion, moderate	**-**	**-**	**-**	**-**	**-**	**0**	**0**	**0**	**0**	**3**

Glandular Stomach

	MALES					FEMALES				
	Con	**LD**	**MD**	**MHD**	**HD**	**Con**	**LD**	**MD**	**MHD**	**HD**
Inflammation, acute, mucosa	**0**	**1**	**0**	**0**	**5**	**0**	**0**	**0**	**0**	**2**
Minimal	0	1	0	0	0	-	-	-	-	-
Moderate	0	0	0	0	2	-	-	-	-	-
Marked	0	0	0	0	3	0	0	0	0	2
Erosion, mucosa	**2**	**1**	**0**	**0**	**1**	**2**	**1**	**1**	**0**	**1**
Minimal	-	-	-	-	-	0	1	1	0	0
Mild	2	1	0	0	0	2	0	0	0	1
Moderate	0	0	0	0	1	-	-	-	-	-
Erosion, hemorrhagic, mucosa, marked	**-**	**-**	**-**	**-**	**-**	**0**	**0**	**0**	**1**	**0**
Ulceration, moderate	**-**	**-**	**-**	**-**	**-**	**0**	**0**	**0**	**1**	**1**
Necrosis	**0**	**0**	**0**	**0**	**1**	**0**	**0**	**0**	**0**	**1**
Moderate	-	-	-	-	-	0	0	0	0	1
Marked	0	0	0	0	1	-	-	-	-	-
Hyperplasia, cystic, mucosa	**26**	**30**	**38**	**25**	**21**	**25**	**22**	**15**	**19**	**28**
Minimal	11	16	23	8	6	12	13	10	14	10
Mild	10	8	6	6	9	11	7	4	1	9
Moderate	4	2	5	6	5	2	2	1	4	7
Marked	1	4	4	5	1	0	0	0	0	2

In HDM, hyperkeratosis and hyperplasia were seen, although at relatively low incidence, in the esophagus.

Observations: Neo-Plastic and Non Neo-Plastic	MALES				
Removal Reasons: All of those SELECTED	0 mg/kg/d	25 mg/kg/d	75 mg/kg/d	200 mg/kg/d	600 /400 mg
Number of Animals on Study :	75	75	75	75	75
Number of Animals Completed:	(75)	(75)	(75)	(75)	(75)
ESOPHAGUS;					
Examined	(75)	(74)	(75)	(75)	(75)
Within Normal Limits	74	73	75	73	70
Not Examined: NOT FOUND AT TRIMMING	0	1	0	0	0
Hyperplasia; epithelial	(0)	(0)	(0)	(1)	(3)
minimal	0	0	0	0	1
mild	0	0	0	1	1
moderate	0	0	0	0	1
Hyperkeratosis	(0)	(0)	(0)	(0)	(5)
mild	0	0	0	0	4
moderate	0	0	0	0	1

In the kidney, BG-12 induced a dose-related increased incidence and/or severity in morphological changes associated with nephropathy in aging mice, which was particularly evident in males (see the sponsor's summary table, below). These changes included glomerular hypertrophy, glomerular atrophy, and sclerosis, Bowman's capsule hyperplasia and adhesions, tubular basophilia, thickened basement membranes, interstitial inflammation and fibrosis, tubular casts, and cysts. In addition to the nephropathy, the pathologist stated that there were observed changes not typically associated with nephropathy that may be attributable to DMF; these included tubular dilatation and degeneration of the tubules of the outer stripe and medullary ray. The pathologist stated that the morphological changes were not individually diagnosed, but were given the summary diagnosis of nephropathy and an overall severity grade; changes possibly attributable to drug were "not graded separately from the nephropathy." Other possible drug-related findings in kidney included: hydronephrosis; tubular cyst, dilatation, mineralization, necrosis and/or hyperplasia; congestion, and/or pyelonephritis (see reviewer's table, below).

Incidence of Nephropathy in the Kidneys

Sex	Males (N=75/Group)					Females (N=75/Group)				
Group	1	2	3	4	5	1	2	3	4	5
Dose (mg/kg/day)	0	25	75	200	600/400*	0	25	75	200	600/400*
Nephropathy (Total)	**68**	**69**	**72**	**72**	**58**	**69**	**70**	**72**	**69**	**66**
Nephropathy, minimal	18	14	8	3	2	13	34	14	21	13
Nephropathy, mild	21	30	35	12	19	41	25	45	34	29
Nephropathy, moderate	27	23	26	45	27	12	8	11	12	22
Nephropathy, marked	2	2	3	12	10	3	3	2	2	2

* Due to mortality observed in the high-dose group, the dose level for Group 5 was decreased to 400 mg/kg/day (40 mg/mL) beginning on Day 9.

	MALES					FEMALES				
	Con	**LD**	**LMD**	**HMD**	**HD**	**Con**	**LD**	**LMD**	**HMD**	**HD**
Hyperplasia, tubular	**1**	**7**	**16**	**40**	**15**	**0**	**7**	**8**	**13**	**13**
Minimal	1	4	13	27	7	0	5	8	10	6
Mild	0	3	3	13	7	0	2	0	2	7
Moderate	0	0	0	0	1	0	0	0	1	0
Cyst, tubular	**7**	**2**	**1**	**4**	**11**	**4**	**0**	**0**	**2**	**1**
Minimal	6	2	1	4	8	4	0	0	1	1
Mild	0	0	0	0	2	0	0	0	1	0
Moderate	1	0	0	0	1	-	-	-	-	-
Cyst, tubular, multifocal	**26**	**36**	**53**	**59**	**39**	**13**	**21**	**17**	**37**	**34**
Minimal	3	5	6	4	1	2	6	2	8	8
Mild	5	16	17	6	4	5	8	10	17	7
Moderate	15	13	27	41	26	6	7	5	11	19
Marked	3	2	3	8	8	0	0	0	1	0
Mineralization, tubular	**16**	**22**	**28**	**40**	**24**	**2**	**3**	**4**	**14**	**31**
Minimal	14	20	27	34	15	2	3	3	13	27
Mild	2	2	1	6	8	0	0	1	0	3
Moderate	0	0	0	0	1	0	0	0	1	1
Dilatation, tubular	**0**	**0**	**0**	**0**	**2**	**0**	**0**	**1**	**0**	**0**
Mild	0	0	0	0	1	-	-	-	-	-
Moderate	0	0	0	0	1	0	0	1	0	0
Necrosis, tubular, mild	**0**	**0**	**0**	**0**	**1**	**0**	**0**	**0**	**1**	**0**
Congestion, mild	**0**	**0**	**0**	**0**	**2**	**-**	**-**	**-**	**-**	**-**
Pyelonephritis	**3**	**6**	**6**	**6**	**9**	**0**	**1**	**0**	**0**	**0**
Minimal	0	0	0	0	1	-	-	-	-	-
Mild	0	1	1	1	1	-	-	-	-	-
Moderate	0	1	1	1	3	0	1	0	0	0
Marked	3	4	4	4	4	-	-	-	-	-
Hydronephrosis	**8**	**16**	**15**	**12**	**7**	**5**	**7**	**7**	**0**	**4**
Minimal	0	0	0	0	2	0	1	0	0	0
Mild	3	5	5	3	2	4	3	3	0	1
Moderate	5	11	8	8	3	1	3	4	0	3
Marked	0	0	2	1	0	-	-	-	-	-

In addition to alterations in stomach and kidney, the pathologist noted that other tissues showed effects attributed to secondary effects of those toxicities. Animals with chronic blood loss or severe inflammatory lesions developed an earlier onset of extramedullary hematopoiesis of the spleen and granulopoiesis or erythropoiesis of the bone marrow (in males). In females, depletion and/or necrosis were noted in bone marrow.

Observations: Neo-Plastic and Non Neo-Plastic — MALES

Removal Reasons: All of those SELECTED

	0 mg/kg/d	25 mg/kg/d	75 mg/kg/d	200 mg/kg/d	600 /400 mg
Number of Animals on Study :	75	75	75	75	75
Number of Animals Completed:	(75)	(75)	(75)	(75)	(75)
BONE MARROW, FEMUR;					
Examined	(75)	(75)	(75)	(75)	(75)
Within Normal Limits	42	53	59	53	35
Granulopoiesis	(19)	(12)	(13)	(19)	(36)
minimal	0	0	0	1	4
mild	5	4	3	7	20
moderate	14	8	10	10	12
marked	0	0	0	1	0
BONE MARROW, STERNUM;					
Examined	(75)	(75)	(75)	(74)	(74)
Granulopoiesis	(19)	(12)	(13)	(19)	(35)
minimal	0	1	0	2	3
mild	5	3	2	6	20
moderate	14	8	11	10	12
marked	0	0	0	1	0

Observations: Neo-Plastic and Non Neo-Plastic — FEMALES

Removal Reasons: All of those SELECTED

	0 mg/kg/d	25 mg/kg/d	75 mg/kg/d	200 mg/kg/d	600 /400 mg
Number of Animals on Study :	75	75	75	75	75
Number of Animals Completed:	(75)	(75)	(75)	(75)	(75)
BONE MARROW, FEMUR;					
Examined	(75)	(75)	(75)	(75)	(75)
Depletion	(2)	(5)	(5)	(9)	(4)
mild	1	3	4	5	3
moderate	1	2	1	4	1
Necrosis	(0)	(0)	(0)	(0)	(3)
moderate	0	0	0	0	3

Dose-related increases in the incidence and/or severity of retinal degeneration was observed in both sexes at HMD and HD but particularly in females (see the sponsor's summary table, below). This degeneration ranged from minimal to mild loss of photoreceptors and the outer nuclear layers to severe changes with loss of all layers including the inner nuclear layers and the ganglion cells; morphologically, the lesions were similar to those observed in controls, but with increased incidence and/or severity. Corneal inflammation was also observed with increased incidence (see reviewer's table, next page).

Incidence of Degeneration of the Retina of the Eye

Sex	Males					Females				
Group	1	2	3	4	5	1	2	3	4	5
Dose (mg/kg/day)	0	25	75	200	400/600	0	25	75	200	400/600
Degeneration (Total)	7	0	2	9	12	4	4	2	11	23
Degeneration, minimal	1	0	0	0	0	0	1	1	3	2
Degeneration, mild	2	0	0	1	3	2	0	0	2	5
Degeneration, moderate	3	0	2	8	7	1	3	1	6	9
Degeneration, marked	1	0	0	0	2	1	0	0	0	7

	MALES					FEMALES				
	Con	LD	LMD	HMD	HD	Con	LD	LMD	HMD	HD
Cornea, chronic-active inflammation	2	7	9	9	3	4	9	7	5	2
Minimal	0	1	2	3	1	0	0	1	1	0
Mild	1	3	5	4	0	2	4	5	3	2
Moderate	0	2	1	1	1	1	4	1	1	0
Marked	1	1	1	1	1	1	1	0	0	0

The sponsor noted that all other histologic lesions observed were considered spontaneous, incidental, and/or common in aging CD-1 mice of this stock. However, there were a few findings not noted by the sponsor that appear dose-related (for some, taking into account the early mortality in HD males and females); see the excerpts from the sponsor's summary table, below, for details. Effects were clearly observed in the spleen, thymus, and lymph nodes. Other effects of possible relationship to drug were generally of low incidence. Alterations were observed in heart and liver in both sexes. In males, observations were recorded in pituitary, skin, ureter, and reproductive organs. In females, observations were recorded in the lung, sciatic nerve, skeletal muscle, urinary bladder, mammary gland, and reproductive organs.

Observations: Neo-Plastic and Non Neo-Plastic

Removal Reasons: All of those SELECTED

	MALES				
	0 mg/kg/d	25 mg/kg/d	75 mg/kg/d	200 mg/kg/d	600 /400 mg
Number of Animals on Study :	75	75	75	75	75
Number of Animals Completed:	(75)	(75)	(75)	(75)	(75)
SPLEEN;					
Examined	(75)	(75)	(74)	(75)	(75)
Depletion; lymphoid	(15)	(16)	(21)	(29)	(32)
minimal	0	0	2	0	0
mild	1	4	0	0	5
moderate	13	11	16	22	12
marked	1	1	3	7	15
Fibrosis; Capsule	(3)	(0)	(3)	(0)	(19)
minimal	0	0	0	0	1
mild	1	0	1	0	4
moderate	2	0	2	0	13
marked	0	0	0	0	1
Necrosis	(2)	(1)	(0)	(1)	(12)
mild	0	0	0	1	1
moderate	2	1	0	0	8
marked	0	0	0	0	3
Fibroplasia; Capsule	(1)	(0)	(0)	(0)	(4)
mild	0	0	0	0	2
moderate	1	0	0	0	2
THYMUS;					
Examined	(75)	(75)	(75)	(75)	(73)
Depletion; lymphoid	(0)	(0)	(0)	(0)	(5)
marked	0	0	0	0	5
LYMPH NODE, MEDIASTINAL;					
Examined	(69)	(75)	(74)	(74)	(72)
Hyperplasia; lymphoid	(4)	(5)	(4)	(10)	(6)
mild	1	4	2	8	5
moderate	3	1	2	2	1
Erythrophagocytosis	(7)	(14)	(9)	(7)	(9)
minimal	1	2	1	2	4
mild	6	12	7	5	3
moderate	0	0	1	0	2
LYMPH NODE, MESENTERIC;					
Examined	(74)	(74)	(74)	(75)	(75)
Erythrophagocytosis	(8)	(10)	(12)	(13)	(13)
minimal	0	3	1	1	4
mild	6	5	9	12	6
moderate	2	2	2	0	3
Hyperplasia; lymphoid	(8)	(9)	(12)	(9)	(13)
minimal	1	1	1	0	0
mild	6	4	8	6	10
moderate	1	4	3	2	2
marked	0	0	0	1	1

Observations: Neo-Plastic and Non Neo-Plastic	MALES				
Removal Reasons: All of those SELECTED	0 mg/kg/d	25 mg/kg/d	75 mg/kg/d	200 mg/kg/d	600 /400 mg
Number of Animals on Study :	75	75	75	75	75
Number of Animals Completed:	(75)	(75)	(75)	(75)	(75)
LYMPH NODE, RENAL;					
Examined	(3)	(3)	(7)	(2)	(0)
Hyperplasia; lymphoid	(1)	(0)	(3)	(1)	(0)
mild	0	0	1	0	0
moderate	1	0	2	0	0
marked	0	0	0	1	0
LYMPH NODE, INGUINAL;					
Examined	(1)	(1)	(4)	(0)	(0)
Hyperplasia; lymphoid	(1)	(1)	(3)	(0)	(0)
minimal	0	0	1	0	0
moderate	0	1	2	0	0
marked	1	0	0	0	0
LYMPH NODE, ILIAC;					
Examined	(2)	(4)	(10)	(2)	(0)
Hyperplasia; lymphoid	(2)	(2)	(6)	(1)	(0)
moderate	1	1	5	0	0
marked	1	1	1	1	0
LYMPH NODE, PANCREATIC;					
Examined	(3)	(0)	(1)	(2)	(1)
Hyperplasia; lymphoid	(1)	(0)	(0)	(2)	(0)
moderate	1	0	0	1	0
marked	0	0	0	1	0
HEART;					
Examined	(75)	(75)	(75)	(75)	(75)
Cardiomyopathy	(57)	(67)	(58)	(69)	(42)
minimal	19	16	13	11	15
mild	26	37	32	41	22
moderate	12	14	11	13	5
marked	0	0	2	4	0
Thrombosis; Atrium	(3)	(2)	(4)	(10)	(1)
minimal	0	0	1	2	0
mild	0	0	1	0	0
moderate	0	1	1	6	1
marked	3	1	1	2	0
LIVER;					
Examined	(75)	(75)	(75)	(75)	(75)
Congestion; Centrilobular	(2)	(0)	(2)	(7)	(3)
mild	0	0	1	1	0
moderate	2	0	1	6	3
PITUITARY GLAND;					
Examined	(74)	(74)	(75)	(75)	(75)
Cyst; Pars Distalis	(0)	(4)	(5)	(1)	(2)
minimal	0	2	5	1	2
mild	0	1	0	0	0
moderate	0	1	0	0	0
URETER;					
Examined	(1)	(1)	(3)	(2)	(0)
Within Normal Limits	1	0	0	0	0
Dilation	(0)	(1)	(3)	(2)	(0)
moderate	0	0	3	1	0
marked	0	1	0	1	0
SKIN;					
Examined	(75)	(75)	(75)	(75)	(75)
Inflammation; chronic-active	(4)	(5)	(7)	(9)	(3)
minimal	0	2	0	0	0
mild	1	0	1	1	0
moderate	3	2	5	6	3
marked	0	1	1	2	0
EPIDIDYMIS;					
Examined	(75)	(75)	(75)	(75)	(75)
Within Normal Limits	45	47	50	33	45
Atrophy	(25)	(21)	(19)	(36)	(27)
minimal	5	9	2	10	5
mild	10	4	10	13	12
moderate	10	8	7	13	10
SEMINAL VESICLE;					
Examined	(75)	(75)	(75)	(75)	(75)
Dilation	(18)	(32)	(22)	(37)	(8)
mild	1	16	14	11	3
moderate	17	16	8	25	5
marked	0	0	0	1	0

Observations: Neo-Plastic and Non Neo-Plastic	FEMALES				
Removal Reasons: All of those SELECTED	0 mg/kg/d	25 mg/kg/d	75 mg/kg/d	200 mg/kg/d	600 /400 mg
Number of Animals on Study :	75	75	75	75	75
Number of Animals Completed:	(75)	(75)	(75)	(75)	(75)
SPLEEN;					
Examined	(75)	(74)	(75)	(75)	(75)
Depletion; lymphoid	(9)	(10)	(10)	(5)	(22)
mild	2	2	1	1	5
moderate	6	6	7	3	5
marked	1	2	2	1	12
Fibrosis; Capsule	(1)	(0)	(1)	(1)	(20)
mild	0	0	0	0	1
moderate	1	0	0	1	16
marked	0	0	1	0	3

(continued, on next page)

Observations: Neo-Plastic and Non Neo-Plastic Removal Reasons: All of those SELECTED	FEMALES				
	0 mg/kg/d	25 mg/kg/d	75 mg/kg/d	200 mg/kg/d	600 /400 mg
Number of Animals on Study :	75	75	75	75	75
Number of Animals Completed:	(75)	(75)	(75)	(75)	(75)
SPLEEN: (continued)					
Necrosis	(2)	(2)	(0)	(0)	(11)
minimal	1	0	0	0	0
mild	1	0	0	0	0
moderate	0	2	0	0	9
marked	0	0	0	0	2
Fibroplasia; Capsule	(0)	(0)	(0)	(0)	(1)
moderate	0	0	0	0	1
Inflammation, Acute; Capsule	(0)	(0)	(0)	(0)	(1)
marked	0	0	0	0	1
Adhesion; Capsule	(0)	(1)	(0)	(0)	(3)
moderate	0	1	0	0	3
THYMUS;					
Examined	(74)	(75)	(75)	(75)	(75)
Depletion; lymphoid	(0)	(0)	(1)	(0)	(11)
marked	0	0	1	0	11
LYMPH NODE, MEDIASTINAL;					
Examined	(75)	(73)	(73)	(75)	(75)
Depletion; lymphoid	(2)	(1)	(0)	(0)	(11)
mild	1	0	0	0	5
moderate	1	1	0	0	5
marked	0	0	0	0	1
LYMPH NODE, MESENTERIC;					
Examined	(75)	(75)	(73)	(73)	(74)
Depletion; lymphoid	(7)	(2)	(0)	(1)	(18)
mild	2	0	0	1	6
moderate	4	2	0	0	11
marked	1	0	0	0	1
Histiocytosis	(1)	(0)	(0)	(2)	(6)
mild	1	0	0	2	2
moderate	0	0	0	0	3
marked	0	0	0	0	1
Hyperplasia; lymphoid	(4)	(9)	(7)	(11)	(15)
minimal	0	1	1	2	0
mild	3	5	6	9	12
moderate	1	3	0	0	2
marked	0	0	0	0	1
LYMPH NODE, MANDIBULAR;					
Examined	(75)	(75)	(75)	(75)	(75)
Depletion; lymphoid	(0)	(0)	(0)	(0)	(7)
mild	0	0	0	0	1
moderate	0	0	0	0	6
Hyperplasia; lymphoid	(4)	(12)	(11)	(9)	(12)
minimal	0	2	2	0	2
mild	4	10	8	8	8
moderate	0	0	1	1	2
HEART;					
Examined	(75)	(75)	(75)	(75)	(75)
Inflammation; chronic-active; Pericardium	(0)	(0)	(2)	(2)	(2)
minimal	0	0	0	1	0
mild	0	0	1	1	0
moderate	0	0	1	0	2
Mineralization	(1)	(2)	(0)	(1)	(3)
minimal	1	0	0	0	0
mild	0	2	0	1	0
moderate	0	0	0	0	3
Necrosis; focal	(1)	(0)	(0)	(1)	(1)
minimal	1	0	0	0	0
mild	0	0	0	1	1
HEART;					
Examined	(75)	(75)	(75)	(75)	(75)
Cardiomyopathy	(58)	(59)	(63)	(58)	(51)
minimal	7	17	14	19	17
mild	32	31	30	22	21
moderate	18	10	18	16	13
marked	1	1	1	1	0
Thrombosis; Aortic Valve	(0)	(0)	(0)	(1)	(0)
moderate	0	0	0	1	0
Thrombosis; Atrium	(6)	(0)	(2)	(2)	(3)
minimal	0	0	0	0	1
mild	0	0	0	1	1
moderate	4	0	0	1	1
marked	2	0	2	0	0
LUNG WITH BRONCHI;					
Examined	(75)	(75)	(75)	(75)	(75)
Fibrosis; pleural; focal	(1)	(1)	(1)	(5)	(3)
minimal	0	1	0	1	0
mild	1	0	1	2	2
moderate	0	0	0	2	1
LIVER;					
Examined	(75)	(75)	(75)	(75)	(75)
Congestion; Centrilobular	(0)	(0)	(2)	(2)	(2)
mild	0	0	0	0	2
moderate	0	0	2	2	0
Degeneration; Centrilobular	(0)	(0)	(1)	(2)	(0)
moderate	0	0	1	2	0
Necrosis; Centrilobular	(0)	(0)	(0)	(2)	(0)
minimal	0	0	0	1	0
moderate	0	0	0	1	0
Cyst; Bile Duct	(0)	(5)	(2)	(3)	(2)
minimal	0	0	0	1	0
mild	0	5	1	1	1
moderate	0	0	0	1	1
marked	0	0	1	0	0

(continued on next page)

Observations: Neo-Plastic and Non Neo-Plastic	FEMALES				
Removal Reasons: All of those SELECTED	0 mg/kg/d	25 mg/kg/d	75 mg/kg/d	200 mg/kg/d	600 /400 mg
Number of Animals on Study :	75	75	75	75	75
Number of Animals Completed:	(75)	(75)	(75)	(75)	(75)
PANCREAS;					
Examined	(75)	(75)	(74)	(75)	(75)
Inflammation; acute; focal	(0)	(0)	(0)	(0)	(3)
moderate	0	0	0	0	3
URINARY BLADDER;					
Examined	(75)	(73)	(74)	(75)	(75)
Infiltration; lymphohistiocytic	(2)	(4)	(11)	(0)	(0)
minimal	2	4	11	0	0
NERVE, SCIATIC;					
Examined	(75)	(75)	(75)	(75)	(75)
Degeneration	(8)	(10)	(13)	(4)	(4)
minimal	8	10	12	4	4
mild	0	0	1	0	0
SKELETAL MUSCLE (THIGH);					
Examined	(75)	(75)	(75)	(74)	(75)
Atrophy	(13)	(8)	(15)	(10)	(17)
minimal	6	4	9	4	5
mild	7	4	6	6	9
moderate	0	0	0	0	3
OVARY;					
Examined	(75)	(75)	(75)	(75)	(75)
Cyst; Bursa	(56)	(59)	(58)	(62)	(38)
minimal	1	0	0	0	0
mild	0	6	0	4	0
moderate	49	46	50	47	32
marked	6	7	8	11	6
Cyst; hemorrhagic; Bursa	(0)	(0)	(0)	(0)	(2)
marked	0	0	0	0	2
Hyperplasia; cystic; papillary	(0)	(2)	(0)	(5)	(0)
mild	0	2	0	3	0
moderate	0	0	0	2	0
UTERUS;					
Examined	(75)	(75)	(75)	(75)	(75)
Within Normal Limits	0	2	0	1	13
Atrophy	(1)	(3)	(5)	(10)	(10)
mild	0	2	1	1	0
moderate	1	1	4	9	10
Thrombosis	(2)	(4)	(4)	(1)	(0)
mild	0	1	0	0	0
moderate	1	2	1	0	0
marked	1	1	3	1	0
VAGINA;					
Examined	(75)	(75)	(75)	(75)	(75)
Fibrosis; stromal	(0)	(2)	(4)	(1)	(1)
moderate	0	2	2	1	1
marked	0	0	2	0	0
MAMMARY GLAND;					
Examined	(75)	(74)	(75)	(75)	(74)
Ectasia; Duct	(2)	(11)	(6)	(5)	(0)
mild	1	8	3	3	0
moderate	1	3	3	2	0
Hyperplasia; lobular	(0)	(2)	(1)	(1)	(0)
mild	0	0	1	0	0
moderate	0	2	0	1	0

Toxicokinetics [see sponsor's table, below]

The sponsor stated that DMF undergoes rapid and extensive metabolism by non-specified esterase and was not detectable in the majority of *in vivo* and *in vitro* ADME studies; therefore, MMF (the primary, active, metabolite) was measured in these studies. Blood samples (0.2 mL/sample) were collected from the orbital plexus (under isoflurane anesthesia) in 3/sex/time point for up to 180 min post dose (see sponsor's design table, below).

TK Sample Collection Schedule

Group/ Subgroup	No. of Animals/Sex/Time Point Following Dosing on Days 1, 90, and 180							
	5 min	10 min	20 min	40 min	60 min	90 min	120 min	180 min
1 / A Vehicle		3/3						
1 / B Vehicle					3/3			
2-5 / A	3/3							
2-5 / B		3/3						
2-5 / C			3/3					
2-5 / D				3/3				
2-5 / E					3/3			
2-5 / F						3/3		
2-5 / G							3/3	
2-5 / H								3/3

LMD animals had an unusually low plasma exposure compared to the rest of the groups on Day 180 that the sponsor could not explain; therefore, those data were excluded from further analysis. After oral administration of DMF, MMF T_{max} was early, ranging from 5 to 20 minutes post dose. MMF AUC_{∞} increased in a greater than dose-proportional manner in both females and males. A slightly longer terminal $T_{1/2}$ (range 17 - 31 minutes) was found at ≥ MHD as compared to the terminal $T_{1/2}$ (range 8 to 19 minutes) in ≤ LMD. No consistent sex differences were reported. MMF concentrations in the control groups and in the pre-dose samples on Day 1 in the treated groups were all BLOQ. See the sponsor's summary data table, below.

Table 4 Toxicokinetic parameter estimates of MMF on Days 1, 90 and 180 after daily oral administration of BG00012 to CD-1 mice for 6 months at 25, 75, 200, 400 or 600 mg/kg/day.

Dose (mg/kg)	Gender	Study Day	$T_{1/2}$ (min)	T_{max} (min)	C_{max} (µg/mL)	AUC_{inf} (hr*µg/mL)	AUC_{last} (hr*µg/mL)	Cl/F (mL/min/kg)	Vz/F (mL/kg)
25	Female	1	9	5	12.7	3.4	3.4	123	1559
		90	13	5	14.0	3.6	3.6	115	2119
		180	12	5	9.5	2.6	2.6	159	2678
	Male	1	15	10	9.4	3.0	2.9	139	3108
		90	8	5	11.2	2.6	2.6	160	1799
		180	8	10	8.8	3.0	3.0	140	1635
75	Female	1	10	10	28.9	10.4	10.4	120	1657
		90	13	10	37.1	10.8	10.8	116	2100
	Male	1	15	10	33.4	12.8	12.7	98	2117
		90	19	5	36.4	9.8	9.8	127	3425
200	Female	1	20	20	89.7	42.5	42.4	78	2232
		90	22	5	82.6	37.4	37.0	89	2874
		180	17	10	76.1	33.6	33.6	99	2469
	Male	1	24	20	61.2	32.8	32.7	102	3556
		90	20	5	90.7	35.4	35.3	94	2691
		180	23	5	63.4	31.5	31.4	106	3519
400	Female	90	31	10	159.2	102.7	99.7	65	2905
		180	31	5	197.0	93.5	91.8	71	3180
	Male	90	25	10	182.9	107.6	106.6	62	2200
		180	17	5	212.0	110.4	110.2	60	1439
600	Female	1	NR*	20	156.0	119.2	112.2	NR*	NR*
	Male	1	31	10	197.3	106.1	105.0	94	4216

* NR - Not Reportable. For the 600 mg/kg dose group, Females on Day 1, the terminal phase could not be accurately determined due to fluctuation of concentration in the terminal phase, so the terminal half life, Cl/F, and Vz/F were not reported for this group.

Dosing Solution Analysis

Ten test article dosing samples were analyzed (except for HD, only 8 samples were collected due to discontinuation of dosing); all samples were within the protocol-specified limits of ±15% of the intended concentration. With regard to homogeneity, all of the results were within the acceptable range of ±5% RSD except for the MHD formulation (20 mg/mL) prepared in month 24, which was slightly above the acceptable limit for homogeneity (5.3 - 5.6%). The sponsor indicated that this deviation did not affect the validity of the study because the RSD was only slightly above that specified by protocol and the Group 4 formulation had consistently met homogeneity specifications at previous sampling intervals. Stability of the formulations was previously demonstrated in studies EBA00016AO7 and EBA00124AO8 at concentrations and storage conditions bracketing those used in this study (confirmed for 24 hr at 2 ± 5°C and 11 days at 5 ± 3°C).

EXPERT REPORT ON HISTOPATHOLOGICAL RE-EVALUATION OF RAT AND MOUSE KIDNEY FROM CARCINOGENICITY STUDIES WITH ORALLY ADMINISTERED BG00012 (DIMETHYL FUMARATE)

Non-GLP, Performed by (b) (4) on March 16-29, 2012, report dated 5/11/12.
Individual and comparative data tables (dated 10/20/12) provided upon Agency request

(b) (4) generated new data based on the re-examination of the renal histopathology occurring in the mouse (and rat; see review for rat above following the review of the rat 2-yr bioassay) carcinogenicity study of DMF. The sponsor requested that (b) (4) "identify the nature of the lesions reported, and to attempt to determine key histopathological events that would support mode(s) of action underlying the development of renal tubule tumors in… [the] carcinogenicity studies." The observed renal tubule hyperplasia was evaluated for the atypical tubule hyperplasia (ATH) form, which is accepted as being on the developmental continuum with renal tubule adenoma and carcinoma (Hard, 1987, Lipsky and Trump, 1988; Dietrich and Swenberg, 1991; Nogueira et al., 1993).

The previously created histology slides of mouse kidney stained with hematoxylin and eosin (H&E) were examined. (b) (4) used the criteria recommended by WHO/IARC (Hard et al., 1998) to diagnose mouse kidney lesions. The criteria of Hard et al. (2005, 2006) were used for identifying "non-biologically significant, proliferative lesions occurring in chronic progressive nephropathy (CPN) and distinguishing them from ATH and adenoma." (b) (4) noted that spontaneous nephropathy in mice (mouse CPN) "has not been as well characterized as rat CPN, but comprises a similar spectrum of components" (Wolf and Hard, 1996). The severity of the observed mouse CPN was graded on a scale of 1-4, based on increasing frequency/severity of lesions. Other "renal lesions of importance" occurring in mice, such as cysts, were also graded on a 1 to 4 scale; cyst severity was based on a qualitative impression of increasing numbers of small and large cysts. See the sponsor's summary tables of the original pathologist's findings and (b) (4) re-evaluation, below.

Incidence (and Severity) of Nephropathy, Renal Hyperplasia, Adenoma and Carcinoma in Mice of 2-Year Carcinogenicity Study – Results from the Final Study Report

Gender	Male					Female				
Dosage (mg/kg/day)	0	25	75	200	400 [a]	0	25	75	200	400 [a]
Nephropathy, Incidence	68	69	72	72	58	69	70	72	69	66
Average severity score [b]	2.19	2.19	2.33	2.92	2.78	2.07	1.71	2.01	1.93	2.20
Hyperplasia, tubule	1	7	16	40	15	0	7	8	13	13
Adenoma, tubule	1	2	0	5	3	0	0	0	2	4
Carcinoma, tubule	0	0	2	4	3	0	0	0	0	1

a: Dose level was reduced from 600 to 400 mg/kg/day on Day 9 due to high mortality rate during the first week of treatment.
b: Severity score based on following grades: 0 = absent, 1= minimal, 2 = mild, 3 = moderate, 4 = marked

Incidence (and Severity) of Nephropathy, Renal Hyperplasia, Adenoma and Carcinoma in Mice of 2-Year Carcinogenicity Study – Results from (b) (4) Evaluation

Gender	Male					Female				
Dosage (mg/kg/day)	0	25	75	200	400 [a]	0	25	75	200	400 [a]
Nephropathy, Incidence	65	69	73	71	60	61	65	72	72	61
Average severity score [b]	2.0	1.9	2.4	3.0	2.8	1.5	1.7	2.1	2.2	2.4
Hyperplasia (ATH), tubule [c]			2	3					1	1
Adenoma, tubule	2	2[d]		5	3	0	0	0	2	4[d]
Carcinoma, tubule			2	4	3	0	0	0	0	1

a: Dose level was reduced from 600 to 400 mg/kg/day on Day 9 due to high mortality rate during the first week of treatment.
b: Severity score based on following grades: 0 = absent, 1= minimal, 2 = mild, 3 = moderate, 4 = marked
c: Hyperplasia noted were described as atypical tubule hyperplasia (ATH).
d: Two adenomas present in one animal.

Renal tubule cells of early decedent mice, and mice with no/low grade CPN, did not show evidence of drug-related cytotoxicity. Drug-related exacerbation of mouse CPN was observed in the study. (b) (4) observed that an unusual feature of the mouse kidney in this study was an increase in epithelial cysts, mainly in the cortex (see (b) (4) Table 5, below). The cysts were described, as follows: " …varied in size from small to large, and had a single cell lining of epithelial cells that could range from flattened to plump cuboidal shape. A number of large cysts contained a glomerular tuft, while most small developing cysts also contained a glomerulus, indicating that cyst development represented expansion of Bowman's space, rather than expansion of proximal tubule lumens. The cysts appeared not to be associated with amyloidosis, an intercurrent disease that was prominent in this study." He stated that cyst development generally paralleled the CPN severity. "Often" the grades of each change were the same in each animal, or cysts were one grade lower than CPN grade. (b) (4) noted that 3 cases of ATH and 5 adenomas were associated with cysts (see (b) (4) Table 8, below).

Although most of the pre/neoplastic lesions occurred in kidneys with grade 3 or 4 CPN, there also appeared to be an association between the severity of CPN and that of cyst formation (cyst severity 3 or 4).

Table 5. Grade of cyst formation severity in male and female mice according to dose in the mouse carcinogenicity study with BG00012

Dose (mg/kg/d)	Animals Assessed*	Grade of cyst formation severity					Mean grade
		0	1	2	3	4	
Males							
0	73	**30**	26	8	8	1	1.0
25	70	19	**24**	16	9	2	1.3
75	73	11	12	**26**	21	3	1.9
200	71	3	11	16	**34**	7	2.4
600/400	59**	2	7	14	**23**	13	2.6
Females							
0	65	**34**	17	11	3	0	0.7
25	65	**30**	21	9	5	0	0.8
75	72	13	**27**	19	10	2	1.4
200	72	7	**26**	23	14	2	1.7
600/400	62**	10	14	16	**21**	1	1.8

The modes for each grade are emboldened
* animals in which cysts may have been obscured because of severe amyloidosis or pyelonephritis have been excluded
** in addition, 12 male and female animals dying in the first week of treatment have been excluded

TABLE 8. Incidence of ATH, adenoma, and carcinoma in the mouse carcinogenicity study with BG00012, recorded as severity grade of cyst formation in each mouse with an ATH, adenoma, or carcinoma

Dose (mg/kg/d)	Lesion type		
	ATH	Adenoma	Carcinoma
Males			
0		3, X	
25		3, 3*	
75	4*, 3*		3, 3
200	4, 3, 4*	3*, 2, 4*, 3, 2	3, 3, 3, 4
600/400		4, 3, 3	2, 3, 3
Females			
0			
25			
75			
200	2	2, 3	
600/400	3	3, 3, 3*, 3*	3

ATH, atypical tubule hyperplasia
X kidneys could not be assessed for CPN because of severe amyloidosis
* suggestion of an association with a cyst

Although (b) (4) stated that mouse CPN "has not been well characterized and any possible association between exacerbation of mouse CPN and increase in renal tubule tumor incidence has not been studied," he concluded that exacerbation of CPN was observed. He also noted that there was a parallel increase in epithelium-lined cysts, and that those cysts were often related to the pre/neoplastic lesions. (b) (4) stated,

> "For several ATH and several adenomas, growth appeared to arise from cyst lining or within a cyst. It is not certain whether the cyst formation was part of the CPN, but there was a close parallel in increasing severity between the two pathologies. Given that some ATH and adenomas were intimately linked with cyst formation, it is suggestive of the possibility that the tumor response in mice was secondary to the exacerbation by BG00012 of CPN coupled with cyst formation. This was also supported by the site concordance in the cortex of the cysts and the ATH and small adenomas."

It was notable that a few animals demonstrated pre/neoplastic lesions without increased severity of CPN and/or cyst formation (i.e., HMDM269 showed adenoma with grade 2 CPN and grade 1 cyst; HDM861 showed a carcinoma with grade 2 CPN and grade 3 cyst; HMDF537 showed an adenoma with grade 2 CPN and 0 grade cyst; and HMDF578 showed ATH with grade 2 CPN and grade 1 cyst).

Histopathology Inventory- Carcinogenicity- 2 year Bioassays

Study	Carc P00012-04-11	Carc P00012-05-03
Species	Rat	Mouse
Adrenals	X	X
Aorta	X	X
Bone Marrow smear	X (scheduled only, for "possible evaluation")	X (scheduled only, for "possible evaluation")
Bone (femur)	X	X
Brain	X	X
Cecum	X	X
Cervix		
Colon	X	X
Duodenum	X	X
Epididymis	X	X
Esophagus	X	X
Eye	X	X
Fallopian tube		
Gall bladder	n/a	X
Gross lesions	X	X
Harderian gland	X	X
Heart	X	X
Ileum	X	X
Injection site		

Jejunum	X	X
Kidneys	X	X
Lachrymal gland		
Larynx	X	X
Liver	X	X
Lungs	X	X
Lymph nodes, cervical		
Lymph nodes mandibular	X	X
Lymph nodes, mesenteric	X	X
Mammary Gland	X	X
Nasal cavity		
Optic nerves		X (w/eye)
Ovaries	X	X
Pancreas	X	X
Parathyroid	X (w/thyroid)	X
Peripheral nerve	X (sciatic)	X (sciatic)
Pharynx		
Pituitary	X	X
Prostate	X	X
Rectum	X	X
Salivary gland	X	X
Sciatic nerve	X	X
Seminal vesicles	X	X
Skeletal muscle	X (thigh)	X (thigh)
Skin	X	X
Spinal cord	X	X
Spleen	X	X
Sternum		X
Stomach	X	X
Testes	X	X
Thymus	X	X
Thyroid	X	X
Tongue	X	X
Trachea	X	X
Urinary bladder	X	X
Uterus	X	X
Vagina	X	X
Zymbal gland		

X, histopathology performed
*, organ weight obtained

Also: Lymph node, mediastinal (mouse)
Tissue masses and/or suspect tumors (rat & mouse)

9 Reproductive and Developmental Toxicology

9.1 Fertility and Early Embryonic Development

Study title: ORAL (GAVAGE) FERTILITY AND GENERAL REPRODUCTION TOXICITY STUDY OF BG00012 IN MALE RATS

Study no.: P00012-04-03, (b) (4) # EBA00010

Conducting laboratory and location: (b) (4)

Date of study initiation: 5/27/04

GLP compliance: Yes, Appx. I

QA statement: Yes, Appx. J

Drug, lot #, and % purity: DMF, Lot F1177170, 99.6% pure (MMF <0.1%)

Methods: see details in the sponsor's table, below.

Frequency of dosing: Daily, for 70 days before cohabitation and through a 14-day cohabitation period until the day before sacrifice (~14 weeks)

Route of administration: oral gavage

Formulation/Vehicle: 0.8% (w/v) hydroxypropylmethylcellulose (HPMC) in R.O. deionized water

Species/Strain: Crl:CD®(SD)IGS BR VAF/Plus® male rats
62 days of age at arrival, 325-356 g at initiation
(female rats were used for mating but not dosed)

Dosage Group	Dosage[a] (mg/kg/day)	Concentration (mg/mL)	Dosage Volume (mL/kg)	Number of Rats per Sex[b]	Assigned Rat Numbers	
					Male Rats	Female Rats
I	0 (Vehicle)	0	10	25	12301 - 12325	14401 - 14425
II	75	7.5	10	25	12326 - 12350	14426 - 14450
III	250	25	10	25	12351 - 12375	14451 - 14475
IV	375	37.5	10	25	12376 - 12400	14476 - 14500

a. The test article was considered to be 100% active for the purpose of dosage calculations.
b. Only male rats were administered the test article or vehicle.

Observations and Results

Mortality

The sponsor reported no drug-related deaths. Two animals (one control, one HD) were euthanized on day 63 due to injuries to the snout. However, the HDM also showed other effects at necropsy, some of which may have contributed to the animal's poor condition: all regions of the stomach were thick, the cardiac region of the stomach was white with accentuated rough ridges, and the testes and epididymides were small.

Clinical Signs

Excessive salivation and a red perioral substance occurred in a roughly dose-related manner ([ss] in MDM and HDM); see excerpt from sponsor's table, below. Red substance at the penis was observed with low incidence in all treated groups. Ungroomed coat was seen at MD and HD. Dehydration (6/25) and swollen pinna (3/25) were significantly increased in HDM.

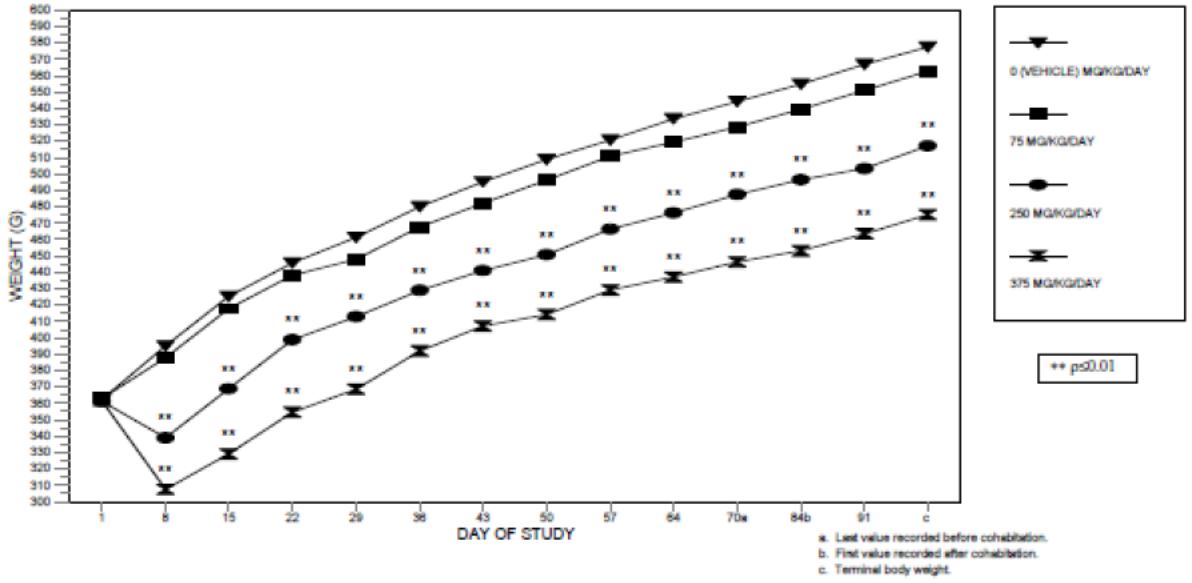
TABLE B1 (PAGE 1): CLINICAL OBSERVATIONS - SUMMARY - MALE RATS
(See footnotes on the last page of this table.)

DOSAGE GROUP DOSAGE (MG/KG/DAY)	I 0(VEHICLE)	II 75	III 250	IV 375
MAXIMUM POSSIBLE INCIDENCE	2307/ 25	2337/ 25	2338/ 25	2309/ 25
MORIBUND SACRIFICED	1a	0	0	1b
EXCESS SALIVATION	0/ 0	14/ 5	230/ 19**	539/ 25b**
RED PERIORAL SUBSTANCE	0/ 0	11/ 9	107/ 18**	110/ 22b**
DEHYDRATION	1/ 1a	0/ 0	5/ 1	111/ 6b**
RIGHT PINNA: SWOLLEN	0/ 0	0/ 0	0/ 0	71/ 3**
UNGROOMED COAT	0/ 0	0/ 0	7/ 3	8/ 2
SNOUT: SWOLLEN	18/ 1a	0/ 0	0/ 0	8/ 2b
PENIS: RED SUBSTANCE	0/ 0	4/ 4	1/ 1	1/ 1

Body Weight

Significant body weight losses occurred on days 1 to 8 in MDM and HDM. These losses resulted in mean body weights that were reduced at MD and HD from day 8 until termination. Mean body weights at termination were 97.4%, 89.6%, and 82.3% of controls for LD, MD, and HD males.

BODY WEIGHTS
MALE RATS
Figure 1



Food Consumption

Absolute food consumption was generally reduced in all treated groups. Relative food consumption values were significantly reduced for all treated groups on D1 to D8 but were increased at later timepoints for MDM and HDM due to the reductions in body weight.

Toxicokinetics

Not performed.

Dosing Solution Analysis

All dosing formulations tested were within ±15% of nominal. With regard to homogeneity, all of the results were within the acceptable range of ≤5% RSD.

Necropsy

Terminal body weights were significantly reduced at MD and HD. Absolute and relative (to body weight) kidney (up to ~60%), stomach (up to ~2.7x), and pancreas weights (up to ~30%) were significantly increased in all treated groups. Absolute testes weight was increased at HD (~10%). Relative weights of the cauda epididymides (weighed separately) were slightly increased at MD and/or HD. The relative weight of the seminal vesicles with and without fluid were slightly (10-20%, [ss]) increased at HD.

Grossly, a thick region in the fundus of the stomach was observed in all treated males. Large pancreatic lymph node and a few observations in the kidney, epididymis and testes were detected.

	Control	LD	MD	HD
"Appeared normal"	24	0	0	0
STOMACH				
Fundic region thick and/or one red erosion	0	25	25	24
All regions, and cardiac region, white rough ridges accentuated and/or thick				1*
PANCREATIC LYMPH NODE				
Large	0	2	25	24
EPIDIDYMIS				
Small, bilateral	0	0	0	1*
KIDNEYS				
R, pelvis dilatation	0	1	0	0
Large or pitted areas, bilateral	0	0	0	2
TESTES				
L, small, purple & flaccid	0	1	0	0
Small, bilateral	0	0	1	1*

* indicates an animal that was euthanized early.

The testes, epididymides, prostate, seminal vesicles, kidneys, pancreas, pancreatic lymph nodes, and stomach were processed for histopathology from all male animals. Treatment-related microscopic changes were observed in the forestomach, kidneys, and testes of all treated groups; microscopic changes were also observed in the glandular stomach of the MD and HD groups.

In the forestomach, the most common change was thickening of the squamous mucosa due to hyperplasia and hyperkeratosis. Other drug-related changes in the forestomach included: microabscess/necrosis/vacuolar degeneration of the superficial epithelium/keratin layer, ulcers, pleocellular inflammation, and squamous cell carcinomas (lesions were focal, endophytic, well-differentiated and keratinizing squamous epithelial proliferations that invaded the submucosa). Additionally, focal atypical hyperplasia of the forestomach mucosa and a squamous papilloma each occurred in 1 HDM. Treatment-related changes also were seen at MD and HD in the glandular stomach, consisting of hyperplasia of the superficial/foveolar epithelium, glandular hyperplasia, pleocellular inflammation, and erosions. See the sponsor's summary tables, below.

Dose Group:	I	II	III	IV
Sex:	M	M	M	M
Number of Rats/Group:	25	25	25	25
STOMACH (FORESTOMACH):				
-carcinoma, squamous-cell	0	1	5	5
-hyperplasia, atypical, squamous epithelium, focal				
mild	0	0	0	1
Total Incidence, All Grades	0	0	0	1
-hyperplasia/hyperkeratosis, mucosa, diffuse				
moderate	0	13	0	1
marked	0	12	25	24
Total Incidence, All Grades	0	25	25	25
-inflammation, pleocellular				
minimal	0	5	4	5
mild	0	2	14	8
moderate	0	0	3	12
Total Incidence, All Grades	0	7	21	25
-microabscess/necrosis/vacuolar degeneration, superficial squamous epithelium/keratin layer				
minimal	0	3	10	9
mild	0	0	6	6
Total Incidence, All Grades	0	3	16	15
-papilloma, squamous	0	0	0	1
-ulcer(s)				
minimal	0	1	3	2
mild	0	0	1	4
moderate	0	0	0	5
Total Incidence, All Grades	0	1	4	11

Dose Group:	I	II	III	IV
Sex:	M	M	M	M
Number of Rats/Group:	25	25	25	25
STOMACH (GLANDULAR):				
-erosion(s), mucosal				
minimal	0	0	1	5
mild	0	0	0	3
Total Incidence, All Grades	0	0	1	8
-hyperplasia, surface/foveolar epithelium				
mild	0	0	4	22
Total Incidence, All Grades	0	0	4	22
-hyperplasia, glandular				
minimal	0	0	6	8
mild	0	0	2	11
moderate	0	0	0	1
Total Incidence, All Grades	0	0	8	20
-inflammation, pleocellular				
minimal	0	0	4	15
mild	0	0	2	5
moderate	0	0	1	1
Total Incidence, All Grades	0	0	7	21

Drug-related microscopic changes in the kidneys included a dose-related increased incidence and/or severity of: dilatation of cortical tubules, nephropathy, nuclear and

cellular hypertrophy of the cortical tubular epithelium, and segmental regeneration of the cortical tubular epithelium. See the excerpt from the sponsor's table, below. Hyaline droplet accumulations in cortical tubules occurred with increased incidence and severity in treated groups.

Dose Group:	I	II	III	IV
Sex:	M	M	M	M
Number of Rats/Group:	25	25	25	25
KIDNEYS:				
-dilatation, cortical tubules				
minimal	4	18	14	11
mild	0	0	11	11
moderate	0	0	0	2
Total Incidence, All Grades	4	18	25	24
-hyaline droplets, cortical tubules, multifocal				
minimal	7	15	17	10
mild	0	2	3	2
Total Incidence, All Grades	7	17	20	12
-nephropathy				
minimal	18	21	17	16
mild	0	1	5	4
moderate	0	0	0	2
Total Incidence, All Grades	18	22	22	22
-nuclear/cellular hypertrophy, cortical tubular epithelium				
minimal	0	1	20	8
mild	0	1	5	16
Total Incidence, All Grades	0	2	25	24
-regeneration, tubular epithelium, segmental, multifocal				
minimal	1	5	14	17
mild	0	3	7	3
moderate	0	0	0	2
Total Incidence, All Grades	1	8	21	22

In the testes, a low incidence of minimal to mild multifocal interstitial-cell hyperplasia occurred in the testes of all treated groups. The sponsor reported no drug-related effects observed in the epididymides, prostate, seminal vesicles, or pancreas.

TESTES:				
NO. EXAMINED	25	25	25	25
NO. NORMAL	23	22	15	16
-atrophy, diffuse	[0]	[0]	[0]	[1]
marked	0	0	0	1
-hyperplasia, interstitial-cell, multifocal	[0]	[1]	[9]	[9]
minimal	0	1	8	8
mild	0	0	1	1

Effects in pancreatic lymph nodes were considered secondary to drug-related toxic effects (stomach toxicity).

LYMPH NODE, PANCREATIC:				
NO. EXAMINED	0	3	25	24
NO. NORMAL	0	0	0	0
-dilatation (ectasia), sinuses	[0]	[0]	[15]	[21]
minimal	0	0	9	4
mild	0	0	6	13
moderate	0	0	0	4
-hyperplasia, lymphocytic/plasmacytic	[0]	[2]	[25]	[24]
minimal	0	1	2	1
mild	0	1	17	19
moderate	0	0	6	4
-intrasinus erythrocytes	[0]	[3]	[2]	[4]
minimal	0	3	2	4
-intrasinus neutrophils	[0]	[0]	[0]	[1]
mild	0	0	0	1
-intrasinus neutrophils/eosinophils	[0]	[0]	[1]	[0]
mild	0	0	1	0

Fertility Parameters

Female rats were sacrificed on presumed GD13. Mating and fertility were not affected by BG00012 treatment. The sponsor reported that no drug-related differences were observed for days in cohabitation, rats mating, fertility index (number of pregnancies/number of rats that mated), or rats pregnant/rats in cohabitation. The sponsor reported no drug-related differences among the untreated females for corpora lutea, implantations, litter size, viable or non viable embryos, or dams with non viable embryos. No dam had all non-viable embryos. No effect on sperm motility from the vas deferens or cauda epididymal sperm count was reported; see the sponsor's table, below. However, nonmotile sperm were increased at MD and HD.

TABLE B10 (PAGE 1): SPERM MOTILITY, COUNT AND DENSITY - SUMMARY - MALE RATS

DOSAGE GROUP		I	II	III	IV
DOSAGE (MG/KG/DAY)		0 (VEHICLE)	75	250	375
RATS TESTED	N	24a	25	25	24a
VAS DEFERENS SPERM MOTILITY					
NUMBER MOTILE	MEAN±S.D.	423.29 ± 208.89	405.42 ± 175.60 [24]b	398.29 ± 177.72 [24]b	412.75 ± 183.11
MOTILE PERCENT	MEAN±S.D.	89.50 ± 22.01	91.67 ± 17.72 [24]b	87.67 ± 22.63 [24]b	84.92 ± 18.65
STATIC COUNT (NONMOTILE)	MEAN±S.D.	31.58 ± 40.64	30.21 ± 49.96 [24]b	44.38 ± 62.17 [24]b	61.25 ± 54.43
TOTAL COUNT c	MEAN±S.D.	454.88 ± 196.72	435.63 ± 163.99 [24]b	442.67 ± 155.45 [24]b	474.00 ± 176.89
CAUDA EPIDIDYMAL SPERM COUNT					
SPERM COUNT d	MEAN±S.D.	193.00 ± 62.46	158.60 ± 58.97	140.88 ± 56.82*	177.21 ± 81.55
SPERM DENSITY e	MEAN±S.D.	1461.43 ± 417.77	1242.59 ± 509.00	1146.08 ± 403.06*	1469.19 ± 582.15

[ ] = NUMBER OF VALUES AVERAGED

a. Excludes rats that were moribund sacrificed.
b. Excludes values for rats that had motility data that reflected drifting debris.
c. Sum of number motile and static count. Groups of five fields were evaluated until a sperm count of at least 200 was achieved or 20 fields were evaluated.
d. Sperm count used in the calculation of sperm density. Ten fields were evaluated.
e. The sperm density was calculated by dividing the sperm count by the volume in the image area (34.3×10^{-6} mL), multiplying by 2 (dilution factor) and multiplying by 10^{-6} to obtain the sperm concentration. The calculated sperm concentration value (rounded to 1 decimal place) was multiplied by 50 (volume) and divided by the weight of the left cauda epididymis (see Table B16 for the weight of the left cauda epididymis) to obtain the sperm density. The calculated value will vary by approximately 0.8% from the Computer Automated Sperm Analysis because the digital image evaluated is slightly smaller (4 pixels) than the actual field causing a slight underestimate of the actual volume and an overestimate of the concentration.

* Significantly different from the vehicle control group value ($p \leq 0.05$).

Study title: BG00012: Oral (Gavage) Fertility and General Reproduction Toxicity Study of BG00012 in Female Rats

Study no.:	P00012-10-01, (b) (4) # EBA00421
Conducting laboratory and location:	(b) (4)
Date of study initiation:	2/3/10
GLP compliance:	Yes, Pg 8
QA statement:	Yes, Pg 9
Drug, lot #, and % purity:	DMF, lot 1427169, 100.2% pure (0.02% MMF)

Methods:

Doses:	0, 25, 100, and 250 mg/kg/day
Frequency of dosing:	once daily beginning 15 days before cohabitation (maximum 21 days) and continuing through GD7
Dose volume:	10 mL/kg
Route of administration:	Oral gavage
Formulation/Vehicle:	Hypromellose 0.8% in R.O. deionized water
Species/Strain:	presumed-pregnant Crl:CD(SD) female rats, (b) (4) 66 days of age at arrival, 227-266 g at initiation
Number/Sex/Group:	25/group
Satellite groups:	TK: 5/gp

Observations and Results

Mortality

No drug-related deaths were reported. One control (GD7) and 2 HDF (pre-mating) rats died prior to scheduled sacrifice; these deaths were attributed to intubation accidents.

Clinical Signs

Excessive salivation, dehydration, a red perioral substance, sparse hair coat, chromorhinorrhea, and ungroomed coat were increased at HD during the pre-mating dosing period. Excess salivation, dehydration, and sparse hair coat continued to be increased at HD during the gestation period; 3-4 MDF rats also showed excess salivation and/or sparse hair coat during the gestation period. See excerpts from the sponsor's Table 1, below.

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TABLE 1 (PAGE 1): CLINICAL OBSERVATIONS - SUMMARY - FEMALE RATS
(See footnotes on the last page of this table.)

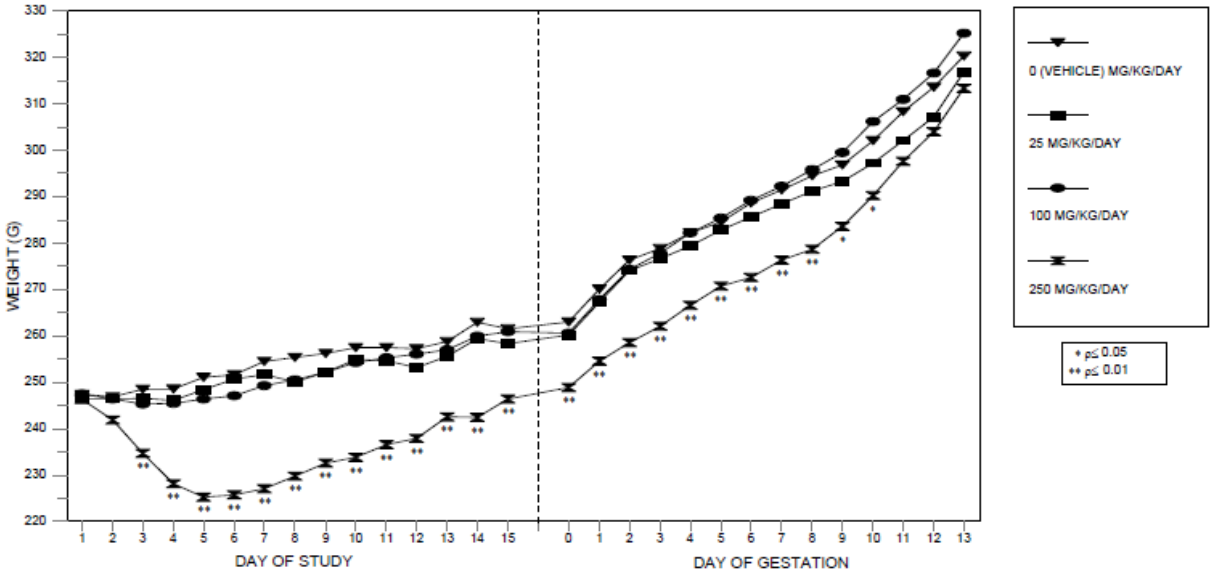
DOSAGE GROUP	I	II	III	IV
DOSAGE (MG/KG/DAY)a	0 (VEHICLE)	25	100	250
FOUND DEAD	1b	0	0	2c,d
PREMATING:				
MAXIMUM POSSIBLE INCIDENCE	400/ 25	415/ 25	420/ 25	394/ 25
EXCESS SALIVATION: TOTAL	0/ 0	0/ 0	3/ 1	100/ 23**
SLIGHT	0/ 0	0/ 0	2/ 1	56/ 20**c
MODERATE	0/ 0	0/ 0	1/ 1	40/ 11**c
EXTREME	0/ 0	0/ 0	0/ 0	7/ 2
DEHYDRATION: TOTAL	0/ 0	0/ 0	0/ 0	78/ 15**
MILD	0/ 0	0/ 0	0/ 0	53/ 13**c
MODERATE	0/ 0	0/ 0	0/ 0	25/ 7**c
RED PERIORAL SUBSTANCE	0/ 0	0/ 0	0/ 0	9/ 6**c
SPARSE HAIR COAT: TOTAL	0/ 0	0/ 0	2/ 1	18/ 5**
LIMB(S)	0/ 0	0/ 0	2/ 1	8/ 2
BACK	0/ 0	0/ 0	0/ 0	7/ 2
NECK	0/ 0	0/ 0	0/ 0	3/ 1
CHROMORHINORRHEA	0/ 0	0/ 0	0/ 0	11/ 4**c
UNGROOMED COAT	0/ 0	0/ 0	0/ 0	12/ 3**c
PRESUMED GESTATION:e				
MAXIMUM POSSIBLE INCIDENCE	344/ 25	336/ 24	336/ 24	322/ 23
EXCESS SALIVATION: TOTAL	0/ 0	0/ 0	4/ 3	69/ 16**
SLIGHT	0/ 0	0/ 0	4/ 3	53/ 15**
MODERATE	0/ 0	0/ 0	0/ 0	15/ 8**
EXTREME	0/ 0	0/ 0	0/ 0	2/ 2
DEHYDRATION: TOTAL	0/ 0	0/ 0	0/ 0	132/ 13**
MILD	0/ 0	0/ 0	0/ 0	110/ 13**
MODERATE	0/ 0	0/ 0	0/ 0	23/ 6**
PRESUMED GESTATION:e (CONT.)				
SPARSE HAIR COAT: TOTAL	6/ 2	0/ 0	17/ 4	109/ 12**
HEAD	0/ 0	0/ 0	1/ 1	40/ 7**
LIMB(S)	6/ 2	0/ 0	16/ 3	33/ 3
BACK	0/ 0	0/ 0	0/ 0	28/ 2
NECK	0/ 0	0/ 0	0/ 0	14/ 1
UNDERSIDE	0/ 0	0/ 0	0/ 0	4/ 1

STATISTICAL ANALYSES OF CLINICAL OBSERVATION DATA WERE RESTRICTED TO THE NUMBER OF RATS WITH OBSERVATIONS.
MAXIMUM POSSIBLE INCIDENCE = (DAYS x RATS)/NUMBER OF RATS EXAMINED PER GROUP
N/N = TOTAL NUMBER OF OBSERVATIONS/NUMBER OF RATS WITH OBSERVATION
a. Dosage occurred on day 1 of study through day 7 of presumed gestation.
b. Dam 4603 was found dead on day 7 of gestation.
c. Rat 4686 was found dead on day 10 of study.
d. Rat 4698 was found dead on day 7 of study.
e. Restricted to rats with a confirmed mating date.
** Significantly different from the vehicle control group value (p≤0.01).

Body Weight

Reduced body weights and body weight gains during the pre-mating period were observed at HD. During the first week of dosing, body weight gains were significantly reduced at LD and MD (~60%), and significant body weight loss (~8%) occurred at HD; body weight gains were increased during the second week of dosing. During the entire pre-mating period (D1 to D15), a significant body weight loss occurred at HD. Body weight gains during the gestation dosing period were comparable among groups. After dosing, body weight gains were significantly increased for the entire gestation dosing period at MD and HD; however, body weights were significantly reduced in the HD group from D3 to D15 and through GD10.

BODY WEIGHTS - FEMALE RATS
Figure 1



Food Consumption

Reduced absolute and relative food consumption was observed at HD during the entire pre-mating period (D1-D15; reductions of 55% the first week, and 10% the second week). During the first week of dosing during the pre-mating period, transient reductions in absolute (reduced ~15%) and relative food consumption also occurred in the MD group, reflecting reduced body weight gains. Absolute and relative food consumption values were comparable among the groups during the gestation dosing period (GD0 to GD8), and were increased at MD and HD during the gestation post-dosing period (up to ~20%; GD8 to GD13) and the overall gestation period (up to 10%; GD0 to GD13).

Toxicokinetics

Exposure to BG00012 (as measured by MMF plasma concentration) was confirmed in a subset of five female rats per group at approximately 30 minutes postdose on GD7, the last day of dosing. However, there were problems with the assay and these analyses were not reliable (only individual plasma MMF concentrations were available). The control animal values were BQL.

Dosing Solution Analysis

The first and last preparations of dose formulations were analyzed for concentration and homogeneity. Mean BG00012 concentrations were within the acceptable limits (± 15% of nominal). Homogeneity (as assessed by measurement at the top, middle and bottom of each formulation concentration) was acceptable (≤ 5% RSD).

Necropsy

At terminal necropsy, the incidence of numerous white raised areas on the mucosal surface of the cardiac region of the stomach and thickened walls of the stomach were significantly increased in the HD group. Heart and lung findings observed in the early HD mortalities support the sponsor's attribution of these deaths to gavage error. Histopathological assessment was not performed.

TABLE 11 (PAGE 1): NECROPSY OBSERVATIONS - SUMMARY - FEMALE RATS

DOSAGE GROUP		I	II	III	IV
DOSAGE (MG/KG/DAY)a		0 (VEHICLE)	25	100	250
RATS EXAMINED b	N	25	25	25	25
FOUND DEAD	N	1c	0	0	2d,e
APPEARED NORMAL	N	23	25	22	10**
HEART:					
ADHERED TO RIGHT APICAL AND DIAPHRAGMATIC LUNG LOBES	N	0	0	0	1d
LUNGS:					
RIGHT APICAL, CARDIAC AND DIAPHRAGMATIC LOBES ADHERED TO EACH OTHER AND ENCASED IN FIBROUS MATERIAL	N	0	0	0	1d
ALL LOBES, MOTTLED RED AND DARK RED	N	0	0	0	1e
RIGHT AXILLARY AND/OR VENTRAL THORACIC REGION(S):					
RED GELATINOUS MATERIAL PRESENT SUBCUTANEOUSLY	N	1c	0	2	0
SPLEEN:					
LARGE; ACCESSORY SPLEEN	N	0	0	0	1
STOMACH:					
FUNDIC AND PYLORIC REGIONS, MUCOSAL SURFACE, NUMEROUS RED AREAS	N	1c	0	0	0
CARDIAC REGION, MUCOSAL SURFACE, NUMEROUS WHITE RAISED AREAS	N	0	0	1	11**
WALLS THICK	N	0	0	0	9**
INTESTINES:					
DUODENUM, DIVERTICULUM	N	1	0	0	0

a. Dosage occurred on day 1 of study through day 7 of presumed gestation.
b. Refer to the individual clinical observations table (Table 13) for external observations confirmed at necropsy.
c. Dam 4603 was found dead on day 7 of gestation.
d. Rat 4686 was found dead on day 10 of study.
e. Rat 4698 was found dead on day 7 of study.
** Significantly different from the vehicle control group value ($p \leq 0.01$).

Fertility Parameters (Mating/Fertility Index, Corpora Lutea, Preimplantation Loss, etc.)

The number of estrous cycles per 14 days was significantly reduced and the number of rats with six or more days of diestrus was significantly increased at HD. Days in cohabitation was slightly increased in HD females. See the sponsor's Table 10, below. However, this effect did not translate into an effect on fertility, as measured. Pregnancy occurred in 96%, 100%, 100%, and 100% of rats in the Control, LD, MD, and HD groups, respectively. Caesarean-sectioning and litter observations were based on 23, 25, 25 and 23 pregnant rats on GD13; the sponsor reported no Caesarean-sectioning or litter parameters affected by BG-12. However, there were slight changes in postimplantation loss percentage, viable embryo count, nonviable embryo count, and number of dams with any nonviable embryo count at HD. See sponsor's Table 12, below, for details.

TABLE 10 (PAGE 1): ESTROUS CYCLING, MATING AND FERTILITY - SUMMARY - FEMALE RATS

DOSAGE GROUP DOSAGE (MG/KG/DAY)a		I 0 (VEHICLE)	II 25	III 100	IV 250
ESTROUS CYCLING OBSERVATIONS					
PREDOSAGE ESTROUS CYCLING					
RATS EVALUATED	N	25	25	25	25
ESTROUS STAGES/ 14 DAYS	MEAN±S.D.	3.8 ± 0.4	3.8 ± 0.4	3.6 ± 0.5	3.6 ± 0.6
RATS WITH 6 OR MORE CONSECUTIVE DAYS OF DIESTRUS	N(%)	0(0.0)	0(0.0)	0(0.0)	0(0.0)
RATS WITH 6 OR MORE CONSECUTIVE DAYS OF ESTRUS	N(%)	0(0.0)	0(0.0)	0(0.0)	0(0.0)
PRECOHABITATION ESTROUS CYCLING					
RATS EVALUATED	N	25	25	25	25
INCLUDED IN ANALYSES	N	25	25	25	23b
ESTROUS STAGES/ 14 DAYS	MEAN±S.D.	3.3 ± 0.5	3.2 ± 0.4	3.2 ± 0.6	2.5 ± 0.9**
RATS WITH 6 OR MORE CONSECUTIVE DAYS OF DIESTRUS	N(%)	0(0.0)	0(0.0)	0(0.0)	5(21.7)**
RATS WITH 6 OR MORE CONSECUTIVE DAYS OF ESTRUS	N(%)	0(0.0)	0(0.0)	0(0.0)	0(0.0)

a. Dosage occurred on day 1 of study through day 7 of gestation.
b. Excludes rats 4686 and 4698, which were found dead during estrous evaluations.
** Significantly different from the vehicle control group value (p≤0.01).

TABLE 10 (PAGE 2): ESTROUS CYCLING, MATING AND FERTILITY - SUMMARY - FEMALE RATS

DOSAGE GROUP DOSAGE (MG/KG/DAY)a		I 0 (VEHICLE)	II 25	III 100	IV 250
MATING OBSERVATIONS					
RATS IN COHABITATION	N	25	25	25	23b
DAYS IN COHABITATION c	MEAN±S.D.	2.0 ± 1.0	1.8 ± 1.0 [24]	2.0 ± 1.0 [24]	2.4 ± 1.3
RATS THAT MATED	N(%)	25(100.0)	25(100.0)	25(100.0)	23(100.0)
FERTILITY INDEX d	N/N (%)	24/25 (96.0)	25/25 (100.0)	25/25 (100.0)	23/23 (100.0)
RATS WITH CONFIRMED MATING DATES	N	25	24	24	23
MATED BY FIRST MALE e					
DAYS 1-7	N(%)	25(100.0)	24(100.0)	24(100.0)	23(100.0)
DAYS 8-14	N(%)	0(0.0)	0(0.0)	0(0.0)	0(0.0)
RATS PREGNANT/RATS IN COHABITATION	N/N (%)	24/25 (96.0)	25/25 (100.0)	25/25 (100.0)	23/23 (100.0)

[ ] = NUMBER OF VALUES AVERAGED
a. Dosage occurred on day 1 of study through day 7 of gestation.
b. Excludes values for rats 4686 and 4698, which were found dead before mating.
c. Restricted to rats with a confirmed mating date and rats that did not mate.
d. Number of pregnancies/number of rats that mated.
e. Restricted to rats with a confirmed mating date.

TABLE 12 (PAGE 1): CAESAREAN-SECTIONING OBSERVATIONS - SUMMARY - FEMALE RATS

DOSAGE GROUP		I	II	III	IV
DOSAGE (MG/KG/DAY)a		0 (VEHICLE)	25	100	250
RATS TESTED	N	25	25	25	23b
PREGNANT	N(%)	24(96.0)	25(100.0)	25(100.0)	23(100.0)
FOUND DEAD	N(%)	1(4.2)	0(0.0)	0(0.0)	0(0.0)
RATS PREGNANT AND CAESAREAN-SECTIONED ON DAY 13 OF GESTATION	N	23	25c	25c	23
CORPORA LUTEA	MEAN±S.D.	16.0 ± 2.1	15.0 ± 1.7	17.7 ± 2.3*	15.5 ± 2.7
IMPLANTATIONS	MEAN±S.D.	15.4 ± 2.3	14.5 ± 1.6	17.2 ± 2.3*	14.9 ± 3.6
% PREIMPLANTATION LOSS	MEAN±S.D.	3.4 ± 4.5	2.8 ± 3.7	3.0 ± 5.4	6.0 ± 15.6
VIABLE EMBRYOS	N	338	338	405	312
	MEAN±S.D.	14.7 ± 2.4	13.5 ± 1.7	16.2 ± 2.5	13.6 ± 3.9
NONVIABLE EMBRYOS	N	17	25	25	31
	MEAN±S.D.	0.7 ± 0.8	1.0 ± 1.0	1.0 ± 1.1	1.3 ± 1.2
% POSTIMPLANTATION LOSS	MEAN±S.D.	5.0 ± 5.6	6.8 ± 6.7	5.9 ± 6.3	10.8 ± 12.3
DAMS WITH ANY NONVIABLE EMBRYOS	N(%)	12(52.2)	16(64.0)	15(60.0)	18(78.3)
DAMS WITH ALL NONVIABLE EMBRYOS	N(%)	0(0.0)	0(0.0)	0(0.0)	0(0.0)
DAMS WITH VIABLE EMBRYOS	N(%)	23(100.0)	25(100.0)	25(100.0)	23(100.0)
PLACENTAE APPEARED NORMAL	N(%)	23(100.0)	25(100.0)	25(100.0)	23(100.0)

% PREIMPLANTATION LOSS = [(NUMBER OF CORPORA LUTEA - NUMBER OF IMPLANTATIONS) / NUMBER OF CORPORA LUTEA] x 100
% POSTIMPLANTATION LOSS = [(NUMBER OF IMPLANTATIONS - NUMBER OF LIVE EMBRYOS) / NUMBER OF IMPLANTATIONS] x 100
a. Dosage occurred on day 1 of study through day 7 of gestation.
b. Excludes values for rats 4686 and 4698, which were found dead before mating.
c. Includes values for dams that did not have confirmed mating dates.
* Significantly different from the vehicle control group value (p≤0.05).

9.2 Embryonic Fetal Development

Study title: ORAL (STOMACH TUBE) DEVELOPMENTAL TOXICITY STUDY OF DIMETHYL FUMARATE (DMF) IN RABBITS

Study no.: P00012-06-01. (b) (4) #EBA00154

Conducting laboratory and location: (b) (4)

Date of study initiation: 2/17/06

GLP compliance: Yes, Appx I

QA statement: Yes, Appx J

Drug, lot #, and % purity: As in sponsor's table below; 33004999 was 99.8% pure (0.03% MMF); 33004998 was 100.2% pure (0.02% MMF)

Test Article Information			
Name:	Dimethyl fumarate (DMF) (BG00012)		
Storage:	Room temperature, protected from light		
Description:	White powder		
Lot Number	Date Received	Supplier	Expiration Date
1102643 "33004999"	16 FEB 06	Sponsor[a]	14 SEP 06
1102642 "33004998"	17 FEB 06	(b) (4)	TBA

a. Received from (b) (4)
b. Received from
TBA - To be added prior to report finalization.

Methods: Details as in sponsor's table, below

Doses: 0 (Vehicle), 25, 75, and 150 mg/kg/day
Frequency of dosing: once daily on days 7 through 19 of presumed gestation
Route of administration: orally (via stomach tube)
Formulation/Vehicle: hydroxypropylmethylcellulose (HPMC) 0.8% in R.O. deionized water
Species/Strain: timed-mated female Hra:(NZW)SPF rabbits, 5 mo. of age, 2.6-3.8 kg, (b) (4)

Dosage Group	Dosage (mg/kg/day)	Concentration (mg/mL)	Dosage Volume (mL/kg)	Number of Rabbits	Assigned Rabbit Numbers	
					Main Study	Toxicokinetic Study
I	0 (Vehicle)	0	10	20	6001 - 6020	NA
II	25	2.5	10	20 + 3[a]	6021 - 6040	5891 - 5893
III	75	7.5	10	20 + 3[a]	6041 - 6060	5894 - 5896
IV	150	15	10	20 + 3[a]	6061 - 6079, 370[b]	5897 - 5899

The test article was considered 100% active for the purpose of dosage calculations.
NA - not applicable.
a. Three rabbits assigned to toxicokinetic sample collection.
b. Rabbit 6080 was removed from the study on DG 5 before the first day of dosage administration due to adverse clinical observations and was replaced with rabbit 370.

Observations and Results

Mortality

No does died on study; however, an increased number of HD does (4/20, [ss]) aborted and were sacrificed. These abortions were accompanied by significant decreases in body weights and food consumption during the dosing period. Each of these does is described below; all other does survived to scheduled sacrifice. (Details about replaced HD doe 6080 were not available.)

Doe 6067 aborted and was sacrificed on GD22. Adverse clinical signs included scant feces from GDs14 to 21, sparse hair coat from GDs 19 to 22 and a red substance in the cage pan on GD22. This doe generally lost weight after GD8. Food consumption was severely reduced from GDs 13 to 21. All tissues appeared normal at necropsy. The litter consisted of one aborted fetus, seven fetuses (viability at the time of abortion "could not be determined"), and two late resorptions *in utero*. There were no gross or soft tissue alterations and no skeletal alterations except for non-ossified pubis bones.

Doe 6071 aborted and was sacrificed on GD22. Adverse clinical signs included scant feces (GD 11 - 22). This doe generally lost weight after GD7. Food consumption was severely reduced from GDs 8 to 21. Gross necropsy revealed red material in the

stomach; all other tissues appeared normal. The litter consisted of nine aborted fetuses and three early resorptions. Gross and soft tissue examination revealed no alterations. Skeletal examination revealed several fetuses with non-ossified pubes, one fetus with a split rib and non-ossified pubes and ischia, and one fetus with non-ossified pubes and ischia.

Doe 6075 aborted and was sacrificed on GD19. Adverse clinical signs included scant feces (GD 13 - 19). This doe generally lost weight after GD7; food consumption was severely reduced (GD8 - 17). Gross necropsy revealed a white firm mass measuring 3.3 cm in diameter on the left kidney; the cut surface of this mass revealed a white firm material. All other tissues appeared normal. The litter consisted of two late resorptions and seven (six late and one early) resorptions *in utero*. Gross examination of one late resorption revealed a short right forelimb with short digits and a rotated left forepaw. All other resorptions were too autolyzed for further analysis.

Doe 6076 aborted and was sacrificed on GD25. Adverse clinical signs included scant feces (GD 12 - 20, 22 - 24), no feces in the cage pan (GD21), and a red substance in the cage pan (GD25). This doe lost weight on GD7 to GD24. Food consumption was severely reduced from GDs 8 to 22. Gross necropsy revealed no lesions. The litter consisted of six dead aborted fetuses and three early resorptions *in utero*. At gross external and soft tissue examination, four fetuses were partially cannibalized; the other two fetuses appeared normal. At skeletal examination, four fetuses had non-ossified pubes.

Clinical Signs

Scant feces were observed in a dose-dependent manner; at HD, the increased incidence was statistically significant. Sparse hair coat was also observed at HD. One MD doe also showed no feces on one occasion. See the sponsor's summary table, below.

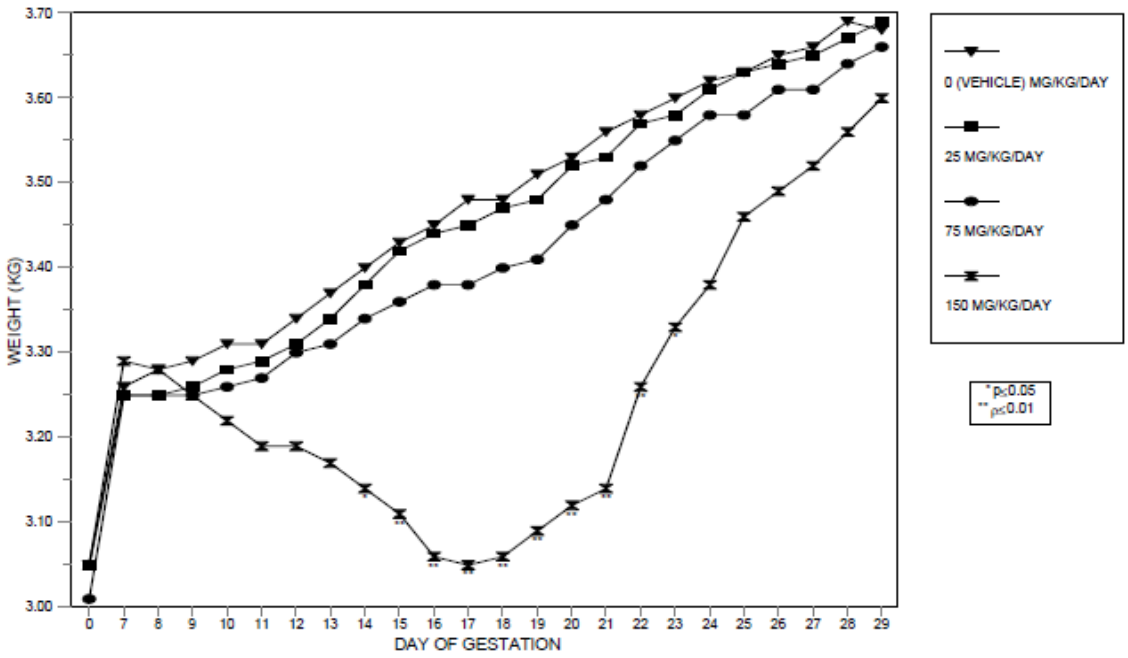
DOSAGE GROUP DOSAGE (MG/KG/DAY)a	I 0 (VEHICLE)	II 25	III 75	IV 150
MAXIMUM POSSIBLE INCIDENCE	460/ 20	460/ 20	460/ 20	432/ 20
ABORTED AND SACRIFICED	0	0	0	4b-e**
SCANT FECES	1/ 1	7/ 3	11/ 3	99/ 15b-e**
SPARSE HAIR COAT	2/ 1	0/ 0	2/ 1	28/ 4b
RED SUBSTANCE IN CAGE PAN	0/ 0	0/ 0	0/ 0	2/ 2b,e
NO FECES IN CAGE PAN	0/ 0	0/ 0	1/ 1	1/ 1e
SOFT OR LIQUID FECES	1/ 1	0/ 0	1/ 1	0/ 0
LOCALIZED ALOPECIA: LIMB(S)	0/ 0	0/ 0	1/ 1	0/ 0
UNGROOMED COAT	3/ 2	3/ 1	0/ 0	0/ 0

STATISTICAL ANALYSES OF CLINICAL OBSERVATION DATA WERE RESTRICTED TO THE NUMBER OF RABBITS WITH OBSERVATIONS.
MAXIMUM POSSIBLE INCIDENCE = (DAYS x RABBITS)/NUMBER OF RABBITS EXAMINED PER GROUP ON DAYS 7 THROUGH 29 OF PRESUMED GESTATION.
N/N = TOTAL NUMBER OF OBSERVATIONS/NUMBER OF RABBITS WITH OBSERVATION.
a. Dosage occurred on days 7 through 19 of presumed gestation.
b. Doe 6067 aborted and was sacrificed on day 22 of gestation.
c. Doe 6071 aborted and was sacrificed on day 22 of gestation.
d. Doe 6075 aborted and was sacrificed on day 19 of gestation.
e. Doe 6076 aborted and was sacrificed on day 25 of gestation.
** Significantly different from the vehicle control group value ($p \leq 0.01$).

Body Weight

Mean body weights were statistically significantly reduced at HD on GDs 14 to 23; from GD7 to GD19, body weight losses (~6%) occurred at HD. Body weight gains for the entire dosing period (calculated as GDs 7 to 20) were reduced 30% at MD [ss]; however, the mean body weight was only decreased ~3% on GD19. There was no effect at LD. After the dosing period (GDs 20 to 29), significant increases in body weight gain [ss] occurred at HD. See the sponsor's Figure 1 for details.

MATERNAL BODY WEIGHTS
Figure 1



Food Consumption

Absolute and relative food consumption values were significantly reduced [ss] at HD for the entire dosing period (averaging 44% of control) and at each interval evaluated during the dosing period. After the dosing period, absolute and relative food consumption values were significantly increased [ss] at HD.

Toxicokinetics (Maternal GDs 7 and 19, Fetal GD20)

MMF exposure was confirmed for 3 hr at LD and for 6 hr at MD and HD. See sponsor's Tables 2 and 3. MMF did not show accumulation in systemic circulation. MMF plasma exposures increased with dose in a slightly more-than-dose-proportional manner between LD and MD, and in an approximately dose-proportional manner between MD and HD. The half-life was 0.4-1 hr, relatively constant upon repeat dosing, and dose-independent. MMF crossed the placenta membrane into fetal blood circulation, and the MMF plasma concentration ratio between fetal and maternal plasma was approximately 0.1.

Table 2. Toxicokinetic Parameter Estimates of MMF on Days 7 and 19 in Presumed Pregnant Rabbits Following a Daily Oral Administration.

Dose (mg/kg)	Study Day	Animal ID	AUCinf (hour*ng/mL)	AUClast [a] (hour*ng/mL)	Cmax (ng/mL)	Tmax (hour)	T1/2 (hour)
25	Day 7	5891	17607	17313[a1]	18400	0.25	0.5
		5892	12110	12047[a1]	18700	0.25	0.4
		5893	12064	12041[a1]	13900	0.25	0.3
		Mean	**13927**	**13800**	**17000**	**0.25**	**0.4**
		SD	3187	3042	2688.87	0.00	0.1
		CV%	23	22	16	0	22
	Day 19	5891	12752	12676	12800	0.25	0.8
		5892	9999	8740[a2]	19000	0.25	0.3
		5893	11679	11650[a1]	18200	0.25	0.3
		Mean	**11477**	**11022**	**16667**	**0.25**	**0.5**
		SD	1388	2041	3372.44	0.00	0.3
		CV%	12	19	20	0	58
75	Day 7	5894	67069	66930	58000	0.50	0.6
		5895	49627	49563	42600	0.50	0.6
		5896	58752	58660	69000	0.25	0.7
		Mean	**58483**	**58384**	**56533**	**0.42**	**0.6**
		SD	8724	8687	13260.97	0.14	0.0
		CV%	15	15	23	35	6
	Day 19	5894	57117	57032	55300	0.25	0.6
		5895	64691	64353	57300	0.25	0.7
		5896	43414	42402[a1]	41700	0.25	0.5
		Mean	**55074**	**54595**	**51433**	**0.25**	**0.6**
		SD	10785	11177	8488.42	0.00	0.1
		CV%	20	20	17	0	17
150	Day 7	5897	111686	111565	93500	0.25	0.6
		5898	105620	104042	64600	0.50	1.0
		5899	103465	103108	104000	0.25	0.7
		Mean	**106924**	**106239**	**87367**	**0.33**	**0.8**
		SD	4262	4637	20403.51	0.14	0.2
		CV%	4	4	23	43	26
	Day 19	5897	129456	127464	98100	0.25	0.9
		5898	151417	147641	123000	0.25	1.1
		5899	103728	103660	97900	0.50	0.5
		Mean	**128201**	**126255**	**106333**	**0.33**	**0.9**
		SD	23869	22015	14434.1	0.14	0.3
		CV%	19	17	14	43	33

[a] The last time point with detectable MMF concentration was 6 hours post-dosage for most animals with the exceptions noted as following: [a1] 3 hours and [a2] 1 hour.

Table 3. Comparison of Mean Plasma Concentrations of MMF in Fetus (Day 20) and Doe (Day 19) at 30 Minutes Post Dosage.

Animal	Animal Group	Fetal Plasma Concentration Day 20 (ng/mL)	Maternal Plasma Concentration Day 19 (ng/mL)	Concentration Ratio (fetal: maternal)
5891	2	1110	9280	0.12
5892	2	1540	9800	0.16
5893	2	1600	12300	0.13
Mean		**1417**	**10460**	**0.14**
SD		267	1615	0.02
%CV		19	15	14
5894	3	3260	48800	0.07
5895	3	5750	48300	0.12
5896	3	4070	35300	0.12
Mean		**4360**	**44133**	**0.10**
SD		1270	7654	0.03
%CV		29	17	29
5897	4	13500	90200	0.15
5898	4	9650	72200	0.13
5899	4	[a]	97900	NA
Mean		**11575**	**86767**	**0.14**
SD		NA	13190	NA
%CV		NA	15	NA

[a]: The doe was found not pregnant; therefore, no fetal plasma sample was obtained.
NA: not applicable.

Dosing Solution Analysis

Suspensions of the test article were prepared at least once weekly, stored refrigerated (2 - 8°C), and protected from light. Concentration and homogeneity analyses were conducted, indicating that there was no test article contamination of the vehicle and the formulations were within ±15% of target and homogenous (≤ 5% RSD).

Necropsy (GD29)

The sponsor noted no drug-related necropsy observations. Red material in the stomach and white kidney mass were observed in does that aborted. Bilateral multiple (4-8, 0.2-0.3 cm in diameter) parovarian cysts were observed in one HD doe.

Cesarean Section Data

Pregnancy occurred in 19 - 20 does in each dose group. No Caesarean-sectioning or litter parameters were affected. The sponsor reported no changes in litter averages for corpora lutea, implantations, litter sizes, live fetuses, early and late resorptions, fetal body weights, percent resorbed conceptuses, or percent live male fetuses. All

placentae appeared normal. There was a slight suggestion of increased resorptions, but this was mostly a result of early resorptions (see the sponsor's Table 8, below).

TABLE 8 (PAGE 1): CAESAREAN-SECTIONING OBSERVATIONS - SUMMARY

DOSAGE GROUP		I	II	III	IV
DOSAGE (MG/KG/DAY)a		0 (VEHICLE)	25	75	150
RABBITS TESTED	N	20	20	20	20
PREGNANT	N(%)	19(95.0)	20(100.0)	20(100.0)	20(100.0)
ABORTED AND SACRIFICED	N(%)	0(0.0)	0(0.0)	0(0.0)	4(20.0)**
RABBITS PREGNANT AND CAESAREAN-SECTIONED ON DAY 29 OF GESTATION	N	19	20	20	16
CORPORA LUTEA	MEAN±S.D.	8.8 ± 1.6	9.1 ± 1.6	8.2 ± 1.8	9.1 ± 1.7
IMPLANTATIONS	MEAN±S.D.	8.8 ± 1.7	9.0 ± 1.7	8.2 ± 1.8	9.0 ± 1.8
LITTER SIZES	MEAN±S.D.	8.5 ± 1.4	8.7 ± 1.7	8.0 ± 1.8	8.2 ± 2.2
LIVE FETUSES	N	161	174	159	131
	MEAN±S.D.	8.5 ± 1.4	8.7 ± 1.7	8.0 ± 1.8	8.2 ± 2.2
DEAD FETUSES	N	0	0	0	0
RESORPTIONS	MEAN±S.D.	0.3 ± 0.7	0.4 ± 0.7	0.2 ± 0.6	0.8 ± 1.4
EARLY RESORPTIONS	N	4	2	4	9
	MEAN±S.D.	0.2 ± 0.4	0.1 ± 0.3	0.2 ± 0.5	0.6 ± 1.4
LATE RESORPTIONS	N	2	5	1	4
	MEAN±S.D.	0.1 ± 0.3	0.2 ± 0.6	0.0 ± 0.2	0.2 ± 0.4
DOES WITH ANY RESORPTIONS	N(%)	4(21.0)	5(25.0)	4(20.0)	6(37.5)
DOES WITH ALL CONCEPTUSES RESORBED	N(%)	0(0.0)	0(0.0)	0(0.0)	0(0.0)
DOES WITH VIABLE FETUSES	N(%)	19(100.0)	20(100.0)	20(100.0)	16(100.0)
PLACENTAE APPEARED NORMAL	N(%)	19(100.0)	20(100.0)	20(100.0)	16(100.0)

a. Dosage occurred on days 7 through 19 of gestation.
** Significantly different from the vehicle control group value ($p \leq 0.01$).

Offspring

Fetal evaluations were based on 161, 174, 159, and 131 live fetuses in 19, 20, 20, and 16 litters in the control, LD, MD and HD groups, respectively. Each of these fetuses was examined for gross external, soft tissue and skeletal alterations, and fetal ossification site averages. There were no drug-related gross external, soft tissue, or skeletal fetal alterations (malformations or variations). There were no dose-dependent or significant differences in the litter or fetal incidences of any gross external, soft tissue, or skeletal alterations; see the sponsor's summary Table 10, below. The average numbers of ossification sites per fetus per litter were comparable among the groups.

TABLE 10 (PAGE 1): FETAL ALTERATIONS - SUMMARY

DOSAGE GROUP		I	II	III	IV
DOSAGE (MG/KG/DAY)a		0 (VEHICLE)	25	75	150
LITTERS EVALUATED	N	19	20	20	16
FETUSES EVALUATED	N	161	174	159	131
LIVE	N	161	174	159	131
LITTERS WITH FETUSES WITH ANY ALTERATION OBSERVED	N(%)	7(36.8)	12(60.0)	10(50.0)	7(43.8)
FETUSES WITH ANY ALTERATION OBSERVED	N(%)	18(11.2)	25(14.4)	16(10.1)	12(9.2)
% FETUSES WITH ANY ALTERATION/LITTER	MEAN±S.D.	10.7 ± 15.2	14.6 ± 14.7	10.8 ± 14.6	10.1 ± 16.1

a. Dosage occurred on days 7 through 19 of gestation.

Gross Alterations
One HD fetus (6070-10) had a cleft palate. This was the only externally malformed fetus. Skeletal examination of the fetus confirmed the incompletely ossified palate and revealed bilateral angulated hyoid ala and an irregularly shaped xiphoid.

Visceral Alterations
There were no clear drug-related visceral malformations.

Skeletal Alterations
A number of skeletal alterations occurred that did not appear dose-related. The HD fetus previously noted (6070-10) showed an incompletely ossified palate. One MD fetus (6041-1) had interrelated axial malformations consisting of fused arches of the 10th and 11th, bifid centrum of the 11th, fused centra of the 11th and 12th thoracic vertebra and a split right 10th rib. There were a few sporadic variations. One HD fetus (370-2) had thickened ribs; this fetus had no additional alterations. Another HD fetus (6068-5) had an asymmetric sternal centra, with no additional alterations.

Study title: ORAL (GAVAGE) DEVELOPMENTAL TOXICITY STUDY OF DIMETHYL FUMARATE (DMF) IN RATS

Study no.:	P00012-06-02. (b) (4) # EBA00153
Conducting laboratory and location:	(b) (4)
Date of study initiation:	2/24/06
GLP compliance:	Yes, Appx. I
QA statement:	Yes, Appx. J
Drug, lot & purity	As below, 1102642 (also 33004998), 100.2% pure

Test Article Information		
Name:	Dimethyl fumarate (DMF) (BG00012)	
Storage:	Room temperature, protected from light	
Description:	White powder	
Lot Number	Date Received	Supplier
1102642	16 FEB 06	(b) (4)

(b) (4)

Methods: Details in the sponsor's table, below

Doses:	0, 25, 100, and 250 mg/kg/day
Frequency of dosing:	once daily on GD7 - GD17 of presumed gestation
Route of administration:	orally (via gavage)
Formulation/Vehicle:	HPMC 0.8% in R.O. deionized water
Species/Strain:	mated female Crl:CD(SD) rats, 62 days of age, 213-255 g, (b) (4)

Dosage Group	Dosage (mg/kg/day)	Concentration (mg/mL)	Dosage Volume (mL/kg)	Number of Rats	Assigned Rat Numbers	
					Main Study	Toxicokinetic Study
I	0 (Vehicle)	0	10	25 + 3[a]	8701 - 8725	10744[b], 10745[b], 8803
II	25	2.5	10	25 + 6[c]	8726 - 8750	8804 - 8809
III	100	10	10	25 + 6[c]	8751 - 8775	8810 - 8815
IV	250	25	10	25 + 6[c]	8776 - 8779, 4295[d], 8781 - 8800	8816 - 8821

The test article was considered 100% active for the purpose of dosage calculations.

a. Three rats assigned to toxicokinetic sample collection.
b. Rats 8801 and 8802 were removed from the study on 7 March 2006 (DG 7) because the 15 minutes postdosage timepoint blood collection was missed, and were replaced with rats 10744 and 10745, respectively.
c. Six rats assigned to toxicokinetic sample collection.
d. Rat 8780 had ten or fewer sperm present in the cohabitation smear and was inadvertently assigned to study. Rat 8780 was removed from the study on 3 March 2006 (DG 3) before the first day of dosage administration and was replaced with rat 4295.

Observations and Results

Mortality

All dams survived to scheduled sacrifice.

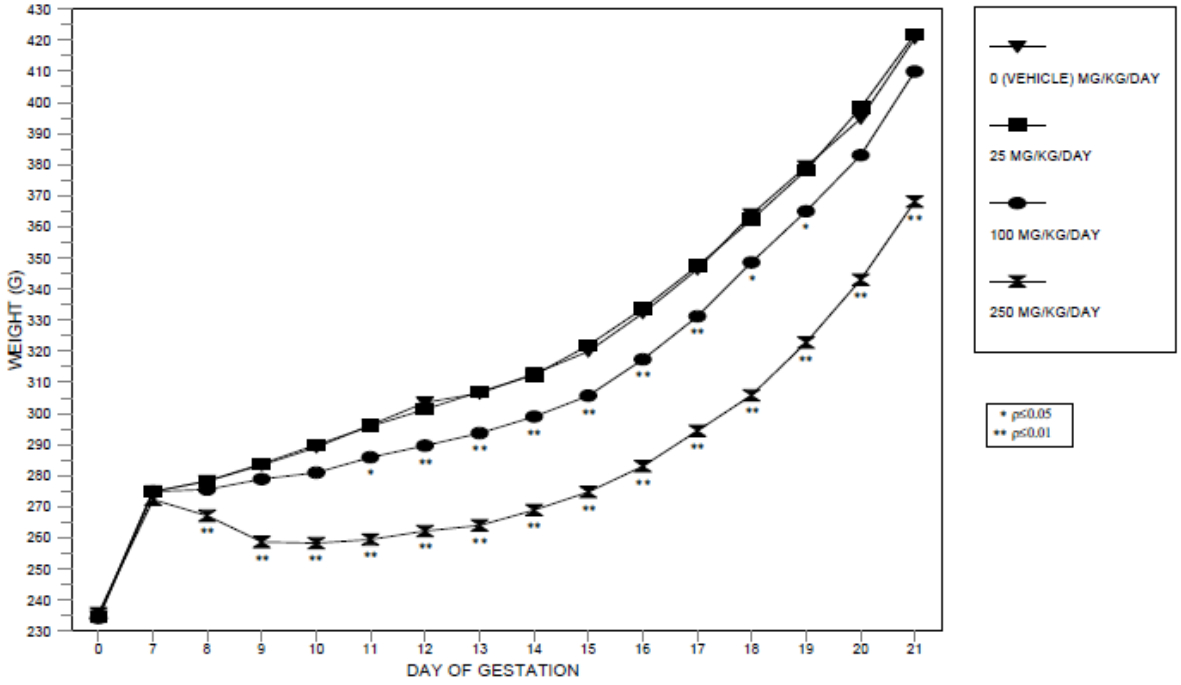
Clinical Signs

HD dams showed slight excess salivation (9/25, [ss]) and sparse hair coat (5/25). At MD, two dams exhibited slight excess salivation. One HD dam also showed localized alopecia (underside), red peri-oral substance, and dehydration.

Body Weight

Body weights were significantly reduced at HD (GD 8 - 21, ~12%, [ss]) and MD (GD 11 - 19, ~4%, [ss]); reflecting this, significant reductions in body weight gains occurred for the entire dosing period at MD and HD. Body weight loss (~5%) occurred at HD on GD 7 - 10, followed by reduced body weight gains (that were also observed at MD). After the dosing period, body weight gains were comparable among the groups; this resulted in a consistently lower average body weight in the HD group.

MATERNAL BODY WEIGHTS
Figure 1



Food Consumption

Absolute and relative food consumption values were significantly reduced during the entire dosing period (GD 7 - 18) at MD (9%) and HD (34%), and remained reduced through GD21 (6% and 25% at MD and HD, respectively).

Toxicokinetics (Maternal on GDs 7 and 17, Fetal on GD18)

MMF exposure was confirmed for 3 hours at LD and for at least 6 hours at MD and HD. Accumulation of MMF in the systemic circulation was not observed for the LD and MD groups; a slight accumulation was observed at HD. MMF plasma exposures increased in a slightly more-than-dose-proportional manner between LD and MD, and in an approximately dose-proportional manner between MD and HD. The half-life was 0.4 to 1.5 hr, relatively constant upon repeat dosing, and dose-independent. See the sponsor's summary Table 3, below. Assessed on day 18, MMF crossed the placental membrane into fetal blood circulation, with a fetal:maternal plasma ratio of 0.48 to 0.64. See the sponsor's Table 4, below.

Table 3. Toxicokinetic Parameter Estimates of MMF on Days 7 and 17 in Presumed Pregnant Sprague Dawley Rats Following a Daily Oral (Gavage) Administration.

Dose (mg/kg)	Study Day	AUCinf (hour*ng/mL)	AUClast[a] (hour*ng/mL)	Cmax (ng/mL)	Tmax (hour)	T1/2 (hour)
25	Day 7	4262	4228	4610	0.25	0.4
	Day 17	4849	4784	5043	0.25	0.5
100	Day 7	28836	28652	21167	0.5	0.8
	Day 17	29947	29925	27800	0.25	0.6
250	Day 7	73733	69020	40667	0.5	1.5
	Day 17	86521	85950	63100	0.5	0.8

[a] The last time point with detectable MMF concentration was 3 hours post-dosage for the 25 mg/kg dose group and 6 hours for the other two dose groups.

Table 4. Comparison of Mean Plasma Concentrations of MMF in Fetus and Dam at 30 Minutes Post Dosage.

Dose (mg/kg)	Fetal Mean Plasma Concentration (ng/mL)	Maternal Mean Plasma Concentration (ng/mL)	Concentration Ratio (Fetal:Maternal)
25	2438 ± 373	4380 ± 1696	0.56
100	13408 ± 8750	21067 ± 3436	0.64
250	30150 ± 11174	63100 ± 17952	0.48

Dosing Solution Analysis

There was no drug contamination of the vehicle. The formulation analyses results for concentration and homogeneity showed results that were within ±15% of nominal and homogenous (≤ 5% RSD).

Necropsy (GD21)

There were no necropsy observations.

Cesarean Section Data

Observations were based on 24 (96.0%), 25 (100%), 24 (96.0%), and 25 (100%) pregnant rats with one or more live fetuses in the control, LD, MD, and HD groups respectively. The litter averages for corpora lutea, implantations, and percent live male fetuses were similar among the groups. A very slight decrease in litter size and live fetuses at HD appeared to result from a slight increase in early resorptions and the number of dams with any resorptions (see excerpt from sponsor's Table 6, below). No dam had a litter consisting of only resorbed conceptuses, and there were no dead fetuses. All placentae appeared normal. Fetal weights (total, male, and female) were significantly reduced at HD, as compared to the control group values; see sponsor's Table 7, below.

TABLE 6 (PAGE 1): CAESAREAN-SECTIONING OBSERVATIONS - SUMMARY

DOSAGE GROUP DOSAGE (MG/KG/DAY)a		I 0 (VEHICLE)	II 25	III 100	IV 250
RATS TESTED	N	25	25	25	25
PREGNANT	N	24(96.0)	25(100.0)	24(96.0)	25(100.0)
RATS PREGNANT AND CAESAREAN-SECTIONED ON DAY 21 OF GESTATION	N	24	25	24	25
CORPORA LUTEA	MEAN±S.D.	15.4 ± 1.8	16.2 ± 2.7	15.2 ± 2.1	15.4 ± 2.4
IMPLANTATIONS	MEAN±S.D.	14.4 ± 2.5	14.8 ± 1.9	14.5 ± 2.5	14.3 ± 2.2
LITTER SIZES	MEAN±S.D.	14.1 ± 2.6	14.0 ± 2.9	14.0 ± 2.6	13.6 ± 2.2
LIVE FETUSES	N	339	349	337	340
	MEAN±S.D.	14.1 ± 2.6	14.0 ± 2.9	14.0 ± 2.6	13.6 ± 2.2
DEAD FETUSES	N	0	0	0	0
RESORPTIONS	MEAN±S.D.	0.3 ± 0.7	0.8 ± 1.7	0.4 ± 0.6	0.7 ± 0.7
EARLY RESORPTIONS	N	8	18	10	18
	MEAN±S.D.	0.3 ± 0.7	0.7 ± 1.7	0.4 ± 0.6	0.7 ± 0.7
LATE RESORPTIONS	N	0	2	1	0
	MEAN±S.D.	0.0 ± 0.0	0.1 ± 0.3	0.0 ± 0.2	0.0 ± 0.0
DAMS WITH ANY RESORPTIONS	N(%)	6(25.0)	9(36.0)	9(37.5)	14(56.0)
DAMS WITH ALL CONCEPTUSES RESORBED	N(%)	0(0.0)	0(0.0)	0(0.0)	0(0.0)
DAMS WITH VIABLE FETUSES	N(%)	24(100.0)	25(100.0)	24(100.0)	25(100.0)
PLACENTAE APPEARED NORMAL	N(%)	24(100.0)	25(100.0)	24(100.0)	25(100.0)

a. Dosage occurred on days 7 through 17 of gestation.

TABLE 7 (PAGE 1): LITTER OBSERVATIONS (CAESAREAN-DELIVERED FETUSES) - SUMMARY

DOSAGE GROUP DOSAGE (MG/KG/DAY)a		I 0 (VEHICLE)	II 25	III 100	IV 250
LITTERS WITH ONE OR MORE LIVE FETUSES	N	24	25	24	25
IMPLANTATIONS	MEAN±S.D.	14.4 ± 2.5	14.8 ± 1.9	14.5 ± 2.5	14.3 ± 2.2
LIVE FETUSES	N	339	349	337	340
	MEAN±S.D.	14.1 ± 2.6	14.0 ± 2.9	14.0 ± 2.6	13.6 ± 2.2
LIVE MALE FETUSES	N	158	174	171	177
% LIVE MALE FETUSES/LITTER	MEAN±S.D.	45.5 ± 15.1	50.0 ± 12.6	51.6 ± 14.5	51.9 ± 14.2
LIVE FETAL BODY WEIGHTS (GRAMS)/LITTER	MEAN±S.D.	5.45 ± 0.25	5.54 ± 0.30	5.50 ± 0.33	5.00 ± 0.34**
MALE FETUSES	MEAN±S.D.	5.59 ± 0.30 [23]b	5.69 ± 0.31	5.64 ± 0.35	5.13 ± 0.34**
FEMALE FETUSES	MEAN±S.D.	5.34 ± 0.24	5.40 ± 0.32	5.36 ± 0.32	4.87 ± 0.35**
% RESORBED CONCEPTUSES/LITTER	MEAN±S.D.	2.3 ± 4.9	6.0 ± 13.8	3.2 ± 4.6	4.9 ± 5.1

[ ] = NUMBER OF VALUES AVERAGED
a. Dosage occurred on days 7 through 17 of gestation.
b. Litter 8713 had no male fetuses.
** Significantly different from the vehicle control group value ($p \leq 0.01$).

Offspring (Malformations, Variations, etc.)

Each fetus was weighed and examined for sex and extenal lesions. Fetal evaluations were based on 339, 349, 337, and 340 live fetuses in 24, 25, 24 and 25 litters in the control, LD, MD, and HD groups, respectively. Of these respective fetuses, 163, 167, 162, and 163 fetuses, respectively, were examined for soft tissue alterations (and the heads of these fetuses were subsequently examined by free-hand sectioning), and 176, 182, 175, and 177 fetuses, respectively, were examined for skeletal alterations and fetal

ossification site averages. There were no clearly drug-related malformations; however, the overall numbers of both fetuses and litters with alterations were significantly increased in the HD group. The statistically significant increase in alterations did not result from an increased incidence of any particular alteration; the sponsor attributed the increase in alterations to developmental delays, related to the reduction in fetal weight. HD fetus 8800-8 exhibited a thread-like and short tail, only four lumbar and no ossified sacral or caudal vertebrae, and a number of other skeletal alterations (including small ossification site below the 4th lumbar vertebra). Another HD fetus 8794-4 had a folded retina. HD fetus (8776-3) had a hole in the parietal bone of the skull (the sponsor considered this a variation). Variation "cervical rib present at the 7th cervical vertebrae" was increased in treated groups, particularly at HD. See sponsor's Table 11 for details. While skeletal ossification findings were generally comparable among groups (see sponsor's Table 12; however, note that these values did not include data from fetus 8800-8), statistically significant reductions in the numbers of ossified metatarsals and hindlimb phalanges were observed. Inclusion of HD fetus 8800-8 would have resulted in reductions of the numbers of ossified lumbar, sacral and caudal vertebrae as well.

TABLE 8 (PAGE 1): FETAL ALTERATIONS - SUMMARY

DOSAGE GROUP		I	II	III	IV
DOSAGE (MG/KG/DAY)a		0 (VEHICLE)	25	100	250
LITTERS EVALUATED	N	24	25	24	25
FETUSES EVALUATED	N	339	349	337	340
LIVE	N	339	349	337	340
LITTERS WITH FETUSES WITH ANY ALTERATION OBSERVED	N(%)	4(16.7)	3(12.0)	1(4.2)	10(40.0)**
FETUSES WITH ANY ALTERATION OBSERVED	N(%)	4(1.2)	3(0.8)	1(0.3)	12(3.5)**
% FETUSES WITH ANY ALTERATION/LITTER	MEAN±S.D.	1.1 ± 2.6	1.5 ± 5.2	0.2 ± 1.3	3.8 ± 5.4

a. Dosage occurred on days 7 through 17 of gestation.
** Significantly different from the vehicle control group value ($p \leq 0.01$).

TABLE 11 (PAGE 1): FETAL SKELETAL ALTERATIONS - SUMMARY
(See footnotes on the last page of this table.)

DOSAGE GROUP		I	II	III	IV
DOSAGE (MG/KG/DAY)a		0 (VEHICLE)	25	100	250
LITTERS EVALUATED	N	24	25	24	25
FETUSES EVALUATED	N	176	182	175	177
LIVE	N	176	182	175	177
SKULL: PARIETAL, CONTAINS A HOLE					
LITTER INCIDENCE	N(%)	0(0.0)	0(0.0)	0(0.0)	1(4.0)
FETAL INCIDENCE	N(%)	0(0.0)	0(0.0)	0(0.0)	1(0.6)
CERVICAL VERTEBRAE: CERVICAL RIB PRESENT AT 7TH CERVICAL VERTEBRAE					
LITTER INCIDENCE	N(%)	0(0.0)	2(8.0)	1(4.2)	3(12.0)
FETAL INCIDENCE	N(%)	0(0.0)	2(1.1)	1(0.6)	4(2.2)
THORACIC VERTEBRAE: CENTRUM, BIFID					
LITTER INCIDENCE	N(%)	3(12.5)	1(4.0)	0(0.0)	2(8.0)
FETAL INCIDENCE	N(%)	3(1.7)	1(0.5)	0(0.0)	2(1.1)
LUMBAR VERTEBRAE: CENTRUM, BIFID					
LITTER INCIDENCE	N(%)	0(0.0)	0(0.0)	0(0.0)	1(4.0)
FETAL INCIDENCE	N(%)	0(0.0)	0(0.0)	0(0.0)	1(0.6)b
LUMBAR VERTEBRAE: SMALL OSSIFICATION SITE					
LITTER INCIDENCE	N(%)	0(0.0)	0(0.0)	0(0.0)	1(4.0)
FETAL INCIDENCE	N(%)	0(0.0)	0(0.0)	0(0.0)	1(0.6)b
LUMBAR VERTEBRAE: 4 PRESENT					
LITTER INCIDENCE	N(%)	0(0.0)	0(0.0)	0(0.0)	1(4.0)
FETAL INCIDENCE	N(%)	0(0.0)	0(0.0)	0(0.0)	1(0.6)b
SACRAL VERTEBRAE: 0 PRESENT					
LITTER INCIDENCE	N(%)	0(0.0)	0(0.0)	0(0.0)	1(4.0)
FETAL INCIDENCE	N(%)	0(0.0)	0(0.0)	0(0.0)	1(0.6)b
CAUDAL VERTEBRAE: 0 PRESENT					
LITTER INCIDENCE	N(%)	0(0.0)	0(0.0)	0(0.0)	1(4.0)
FETAL INCIDENCE	N(%)	0(0.0)	0(0.0)	0(0.0)	1(0.6)b
RIBS: SHORT					
LITTER INCIDENCE	N(%)	0(0.0)	0(0.0)	0(0.0)	1(4.0)
FETAL INCIDENCE	N(%)	0(0.0)	0(0.0)	0(0.0)	1(0.6)
STERNAL CENTRA: ASYMMETRIC					
LITTER INCIDENCE	N(%)	0(0.0)	0(0.0)	0(0.0)	1(4.0)
FETAL INCIDENCE	N(%)	0(0.0)	0(0.0)	0(0.0)	1(0.6)
STERNAL CENTRA: INCOMPLETELY OSSIFIED					
LITTER INCIDENCE	N(%)	0(0.0)	0(0.0)	0(0.0)	1(4.0)
FETAL INCIDENCE	N(%)	0(0.0)	0(0.0)	0(0.0)	1(0.6)
PELVIS: ILIUM, CLOSE SET					
LITTER INCIDENCE	N(%)	0(0.0)	0(0.0)	0(0.0)	1(4.0)
FETAL INCIDENCE	N(%)	0(0.0)	0(0.0)	0(0.0)	1(0.6)b
PELVIS: ISCHIUM, CLOSE SET					
LITTER INCIDENCE	N(%)	0(0.0)	0(0.0)	0(0.0)	1(4.0)
FETAL INCIDENCE	N(%)	0(0.0)	0(0.0)	0(0.0)	1(0.6)b
PELVIS: PUBIS, CLOSE SET					
LITTER INCIDENCE	N(%)	0(0.0)	0(0.0)	0(0.0)	1(4.0)
FETAL INCIDENCE	N(%)	0(0.0)	0(0.0)	0(0.0)	1(0.6)b

a. Dosage occurred on days 7 through 17 of gestation.
b. Fetus 8800-8 had other skeletal alterations.

TABLE 12 (PAGE 1): FETAL OSSIFICATION SITES - CAESAREAN-DELIVERED LIVE FETUSES (DAY 21 OF GESTATION) - SUMMARY

DOSAGE GROUP		I	II	III	IV
DOSAGE (MG/KG/DAY) a		0 (VEHICLE)	25	100	250
LITTERS EXAMINED	N	24	25	24	25
FETUSES EXAMINED	N	176	182	175	177
OSSIFICATION SITES PER FETUS PER LITTER					
HYOID	MEAN±S.D.	0.99 ± 0.09	1.00 ± 0.00	0.99 ± 0.04	0.99 ± 0.04
VERTEBRAE					
CERVICAL	MEAN±S.D.	7.00 ± 0.00	7.00 ± 0.00	7.00 ± 0.00	7.00 ± 0.00
THORACIC	MEAN±S.D.	13.03 ± 0.09	13.02 ± 0.05	13.10 ± 0.17	13.09 ± 0.16
LUMBAR	MEAN±S.D.	5.97 ± 0.09	5.97 ± 0.07	5.89 ± 0.18	5.90 ± 0.17
SACRAL	MEAN±S.D.	3.00 ± 0.00	3.00 ± 0.00	3.00 ± 0.00	3.00 ± 0.00
CAUDAL	MEAN±S.D.	7.60 ± 0.90	7.62 ± 0.79	8.06 ± 0.86	7.70 ± 0.67
RIBS (PAIRS)	MEAN±S.D.	13.02 ± 0.07	13.02 ± 0.04	13.09 ± 0.15	13.07 ± 0.13
STERNUM					
MANUBRIUM	MEAN±S.D.	1.00 ± 0.00	1.00 ± 0.00	1.00 ± 0.00	1.00 ± 0.00
STERNAL CENTERS	MEAN±S.D.	3.99 ± 0.03	4.00 ± 0.00	3.99 ± 0.04	3.97 ± 0.07
XIPHOID	MEAN±S.D.	1.00 ± 0.00	1.00 ± 0.00	1.00 ± 0.00	1.00 ± 0.00
FORELIMB b					
CARPALS	MEAN±S.D.	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
METACARPALS	MEAN±S.D.	4.00 ± 0.02	4.00 ± 0.00	4.00 ± 0.00	4.00 ± 0.00
DIGITS	MEAN±S.D.	5.00 ± 0.00	5.00 ± 0.00	5.00 ± 0.00	5.00 ± 0.00
PHALANGES	MEAN±S.D.	8.32 ± 0.53	8.27 ± 0.80	8.35 ± 0.46	7.92 ± 0.72
HINDLIMB b					
TARSALS	MEAN±S.D.	0.02 ± 0.05	0.03 ± 0.07	0.02 ± 0.10	0.00 ± 0.00
METATARSALS	MEAN±S.D.	4.87 ± 0.17	4.85 ± 0.20	4.83 ± 0.20	4.43 ± 0.30**
DIGITS	MEAN±S.D.	5.00 ± 0.00	5.00 ± 0.00	5.00 ± 0.00	5.00 ± 0.00
PHALANGES	MEAN±S.D.	6.32 ± 1.03	6.46 ± 1.02	6.36 ± 1.06	5.57 ± 0.82*

a. Dosage occurred on days 7 through 17 of gestation.
b. Calculated as average per limb.
* Significantly different from the vehicle control group value ($p \leq 0.05$).
** Significantly different from the vehicle control group value ($p \leq 0.01$).

9.3 Prenatal and Postnatal Development

Study title: BG00012: Oral (Gavage) Developmental and Perinatal/Postnatal Reproduction Toxicity Study of BG00012 in Rats, Including a Postnatal Behavioral/Functional Evaluation

Study no.:	P00012-09-02, (b) (4) No. EBA00286
Conducting laboratory and location:	(b) (4)
Date of study initiation:	11/24/09
GLP compliance:	Yes, pg. 12 (except CoA and bioanalysis)
QA statement:	Yes, pg. 13-15
Drug, lot #, and % purity:	BG00012, lot 1427169 21309928), 100.2% (MMF 0.02%)

Methods (See details in the sponsor's tables, below)

Frequency of dosing:	1x/day, GD7 to LD20
Route of administration:	Oral gavage
Formulation/Vehicle:	Hypromellose 0.8% in R.O. deionized water
Species/Strain:	F Rat/Crl:CD(SD), (b) (4)
Deviation from study protocol:	Deviations noted included late clinical observation times, lack of recorded feed lot numbers on several occasions, and discrepancies in the amount of milk sampled.

4.5.1. F0 Generation Rats

Dosage Group	Dosage[a] (mg/kg/day)	Concentration (mg/mL)	Dosage Volume (mL/kg)	Number of Rats	Assigned F0 Generation Rat Numbers
I	0 (Vehicle)	0	10	25	1901 - 1925
II	25	2.5	10	25	1926 - 1950
III	100	10	10	25	1951 - 1975
IV	250	25	10	25	1976 - 2000

a. The test article was considered 100% active/pure for the purpose of dosage calculations.

4.5.2. F1 Generation Rats

Dosage Group	Maternal Dosage (mg/kg/day)	Number of Rats Per Sex	Assigned F1 Generation Rat Numbers	
			Male Rats	Female Rats
I	0 (Vehicle)	25	301 - 325	401 - 425
II	25	25	326 - 350	426 - 450
III	100	25	351 - 375	451 - 475
IV	250	25	376 - 400	476 - 500

Observations and Results

F_0 Dams

Survival:

There were no mortalities.

Clinical signs:

During the dosing period at HD, the number of rats with slight excess salivation, mild dehydration and rales (presumed gestation period only) was significantly increased [ss]. Additionally, the number of rats with urine-stained abdominal fur was increased at HD (lactation period only; [nss]).

Body weight:

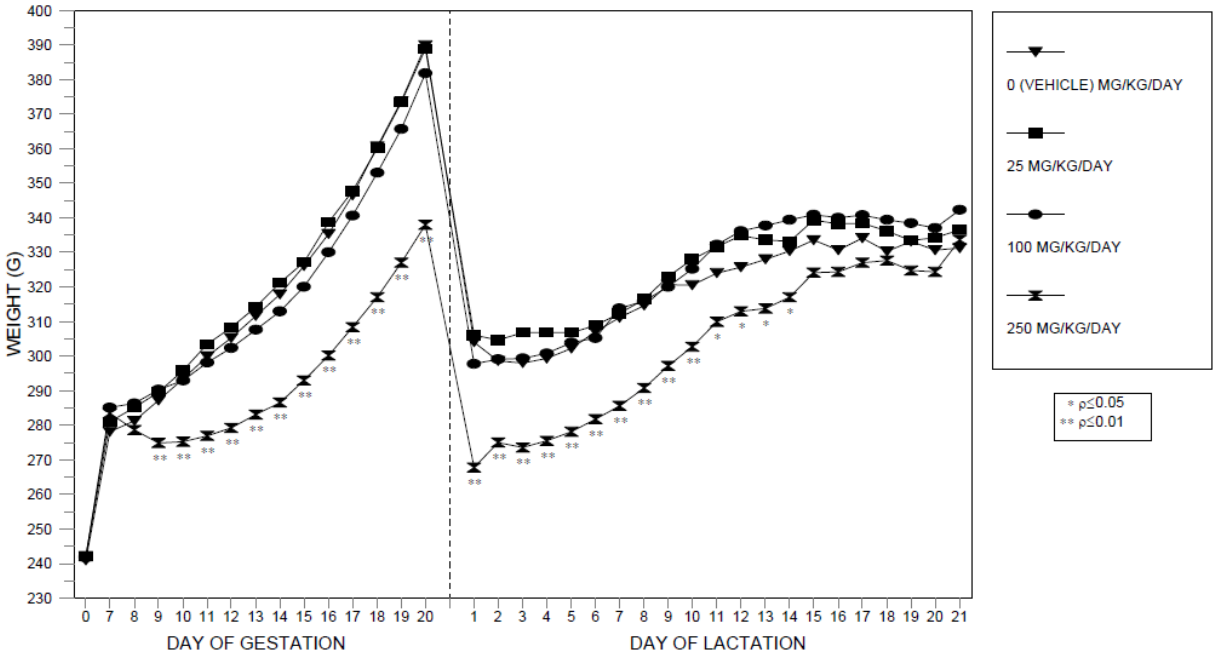
Average body weight during gestation was significantly reduced in the HD group (up to ~13% lower than controls; GD9 - GD 20, [ss]); likewise, body weight gains were also significantly reduced (with body weight loss occurring on GD7 to GD10) at HD at each interval and for the entire gestation dosing period (calculated as GD7 to GD20). At MD, a significant decrease in average body weight was observed from GD7 to GD10 (the first three days of dosing), and the overall body weight gain (from GD7 to GD20) was

significantly reduced, although this likely reflected the reduction from GD7 to GD10. See sponsor's Figure 1, below.

During the lactation period, average body weights continued to be significantly reduced from LD0 to LD14 at HD (~5% reduction). However, body weight gains were significantly increased from LD1 to LD4, and over the entire lactation period (LD1 to LD21) in the MD and HD groups.

MATERNAL BODY WEIGHTS - F0 GENERATION FEMALE RATS

Figure 1



Food consumption:

At MD and HD, absolute and relative food consumption was significantly reduced during the gestation period (on average ~30% lower than control; GD 7 to 10 and GD10 to 12, as well as GD7 to 20 at MD, and at all measured intervals GD7 to GD20 at HD). There was no effect at LD.

During the lactation period, absolute and relative food consumption was not clearly affected.

Uterine content:

Pregnancy occurred in 23, 25, 24, and 25 rats in the control, LD, MD, and HD groups, respectively. All pregnant dams delivered litters. See sponsor's Table A11. Pup weights in the HD group were reduced from PND1 to PND21 [ss]. The average duration of gestation was very slightly (but [ss]) increased (see sponsor's Table A11). The viability index was slightly decreased at HD (see sponsor's Table A12).

TABLE A11 (PAGE 1): NATURAL DELIVERY OBSERVATIONS - SUMMARY - F0 GENERATION FEMALE RATS

DOSAGE GROUP DOSAGE (MG/KG/DAY)a		I 0 (VEHICLE)	II 25	III 100	IV 250
RATS ASSIGNED TO NATURAL DELIVERY	N	25	25	25	25
PREGNANT	N	23	25	24	25
DELIVERED LITTERS	N(%)	23(100.0)	25(100.0)	24(100.0)	25(100.0)
DURATION OF GESTATION b	MEAN±S.D.	22.3 ± 0.5	22.7 ± 0.4*	22.7 ± 0.5*	22.9 ± 0.5**
IMPLANTATION SITES PER DELIVERED LITTER	N MEAN±S.D.	329 14.3 ± 1.6	363 14.5 ± 2.1	342 14.2 ± 1.6	362 14.5 ± 1.6
DAMS WITH STILLBORN PUPS	N(%)	2(8.7)	4(16.0)	0(0.0)	2(8.0)
DAMS WITH NO LIVEBORN PUPS	N	0	0	0	0
GESTATION INDEX c	% N/N	100.0 23/ 23	100.0 25/ 25	100.0 24/ 24	100.0 25/ 25
DAMS WITH ALL PUPS DYING DAYS 1-4 POSTPARTUM	N(%)	0(0.0)	0(0.0)	0(0.0)	0(0.0)
DAMS WITH ALL PUPS DYING DAYS 5-21 POSTPARTUM	N(%)	0(0.0)	0(0.0)	0(0.0)	0(0.0)

a. Dosage occurred on day 7 of gestation through day 20 postpartum.
b. Calculated (in days) as the time elapsed between confirmed mating (arbitrarily defined as day 0 of gestation) and the day the first pup was delivered.
c. Number of rats with live offspring/number of pregnant rats.
* Significantly different from the vehicle control group value (p≤0.05).
** Significantly different from the vehicle control group value (p≤0.01).

TABLE A12 (PAGE 1): LITTER OBSERVATIONS (NATURALLY DELIVERED PUPS) - SUMMARY - F1 GENERATION LITTERS

DOSAGE GROUP DOSAGE (MG/KG/DAY)a		I 0 (VEHICLE)	II 25	III 100	IV 250
DELIVERED LITTERS WITH ONE OR MORE LIVEBORN PUPS	N	23	25	24	25
PUPS DELIVERED (TOTAL)	N MEAN±S.D.	313 13.6 ± 1.7	332 13.3 ± 2.1	317 13.2 ± 2.6	335 13.4 ± 1.8
LIVEBORN	MEAN±S.D. N(%)	13.5 ± 1.7 310(99.0)	12.9 ± 2.3 323(97.3)**	13.2 ± 2.6 317(100.0)	13.2 ± 2.0 331(98.8)
STILLBORN	MEAN±S.D. N(%)	0.1 ± 0.4 3(1.0)	0.4 ± 1.0 9(2.7)**	0.0 ± 0.0 0(0.0)	0.1 ± 0.4 3(0.9)
UNKNOWN VITAL STATUS b	N	0	0	0	1
UNSCHEDULED SACRIFICE	N	1	0	0	0
SACRIFICED FOR TERMINAL BLOOD COLLECTION	N	10	10	10	10
PUPS FOUND DEAD, PRESUMED CANNIBALIZED OR UNSCHEDULED SACRIFICED					
DAY 1	N/N(%)	2/310(0.6)	0/323(0.0)	1/317(0.3)	2/331(0.6)
DAYS 2- 4	N/N(%)	5/308(1.6)	3/323(0.9)	6/316(1.9)	11/329(3.3)
DAYS 5- 7	N/N(%)	1/303(0.3)	0/320(0.0)	1/310(0.3)	2/318(0.6)
DAYS 8-14c	N/N(%)	1/302(0.3)	0/320(0.0)	2/309(0.6)	1/316(0.3)
DAYS 15-18	N/N(%)	0/291(0.0)	0/310(0.0)	0/297(0.0)	1/305(0.3)
DAYS 19-21	N/N(%)	0/291(0.0)	0/310(0.0)	0/297(0.0)	0/304(0.0)
VIABILITY INDEX d	% N/N	97.7 303/310	99.1 320/323	97.8 310/317	96.1 318/331
LACTATION INDEX e	% N/N	95.7 291/303	96.9 310/320	95.8 297/310	95.6 304/318

DAY(S) = DAY(S) POSTPARTUM
a. Dosage occurred on day 7 of gestation through day 20 postpartum.
b. Degree of cannibalization precluded identification of vital status at birth.
c. On day 14 postpartum, five female and five male pups per dosage group were sacrificed for terminal blood collection.
d. Number of live pups on day 4 postpartum/number of liveborn pups on day 1 postpartum.
e. Number of live pups on day 21 (weaning) postpartum/number of live pups on day 4 postpartum.

Necropsy observation:

No drug-related observations were recorded at LD in dams. At MD and HD, stomach lesions consisting of thickened walls in the stomach and/or white walls and/or raised white areas were significantly increased (see sponsor's table A2, below). Also, a

significantly increased number of HD dams had pink areas on the mucosal surface in the cardiac region of the stomach. Observations of red areas on the mucosal surface in the cardiac region of the stomach in 1 MD dam and a white mass on the serosal surface of the fundic or cardiac region of the stomach in 2 HD dams were observed.

TABLE A2 (PAGE 1): NECROPSY OBSERVATIONS - SUMMARY - F0 GENERATION FEMALE RATS

DOSAGE GROUP		I	II	III	IV
DOSAGE (MG/KG/DAY)a		0 (VEHICLE)	25	100	250
RATS EXAMINED b	N	25	25	25	25
MORTALITY	N	0	0	0	0
APPEARED NORMAL	N	24	25	5**	1**
STOMACH:					
WALLS, THICK; CARDIAC REGION, WALLS THICK OR CARDIAC REGION, MUCOSAL SURFACE, WALLS THICK	N	0	0	18**	16**
WALLS, WHITE; CARDIAC REGION, WALLS, WHITE; CARDIAC REGION, MUCOSAL SURFACE, WALLS, WHITE; CARDIAC REGION, MUCOSAL SURFACE, WHITE AREA(S) OR CARDIAC REGION, MUCOSAL SURFACE, WHITE RAISED AREA(S)	N	0	0	16*	24**
CARDIAC REGION, MUCOSAL SURFACE, RED AREAS	N	0	0	1	0
FUNDIC REGION, SEROSAL SURFACE, MASS OR CARDIAC REGION, SEROSAL SURFACE, WHITE MASS	N	0	0	0	2
CARDIAC REGION, MUCOSAL SURFACE, PINK AREA	N	0	0	0	3**
UTERUS:					
RIGHT HORN, FLUID-FILLED CYST	N	1	0	0	0

a. Dosage occurred on day 7 of presumed gestation through day 20 postpartum or day 24 of presumed gestation (rats that did not deliver a litter).
b. Refer to the individual clinical observations table (Table A15) for external observations confirmed at necropsy.
* Significantly different from the vehicle control group value (p≤0.05).
** Significantly different from the vehicle control group value (p≤0.01).

Toxicokinetics:

There were issues with the plasma analysis for MMF; the internal standard was flawed, the method of calculation used was not validated, and the raw data and report were not QAU audited. Notably, the control dams showed MMF levels similar to (and sometimes higher than) the LD dams. The pooled data for pup plasma levels of MMF were BLOQ for all groups. The sponsor indicated that the control dams appeared to have been mis-dosed, but argued that this only occurred on one day (based on their reviews of the dosing times for the F_0 dams, the test article analyses, and the results of the study).

Dosing Solution Analysis

Samples from the dose formulations used on the first and last days of dosing were analyzed for BG00012 concentration by high-performance liquid chromatography with ultraviolet detection (HPLC-UV). The formulations were within ±15% of nominal, and the homogeneity was acceptable (≤5% relative standard deviation).

F_1 Generation

Survival:

The viability index was slightly reduced at HD (see sponsor's Table A12 above). At birth, there were 3, 9, 0, and 3 stillbirths in the control, LD, MD, and HD groups,

respectively. There was one unscheduled sacrifice in the control group, and one HD pup was cannibalized. From PND1 to PND21, there were additional pup deaths in each group but slightly more at HD (totals of 9, 3, 10, and 17 in the control, LD, MD, and HD groups, respectively; see sponsor's Table A12 for details).

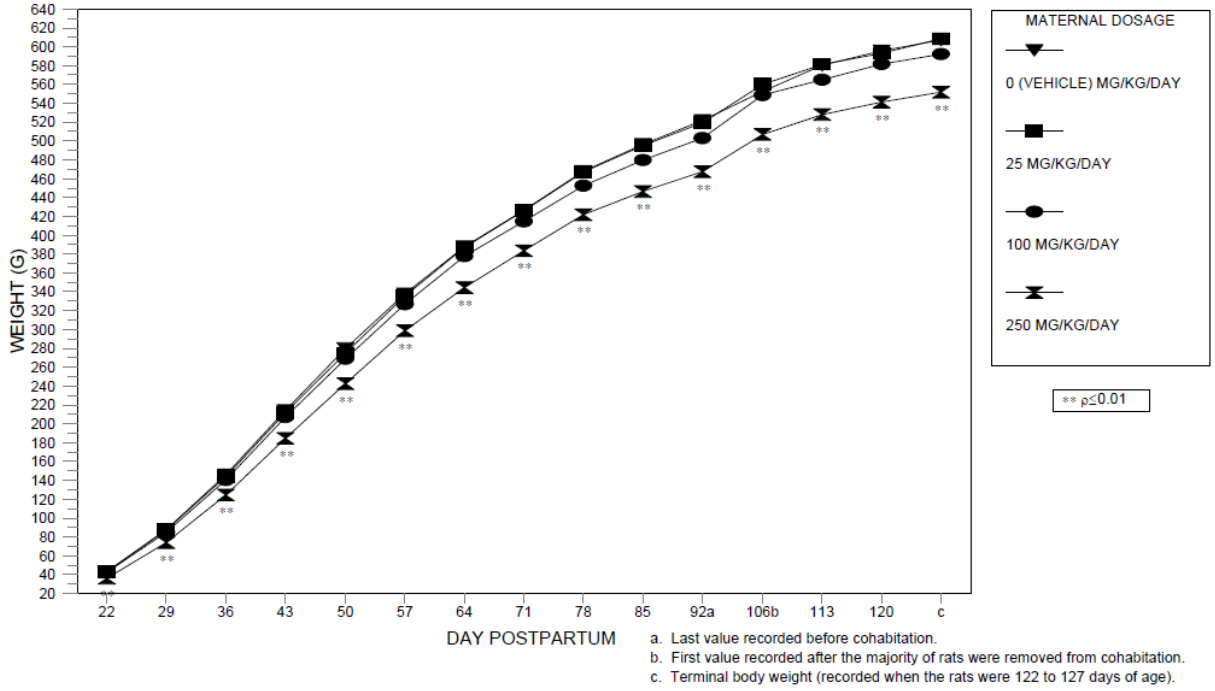
Post-weaning, 1, 0, 2, and 1 male rats in the control, LD, MD, and HD groups did not survive to scheduled sacrifice. These deaths were attributed to "failure to thrive." One control male pup was found dead on PND 25, after exhibiting adverse clinical signs. Two MD male pups (354 and 356) were found dead on PND 26 and 27; these pups did show exhibit adverse clinical signs but were of comparable weight to other pups in the group, and either did not show abnormalities at necropsy or revealed the gas-distended intestines. HD male pup (383) was found dead on PND 95; this male showed a number of adverse clinical signs starting PND 85 that lead up to death (i.e., dyspnea, substance in the cage pan and torn toe nails on PND 85, hyperactivity on PNDs 85 and 87, rough hair coat on PNDs 85, 87, and 93, dehydration on PNDs 85, 87, 89, 92, and 93, teeth chattering on PNDs 85 and 89, gasping on PNDs 85 and 92, piloerection on PNDs 85 and 93, hyperpnea and vocalization on PND 87, scant feces on PNDs 87 and 93, excess salivation on PNDs 87, 89, and 92, and thin body condition, chromorhinorrhea and ungroomed coat on PND 93). No cause of death was determined; its weight was similar to that of other in the group, and all tissues appeared normal at necropsy, although there was a moderate degree of autolysis.

Clinical signs:
At birth, the number of pups that were cold to touch was significantly increased at HD compared to controls. The sponsor reported no other drug-related clinical observations in the F1 generation pups. Also, no necropsy observations in the F1 generation pups were attributed to drug. No milk in the stomach occurred in 2, 1, 4 and 0 found dead pups in the control, LD, MD, and HD groups, respectively; this finding resulted in a significant reduction in the number of MD pups that appeared normal at necropsy.

Body weight:
In F1 males, average body weights were significantly reduced (~10-15%) from weaning to PND 120 at HD. See sponsor's Figure 2, below. These pups were smaller pre-weaning, and body weight gains were initially reduced during the post-weaning period (PND 22 to 50). However, after this interval, body weight gains were comparable to control pups for the rest of the post-weaning period up to pre-cohabitation. The average body weights and body weight gains of LD and males appeared dose-related (with little/no effect at LD).

BODY WEIGHTS - F1 GENERATION MALE RATS

Figure 2

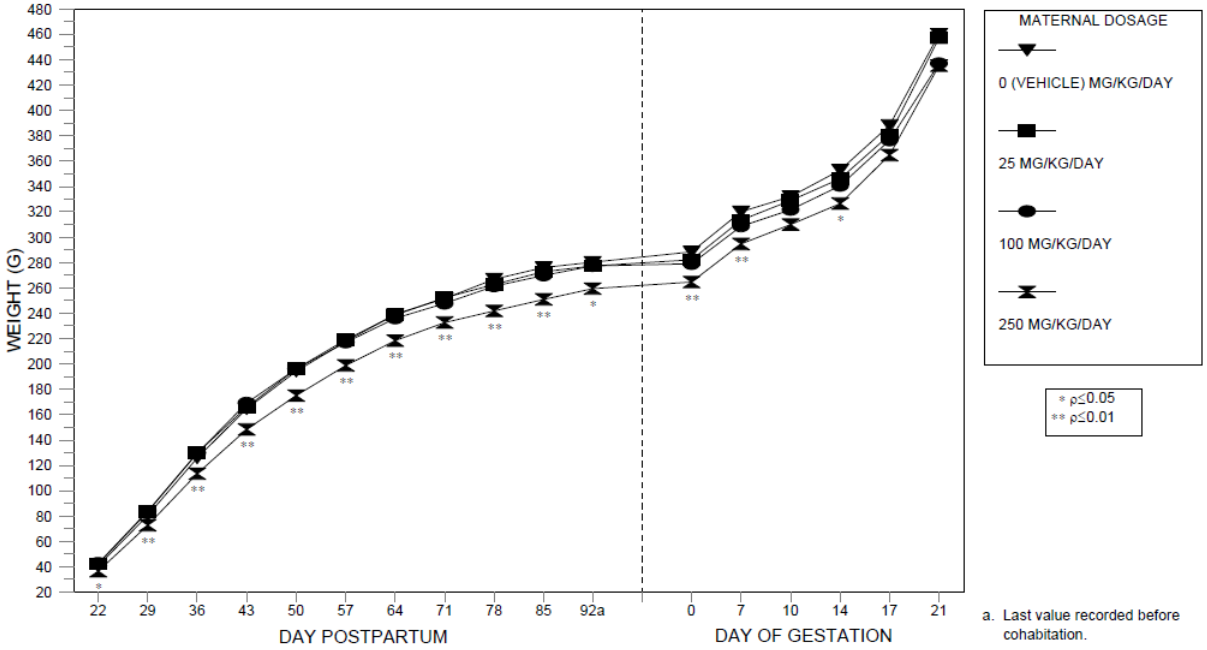


a. Last value recorded before cohabitation.
b. First value recorded after the majority of rats were removed from cohabitation.
c. Terminal body weight (recorded when the rats were 122 to 127 days of age).

Body weights for F1 females were significantly reduced (~10%) from weaning to PND 92, and also at pre-cohabitation at HD. See sponsor's Figure 3, below. These pups were smaller pre-weaning, and body weight gains were initially reduced in the post-weaning period from PND 22 to 43. Generally, following this post-weaning interval, body weight gains were comparable to control pups. Overall body weight gains (PND 22 to pre-cohabitation) were not altered. During the F_1 gestation period, average body weights in HD group F_1 dams were significantly reduced on GDs 0, 7, and 14 compared to the F_1 controls (~5-10%). Body weight gains did not differ among the groups during gestation. Average body weights and body weight gains of the F_1 females were unaffected at LD or MD; the F_1 gestation body weights appeared dose-related (with little/no change at LD).

BODY WEIGHTS - F1 GENERATION FEMALE RATS

Figure 3



Food consumption:

Absolute, but not relative, food consumption (reflecting the lower body weights at weaning), was significantly reduced (~10-15%) in the HD F_1 male and female groups for the majority of the weekly intervals evaluated. The effect appeared dose-related, with little/no effect at LD.

Physical development:

Terminal body weights were significantly reduced in the HD F_1 group compared to the control F_1 group; generally, the weight reduction appeared dose-related, with a small effect at MD and little/no effect at LD. Reflecting the lowered terminal body weight and generally lower body weights from weaning, the paired testes absolute weight was significantly reduced (~10%) in the HD F_1 group compared to the control F_1 group. See sponsor's Table B3, below. Epididymal weights did were not affected.

TABLE B3 (PAGE 1): TERMINAL BODY WEIGHTS AND ORGAN WEIGHTS - SUMMARY - F1 GENERATION MALE RATS

MATERNAL DOSAGE GROUP		I	II	III	IV
MATERNAL DOSAGE (MG/KG/DAY)		0 (VEHICLE)	25	100	250
RATS TESTED	N	24	25	23	24
TERMINAL BODY WEIGHT	MEAN±S.D.	607.0 ± 77.7	608.4 ± 66.4	592.3 ± 66.2	551.7 ± 61.4**
EPIDIDYMIDES PAIRED (G)	MEAN±S.D.	1.53 ± 0.12	1.51 ± 0.13	1.57 ± 0.13	1.48 ± 0.15
EPIDIDYMIDES PAIRED (%)	MEAN±S.D.	0.253 ± 0.029	0.251 ± 0.033	0.266 ± 0.022	0.269 ± 0.031
TESTES PAIRED (G)	MEAN±S.D.	3.71 ± 0.37	3.58 ± 0.25	3.64 ± 0.28	3.36 ± 0.36**
TESTES PAIRED (%)	MEAN±S.D.	0.617 ± 0.074	0.594 ± 0.072	0.618 ± 0.064	0.613 ± 0.076

ALL WEIGHTS WERE RECORDED IN GRAMS (G). RATIOS (%) = (ORGAN WEIGHT/TERMINAL BODY WEIGHT) X 100.
** Significantly different from the vehicle control group value (p<=0.01).

Reflecting the significantly lower body weights for the F_1 generation male and female rats at weaning, the average day that sexual maturation was achieved was slightly delayed (see sponsor's Table B18, below). In males, the day of preputial separation was significantly increased in the HD group (a maternal dose-related effect is suggested at MD and HD). Vaginal patency also was very slightly delayed in the HD group. Notably, average body weights on the day that sexual maturation was achieved in the HD groups were significantly lower than those of the control groups.

TABLE B18 (PAGE 1): SEXUAL MATURATION - SUMMARY - F1 GENERATION RATS

MATERNAL DOSAGE GROUP		I	II	III	IV
MATERNAL DOSAGE (MG/KG/DAY)		0 (VEHICLE)	25	100	250
MALE RATS	N	25	25	25	25
PREPUTIAL SEPARATION a	MEAN±S.D.	47.1 ± 2.6	47.1 ± 2.7	47.9 ± 2.7	49.7 ± 3.3*
BODY WEIGHT AT SEPARATION (G)b	MEAN±S.D.	251.6 ± 24.0	250.3 ± 28.6	256.4 ± 32.9	240.1 ± 26.7
FEMALE RATS	N	25	24	25	25
VAGINAL PATENCY c	MEAN±S.D.	32.6 ± 1.7	32.2 ± 1.4	32.6 ± 1.7	33.0 ± 2.3
BODY WEIGHT AT VAGINAL PATENCY (G)d	MEAN±S.D.	102.1 ± 10.8	104.3 ± 12.0	106.4 ± 11.7	94.9 ± 12.7*

a. Average day postpartum that the prepuce was observed to be separated.
b. Average body weight on day prepuce was first observed to be separated.
c. Average day postpartum that the vagina was observed to be patent.
d. Average body weight on day vagina was first observed to be patent.
* Significantly different from the vehicle control group value (p<=0.05).

Neurological assessment:

There were no clear drug-related differences in the performance of the passive avoidance task in the F1 pups. See sponsor's Table B19, below.

TABLE B19 (PAGE 1): PASSIVE AVOIDANCE - SUMMARY - F1 GENERATION RATS

MATERNAL DOSAGE GROUP		I	II	III	IV
MATERNAL DOSAGE (MG/KG/DAY)		0 (VEHICLE)	25	100	250
MALE RATS					
SESSION 1a	N	23	25	24	25
TRIALS TO CRITERION	MEAN ± S.D.	4.57 ± 1.59	4.12 ± 1.59	4.67 ± 1.86	4.20 ± 0.91
LATENCY TRIAL 1b	MEAN ± S.D.	7.43 ± 5.26	6.68 ± 4.69	7.13 ± 4.70	6.48 ± 3.78
LATENCY TRIAL 2b	MEAN ± S.D.	27.83 ± 23.12	23.52 ± 20.75	32.63 ± 23.86	32.40 ± 24.62
FAILED TO LEARN c	MEAN ± S.D.	0(0.0)	0(0.0)	0(0.0)	0(0.0)
SESSION 2a	N	22d	25	22d	25
TRIALS TO CRITERION	MEAN ± S.D.	3.14 ± 0.94	3.60 ± 2.75	3.41 ± 2.68	2.72 ± 0.68
LATENCY TRIAL 1b	MEAN ± S.D.	27.23 ± 24.84	30.92 ± 24.07	28.55 ± 22.47	36.76 ± 23.71
FEMALE RATS					
SESSION 1a	N	23	24	24	25
TRIALS TO CRITERION	MEAN ± S.D.	4.43 ± 1.12	4.50 ± 1.38	4.67 ± 1.86	4.56 ± 1.08
LATENCY TRIAL 1b	MEAN ± S.D.	7.00 ± 7.05	9.13 ± 6.36	8.92 ± 9.17	10.04 ± 12.08
LATENCY TRIAL 2b	MEAN ± S.D.	32.96 ± 21.54	32.42 ± 22.25	29.29 ± 22.37	30.00 ± 23.17
FAILED TO LEARN c	MEAN ± S.D.	0(0.0)	0(0.0)	0(0.0)	0(0.0)
SESSION 2a	N	23	24	24	25
TRIALS TO CRITERION	MEAN ± S.D.	2.87 ± 0.55	3.25 ± 1.59	3.83 ± 2.51	2.80 ± 0.76
LATENCY TRIAL 1b	MEAN ± S.D.	28.57 ± 21.95	29.67 ± 22.46	25.92 ± 20.61	31.20 ± 23.32

a. Sessions 1 (Learning Phase) and 2 (Retention Phase) of testing were separated by a one-week interval.
b. The latency was recorded in seconds.
c. Number of rats that did not meet the criterion in Session 1 (Learning Phase); Session 2 (Retention Phase) values for these rats were excluded from summarization and statistical analyses.
d. Excludes values for rats that were found dead.

Performance in a modified, water-filled M-maze showed differences at MD and/or HD. Generally, treated animals tended to require slightly more trials to learn the task and made more errors in remembering the task; only MDF and HDM had animals fail to learn the task. In males, only the latencies during the first and second sessions were statistically significantly longer in the HD group compared to the control group. In females, the number of trials required to remember the task was significantly increased. The sponsor did not consider the increased number of trials required to meet criterion in session 2 in females to be toxicologically meaningful because the values were within the historical control range for this facility (and the average for the control group was at the low end of the historical control range; see the sponsor's historical control data).

TABLE B20 (PAGE 1): WATERMAZE PERFORMANCE - SUMMARY - F1 GENERATION RATS

MATERNAL DOSAGE GROUP		I	II	III	IV
MATERNAL DOSAGE (MG/KG/DAY)		0 (VEHICLE)	25	100	250
MALE RATS					
SESSION 1a	N	22	25	22	25
TRIALS TO CRITERION	MEAN±S.D.	9.0 ± 2.7	9.7 ± 3.0	10.6 ± 3.6	10.2 ± 2.8
ERRORS PER TRIAL	MEAN±S.D.	0.37 ± 0.20	0.40 ± 0.25	0.43 ± 0.18	0.47 ± 0.25
LATENCY TRIAL 2b	MEAN±S.D.	10.5 ± 7.2	12.6 ± 10.9	12.2 ± 6.7	19.0 ± 12.8**
FAILED TO LEARN c	N(%)	0(0.0)	3(12.0)	4(18.2)	2(8.0)
SESSION 2a	N	22	22	18	23
TRIALS TO CRITERION	MEAN±S.D.	6.2 ± 2.3	6.1 ± 2.4	7.1 ± 3.3	6.4 ± 2.0
ERRORS PER TRIAL	MEAN±S.D.	0.04 ± 0.10	0.11 ± 0.19	0.16 ± 0.17	0.11 ± 0.12
LATENCY TRIAL 1b	MEAN±S.D.	7.0 ± 3.8	8.6 ± 5.6	9.8 ± 5.8	12.9 ± 7.8**
FEMALE RATS					
SESSION 1a	N	23	24	24	25
TRIALS TO CRITERION	MEAN±S.D.	8.8 ± 2.5	9.2 ± 2.7	10.9 ± 3.2	10.1 ± 2.6
ERRORS PER TRIAL	MEAN±S.D.	0.41 ± 0.21	0.40 ± 0.18	0.47 ± 0.18	0.43 ± 0.16
LATENCY TRIAL 2b	MEAN±S.D.	14.8 ± 7.3	13.6 ± 8.6	13.1 ± 6.6	15.7 ± 9.6
FAILED TO LEARN c	N(%)	0(0.0)	0(0.0)	5(20.8)**	0(0.0)
SESSION 2a	N	23	24	19	25
TRIALS TO CRITERION	MEAN±S.D.	5.5 ± 1.5	6.4 ± 1.9*	7.4 ± 3.3**	7.6 ± 3.1**
ERRORS PER TRIAL	MEAN±S.D.	0.06 ± 0.14	0.15 ± 0.16	0.18 ± 0.18	0.17 ± 0.20
LATENCY TRIAL 1b	MEAN±S.D.	8.8 ± 5.7	12.9 ± 10.2	13.0 ± 8.6	11.6 ± 9.3

a. Sessions 1 (Learning Phase) and 2 (Retention Phase) of testing were separated by a one-week interval.
b. The latency was recorded in seconds.
c. Number of rats that did not meet the criterion in session 1 of testing (learning); session 2 (retention) values for these rats were excluded from group averages and statistical analyses.
* Significantly different from the vehicle control group value ($p<=0.05$).
** Significantly different from the vehicle control group value ($p<=0.01$).

WATERMAZE
HISTORICAL CONTROL DATA
Crl:CD(SD) RATS

CODE:
PROTOCOL:
TYPE OF STUDY: SUMMARY
ROUTE:
FINAL DRAFT REPORT MAIL DATE:

		AVERAGE	MINIMUM	MAXIMUM	NO. STUDIES INCLUDED
FEMALE RATS					
WATERMAZE LEARNING					
FEMALE RATS: SESSION 1	N	21.5	5	33	136
TOTAL TRIALS TO CRITERION	MEAN	8.8	7.0	11.2	136
ERRORS PER TRIAL	MEAN	0.40	0.23	0.54	136
LATENCY TRIAL 2	MEAN	14.6	9.5	20.1	136
FAILED TO LEARN	N	0.6	0	3	136
	%	2.6	0.0	15.8	136
WATERMAZE RETENTION					
FEMALE RATS: SESSION 2	N	21.0	5	30	136
TOTAL TRIALS TO CRITERION	MEAN	7.0	5.4	9.3	136
ERRORS PER TRIAL	MEAN	0.15	0.07	0.45	136
LATENCY TRIAL 1	MEAN	11.2	6.5	21.9	136

Reproduction:
There were no clear drug-related effects on the mating and fertility parameters evaluated in the F_1 males and females. See the sponsor's summary Tables B21 and B22, below. Values for the number of days in cohabitation, the number of rats that mated, the fertility index (number of pregnancies per number of rats that mated), the number of rats with confirmed mating dates during the first and second weeks of cohabitation, and the number of pregnancies per number of rats in cohabitation were comparable among the four dose groups. Pregnancy occurred as a result of mating in F_1 males in 18 (75%), 24 (100%), 18 (78.3%), and 21 (87.5%) of the control, LD, MD and HD groups, respectively. Pregnancy occurred in 20 (80.0%), 24 (100.0%), 20 (80.0%), and 22 (88.0%) of the F_1 females in the control, LD, MD and HD groups, respectively.

TABLE B21 (PAGE 1): MATING AND FERTILITY - SUMMARY - F1 GENERATION MALE RATS

MATERNAL DOSAGE GROUP		I	II	III	IV
MATERNAL DOSAGE (MG/KG/DAY)		0 (VEHICLE)	25	100	250
RATS IN COHABITATION	N	24a	24b	23a	24a
DAYS IN COHABITATION c,d	MEAN±S.D.	3.7 ± 2.7	2.4 ± 1.0	4.0 ± 2.9	3.0 ± 2.1
RATS THAT MATED d	N(%)	23(95.8)	24(100.0)	23(100.0)	24(100.0)
FERTILITY INDEX e,f	N/N	18/ 23	24/ 24	18/ 23	21/ 24
	(%)	(78.3)	(100.0)	(78.3)	(87.5)
RATS WITH CONFIRMED MATING DATES	N	23	24	23	24
MATED WITH FEMALE g					
DAYS 1-7	N(%)	22(95.6)	24(100.0)	20(87.0)	23(95.8)
DAYS 8-14	N(%)	1(4.3)	0(0.0)	3(13.0)	1(4.2)
RATS PREGNANT/RATS IN COHABITATION f	N/N	18/ 24	24/ 24	18/ 23	21/ 24
	(%)	(75.0)	(100.0)	(78.3)	(87.5)

a. Excludes values for rats that were found dead.
b. Excludes values for rat 328, which was not assigned to cohabitation because there were no available female rats.
c. Restricted to rats with a confirmed mating date and rats that did not mate.
d. Includes only one mating for each male rat.
e. Number of pregnancies/number of rats that mated.
f. Includes only one pregnancy for each rat that impregnated more than one female rat.
g. Restricted to rats with a confirmed mating date.

TABLE B22 (PAGE 1): MATING AND FERTILITY - SUMMARY - F1 GENERATION FEMALE RATS

MATERNAL DOSAGE GROUP		I	II	III	IV
MATERNAL DOSAGE (MG/KG/DAY)		0 (VEHICLE)	25	100	250
RATS IN COHABITATION	N	25	24	25	25
DAYS IN COHABITATION a	MEAN±S.D.	3.8 ± 3.5	2.4 ± 1.0	4.0 ± 2.8	3.1 ± 2.1
RATS THAT MATED	N(%)	25(100.0)	24(100.0)	25(100.0)	25(100.0)
FERTILITY INDEX b	N/N	20/ 25	24/ 24	20/ 25	22/ 25
	(%)	(80.0)	(100.0)	(80.0)	(88.0)
RATS WITH CONFIRMED MATING DATES	N	25	24	25	25
MATED BY FIRST MALE c					
DAYS 1-7	N(%)	24(96.0)	24(100.0)	22(88.0)	24(96.0)
DAYS 8-14	N(%)	0(0.0)	0(0.0)	3(12.0)	1(4.0)
MATED BY SECOND MALE c					
DAYS 15-21	N(%)	1(4.0)	0(0.0)	0(0.0)	0(0.0)
RATS PREGNANT/RATS IN COHABITATION	N/N	20/ 25	24/ 24	20/ 25	22/ 25
	(%)	(80.0)	(100.0)	(80.0)	(88.0)

a. Restricted to rats with a confirmed mating date and rats that did not mate.
b. Number of pregnancies/number of rats that mated.
c. Restricted to rats with a confirmed mating date.

F_2 Generation

Survival:

Excluding two MD F_1 dams that delivered on GD20 and GD21, the Caesarean-sectioning observations were based on 20, 24, 18, and 22 F1 dams with one or more live fetuses in the control, LD, MD, and HD groups, respectively (see sponsor's table, below). The litter averages for corpora lutea, implantations, litter sizes, live fetuses, early and late resorptions, and percent resorbed conceptuses did not show significant differences among groups. No dam had a litter consisting of only resorbed conceptuses, and there were no dead fetuses. All placentae appeared normal.

The two MD female F_1 rats that delivered GD 20-21 and were sacrificed did not show abnormal findings (i.e., clinical signs, body weight or necropsy findings). The uterus of one dam contained 16 implantation sites, and there were 16 live born pups that all

appeared grossly normal. The uterus of the second contained 15 implantation sites, and there were 12 live born pups, two fetuses, and one early resorption *in utero* that all appeared grossly normal.

TABLE B23 (PAGE 1): CAESAREAN-SECTIONING OBSERVATIONS - SUMMARY - F1 GENERATION FEMALE RATS

MATERNAL DOSAGE GROUP		I	II	III	IV
MATERNAL DOSAGE (MG/KG/DAY)		0 (VEHICLE)	25	100	250
RATS TESTED	N	25	24	25	25
PREGNANT	N(%)	20(80.0)	24(100.0)	20(80.0)	22(88.0)
DELIVERED AND SACRIFICED	N(%)	0(0.0)	0(0.0)	2(10.0)	0(0.0)
RATS PREGNANT AND CAESAREAN-SECTIONED ON DAY 21 OF GESTATION	N	20	24	18	22
CORPORA LUTEA	MEAN±S.D.	16.4 ± 3.0	15.9 ± 2.3	15.9 ± 3.9	14.8 ± 1.8
IMPLANTATIONS	MEAN±S.D.	14.6 ± 2.4	15.3 ± 2.6	14.0 ± 2.1	13.9 ± 2.4
% PREIMPLANTATION LOSS	MEAN±S.D.	9.2 ± 16.7	4.0 ± 8.9	9.4 ± 13.6	6.2 ± 11.3
LITTER SIZES	MEAN±S.D.	13.9 ± 2.6	14.6 ± 2.9	12.9 ± 3.2	13.4 ± 2.4
LIVE FETUSES	N	278	350	232	294
	MEAN±S.D.	13.9 ± 2.6	14.6 ± 2.9	12.9 ± 3.2	13.4 ± 2.4
DEAD FETUSES	N	0	0	0	0
RESORPTIONS	MEAN±S.D.	0.7 ± 1.2	0.7 ± 0.7	1.1 ± 2.1	0.5 ± 0.7
EARLY RESORPTIONS	N	14	17	18	11
	MEAN±S.D.	0.7 ± 1.2	0.7 ± 0.7	1.0 ± 1.8	0.5 ± 0.7
LATE RESORPTIONS	N	0	0	2	0
	MEAN±S.D.	0.0 ± 0.0	0.0 ± 0.0	0.1 ± 0.5	0.0 ± 0.0
% POSTIMPLANTATION LOSS	MEAN±S.D.	4.8 ± 8.0	5.1 ± 5.2	8.4 ± 15.9	3.6 ± 5.3
DAMS WITH ANY RESORPTIONS	N(%)	7(35.0)	14(58.3)	7(38.9)	9(40.9)
DAMS WITH ALL CONCEPTUSES DEAD OR RESORBED	N(%)	0(0.0)	0(0.0)	0(0.0)	0(0.0)

% PREIMPLANTATION LOSS = [(NUMBER OF CORPORA LUTEA - NUMBER OF IMPLANTATIONS) / NUMBER OF CORPORA LUTEA] x 100
% POSTIMPLANTATION LOSS = [(NUMBER OF IMPLANTATIONS - NUMBER OF LIVE FETUSES) / NUMBER OF IMPLANTATIONS] x 100

RATS TESTED	N	25	24	25	25
PREGNANT	N(%)	20(80.0)	24(100.0)	20(80.0)	22(88.0)
DELIVERED AND SACRIFICED	N(%)	0(0.0)	0(0.0)	2(10.0)	0(0.0)
RATS PREGNANT AND CAESAREAN-SECTIONED ON DAY 21 OF GESTATION	N	20	24	18	22
DAMS WITH VIABLE FETUSES	N(%)	20(100.0)	24(100.0)	18(100.0)	22(100.0)
PLACENTAE APPEARED NORMAL	N(%)	20(100.0)	24(100.0)	18(100.0)	22(100.0)

<u>Body weight:</u>
There was no clear effect on fetal weights.

<u>External evaluation:</u>
There were no clearly drug-related abnormalities.

<u>Male/Female ratio:</u>
The percent live male fetuses/litter was very slightly increased at HD (see sponsor's table, below).

TABLE B24 (PAGE 1): LITTER OBSERVATIONS (CAESAREAN-DELIVERED FETUSES) - SUMMARY - F2 GENERATION LITTERS

MATERNAL DOSAGE GROUP		I	II	III	IV
MATERNAL DOSAGE (MG/KG/DAY)		0 (VEHICLE)	25	100	250
LITTERS WITH ONE OR MORE LIVE FETUSES	N	20	24	18	22
IMPLANTATIONS	MEAN±S.D.	14.6 ± 2.4	15.3 ± 2.6	14.0 ± 2.1	13.9 ± 2.4
LIVE FETUSES	N	278	350	232	294
	MEAN±S.D.	13.9 ± 2.6	14.6 ± 2.9	12.9 ± 3.2	13.4 ± 2.4
LIVE MALE FETUSES	N	146	181	118	159
% LIVE MALE FETUSES/LITTER	MEAN±S.D.	51.4 ± 16.4	51.8 ± 11.7	50.1 ± 11.2	53.6 ± 14.1
LIVE FETAL BODY WEIGHTS (GRAMS)/LITTER	MEAN±S.D.	5.42 ± 0.41	5.36 ± 0.29	5.43 ± 0.41	5.50 ± 0.26
MALE FETUSES	MEAN±S.D.	5.58 ± 0.44	5.52 ± 0.33	5.56 ± 0.52	5.66 ± 0.34
FEMALE FETUSES	MEAN±S.D.	5.28 ± 0.41	5.20 ± 0.26	5.28 ± 0.36	5.33 ± 0.24
% RESORBED CONCEPTUSES/LITTER	MEAN±S.D.	4.8 ± 8.0	5.1 ± 5.2	8.4 ± 15.9	3.6 ± 5.3

10 Special Toxicology Studies

In addition to the standard toxicology package, the sponsor submitted a few studies to address the renal and dermal toxicity of DMF, as well as a study investigating the concentration of fumaric acid in cell nuclei.

Summary Report for Study PD 08-03: Evaluation of potential nephrotoxicity of DMF when administered by oral gavage to CD rats for 14 days with a 14-day recovery

This non-GLP study was conducted in-house to assist in the discrimination of DMF-related related renal lesions from exacerbation/advanced CPN observed in rats in the 14-week and 6-month toxicity and 2-year carcinogenicity studies in rats.

Species: Rat/CD (b) (4), approximately 9 weeks old at initiation of dosing (See the sponsor's summary design table, below.)

Drug: DMF

Measures: Immunohistochemical marker of cellular proliferation (Ki-67), urinary proteins (NAG, β_2M [ALPCO], KIM-1 (b) (4)], and albumin (b) (4)), urinalysis parameters and necropsy (with histopathology)

Group	Treatment	Dose (mg/kg)*, route	Dose concentration (mg/mL)	Interim (2 week) # Males	Terminal (4 week) # Males
1	0.8% HPMC	0, gavage tid	0	5	5
2	DMF	250, gavage qd	25	5	5
3	DMF	83, gavage tid	8.3	5	5
4	Gentamycin	50, qd sc injection	50	5	-

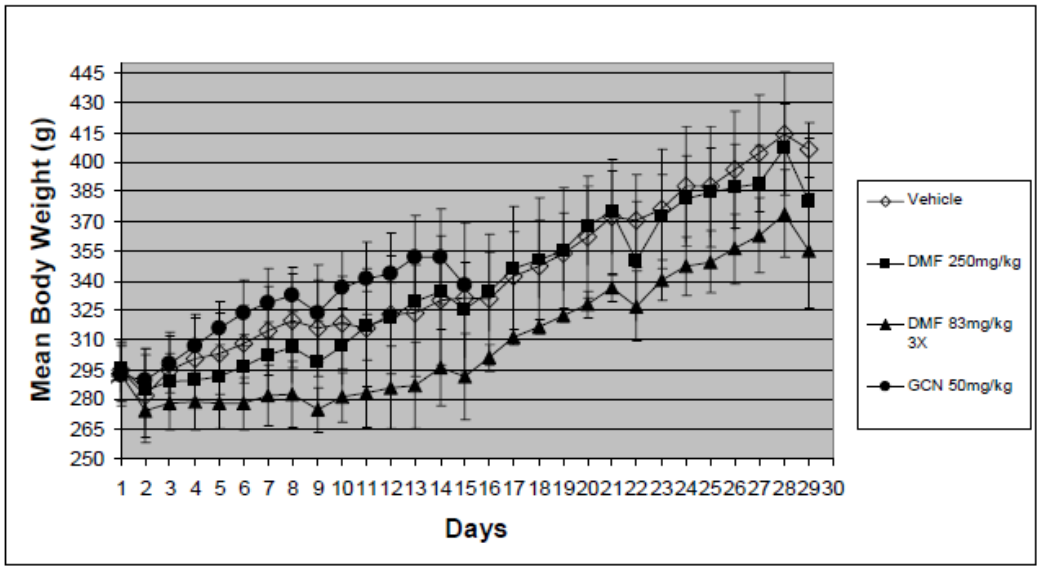
*10 mL/kg dose volume for vehicle and DMF treated groups, 1 mL/kg for Gentamycin sc dosing.

In-life observations were recorded twice daily for mortality, moribundity, and any abnormal clinical observations. Body weights were recorded daily. Urine collections were obtained by placing the animals in metabolism cages overnight, with free access to food and water, once weekly (Days 1, 8, 14, 21, and 28). Urine volume and specific gravity were recorded. Frozen samples were aliquoted, frozen at -70 C, and shipped for analysis. BUN and creatinine was evaluated from blood taken by cardiac puncture at necropsy. Necropsies were performed on Day 15 and 29; following sacrifice, kidneys were weighed and placed into 10% neutral buffered formalin for histopathologic examination and immunohistochemical analysis for a marker of cellular proliferation (Ki-67).

Results

Rats administered DMF showed resistance to dosing beginning on D4, particularly those animals that were dosed three times daily with (83 mg/kg/dose); for this reason, dosing was terminated for the 83 mg/kg TID DMF group on D12 (at this time, the control group was also converted to a once daily regimen). Furthermore, the study design was modified to a 14-day treatment period followed by a 14-day recovery period, and the 28-day repeat dose component was eliminated.

Mean body weight gain in the 250 mg/kg/day DMF group was transiently reduced, and appeared similar to control animals by D9. However, rats dosed with 83 mg/kg TID DMF showed decreased body weights (i.e., reduced gains and/or weight losses) throughout treatment. Gentamicin administration resulted in slightly increased mean body weight and body weight gain. (See sponsor's Figure, below.)



Urine volume was significantly decreased after acute 250 mg/kg DMF and gentamicin treatment. This decrease was transient, as there were no clear differences observed at the D8 or D14 (urine volume appeared increased in the 250 mg/kg DMF group on D14). Although problems occurred with the analysis, specific gravity was increased in the 250 mg/kg DMF and the gentamicin group following D1 treatment; on D8, specific gravity was increased in the gentamicin group only.

According to the sponsor, DMF at 250 mg/kg/day induced transient, statistically significant increases in NAG and KIM-1 (see sponsor's Figures 4.2.5 and 4.2.6) but not albumin or beta-2 microglobulin over the 14-day time course. However, there also appears to be an increase in albumin (250 mg/kg QD and 83 mg/kg TID DMF) on D8 [nss] which resolved in most animals by D21 (see sponsor's Figure 4.2.3). All urinary proteins were generally elevated by gentamicin treatment at the dose and regimen used in this study; although the severity and/or the time course of the observed changes were variable, the results were described as consistent with the expected renal pathology.

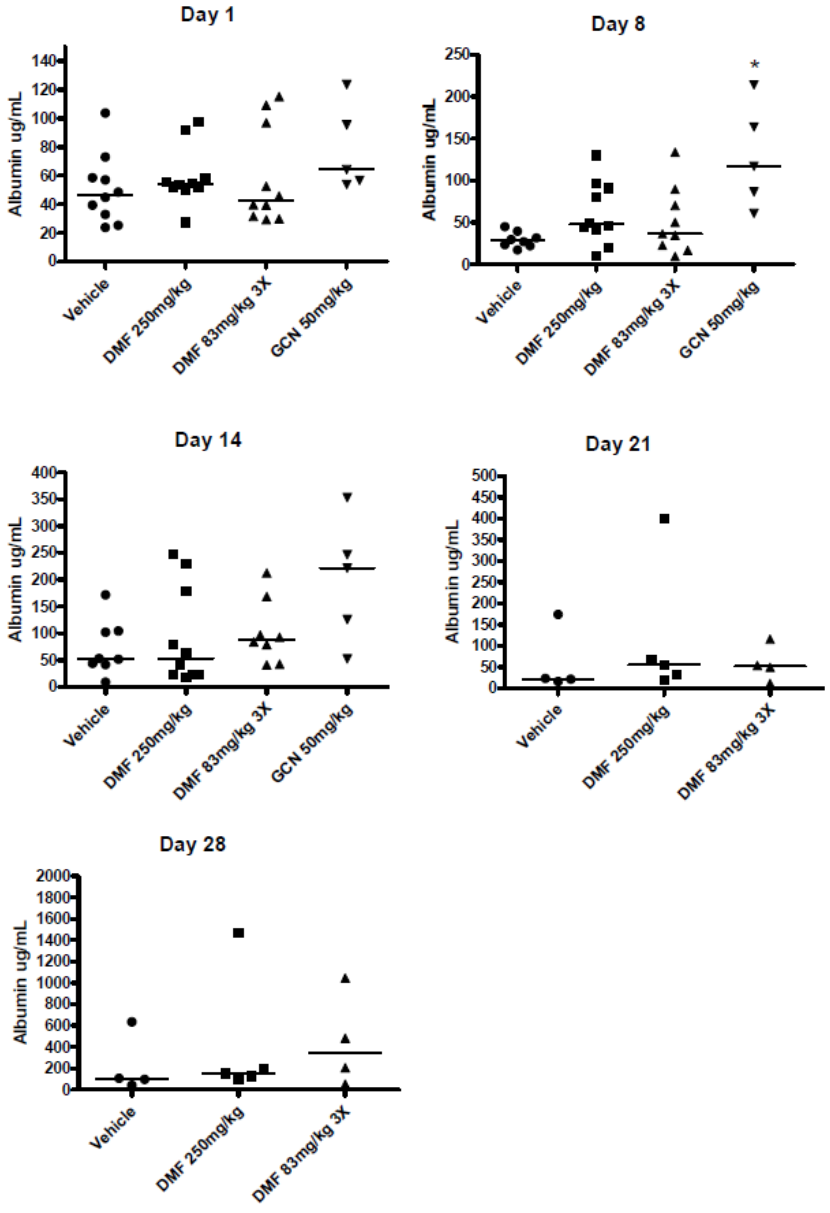


Figure 4.2.3: Albumin data with median value. Statistical comparisons between each BG00012 group to the vehicle control, and GCN to the vehicle control were performed (Kruskal-Wallis ANOVA with Dunn's multiple comparison test, GraphPad PRISM). Significant p-values (<0.05) indicated by an asterisk.

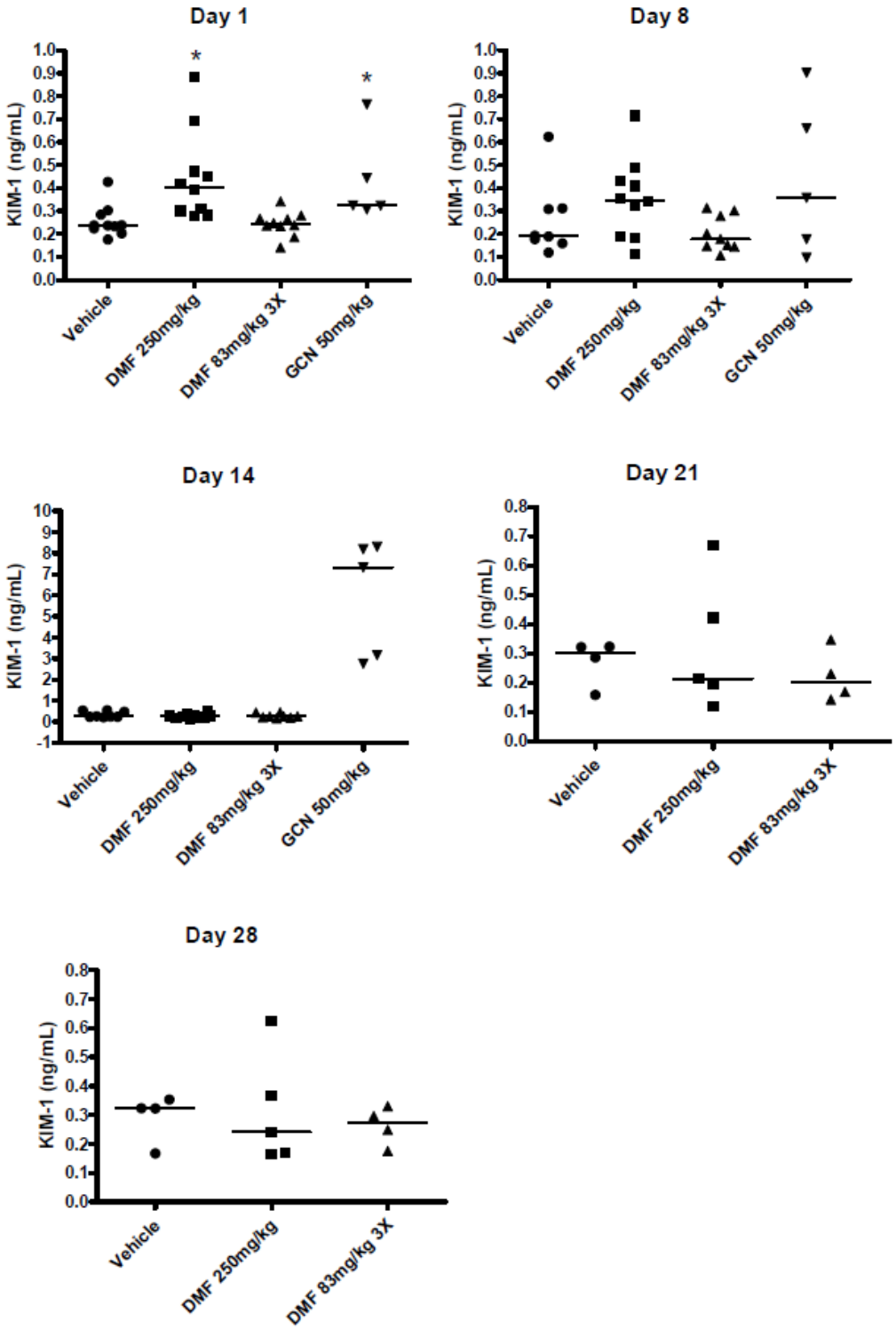


Figure 4.2.5: KIM-1 data with median value. Statistical comparisons between each BG00012 group to the vehicle control, and GCN to the vehicle control were performed (Kruskal-Wallis ANOVA with Dunn's multiple comparison test, GraphPad PRISM). Significant p-values (<0.05) indicated by an asterisk. Note that the scale for KIM-1 on Study Day 14 is different from all other study days to accommodate the Gentamycin data.

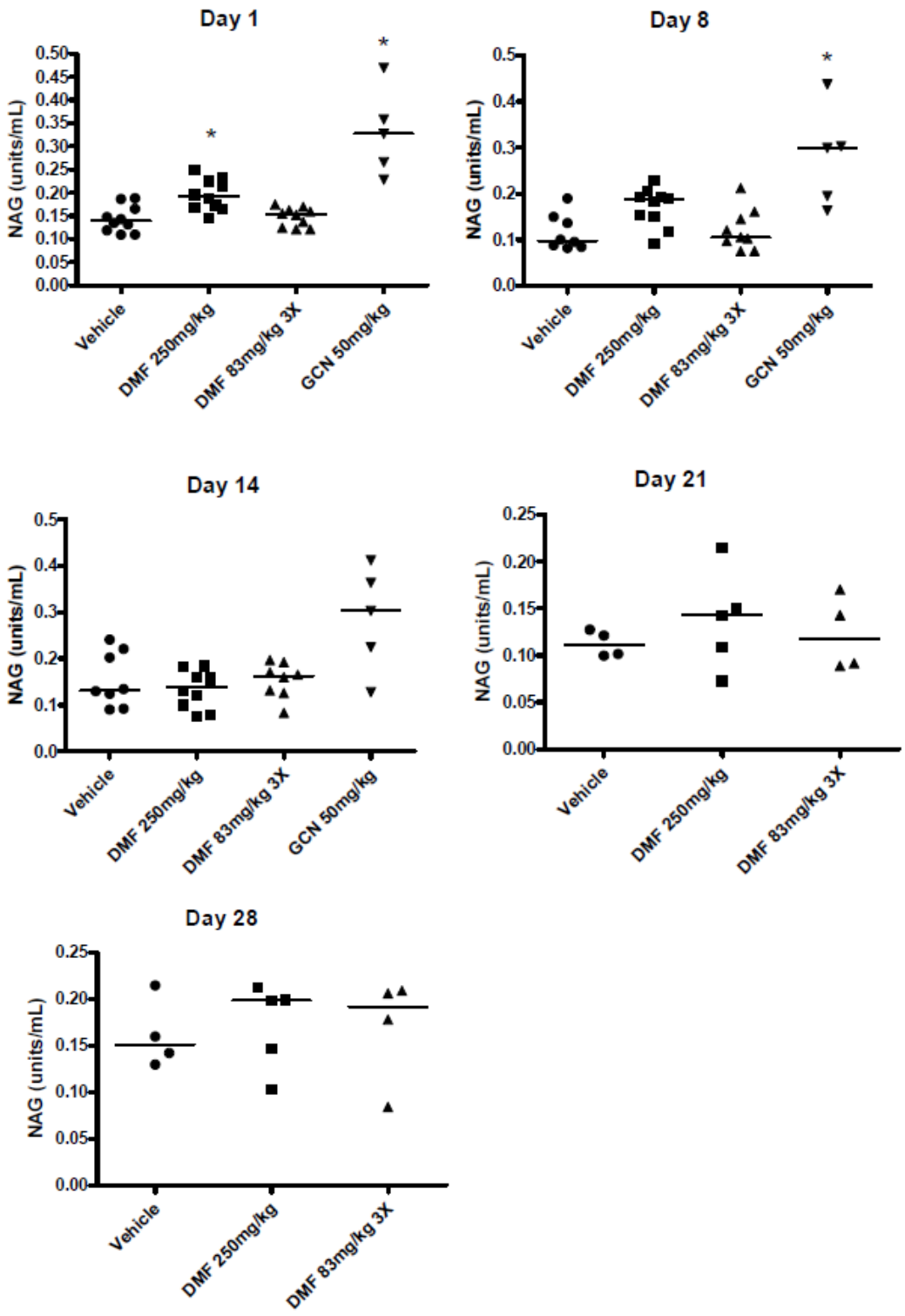


Figure 4.2.6: NAG data with median value. Statistical comparisons between each BG00012 group to the vehicle control, and GCN to the vehicle control were performed (Kruskal-Wallis ANOVA with Dunn's multiple comparison test, GraphPad PRISM). Significant p-values (<0.05) indicated by an asterisk.

DMF was not associated with increases in BUN or creatinine after 14 days of administration or after a 14-day recovery period. Gentamcyin-treated animals showed increased BUN and creatinine after 14 days of administration, as well as increased absolute and relative kidney weights that were histologically correlated with acute tubular damage, inflammation, and tubular regeneration.

Necropsy findings were observed in DMF-treated animals; the gross findings in the kidneys were similar among all groups, but DMF-treated animals also showed changes in the non-glandular stomach that appeared to show recovery (see sponsor's tables, below).

Day 15

Group #	Treatment	Animal #	Gross Observations
1	0.8% HPMC 3X daily IG	1	Both Kidneys with mild multifocal red/tan discoloration
		3	Both Kidneys with mild multifocal red/tan discoloration
		4	Both Kidneys with mild multifocal red/tan discoloration
		5	Both kidneys with multifocal dark foci
2	DMF 250mg/kg 1X daily IG	11	Thickened, granular, and discoloration of the non-glandular stomach
		12	Thickened, granular, and discoloration of the non-glandular stomach
		13	Both Kidneys with mild multifocal red/tan discoloration. Thickened, granular, and discoloration of the non-glandular stomac
		14	Mild thickened lining of the non-glandular stomach. R-Kidney with mild multifocal red/tan discoloration. L-Kidney normal.
		15	Thickened, granular, and discoloration of the non-glandular stomach
3	DMF 83mg/kg 3X daily IG	22	Both Kidneys with mild multifocal red/tan discoloration. Thickened, granular, and discoloration of the non-glandular stomach
		23	Mild thickened lining of the non-glandular stomach with hyperkeratosis. Both kidneys with mild multifocal red/tan discoloration.
		24	Thickened, granular, and discoloration of the non-glandular stomach
		25	Both Kidneys with mild multifocal red/tan discoloration. Thickened, granular, and discoloration of the non-glandular stomach
4	Gentamycin 50mg/kg 1X daily SC	31	No abnormal gross findings
		32	No abnormal gross findings
		33	L-Kidney mild pale discoloration. R-Kidney mild multifocal red/tan discoloration.
		34	Both Kidneys with mild multifocal red/tan discoloration
		35	Both Kidneys with mild multifocal red/tan discoloration

Day 29

Group #	Treatment	Animal #	Gross Observations
1	0.8% HPMC 3X daily IG	6	Both Kidneys with mild multifocal red/tan discoloration
		7	No abnormal gross findings
		8	No abnormal gross findings
		9	Both Kidneys with mild multifocal red/tan discoloration
2	DMF 250mg/kg 1X daily IG	16	Mild thickened and slightly discolored lining of the non-glandular stomach
		17	No abnormal gross findings
		18	Both Kidneys with mild multifocal red/tan discoloration
		19	Both Kidneys with mild multifocal red/tan discoloration
		20	No abnormal gross findings
3	DMF 83mg/kg 3X daily IG	27	Both Kidneys with mild multifocal red/tan discoloration. Mild thickened and slightly discolored lining of the non-glandular stomach
		28	Both Kidneys with mild multifocal red/tan discoloration. Mild thickened and slightly discolored lining of the non-glandular stomach
		29	Both Kidneys with mild multifocal red/tan discoloration
		30	Mild thickened lining of the non-glandular stomach

After 14 days of dosing, DMF-treated (and not gentamicin-treated) rats showed an increased incidence of minimal to mild multifocal nuclear hypertrophy in proximal tubular

epithelium throughout but primarily in the outer two-thirds of the renal cortex. This finding was characterized by proximal tubules containing one or more angular to block shaped nuclei, often with peripheral margination of nuclear chromatin and inconspicuous nucleoli. One animal administered 250 mg/kg/day DMF showed mild focal interstitial fibrosis. Following a 14-day recovery (D29), minimal nuclear hypertrophy was still observed in a few rats from each of the DMF treated groups. See the sponsor's summary table, below.

N=5/group	Vehicle		DMF 250 mg/kg		DMF 83 mg/kg		GCN	
	D15	D29	D15	D29	D15	D29	D15	D29
Nuclear Hypertrophy (PCT)	0	1	4	2	4	2	0	0

In contrast, gentamicin-treated rats showed widespread, mild to moderate tubular injury primarily observed in proximal tubules throughout the cortex and to a lesser degree in straight and collecting tubules along the corticomedullary junction (CMJ). The sponsor also noted that adjacent proximal tubules were frequently lined by “crowded” regenerating epithelial cells with pleomorphic heterochromatic nuclei with loss of polarity and abundant basophilic cytoplasm; the sponsor indicated that these findings were consistent with tubular epithelial regeneration. Mild to moderate tubular dilation was also present throughout the cortex and medulla. A high incidence of granular and protein casts (5/5) were noted within the lumina of affected tubules throughout the cortex and medulla. Occasionally, distal convoluted tubules in the cortex were lined by increased number of prominent cuboidal epithelial cells (“epithelial crowding”). Other findings in the gentamicin-treated rats included mild mononuclear cell infiltrates in the interstitium of these regions and minimal multifocal (scattered) hypertrophy of the parietal epithelium of Bowman’s capsule (glomeruli) in a few animals.

After 14 days of dosing (D15), slight increases in Ki-67 positive nuclei were observed in DMF-treated animals, compared to controls, throughout the cortex and in tubules. See sponsor's summary table, below. In contrast, there was a marked increase (>350%, [ss]) in the mean number of Ki-67 positive nuclei (cortex and tubules) in the gentamicin-treated animals. Following a 14-day recovery period (Day 29), no significant differences were observed between DMF-treated animals and controls.

	Mean Ki-67 Positive Nuclei			
	Treatment		Recovery	
	Mean Ki-67 Positive Nuclei (Cortex)	Mean Ki-67 Positive Nuclei in Tubules Only	Mean Ki-67 Positive Nuclei (Cortex)	Mean Ki-67 Positive Nuclei in Tubules Only
Vehicle 0.8% HPMC TID	16.60	8.32	13.20	6.20
DMF 250 mg/kg daily	20.84	13.36	10.78	5.30
DMF 83 mg/kg TID	21.84	13.52	13.68	6.36
Gentamicin 50 mg/kg daily	80.6* (385.5%)	38.38* (361%)	N/A	

(*p < 0.0001)

Few signs of DMF-induced renal damage were apparent in this study (transient effects on urine volume, urinary albumin, urinary NAG, urinary KIM-1, Ki-67 positive nuclei, and histopathology (partially reversible multifocal nuclear hypertrophy in proximal tubular epithelium throughout the renal cortex). Following longer duration treatment with DMF, however, there is a level of regeneration of tubule epithelium that is evident in rodents, primates and dogs that was not apparent in this short-term study.

Study P00012-08-01 (EBA00528): BG00012: A 14-Week Oral Nephrotoxicity Study in Sprague-Dawley Rats With a 4-Week Recovery Period

This GLP/non-GLP (i.e., CMC, TK, urinary protein analysis, pathology peer review and Ki-67 immunohistochemistry) study was conducted to evaluate the dose and time dependence of the segmental tubule epithelial regeneration observed in nonclinical species, and to correlate renal toxicity markers currently used in the clinical trials with the DMF-related histopathologic lesion observed in rats. Previous rodent studies demonstrated that 14 weeks duration was the earliest timepoint with demonstration of the lesion (Study P00012-04- 03 [male rat fertility], at 250 mg/kg).

Species: Sprague-Dawley rats ([(b) (4)]), 7-8 wks old 170-283 g (See the sponsor's summary design table, below.)
Drug: DMF ([(b) (4)], lot 1253636; 99.3% pure, 0.03% MMF)
Duration; 14 weeks dosing (4-week recovery)
Measures: urinary protein markers: microalbumin, beta-2-microglobulin, and KIM-1; histopathology with standard hematoxylin and eosin; and immunohistochemical evaluation for proliferation marker (Ki67)

Group Number	Number of Animals						Test Article	Dosage Level (mg/kg/dose)	Dose Conc (mg/mL)	Dosage Volume (mL/kg/dose)	Dosing Regimen	Necropsy Day
	Main Study		Recovery		Satellite							
	M	F	M	F	M	F						
1	10	10	5	5	3	3	Vehicle	0	0	5	Twice-daily oral gavage on Days 1 to 98[a]	Main Study: Day 99 Recovery: Day 127
2	10	10	5	5	3	3	BG00012	50	10	5		
3	10	10	5	5	3	3		100	10	10	Once-daily oral gavage on Days 1 to 98	
4	10	10	5	5	3	3		250	25	10		

[a] Doses were administered 6 to 6.5 hours apart between the first and second doses each day.

There were 4 unscheduled HD deaths in the study; in 3 of the 4 animals, the cause of death could not be determined because histopathological assessment was not performed (1 accidental). Dose-dependent clinical signs (e.g., dry red material, salivation, wet fur), average body weight reductions (up to 13%), and decreased food consumption were observed. Decreased RBC parameters (~10%), and increased reticulocyte, platelet (20-30%), and WBC counts (~1.5-3x) were seen in MDM and/or HDM. Decreases in ALT and AST were observed in treated males, and LDF and MDF showed decreases in BUN. (GGT and creatinine data were missing.) At necropsy, only histopathology of the kidneys was assessed. In addition to the gross pathology in nonglandular stomach observed at all doses in both sexes, nephrotoxicity was observed in males at all doses; an increased incidence of nephropathy (described as "consistent" with CPN; including luminal proteinosis, tubular degeneration/regeneration and interstitial fibrosis) and associated segmental tubular regeneration (within the mid to deep renal cortex) was observed in males on D99. Additionally, dose-dependent minimal-mild nuclear hypertrophy of the epithelial cells of the renal proximal tubules was observed in both sexes (with increased incidence at HD), and continued to be observed in females after the recovery period. The pathologist described the hypertrophy as "multifocal and dispersed throughout the cortex and often resulted in abnormally shaped oval to polygonal nuclei. Affected nuclei contained 1 or more prominent nucleoli." See sponsor's Text Tables 4 and 5, for details.

Text Table 4 BG00012-Related Histopathologic Findings at Terminal Necropsy (Day 99)

	Males				Females			
Group	1	2	3	4	1	2	3	4
Dose (mg/kg/dose)	0	50	100	250	0	50	100	250
No. animals examined	10	10	10	10	10	10	10	9
Kidney								
Hypertrophy, nuclear, proximal tubule	**0**	**2**	**4**	**9**	**0**	**2**	**5**	**8**
Minimal	0	2	4	6	0	2	5	6
Mild	0	0	0	3	0	0	0	2
Nephropathy	**1**	**3**	**5**	**9**	**0**	**0**	**0**	**0**
Minimal	1	3	4	2	0	0	0	0
Mild	0	0	1	7	0	0	0	0
Regeneration, tubular, focal								
Mild	1	0	0	0	0	0	0	0
Regeneration, segmental, cortex	**1**	**8**	**7**	**10**	**0**	**0**	**0**	**0**
Minimal	1	5	2	2	0	0	0	0
Mild	0	3	5	8	0	0	0	0

Text Table 5 BG00012-Related Histopathologic Findings at Recovery Necropsy (Day 127)

	Males				Females			
Group	1	2	3	4	1	2	3	4
Dose (mg/kg/dose)	0	50	100	250	0	50	100	250
No. animals examined	5	5	5	3	5	5	5	5
Kidney								
Hypertrophy, nuclear, proximal tubule								
Minimal	0	0	0	0	0	1	1	5
Nephropathy	**0**	**3**	**3**	**3**	**0**	**0**	**0**	**0**
Minimal	0	2	2	2	0	0	0	0
Mild	0	1	1	1	0	0	0	0
Regeneration, tubular, segmental	**0**	**2**	**3**	**3**	**0**	**0**	**0**	**0**
Minimal	0	1	1	3	0	0	0	0
Mild	0	1	2	0	0	0	0	0

Increased urinary albumin and slightly decreased serum albumin concentrations were observed in MDM and HDM. Elevated urinary protein concentration and leukocytes were observed in treated males; MDF and HDF also showed increased urinary protein changes. A reduced A/G ratio (~10%), resulting from slightly increased globulin and slightly decreased serum albumin, was seen in MD and HD males. These clinical chemistry and urinalysis changes did not recover. Dose-dependent increases in kidney weights were observed in all treated males and in HDF; recovery was not observed. Dose-dependent increases in Ki67 immunopositivity in the renal cortex and outer medulla of kidneys (all treated males, MDF and HDF), and an increase in urinary microalbumin levels, were observed. The increase in urinary microalbumin levels was observed as early as Day 42 and continued throughout recovery. After a 4-week treatment-free period, there was a decrease in Ki67 immunopositivity, and a trend

toward recovery of the segmental tubule regeneration. Urinary microalbumin levels, however, did not show recovery in the 4-week treatment-free period. Female rats did not demonstrate histologic evidence of renal tubule regeneration or significant elevations in Ki67 immunopositivity or urinary albumin excretion. However, only females showed urinary KIM-1 elevations on D14-84; this showed recovery.

The sponsor stated that urinary microalbumin testing was included as part of the renal monitoring paradigm in the phase 3 clinical trials due to its presumed sensitivity to early renal tubular injury; persistent abnormal urinalysis findings led to patient discontinuation in all three trials.

Summary Report for Study P00012-09-01: BG00012: An investigative toxicology study to evaluate the time course of renal toxicity and its reversibility in the Sprague Dawley Rat

This non-GLP study was conducted in-house to evaluate the time course for recovery of increased urinary albumin following oral administration of DMF.

Species: Male SD rats/CD (b) (4) ~7 weeks old at assignment, 185-212 g (See the sponsor's summary design table, below.)
Drug: DMF (b) (4), lot 1424853)
Duration; ~10 weeks dosing
Measures: Urinary albumin, immunohistochemical marker of cellular proliferation (Ki-67), and necropsy (with histopathology)

Group	Treatment	Route	#/Animals Terminal Sacrifice	#/Animals Recovery	Dose Level (mg/kg)	Dose Volume (mL/kg)	Dose Conc. (mg/mL)
1	Vehicle	gavage	10 males	10 males	0	10	0
2	DMF	gavage	10 males	10 males	100	10	10

All animals were dosed QD, and overnight urine sample collections were taken to evaluate urinary albumin levels. When an increase in urinary albumin in DMF-treated versus control rats was observed, a terminal necropsy was scheduled. Rats were selected for either the terminal necropsy (N= 4 rats per group) or for necropsy following a treatment-free recovery period (N=4) such that the animals with elevated urinary albumin were evenly distributed between the two sacrifice times. The remaining rats were terminated without further evaluation. On D75, a final dose of DMF or vehicle was administered to the terminal sacrifice animals and a blood sample was collected from the caudal vena cava 30 minutes post-DMF/vehicle administration to confirm exposure to the test article. For the recovery animals, urinary albumin levels were measured on D112; necropsy occurred for this group of animals on D114.

Results

DMF-treated animals showed salivation during dosing (8 animals, 31 observations between D20 and D71). There were no reported drug-related differences in mean body weight.

Urinary albumin was measured weekly until a subset of DMF-treated animals showed consistent increases in urinary microalbumin compared to their pre-dose value, which according to the sponsor occurred on D63 (in 6 of 8) and again on D70. On D63 and D70, the sponsor stated that only two of the 8 vehicle control rats had elevated urinary albumin levels. Treatment was discontinued after the final dose on D71. See the sponsor's Tables 1 and 2 for microalbumin levels. At the terminal necropsy, tan/red mottled color of both kidneys was observed in 4/4 DMF-treated animals (2 mild, 2 moderate) and 2/4 control animals (mild). Relative kidney weight (to body) was increased [ss] in the DMF-treated animals (see sponsor's Figures 5 and 6, below). Histologically (see sponsor's Table 5), DMF-treated animals showed a slight increase in the incidence of mild nuclear hypertrophy in proximal convoluted tubular epithelium within the outer two-thirds of the renal cortex and increases in the incidence and severity of mild hyaline droplet accumulation in cortical tubular cells. These changes were accompanied by a slight but non-statistically significant increase in the median % area of Ki-67 staining (see Sponsor's Figure 9).

Urinary Microalbumin (mg/dL)

			Day							
Treatment Group	Necrospy Group	Animal #	Pre-Dose	28	35	42	49	56	63	70
1 - Vehicle	Terminal	4	BLLQ	BLLQ	BLLQ	BLLQ	8.15	4.24	15.60	4.46
	Terminal	7	18.75	12.41	19.69	27.90	34.00	34.81	44.57	25.20
	Terminal	15	7.92	11.35	5.59	6.30	15.90	28.75	15.08	13.77
	Terminal	20	BLLQ	BLLQ	BLLQ	BLLQ	7.00	12.46	BLLQ	BLLQ
	Recovery	5	BLLQ	4.10	BLLQ	BLLQ	18.83	19.17	6.52	BLLQ
	Recovery	10	1.59	BLLQ	4.90	5.08	10.25	23.33	20.86	BLLQ
	Recovery	14	BLLQ	BLLQ	BLLQ	BLLQ	BLLQ	BLLQ	BLLQ	3.52
	Recovery	18	3.30	BLLQ	BLLQ	BLLQ	12.15	7.10	BLLQ	4.98
2 - DMF 100 mg/kg	Terminal	22	4.86	13.18	2.46	11.45	10.54	11.87	12.78	38.72
	Terminal	27	BLLQ	BLLQ	12.21	4.41	38.78	54.10	34.03	42.81
	Terminal	31	BLLQ	BLLQ	BLLQ	BLLQ	8.54	2.49	9.10	16.28
	Terminal	32	BLLQ	BLLQ	BLLQ	10.43	5.52	BLLQ	BLLQ	BLLQ
	Recovery	24	BLLQ	7.59	BLLQ	12.11	9.37	2.29	9.15	11.65
	Recovery	30	4.30	BLLQ	5.02	5.28	27.36	16.36	16.09	21.72
	Recovery	35	BLLQ	60.56	13.47	36.19	148.15	140.52	60.55	86.06
	Recovery	36	BLLQ	14.50	BLLQ	15.32	1.38	6.06	21.84	13.62

Table 1: Individual animal urinary microalbumin levels from pre-dose to Day 70. BLLQ = below lower limit of quantification.

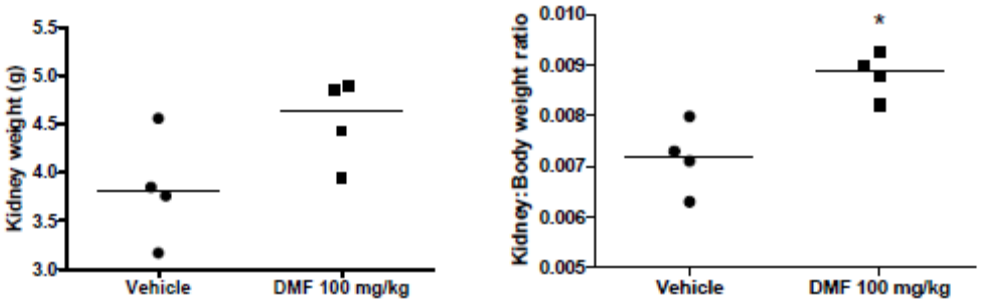


Figure 5 and Figure 6: Kidney weights from the terminal sacrifice animals with median value shown (left) and kidney weight to body weight ratio from the terminal sacrifice animals with median value shown (right). The kidney to body weight ratio was significantly increased (Mann-Whitney test, *p = 0.0286, GraphPad PRISM software V5).

Histopathology Finding		Vehicle (terminal) Day 75	BG00012 (terminal) Day 75	Vehicle (recovery) Day 114	BG00012 (recovery) Day 114
		N = 4	N = 4	N = 4	N = 4
Nuclear Hypertrophy	minimal	2	1	0	1
	mild	0	3	0	0
Hyaline droplet accumulation	minimal	2	1	0	1
	mild	0	3	0	0

Table 5: Summary of Histopathology Findings in Kidney

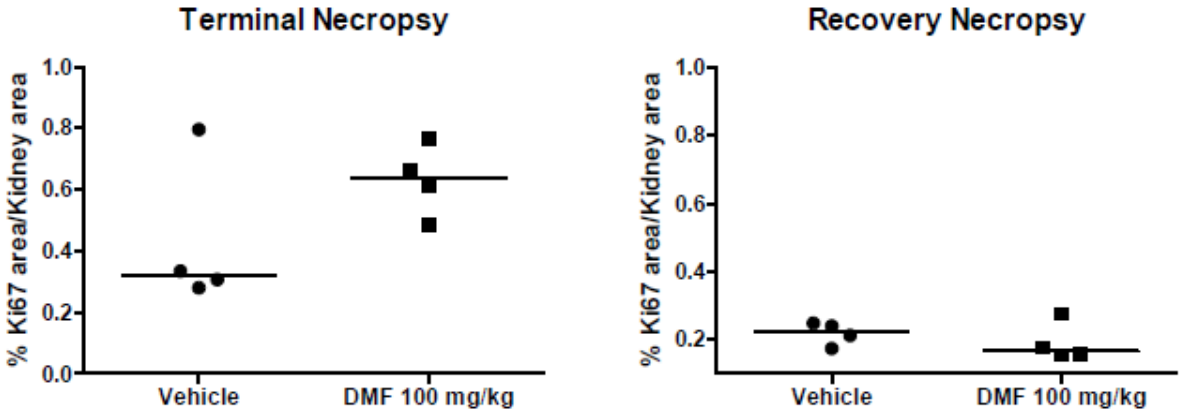


Figure 9 and Figure 10: Median percent (%) area of Ki67 staining from kidneys collected at the terminal necropsy (left) and the recovery necropsy (right).

After the recovery period, microalbumin levels were increased to some degree in all animals. See sponsor's Table 2 for microalbumin levels. There were no gross observations in any animal at the recovery sacrifice. Kidney weights did not differ. Histologically, minimal nuclear hypertrophy (with minimal hyaline droplet accumulation in the cortical tubules) was still observed only in 1/4 DMF-treated rat (see sponsor's Table 5, above). These findings were not observed in control animals at the end of recovery. There was no difference in median percent area of Ki-67 staining in kidneys from DMF-treated and control rats at the end of recovery.

Urinary Microalbumin (mg/dL)

			Day						
Treatment Group	Necrospy Group	Animal #	Pre-Dose	77	84	91	98	105	112
1 - Vehicle	Recovery	5	BLLQ	3.78	9.27	1.91	20.82	30.54	12.06
	Recovery	10	1.59	26.95	45.02	31.64	54.45	76.32	76.73
	Recovery	14	BLLQ	BLLQ	2.52	BLLQ	7.28	5.38	8.04
	Recovery	18	3.30	13.46	16.19	9.79	24.50	28.89	36.45
2 - DMF 100 mg/kg	Recovery	24	BLLQ	14.63	11.38	9.14	17.71	47.48	25.49
	Recovery	30	4.30	3.13	19.84	12.80	BLLQ	19.62	19.76
	Recovery	35	BLLQ	108.14	53.58	134.90	98.68	116.75	112.96
	Recovery	36	BLLQ	40.19	26.32	28.80	24.73	23.82	36.26

Table 2: Individual animal urinary microalbumin levels during the recovery phase. BLLQ = below lower limit of quantification.

The dosing formulation was 77-83% of nominal. Blood samples demonstrated exposure to DMF in treated animals only (7800-13100 ng/mL MMF).

Again, regeneration of the tubule epithelium was not observed in any of the DMF-treated rats in this study; however, the quantitative evaluation of Ki-67 positive tubule epithelial cells demonstrated a difference in the mean/median. The sponsor posited that the onset of a regenerative response requires longer treatment or higher doses of DMF than were evaluated within this design. The sponsor argued that the observed increases in urinary albumin excretion suggested that this change may be an early response that precedes the regeneration. It is of note that the urinary albumin values were variable and inconsistent. The sponsor attributed the increased urinary albumin excretion in both groups at the end of the recovery period to spontaneous chronic progressive nephropathy (CPN) in male rats; however, the Pathologist's report states that "the incidence of nephropathy (CPN) was very low in all groups at both terminal and recovery sacrifices." Based on the observed "reversible" (not in 1/4 animals) histopathologic changes and the Ki-67 data, the sponsor believes these data show that long treatment durations with high doses of DMF are required to elicit a regenerative response (which is believed to indicate previous damage) in the proximal tubule epithelial cells which will be confounded by spontaneous CPN in male rats.

In addition to the studies to further investigate the demonstrated renal toxicity of DMF, the sponsor briefly addressed the dermal toxicity of DMF following cutaneous administration. A study was not conducted with DMF; however, a study using Fumaderm was submitted, and scientific and regulatory literature clearly document toxicity following dermal contact with DMF. Specifically, the sponsor submitted a skin sensitization test for Fumaderm, which will be only briefly reviewed here. Although DMF is only one component of Fumaderm, the study showed clear potential as a sensitizer, which has since been shown for DMF alone in humans (e.g., Rantanen, 2008; Williams et al., 2008). In fact, the European Union has prohibited the use of DMF as a biocide/fungicide for consumer products since 1998 and more recently has banned the import of products containing DMF since 2009 (see EU Commission Decision 2009/251/EC of 17 March 2009, document # C[2009] 1723, establishing a DMF maximum concentration of 0.1 mg/kg [0.1 ppm]), due to cases of severe contact dermatitis that produced a difficult to treat eczema. Concentrations as low as 1 mg/kg (1 ppm) have been shown to produce a reaction.

Study 6053-90: EXAMINATION OF FUMADERM-MIXTURE IN A SKIN SENSITISATION TEST IN GUINEA-PIGS ACCORDING TO MAGNUSSON AND KLIGMAN (Maximisation Test) - orientating study -

GLP, QA (b) (4) Initiated 4/2/90

Drug:	Fumaderm-Mixture, lot 905412 (i.e, dimethyl fumarate 120.0 mg, monoethyl fumarate calcium salts 81.0 mg, monoethyl fumarate magnesium salts 5.0 mg, and monoethyl fumarate zinc salts 3.0 mg)
Vehicle Control:	0.8% aqueous hydroxypropyl methylcellulose gel
Species:	guinea pig, Pirbright white, 23-30 days old, 248-283 g

See excerpts from the sponsor's summary table of the design, below.

Species/Strain: Guinea-pig/Pirbright white			Number of animals: 20	
Procedure	Administration route;site		Day	Vehicle
First induction	intradermal; shoulder		1	0.8 aqueous methyl *
Second induction	epicutaneous; shoulder		8	" " "
First challenge Second challenge Rechallenge	" ; flank		22	" " "
Study group	Control		Test group	
	Concentration of test art.	No. of appl. and ml/appl.	Concentration of test art.	No. of appl. and ml/appl.
First induction	vehicle	two, 0.1 ml plus FCA	0.5 %	two, 0.1 ml plus FCA
Second induction	vehicle	one, 2.0 ml	0.5 %	one, 2.0 ml
First challenge	left flank:0.1%	one, 2.0 ml	left flank:0.1%	one, 2.0 ml
Second challenge Rechallenge	right flank: vehicle	one, 2.0 ml	right flank: vehicle	one, 2.0 ml
Sex (m/f)	m	f	m	f
Number of test animals	10	-	10	-

In the preliminary study, intracutaneous administration of 0.3% and 1.0% aqueous Fumaderm suspension induced slight erythema. There was no reaction to a 0.1% Fumaderm. Based on these results, a 0.5% suspension of Fumaderm was chosen intracutaneous administration in the main experiment.

Topical administration of 0.3%, 1.0%, 3.0%, or 10% aqueous Fumaderm suspension caused concentration-related slight to severe skin reactions; a 0.1% suspension caused no reaction. Based on these results, 0.5% aqueous Fumaderm suspension was chosen for the 2nd induction stage (topical) and for the challenge (topical).

For the intracutaneous induction and topical induction stages, a slightly irritating concentration of 0.5% was used; this caused slight erythema. However, later challenge with a 0.1% aqueous Fumaderm suspension revealed extremely sensitizing properties. All treated animals showed moderate to severe erythemas at 48 and 72 hours; some animals were observed to have necrotic tissue at the challenge site.

Study 119/90: DETERMINATION OF THE FUMARIC ACID CONCENTRATION IN THE CELL NUCLEUS OF V79 CELLS EXPOSED IN *VITRO* TO MONOETHYL FUMARATE AND FUMARIC ACID

GLP, QA [redacted] (b) (4) Initiated 5/14/90

Drugs: Fumaric acid, batch no. 2488391189, strength: > 99.5%

	Monoethyl fumarate, batch no. 7652, strength: 99.5%
Vehicle control:	DMSO (added at a concentration of 1 % (v/v) to the medium
Duration of exposure:	30 minutes 2 hours
Exposure concentration:	1.4 mM monoethyl fumarate 1.4 mM fumaric acid
Cell line:	V79 cells derived from fetal lung of Chinese hamsters, Cultures incubated at 37°C

This study was not reliable. According to the sponsor, the determination of the fumaric acid in the nucleus mixture was found to be extremely difficult. The data suggested that a higher fumaric acid concentration was observed in the cell nuclei sediment of cells exposed to monoethyl fumarate after exposure to equimolar concentrations of fumaric acid or monoethyl fumarate. The sponsor believed the fumaric acid concentration found in the nuclei of the controls was probably caused by contamination. The sponsor believed fumaric acid to be found mainly in mitochondria. The extent of the contamination was believed similar in all 3 groups, and attributed to the difficulty of purification of the nucleus material. The sponsor discontinued the study. See the sponsor's summary table, below.

Exposure	ng fumaric acid/µg protein nucleus material
Control	approx. 0.3
Fumaric acid (1.4 mM)	approx. 0.3
Monoethyl fumarate (1.4 mM)	approx. 0.45

11 Integrated Summary and Safety Evaluation

The mechanism by which DMF acts to treat multiple sclerosis is not fully understood; a number of mechanisms have been suggested, and have been found to have support. DMF is believed to exert anti-inflammatory actions through activation of the Nuclear Factor (Erythroid-derived 2)-like (NFE2L2 or Nrf2) antioxidant response pathway; this pathway is the primary cellular defense system for responding to a variety of potentially toxic stimuli. It has also been suggested that DMF promotes anti-inflammatory cytokine (Th2) expression over pro-inflammatory cytokine (Th1, Th17) expression, and exhibits some cytoprotective responses in CNS cells. Evidence for these effects was demonstrated in *in vitro* assays as well as in *in vivo* models of inflammatory insults (i.e., multiple rodent experimental autoimmune encephalomyelitis (EAE) models and a collagen-induced arthritis [CIA] model). DMF has also been shown to inhibit the NF-κB signaling (believed to result in inhibit expression of pro-inflammatory cytokines and adhesion molecules; e.g., Loewe et al., 2002, Loewe et al., 2001, Vandermeeren et al., 1997) and inhibition of cyclin-dependent kinase was suggested by modeling. Other effects suggested for DMF and/or MMF include inhibition of angiogenesis (Garcia-Caballero et al., 2011) and glutathione depletion (Held, 1991). The combination of all

these effects may very well be relevant to the pathogenesis and treatment of MS, a disease in which immune cell activation and infiltration into the central nervous system (CNS) produces production and release of reactive free radicals and pro-inflammatory stimuli and yields CNS cellular damage.

MMF has also been shown to bind GPR109, a nicotinamide receptor, with good affinity (Tang et al., 2008; Lukasova et al., 2011). It is postulated that this activity may be responsible for the flushing and GI side effects (cf. Hanson et al., 2010) seen in the clinical trials. This activity may also have some role in the retinal effects (cf. Gambhir et al., 2012) and fat distribution effects (e.g., Wanders & Judd, 2011) demonstrated in the 2-year carcinogenicity bioassay in mice.

The safety pharmacology studies showed DMF to have some liability for cardiovascular, but not respiratory, toxicity. The dedicated tests for effects on the QTc interval did not appear to be affected, but the ECG suggested effects on sodium channels; however, QTc effects were seen in a subchronic dog study (possibly secondary to heart rate). CNS toxicity was not directly evaluated.

Studies of the ADME of DMF appeared to have been complicated by methodological problems. Following oral administration, DMF was rapidly absorbed and the radiolabeled material was widely distributed. DMF is rapidly hydrolyzed and was not generally observed in plasma circulation. The metabolism of DMF is mediated by esterases and enzymes involved in the TCA cycle. MMF is considered the "primary metabolite" (at ~5% of the circulating drug-related plasma exposures); MMF was measured in the nonclinical species and humans for the purpose of plasma exposure comparisons. Other metabolic products include glucose (~50%), and fumaric and citric acid (~30%). DMF and MMF are rapidly eliminated by metabolism and are primarily eliminated in expired air; generally, renal elimination plays a minor role. In rats, cysteine or N-acetylcysteine conjugates of monomethyl- and dimethyl- succinate were the major components in urine (representing ~10% of the administered dose).

General Toxicology

DMF has been shown to produce relatively consistent, and sometimes severe, toxicities. Generally, the dose required to produce the toxicities decreased as a function of the duration of exposure. DMF's use as a biocide, and the cutaneous toxicity that has resulted (cf. Williams et al., 2008; Rantanen et al., 2008), suggests that systemic dosing of DMF would result in toxicity due to, at the very least, its irritancy potential. Interestingly, it appears that an overview of the systemic toxicity of DMF has been known for some time; a 1992 EPA report reviewing DMF studies (dated 1951) identified DMF as "moderately toxic", showing severe stomach and kidney damage at doses well-below the acute lethal dose.

Rodent:

Dose-limiting effects in rats have mostly consisted of body weight losses and toxic effects on the stomach (mostly forestomach). Generally, the toxicities were dose- and duration- dependent. Subchronic repeated dose studies showed effects on hematology

(i.e., RBCs, reticulocytes and/or WBCs), clinical chemistry (slightly decreased creatinine BUN), and target organs (i.e., stomach, pancreas, kidney, liver, heart, lymphatic system, and testes). Clearly dose-related toxicity was observed in the nonglandular stomach (e.g., hyperplasia, hyperkeratosis, ulcer, inflammation, and subepithelial granulation), but glandular stomach alterations (minimal-moderate glandular ectasia, as well as low incidence ulceration and erosion) were also observed. Acinar epithelial cell apoptosis and vacuolization was observed in the pancreas. Few histological changes occurred in the kidney (i.e., minimal-mild tubular basophilia, dilatation, mineralization, or proteinosis) in these subchronic studies. Inflammation was observed in several organs, and was often associated with lymphoid hyperplasia in the lymphatic system. Notably, squamous cell carcinoma of the nonglandular stomach was observed in 2 animals in a 3-month toxicity study at 250 mg/kg.

In the pivotal chronic 6-month study in rats, DMF doses of 25, 100 and 200 mg/kg were tested. The study did not demonstrate a NOAEL due to changes in the forestomach and kidney. Slightly reduced body weights, compared to controls, were seen in males at 200 mg/kg. Few significant hematological, clinical chemistry, and urinalysis alterations were observed (e.g., some reductions in serum creatinine and BUN were observed, as well as increased urinary protein and ketones). Identified target organs included stomach, kidney and liver; recovery was incomplete. Squamous epithelial hyperplasia, hyperkeratosis, inflammation, squamous papilloma (1 male at 100 mg/kg) and squamous carcinoma (1 male at 200 mg/kg) were observed in the nonglandular stomach. In the glandular stomach, minimal-mild subacute inflammation was observed. Histopathology in the kidney included increased incidences and severity of nephropathy (greater in males than females) but also included changes not classically seen with nephropathy (e.g., tubular regeneration). In liver, minimal focal/multifocal necrosis and minimal bile duct hyperplasia were observed (greater in females than males). Lymphoid hyperplasia of lymph nodes was often observed. A low incidence of interstitial cell hyperplasia of the testes was observed in males at 200 mg/kg.

Non-rodent:
Monkey
Dose-limiting toxicity was observed as body weight reductions and/or emesis. It is unclear whether a maximally-tolerated dose was tested in the chronic (12-month) toxicity study in monkeys; the study tested DMF doses of 5, 25, and 75 mg/kg BID. The reductions in body weight demonstrated in the dose-ranging study were only observed transiently in the chronic study. However, although there were no premature decedents in the pivotal study, it is possible that much higher doses would not have been tolerated based on the severity of the renal toxicity (and lack of recovery) at 75 mg/kg. Generally, reduced BUN and serum creatinine were observed at 25 and 75 mg/kg. No urinalysis changes were reported. At 75 mg/kg, the demonstrated histologic renal damage (i.e., tubular single cell necrosis, regeneration and atrophy, as well as interstitial fibrosis) was relatively severe and was not always reversible; however, only one case was noted to show clinical chemistry signs of reduced function (i.e., 1 recovery HDM showed increased BUN and creatinine in the presence of renal scarring).

Dog

Dose-limiting toxicity was observed as body weight loss, inappetance, emesis and/or secondary effects attributed to nutritional-related deficiencies. In the 4-week oral gavage study, doses of 50, 100 and 250 mg/kg were tested. The QTc appeared to be increased, but the sponsor believed this may have resulted from the poor nutritional status. Target organs included stomach, thymus, bone marrow and kidney. The chronic 11-month (shortened from 12-months by protocol amendment) dog toxicity study tested BG-12 (b) (4) capsules at doses of 5, 25 and 75/50 mg/kg (HD reduced on day 7). Severe clinical signs (e.g., persistent emesis) were similar, and a number of HD dogs underwent dosing holidays due to body weight loss. Dose-related average body weight reductions were observed in treated groups; this was related to inappetance. In this study, clear ECG alterations were not reported. Although the data were variable, slight reductions in red blood cell parameters were observed at 50 mg/kg, and creatinine and BUN were reduced at 25 and 50 mg/kg. Cholesterol was slightly increased at 50 mg/kg. In the urinalysis, only increased volumes were observed at terminal necropsy at 50 mg/kg. Clear drug-related target organs included kidney, adrenal gland, testes and epididymides. Enlargement of the kidneys correlated with increased kidney weight and hypertrophy of the tubular epithelium (males) and diffuse cortical tubular dilation (males and females). Renal histological findings also included: regeneration of the tubular epithelium, increased pigmentation of the tubular epithelium, atrophy of the cortical parenchyma, cortical thickening of Bowman's capsule basement membrane, hyperplasia of the papillary urothelium, and/or mixed cell infiltrates in the renal papillae. Recovery was not observed for the papillary hyperplasia and tubular epithelium pigmentation. Adrenal enlargement correlated with enlargement of the zona fasciculata; the sponsor attributed this effect to stress. Decreased testes weight correlated with degeneration of the seminiferous tubules at 25 and 50 mg/kg, and decreased epididymides weight correlated with hypospermia at 50 mg/kg. Spermatid giant cells were also observed in the lumen of the seminiferous tubules at 25 and 50 mg/kg.

Reproductive Toxicology

Generally, clear drug-related differences in fertility were not observed in the male and female fertility and early embryonic development studies. In the male study, target organ findings similar to those observed in the toxicity studies were demonstrated (i.e., nonglandular stomach [squamous cell carcinoma, hyperplasia/hyperkeratosis, inflammation, ulcers], glandular stomach [hyperplasia, inflammation and erosions], kidney [nephropathy, hypertrophy, dilatation, hyaline droplet accumulation, and regeneration], testes [interstitial cell hyperplasia], and pancreatic lymph nodes [dilatation and hyperplasia]); in addition to these findings, the number of nonmotile sperm was slightly increased at 250 and 375 mg/kg. In the female study, 250 mg/kg caused maternal toxicity (e.g., body weight loss, stomach alterations), reduced estrous cycling, and increased cohabitation times; however, these effects did not translate into clear effects on fertility, as measured. There were slight increases in postimplantation loss, nonviable embryo count, and the number of dams with any nonviable embryos; a slight decrease was observed for viable embryo count.

The embryofetal development (EFD) studies demonstrated maternal toxicity, and effects on fetal viability. The rabbit EFD study demonstrated maternal toxicity (i.e., body weight losses), increased abortions, and some very slight changes in resorptions and viable fetuses at 150 mg/kg; MMF crossed the placenta and fetal plasma concentrations were about 10% those of the does. The rat EFD study demonstrated maternal toxicity (i.e., transient body weight losses, followed by persistent decreased body weight gains and stomach toxicity) and slight effects on fetal viability (e.g., slightly decreased litter size, increased number of dams with resorptions) and development (i.e., decreased fetal weights) at 250 mg/kg; MMF crossed the placenta and fetal plasma levels were 40-60% those of the dams.

In the pre-/postnatal study, maternal toxicity (i.e., decreased average body weight) was demonstrated at 250 mg/kg/day and fetal viability and development were altered (e.g., slightly increased gestation time, slightly decreased viability index, slightly increased pup deaths, reduced fetal body weights, slightly delayed sexual maturation, and slight effects on learning and memory as measured by water maze performance) at 250 mg/kg.

Genetic Toxicology

DMF

The initial *in vitro* genetic toxicology assays (i.e., bacterial reverse mutation, cell gene mutation, and chromosomal aberration in HPBL assays) were conducted pre-1990, and therefore have inadequacies compared to current methodologies. The bacterial reverse mutation and cell gene mutation assay were negative but lacked one of the required bacterial tester strains and adequate positive controls, and did not evaluate cultures with adequate cytotoxicity (RTGs ~20%), respectively. The initial chromosomal aberration assay also showed methodological inadequacies but showed concentration-related significant increases in the frequency of aberrations excluding gaps.

A chromosomal aberrations assay and an *in vivo* micronucleus assay were conducted more recently (circa 2004). The chromosomal aberrations assay demonstrated that DMF was positive for inducing chromosomal aberrations with a 3-hour treatment under non-activation conditions. The *in vivo* micronucleus assay was reported as negative, but the maximum dose was defined by questionable bone marrow cytotoxicity (particularly since a dose more than double that tested here was tolerated in an acute toxicity study).

Overall, the genetic toxicology results for DMF are equivocal. DMF was shown to induce chromosomal aberrations. Although the *in vivo* micronucleus assay was negative, DMF was not tested at a high enough dose.

MMF

The *in vitro* assays for metabolite MMF were conducted more recently, but the *in vivo* assay was conducted in the 1990s. The bacterial reverse mutation assay was negative and a chromosomal aberration assay with HPBL was positive without metabolic activation (note that the concentrations were lower than nominal).

An *in vivo* micronucleus assay was conducted with MMF in 1995. This nonstandard assay tested only one dose resulting in clinical signs (i.e., reduced mobility, ataxia and dyspnea) at 1 - 3 hr postdose. While the micronucleus assay was negative for inducing micronuclei, the assay was inadequate by design (i.e., single dose) and again may not have tested a high enough dose.

Overall, genetic toxicology testing for DMF and metabolite MMF demonstrated positive results. Although some *in vitro* tests were negative, chromosomal aberration assays were consistently positive, and the *in vivo* assays have methodological flaws that complicate their interpretation.

Carcinogenicity

Two-year rodent carcinogenicity bioassays were conducted in rats and mice. In rats, dimethyl fumarate was administered at oral doses of 25, 50, 100 and 150 mg/kg/day. Dosing was suspended for HDM and HMDM, and those groups were terminated early (week 86 and 88, respectively). A dose-related reduction in survival was observed for males (survival rates: 31%, 27%, 17% [ss], 13% [ss], and 13% [ss], respectively, for control, LD, LMD, HMD, and HD) but not females. Generally, dose-related reductions in body weight were observed (greater in M than F); at termination, average body weights were reduced 4%, 8%, 16% and 17% in males, and were +1%, +1.5%, -7.7%, and -12.4% in females. A dose-related exacerbation of chronic progressive nephropathy was observed; this was a common cause of death, especially in males. Pinworms were found in the main study animals, and the presence appeared dose -related; the sponsor indicated that this did not affect the interpretation of the study. In rats, increased incidences of renal tubular adenomas and carcinomas (at 3 times the RHD, on a mg/m^2 basis; NOEL was ~2x the RHD), testicular interstitial (Leydig) cell adenomas (at 2 times the RHD, on a mg/m^2 basis; NOEL was equal to the RHD), and nonglandular stomach squamous cell papilloma and carcinoma (at less than the RHD, on a mg/m2 basis) were seen. A secondary expert analysis of the renal hyperplasias and neoplasias indicated that the neoplastic effect was not significant, and is possibly attributable to exacerbation of rat CPN.

In mice, dimethyl fumarate was administered at oral doses of 25, 75, 200, and 400 mg/kg/day. The HD was reduced from 600 to 400 mg/kg on D9 due to deaths (15 HDM and 13 HDF) and reduced body weights. Dosing was later suspended in HD animals 72 (males) or week 82 (females). Survival was reduced at HMD (25% in M and 32% in F) and HD (13%). Dose-related toxicity in the stomach and kidney was associated with drug-related mortality. In the kidney, there were dose-dependent increases in nephropathy (esp. males), other renal lesions (including cysts), and neoplasias in both sexes (HMDM, HDM & HDF). In the stomach, many lesions and hyperplasias that extended into the nonglandular submucosa and nonglandular/glandular serosa (HMD & HD) were observed, in addition to observed neoplasias in both sexes. In mice, the incidence of renal tubular adenomas and carcinomas, and nonglandular stomach leiomyosarcoma, papilloma, and squamous cell carcinoma were increased at less than the RHD, on a mg/m^2 basis. A secondary expert analysis indicated that mice showed

an exacerbation of CPN, but relationship between the observed CPN and hyperplasia/neoplasia was not as clear.

Target organs of DMF-related toxicity

Nonglandular stomach

Nonglandular stomach showed clear, drug-related toxicity; severe damage and neoplastic alterations were observed. The incidence of nonglandular stomach tumors both in the carcinogenicity assays and in other nonclinical studies was striking. In addition to tumors (squamous cell adenomas and carcinomas, as well as leiomyosarcomas) observed in the carcinogenicity assays at all doses (and below the RHD on a mg/m2 basis), tumors were also noted at higher doses in a number of shorter duration studies, including: 1 SC carcinoma at 200 mg/kg in 6-month study (M); 2 SC carcinomas at 250 mg/kg in 3-month toxicity study(1/sex); 5 SC carcinomas at 375 mg/kg, 1 SC papilloma at 375 mg/kg, 5 SC carcinomas at 250 mg/kg, and 1 SC carcinoma at 75 mg/kg with 14-weeks dosing (also showed glandular stomach toxicity). Female rats in the reproduction studies testing doses up to 250 mg/kg were only assessed grossly, but had clear macroscopic observations at 100 and 250 mg/kg. The stomach tumors may be explained by the irritancy of DMF and the concentrative-nature of the organ, but even the fact that humans do not possess a direct anatomical correlate to the rodent forestomach is not completely comforting; it is not clear that the same irritancy would not have effects in human esophagus and/or stomach with potentially decades of exposure. The chemist noted that DMF has a low vapor pressure and could potentially evaporate from the capsules, therefore exposing tissues of the skin and GI tract repeatedly. The existing human exposures in the clinical trials describe GI and upper GI discomfort/pain as a common side effect (alternatively, this effect may be related to the binding of MMF to the GPR109A nicotinic acid receptor).

The Nrf2 pathway may play a vital role in the observed GI toxicity, although it is not clear. It has been shown that genetic knockout of KEAP1 in mice leads to postnatal death within 3 weeks of birth due to hyperkeratosis of the esophagus and forestomach (Wakabayashi et al., 2003). This suggests that the effects of the pharmacologic manipulation demonstrated in the 2-year rodent bioassays (i.e., hyperkeratosis of the forestomach at all doses) are not so dissimilar from genetic manipulation, and could argue against simple irritancy as the cause of the forestomach toxicity in rodents. It is not clear whether longer duration, repeated dosing could yield similar effects in humans in anatomically similar sites (e.g., esophagus).

Kidney

Renal toxicity was observed in all nonclinical species examined (monkey, dog, rabbit, rat and mouse), generally with lower margins at increased treatment durations. In rodents, renal neoplasias were also observed. Although exacerbation of rodent chronic progressive nephropathy (CPN) was clearly observed in the studies (which in the long term studies often lead to kidney failure, secondary effects, and death), this is clearly not the sole toxic process occurring and it may not be the process leading to the tumor incidence; for this reason, the human relevance of the toxicity and tumorigenicity is still

in question. Kidney toxicity was not limited to findings classically considered within the spectrum of CPN-related alterations or in typical species; for example, tubular regeneration was observed in multiple species (not just rodents). In nonrodents, toxic effects were observed in the tubules (e.g., pigmentation, single cell necrosis, atrophy), the cortex (e.g., basement membrane thickening, atrophy), and/or in the papillae (e.g., mixed cell infiltrates and urothelium hyperplasia). Although some of these changes demonstrated at least partial recovery, it is notable that evidence of irreversible damage (i.e., interstitial fibrosis) was noted in two recovery monkeys following 12 months of treatment. Additionally, an increased incidence of renal cysts was observed in mice. The observed histopathological alterations were not always accompanied by clear changes in serum chemistry and/or urinalysis. Generally, decreases in BUN and/or creatinine were observed (rats, do, monkey), but urine proteins were only clearly increased in rats. Investigative studies in rats identified urinary microalbumin as a potential early indicator of tubule toxicity. Per the clinicians, demonstrable renal toxicity has not been observed in humans; microalbumin testing (a change noted in rodent toxicity studies and suggested as a possible marker in humans) has not yielded significant findings.

Within an expert opinion by (b) (4) (in a 120-day clinical safety update submission dated 6/26/12; see submission dated 10/23/12) provided by the sponsor, (b) (4) re-examined the renal tissues and altered the histopathological description and interpretations of the renal results of the 2-year rodent bioassays rather dramatically. Based on (b) (4) re-analysis, the prevalence and significance of renal tumors in rats was decreased after a spontaneously-occurring A-V variety was removed. However, (b) (4) observed that increased cyst presence (and not necessarily areas of CPN) may have been related to the tumor incidence in mice. (b) (4) description of renal tumors in mice arising from cysts rather than areas of CPN, as described by the sponsor, is disconcerting. Additionally, from the available data, the severity of the cyst formation might not reliably predict renal tumor presence. In a 10/5/12 submission, the sponsor briefly discussed literature regarding increased intracellular fumaric acid (a potential "oncometabolite") levels, the sponsor's proposed mechanism of action (i.e., Nrf2 transcription factor activation), and the tumor types that are believed to result from such activity (i.e., renal and Leydig cell tumors, as well as leimyosarcomas). Although the sponsor has proposed that all tumors occurred by mechanisms not relevant to humans, it is concerning that the tumors were observed in organs suggested by the discussed mechanism of action (that is, increased activation of Nrf2; e.g.,, kidney, testis, forestomach).

Testes

Both damage and neoplasias were observed in testes. Seminiferous tubule degeneration and atrophy/degeneration of the germinal epithelium were observed in the 11-month dog toxicity study and 2-year rat carcinogenicity bioassay, respectively. Interstitial (Leydig) cell hyperplasia (studies of duration ≥ 14 weeks) and tumors (2-year carcinogenicity bioassay) were observed in rats. The relevance of the observed rodent hyperplasia/neoplasia to humans is unclear. Although the sponsor argued that Leydig cell tumors in rats are common and not relevant to human risk (e.g., Prentice and

Meikle, 1995, Clegg et al., 1997, Cook et al., 1999, Cohen et al., 2003), there is some question about the relevancy based on mechanism. According to expert working groups on the subject (e.g., Clegg et al., 1997, Cook et al., 1999), several hormonal alterations leading to Leydig cell adenomas in rats have the potential to be relevant in humans. One group deemed GnRH agonism and dopamine agonism not relevant to humans, but stated that androgen receptor antagonism, 5α-reductase inhibition, testosterone biosynthesis inhibition, aromatase inhibition, and estrogen agonism (all generating an increase in LH) were at least potentially relevant, albeit with possible quantitative differences existing across species (i.e., rodents being more sensitive). The authors of Cook et al., 1999 also indicated that seminiferous tubule damage would likely lead to Leydig cell hypertrophy, and not hyperplasia, in humans.

Hormonal regulation arguments aside, a specific role for activation of the Nrf2 pathway in testicular Leydig cell toxicity has been demonstrated. Ethane dimethylsulfonate (EDS), a well-known alkylating agent, inhibits testosterone biosynthesis and destroys Leydig cells in a number of species (Lee et al., 2012); gene expression analysis showed induction of genes of the Nrf2 pathway, including gene expression increases in NAD(P)H dehydrogenase, quinone 1 (NQO1), Heme oxygenase (decycling) 1 (Hmox1), Cyclin-dependent kinase inhibitor 1A (p21, Cip1), and Glutathione S-transferase omega 1. This could suggest that either there was too much oxidative damage for to be overcome by activation of the Nrf2 pathway or activation of the Nrf2 pathway may help lead down the pathway to Leydig cell damage. With regard to neoplasia, it is known that Leydig cell tumors are part of the tumor profile believed to result from genetic mutations resulting in low fumarate hydratase and Nrf2 activation (Carvajal-Carmona et al., 2006).

Without evidence suggesting a specific mode of action in the Leydig cell toxicity (or indicating that LH is not increased, therefore being inconsistent with the "potentially human-relevant" modes), the most conservative approach would be to assume relevancy to humans. In the potentially relevant cases, the expert working group felt that a margin of exposure approach should be used for compounds causing Leydig cell adenoma by a hormonal mode that is relevant to humans. In a 6-month study in rats, hyperplasia was demonstrated at 4x the RHD and was not observed at 2x the RHD; however, in the 2-year bioassay, adenomas were observed at 2x the RHD (NOEL at the RHD) and hyperplasia was observed at all doses. It is not clear what mode of action is leading to the tumor formation, but there does not appear to be large margins to the human dose, especially when taken for long periods of time.

Heart

Although few clear signs of toxicity were apparent in heart, the doses tested were generally relatively low multiples of the RHD and minimal signs were observed. Heart weight increases and/or enlargement were observed in rats. In the 2-year carcinogenicity bioassays, a dose-related increased incidence and/or severity of cardiomyopathy was observed, as well as an increased incidence and severity of atrial thrombosis. In the rat, enlarged heart was observed with increased incidence in treated males and HDF; an increased incidence of foci was suggested in HMDM and HDF.

Mineralization and other effects were generally attributed to secondary effects of the renal and/or stomach toxicities leading to morbidity/mortality. However, there is some suggestion that, again, the described mechanism of action of DMF/MMF (i.e., Nrf2 transcriptional activation) could be expected to produce some cardiovascular toxicity. Although the antioxidant activity related to Nrf2 activation is generally thought to be cardioprotective (Ashrafian et al., 2012; using Fh1cardiac KO mice), chronic activation of the pathway has been shown to produce increased protein aggregation and cardiomyopathy in mice (Rajasekaran et al., 2011).

Eye- Retina

Clear, drug-related effects in the retina were only observed in the 2-year carcinogenicity bioassay in mice. Although the potential mechanism underlying the toxicity is unclear, MMF has been shown to be a potent nicotinic acid receptor (i.e., GPR109A) agonist (Tang et al., 2008). Recent studies have also demonstrated the expression and localization of nicotinic acid receptor mRNA and protein in the basolateral membrane of mammalian retinal pigment epithelium (Martin et al., 2009); it was suggested that the presence and activation of this receptor plays a role in diabetic retinopathy. Although a theoretical concern based on the activity of MMF at the GPR109A receptor, the relevance of this finding in humans is unknown.

Conclusions

DMF has been shown to have toxic effects on a number of organs. Dose-limiting effects in the nonclinical species often involved effects on weight (losses/reductions). Generally, the toxicities were dose- and duration- dependent; target organs included stomach, kidney, pancreas, liver, heart, lymphatic system, and testes. Stomach (nonglandular; rodents), kidney (tubular regeneration, damage and/or and a number of effects including tumors; rodents, dog, monkey) and testes (seminiferous tubule degeneration in dog and hyperplasia in rats) most clearly affected with lower margins to human exposures with increased treatment durations. Although kidney was often affected, clinical pathology assessments were often not of assistance in identifying this toxicity.

The majority of the target organs of DMF-related toxicity (i.e., stomach, kidney, testes, and possibly heart) appear to share activation of the Nrf2 transcriptional pathway (i.e., the sponsor's putative mechanism of action of DMF and MMF) as a potential common toxicologic mechanism. While Nrf2 activation is known to affect genes related to detoxification and chemopreventive effects (i.e., protection against cancer development in several models of chemical-induced carcinogenesis; e.g., Yates et al., 2009, Wakabayashi et al., 2010), the Nrf2 pathway appears to act as a double-sided sword generating toxicity (including carcinogenesis) when the activity of the pathway is deregulated. It is possible that the mechanism conferring a protective effect elicited by some stimuli (e.g., a genotoxic agent) may cause toxicity and even tumor formation/promotion when the pathway is activated without the physiological demand or constitutively (such as that demonstrated by genetic knock-outs). Although the exemplary genetic mutations and exploited genetic modifications in animal models of the mechanism typically involve increased intracellular fumarate as a common step, the

tangible differences between these alterations and the demonstrated pharmacological manipulations of the Nrf2 pathway are unknown. It is possible, as the sponsor argued, that the strength of the activation of the pathway would differ based on "pulsatile" changes that might result from the pharmacological manipulation; however, it is not clear that this description is fitting for the downstream effects of DMF/MMF. Most importantly, the intracellular levels of DMF, MMF and/or fumaric acid resulting from DMF treatments are unknown, as is the time course for the resulting downstream effects. The sponsor and others (e.g., Adam et al., 2011; Ooi and Furge, 2012; Lin et al., 2012) have shown that DFM/MMF activate the Nrf2 transcriptional pathway by alteration (alkylation or succination, this is not clear from the literature) of an operative cysteine residue (-38, -151, -241, -319, and/or -613) on the KEAP-1 regulatory enzyme; this disruption in the KEAP1 interaction with Nrf2 leads to accumulation of Nrf2 in cells. The sponsor demonstrated accumulation of Nrf2 protein in the cells and functional activation of the Nrf2 pathway through gene expression analysis following DMF and MMF exposure. In fact, the DMF/MMF-induced gene expression demonstrated by the sponsor varied by gene, tissue, and temporal characteristics; however, it was clear that gene expression in a number of tissues was altered by up to 24 hrs or longer (in contrast to the sponsor's assertion that long-lasting effects would not be expected because of the very short half-life of the drug). Tissues demonstrated to show high exposures (i.e., greater than plasma) following administration of radiolabeled drug included: kidney, stomach, liver, pancreas, brain, small intestine and salivary gland. Although some of the demonstrated toxicity may be explained by other means (e.g., irritancy of the nonglandular stomach lining, exacerbation of CPN in rodents, hormonal alterations leading to Leydig cell hyperplasia and tumorigenesis in rodents), the ability of the pharmacologic mechanism to explain the distribution of the toxicities is striking and troublesome. It is not clear that the sponsor's other explanations of the toxicities are wholly accurate. And in some cases (e.g., stomach toxicity), it is not clear that, with decades of dosing in humans, some of these same toxicities might not arise from the repeated exposures over such a long period of time.

In addition to the Nrf2 mechanism, it is possible that other observed toxicities (i.e., flushing, GI complaints, retinopathy, and possibly reduced fat stores) may be explained by the activity of MMF at the GPR109A nicotinic acid receptor. MMF has been shown to be a potent agonist at the GPR109A receptor (Tang et al., 2008). Others later described flushing in response to MMF (Hanson et al., 2010). A potential role for GPR109A activity in diabetic retinopathy has also been described (cf., Martin et al., 2009, Gambhir et al., 2012); this may play a role in the retinopathy observed in the mouse carcinogenicity assay that was not observed in previous studies. Finally, although not discussed at length as a toxicity of DMF/MMF exposure, the observation of reduced abdominal fat stores in the rodent carcinogenicity assays may also reflect a known function of activation of the GPR109A receptor, lipid effects (Wanders & Judd, 2011).

12 Appendix/Attachments

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/s/

MELISSA K BANKS-MUCKENFUSS
01/27/2013

LOIS M FREED
01/28/2013

PHARMACOLOGY/TOXICOLOGY
FILING CHECKLIST FOR NDA

NDA/BLA Number: 204063 **Applicant: Biogen Idec Inc.** **Stamp Date: 02/27/12**

Drug Name: (b) (4) **(proposed), BG00012, BG-12, dimethyl fumarate** **NDA/BLA Type: Commercial NDA**

On **initial** overview of the NDA/BLA application for filing:

	Content Parameter	Yes	No	Comment
1	Is the pharmacology/toxicology section organized in accord with current regulations and guidelines for format and content in a manner to allow substantive review to begin?	X		It appears adequate.
2	Is the pharmacology/toxicology section indexed and paginated in a manner allowing substantive review to begin?	X		It appears adequate.
3	Is the pharmacology/toxicology section legible so that substantive review can begin?	X		It appears adequate.
4	Are all required (*) and requested IND studies (in accord with 505 b1 and b2 including referenced literature) completed and submitted (carcinogenicity, mutagenicity, teratogenicity, effects on fertility, juvenile studies, acute and repeat dose adult animal studies, animal ADME studies, safety pharmacology, etc)?	X		Generally, the standard studies appear to have been conducted; however, the CNS safety pharmacology studies were conducted with Fumaderm (DMF is one component of this drug). Also, a standard receptor binding screen does not appear to have been conducted.
5	If the formulation to be marketed is different from the formulation used in the toxicology studies, have studies by the appropriate route been conducted with appropriate formulations? (For other than the oral route, some studies may be by routes different from the clinical route intentionally and by desire of the FDA).	X		In most studies, BG00012 was administered to animals by oral gavage (DMF/HPMC formulation), although the clinical drug product was used in the chronic toxicity study in dog.
6	Does the route of administration used in the animal studies appear to be the same as the intended human exposure route? If not, has the applicant submitted a rationale to justify the alternative route?	X		Yes.
7	Has the applicant submitted a statement(s) that all of the pivotal pharm/tox studies have been performed in accordance with the GLP regulations (21 CFR 58) or an explanation for any significant deviations?	X		This statement can be found in the Nonclinical Overview (page 6 of 74).
8	Has the applicant submitted all special studies/data requested by the Division	X		It appears that most standard studies were conducted for BG00012, and

PHARMACOLOGY/TOXICOLOGY
FILING CHECKLIST FOR NDA

	Content Parameter	Yes	No	Comment
	during pre-submission discussions?			genetic toxicology studies were conducted with MMF in addition to DMF, as requested.
9	Are the proposed labeling sections relative to pharmacology/toxicology appropriate (including human dose multiples expressed in either mg/m2 or comparative serum/plasma levels) and in accordance with 201.57?	X		The sponsor has provided labeling, the adequacy of which is a review issue. Notably, the reproductive toxicity study margins were given on a mg/m^2 basis, but were given on an AUC basis for the carcinogenicity studies.
10	Have any impurity – etc. issues been addressed? (New toxicity studies may not be needed.)	X		In a CMC meeting (7/21/11), the sponsor identified the potential to generate (b) (4) The sponsor was advised to monitor for (b) (4) in the drug product, or to provide adequate data and a rationale for its exclusion. The sponsor also identifies (b) (4) as a possible impurity, but indicates that it is not genotoxic. Specified organic impurities include (b) (4); both are identified as organic impurities, degradation products, and metabolites.
11	Has the applicant addressed any abuse potential issues in the submission?	X		This is the purview of CSS, not Pharm/Tox. The Nonclinical Overview states that, "Consistent with ICH M3(R2…), abuse liability studies were not conducted as DMF was not shown to have any CNS activities that would be indicative of abuse or dependency in nonclinical or clinical studies."
12	If this NDA/BLA is to support a Rx to OTC switch, have all relevant studies been submitted?			n/a

IS THE PHARMACOLOGY/TOXICOLOGY SECTION OF THE APPLICATION FILEABLE?
__Yes___

Please identify and list any potential review issues to be forwarded to the Applicant for the 74-day letter:

Please submit standard receptor binding screens for BG-12 and MMF for inclusion in the NDA.

Electronic Signatures:

Melissa K. Banks-Muckenfuss, Ph.D.
Lois M. Freed, Ph.D.

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

/s/

MELISSA K BANKS-MUCKENFUSS
04/30/2012

LOIS M FREED
05/02/2012
CSS is requesting the results of in vitro receptor binding screens for parent and major metabolite.