Observation	Males			Females		
	Early death	Interim Sac	Final Sac	Early Death	Interim Sac	Final Sac
Tumor Observations						
Mammary gland-carcinoma	---	1/15 M, 1/14 H	1/13 H	9/10 H	13/13 H	2/20 L, 2/20 M, 11/11 H
Heart-mal. Schwannoma		1/14 H				2/11 H
Eye-fibrosarcoma	1/8 H					
Seminal vesicles-carcinoma			1/13 H			
Seminal vesicles-fibrosarcoma			1/13 H			
Mandibular salivary gland-sarcoma		1/14 H				
Urinary bladder-benign fibroma		1/14 H				
Skin-basal cell adenoma		1/14 H	3/13 H			
Thyroid-benign follicular adenoma		1/14 H				
Abdominal cavity-fibrosarcoma			3/13 H			
Ovary-benign granuloma/thecal cell tumor					1/13 H	
Harderian gland—mal. Schwannoma					1/13 H	
Optic nerve-mal. Schwannoma					1/13 H	
Subcutaneous tissue, fibrosarcoma			1/13 H			
Adrenal cortex---benign adenoma					1/14 H	
Lung—β-alveolar/bronchiolar adenoma			1/13 H			
Pituitary—benign adenoma						1/11 H
Prostate—invasive fibrosarcoma			1/13 H			
Skin—benign keratoacanthoma	3/8 H	5/14 H	1/19 M, 7/13 H			
Other pathology						
Adrenal cortex—hypertrophy	1/1 L, M	4/14 H	1/19 L, 3/19 M, 4/13 H	6/10 H	1/15 L, 2/15 M, 13/14 H	1/20 L, 5/20 M, 7/11 H
Adrenal cortex-chronic inflammation		1/14 H				
Adrenal cortex—leukocytosis				5/10 H		
Adrenal cortex—congestion						1/11 H
Spleen—lymphoid depletion	1/1 M, 1/8 H					
Spleen—myeloid hyperplasia				5/10 H	4/14 H	8/11 H
Liver—necrosis	1/8 H			3/10 H	1/14 H	2/11 H

Liver-congestion	3/8 H	1/15 L		1/10 H		
Liver—chronic inflammation		1/14 H				2/11 H
Kidney—chronic inflammation	2/8 H	1/15 M				
Kidney—suppurative inflammation	2/8 H					
Pancreas—suppurative inflammation	1/1 M					
Pancreas—chronic inflammation		3/15 M				
Testes—syncytial cells	2/8 H	3/15 M, 13/14 H	6/19 M, 13/13 H			
Epididymis—syncytial cells	1/8 H	9/14 H	1/13 H			
Seminal vesicles—chronic inflammation		1/14 H				
Prostate—suppurative inflammation	1/1 M, 2/8 H		2/13 H			
Urinary bladder—suppurative inflammation	1/1 M, 1/8 H		1/13 H			
Thymus—lymphoid depletion	1/1 L, 1/1 M, 7/8 H	5/15 L, 9/15 M, 11/14 H	6/13 H	8/10 H	14/14L, 15/15 M, 14/14 H	1/11 H
Eye—suppurative inflammation	2/8 H	1/15 C			1/14 H	
Eye—retinal degeneration						1/20 M, 1/11 H
Eye—hemorrhage/cataract/ulcer	1/8 H		1/13 H		4/14 H	1/20 L, 1/20 M
Lymph node—lymphoid hyperplasia				1/10 H		1/11 H
Marrow—myeloid hyperplasia			1/13 H	6/10 H	3/14 H	8/11 H
Stomach—necrosis					1/14 H	
Pituitary—hypertrophy						1/11 H

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Toxicokinetics:

Plasma levels were measured for up to 4 hours after drug administration. The coefficient of variance (%CV) was near 30% for most plasma concentration measurements. Within this error, there were no differences between either Cmax or AUC on day 1 vs day 5 of each cycle; nor were there gender related differences between plasma levels. Tmax was variable. AUC was relatively linear with dose up to 125 mg/m^2. Values are shown in the following table.

Dose (mg/m^2)	Sex	Day	Cmax[a] (µg/ml)	Tmax[a] (hr)	AUC(0-t)[a] (µg-hr/ml)
25	Male	1	2.07	1.00	5.57
		5	2.60	2.00	7.28
		57	1.68	1.00	4.94
		61	2.22	1.00	6.26
		141	1.81	0.25	2.90
		145	2.35	0.25	4.92
	Female	1	2.41	0.50	6.03
		5	2.46	0.50	5.81
		57	3.31	0.25	6.53
		61	3.09	0.50	8.01
		141	2.51	0.25	4.81
		145	3.48	0.50	6.95
50	Male	1	3.59	1.00	9.57
		5	3.72	0.50	9.20
		57	3.58	0.25	8.06
		61	3.25	1.00	8.94
		141	2.85	0.50	7.28
		145	3.58	1.00	8.86
	Female	1	4.57	0.50	11.3
		5	4.20	0.50	10.3
		57	5.53	0.50	11.0
		61	5.48	0.25	11.1
		141	3.56	0.50	8.03
		145	5.11	0.25	11.1
125	Male	1	12.7	1.00	32.9
		5	9.58	0.50	22.9
		57	5.97	1.00	17.0
		61	7.89	0.50	18.8
		141	6.37	1.00	17.1
		145	10.1	0.50	22.5
	Female	1	12.1	0.50	28.2
		5	9.11	1.00	23.1
		57	9.11	0.25	19.3
		61	11.8	0.50	23.6
		141	ND	ND	ND
		145	ND	ND	ND

[a] Pharmacokinetic parameters were determined from mean plasma concentration-time data (n = 5 rats/sex/timepoint)
ND Parameter not determined; no blood samples were collected from female rats

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3. P-5877. Single-cycle oral toxicity study of SCH 52365 in dogs.

Conducted at:
When conducted: March 1993
GLP: YES
Drug Lot #: BA # 28395-103 (AJ-A8.2), INV # 920236001, RIC #17505912
Doses: 0, 200, 500, 1000 mg/m^2 in capsules
Schedule: DX5 with an additional 23 days of observation
Species used: Beagle dogs, 11-12 months old, M: 9.3-12.0 kg, F: 7.3-9.7 kg
#/sex/dose: 7/sex/dose
Final day of observation: day 20 (due to mortality in study)

Measurements and Observations:

Twice daily: mortality and clinical signs
Daily: Food consumption
Pretest, day 4: rectal temperature, blood pressure
Day 1, 8, 15: body weight
Pretest, Day 8: ophthalmoscopy
Pretest, week 1: ECG
Pretest, day 2, 13: hematology, serum chemistry, urinalysis
Days 1 and 5: Predose, 15, 30 minutes, 1, 2, 4 hours post-dose: PK
Termination (interim sac D6 in 3/sex/dose–used sickest dogs from higher doses): gross pathology, organ weights, histopathology

Mortality and clinical signs:

The experiment was terminated on day 20 due to excessive toxicity. The following table summarizes early deaths and clinical signs.

Dose mg/m^2	Males		Females	
	# dead (day)	Observations	# dead (day)	Observations
0	3 (d6 ss) 4 (d20 ss)	soft feces	3 (d6 ss) 4 (d20 ss)	emesis, mucoid feces
200	3 (d 6 ss) 1 (d12 ms) 3 (d13 ms)	emesis, dehydration, anorexia, ataxia, diarrhea, blood from anus, mydriasis, swollen/bruised chest/ abdomen	3 (d6 ss) 4 (d13 ms)	emesis, diarrhea, swollen abdomen, hypoactivity, few/soft/discolored feces, dehydration, swollen limbs, anorexia,
500	3 (d6 ss) 4 (d10 ms)	diarrhea, discolored/reduced feces (black), emesis, hypoactivity	3 (d6 ss) 4 (d9 ms)	dehydration, emesis, few/no/soft/discolored feces, hypoactivity, anorexia
1000	4 (d9, 1 fd/ 3 ms) 3 (d6 1 fd/2 ss)	dehydration, hypoactivity, diarrhea, emesis, discolored feces dyspnea, prostration	3 (d6 2 ss, 1 ms) 1 (d8 ms) 2 (d9 fd) 1 (d7 fd)	dehydration, emesis, hypoactivity, prostration, diarrhea, discolored feces, sore limbs, mydriasis

ss=scheduled sacrifice, fd=found dead, ms=moribund sacrifice

Body weight and food consumption:

By day 8, the HD dogs lost 13% of their initial body weight. In terms of body weight gain, the MD and HD dogs of both sexes lost approximately 1 and 2 kg respectively by day 8. Food consumption was cut in half in the MD males (by 2/3 in the MD females), while at the HD, food consumption decreased by >90%.

Body temperature: There were no remarkable changes in body temperature with treatment; however, just prior to death, several dogs had elevated temperature to 103-104° F.

Ophthalmoscopy: There were no remarkable changes in body temperature with treatment.

Blood pressure and respiration:

Respiratory rate was decreased by 1/3 in 2 HD females at day 4. There were no remarkable changes in blood pressure with treatment at day 4.

ECG: There were no remarkable changes with treatment.

Hematology:

Only the LD dogs were alive at day 13. All blood parameters were decreased in this experiment. Minimal differences were noted between males and females. Platelet # was decreased less at the HD as these animals died earlier. All WBC elements measured decreased (e.g. neutrophils, lymphocytes, monocytes).

	Day 13 (LD only)	Moribund sac
RBC #	↓42% M, ↓26% F	—
Reticulocyte #	↓ >95%	↓ >95%
Platelet #	↓>99%	↓50-90% (less decrement @ HD)
WBC #	↓50-60%	↓>90%

Serum Chemistry:

In the moribund sacrifice animals, no controls were included for comparison and decrements were not dose or gender dependent. Thus, the magnitude of changes are based on pretest and prior control values and are summarized in the following table along with the changes at day 13 in the LD males and females.

	Day 13 Low dose (200 mg/m^2)		Moribund sacrifice
	Males	Females	All doses, both sexes
Glucose	—	—	↑up to 2X
BUN	—	—	↑5-7X
Creatinine	—	—	↑40%-3X
Total cholesterol	—	—	↑up to 3X
AST/ALT	—	—	↑up to 2X
Bilirubin	—	—	↑mostly 4-9X, 1@ 35X
Triglycerides	—	—	↑up to 6X
Na+	—	—	↓up to13 Meq/L
Cl-	—	—	↓up to 40%
ALP	—	↑2.5X	↑2-7X
LDH	↓60%	↓80%	↓10%-90%
Total protein	↓20%	↓20%	↓10%
Albumin	↓20%	↓20%	↓10-20%
Globulin	↓20%	↓20%	↓10-20%
P_I	↓25%	↓15%	↑up to 3X
K+	↓20%	↓15%	↓40%

Urinalysis:

Data was only available on day 2. There were trends toward decreases in urinary electrolytes (Na, K, Cl), creatinine, and an increase in Ca^{++} in males

Gross Pathology:

Macroscopic changes included darkening of the mucosa of the gastrointestinal tract, enlargement of the adrenal cortex, liver, and kidney, and dark/pale areas of the spleen, heart, lymph nodes and lung. Small thymus was also observed. Gelatinous areas of the pancreas and subcutaneous tissue were also observed.

Organ weights:

Organ weight data was difficult to interpret due to differences in timing of moribund sacrifices. There were apparent increases in adrenal liver, and pituitary weights; while thymus and spleen weights diminished.

Histopathology:

The cause of death (or early sacrifice) was primarily marrow atrophy, sepsis, hemorrhage at multiple sites, variable liver damage, and some degree of gi necrosis at the HD (1 male and all 4 females with sacrifice >day 6). Incidences of observations are shown in the table below. Target organs in the dying dogs included liver, kidney, and marrow/thymus. Minimal hemorrhages were also seen in the skin, subcutaneous tissue and various other sites.

Incidence of microscopic observations in the single cycle dog at high doses				
	Males		Females	
	Day 6 N=3	Termination N=4	Day 6 N=3	Termination N=4
Adrenal—congestion/hemorrhage	3H	3M, 1H	3H	1M, 3H
Adrenal—hypertrophy/vacuolization	3H	1L, 2M, 4H	2M, 3H	3M, 4H
Heart—hemorrhage	1H	—	—	1L, 1H
Lung—congestion	1H	1H	—	1L, 1H
Lung—hemorrhage/necrosis/fibrin	—	2L, 1M, 1H	1M	1L, 2H
Spleen—lymphoid depletion	1L, 3M, 3H	2L, 4M, 4H	2L, 3M, 3H	2L, 4M, 4H
Spleen—increased pigment	1M, 2H	1C, 4L, 1M, 4H	1C, L, M; 3H	1L, 1M, 3H
Spleen—edema	—	—	—	2H
Liver—congestion	2M, 2H	1L, 3M, 2H	1L, 2M, 1H	3C, 2L, 3M, 4H
Liver—Periportal inflammation	1H	—	1M, 2H	1H
Liver—bile stasis	—	4M, 3H	—	4M, 4H
Liver necrosis	1H	1H	—	2H
Liver—hypertrophy	—	4M, 3H	—	1L, 4M, 4H
Kidney—tub. Dilatation/necrosis	1M	—	1H	1M, 2H
Kidney—congestion/hemorrhage	—	1H	1H	1L, 2H
Esophagus—mucosal necrosis/ulcer	2M, 3H	1L, 1M, 2H	1M, 3H	2M, 4H
Esophagus—mucosal hypertrophy/regeneration	—	1L, 4M, 3H	—	4M
Esophagus—mucosal degeneration/vacuolization	—	1L, 3M, 2H	—	4M
Esophagus—hemorrhage	—	1M	—	1M, 4H
Stomach—hemorrhage	—	3L, 2M	—	3L, 1H
Stomach—superficial columnar epithelial atrophy	—	1M, 1H	1H	1H
Intestines—crypt gland necrosis	2M, 3H	1C, 2L, 4M&H	1L, 3M, 3H	1L, 3M, 4H
Intestines—decr. Lymphoid tissue	2M, 3H	1L, 4M, 4H	1L, 3M, 3H	4M, 4H
Intestines—hemorrhage	2H	1M, 2H	1M, 3H	1L, 2M, 3H
Intestines—crypt gland regeneration	3M, 3H	1M, 1H	3M, 3H	1M, 2H
Intestines—villous atrophy	3H	1H	1M, 3H	2H
Pancreas—lobule necrosis	—	—	1H	—
Pancreas—atrophy	—	1L	—	1L
LNode—lymphoid depletion	3H	3M, 4H	1M, 3H	2M, 4H
LNode—congestion/hemorrhage	1L, 2H	4L, 4M, 3H	1L, 1H	1C, 4L, 4M, 4H
Testes—syncytial cells	1C, 3H	3M, 4H		
Testes—atrophy	—	2L, 1M		
Urinary bladder—hemorrhage	—	3L, 1M	1M	2L
Urinary bladder—congestion	—	—	1H	1M, 2H
Salivary gland—atrophy	2H	1L, 3H	1M, 3H	1M, 4H
Thymus—lymphoid depletion	2L, 3M, 3H	3L, 3M, 4H	1L, 3M, 3H	4L, 4M, 4H
Thymus—congestion	—	1H	—	1H
Marrow—atrophy	3M, 3H	3L, 4M, 4H	1L, 3M, 3H	4L, 4M, 4H
Retina—outer layer, degeneration/necrosis	3H	3H	3H	4H
Tongue—mucosal epithelial hyperplasia/regeneration	1M, 3H	1C, 2L, 4M, 4H	3M, 1H	4M, 2H

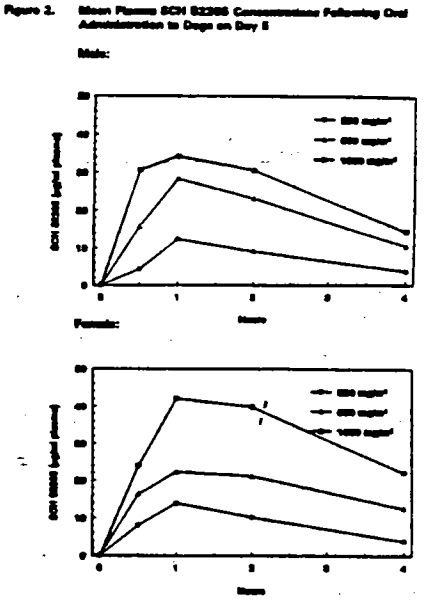
PK:

Samples were analyzed by _ with an internal standard. The lower limit of quantification was 0.10 ug/mL. There were no appreciable differences in AUC_{0-4h} between genders or day 1 vs day 5. Tmax occurred between 0.5 and 2 hours. AUC and Cmax were relatively linear with dose. AUC and Cmax data are summarized below.

AUC (ug•hr/mL) and Cmax (ug/mL) values for Single Cycle dog study at high doses.

Dose mg/m^2	Parameter	Males		Females	
		Day 1	Day 5	Day 1	Day 5
200	Cmax	13.4 ± 2.5	13.2 ± 2.4	15.0 ± 3.6	14.5 ± 1.8
	AUC	30.8 ± 4.0	28.8 ± 3.0	31.4 ± 5.0	33.8 ± 3.6
500	Cmax	28.1 ± 7.3	32.4 ± 7.2	34.0 ± 8.0	29.9 ± 4.6
	AUC	63.5 ± 14.0	73.9 ± 8.9	70.9 ± 12.8	69.2 ± 10.3
1000	Cmax	45.1 ± 12.8	46.4 ± 35.1	47.5 ± 20.3	52.5 ± 35.6
	AUC	101.9 ± 24.7	100.9 ± 72.9	108.3 ± 35.0	125.5 ± 90.5

Values are +/- standard deviation



4. P-5878. Single-cycle oral toxicity study with lower doses of SCH 52365 in dogs.

Conducted at:
When conducted: April-May 1993
GLP: Yes
Route: oral in gelatin capsules
Drug Lot #: INV #920236001, BA #28395-103, RIC # 17505912
Dosing: 0, 25, 50, 125 mg/m^2/day
Schedule: DX5 with 23 days of observation
Species: Beagle dogs; 13 months old; M: 7.5-13.4 kg; F: 6.7-10.9 kg
#/sex/dose: 7/sex/dose
Termination: D6 (interim sac, n=3/sex/dose), D29 (final sac, 4/sex/dose)

Measurements and Observations:

Twice daily: mortality and clinical signs
Pretest, weekly: body weight,
Daily: food consumption, rectal temperature
Pretest, D6, 27: ophthalmoscopy
Day 4, 28: blood pressure
Day 3, 27: ECG
Pretest, alternate days: hematology
Pretest, day 2, 24: serum chemistry, urinalysis
Day 1, 5: blood for PK determinations @ 0.25, 0.5, 1, 2, 4 hr
Termination: gross pathology, organ weights, histopathology

Mortality and clinical signs:

One HD male was a moribund sacrifice on day 11 after exhibiting hypoactivity, emesis, salivation and a temperature of 105.5°F. Signs in the HD groups included pale gums, hypoactivity, emesis, mucoid/soft feces, and alopecia. Emesis was noted at lower doses.

Body weight and food consumption:

There were no meaningful changes in weight or food consumption with treatment.

Body temperature: There were no remarkable changes in all but the single dead dog.

Blood pressure: There were no statistically significant changes in mean arterial blood pressure (MAP).

Hematology:

Reductions were seen primarily at the HD. Changes in RBC # were not statistically significant.

	Day of nadir	% reduction
RBC #	D18-24 M, D8-24: F	10-20%
Platelet #	D8-20	>90%
WBC #	D4-14	30-50%

Serum chemistry: There were no remarkable changes in serum chemistry with treatment.

Urinalysis: There were no remarkable changes in urinalysis with treatment.

Gross Pathology:

In the early death dog, there was an enlarged spleen, and thickened/dark area of duodenum. At interim sacrifice, one MD female had a mass in the uterus. At the final sacrifice, one MD male had a dark area in the ileum, dark area of the lymph node, small prostate. and sore foot.

Organ weights:

There were few statistically significant changes in organ weights, or in other than the HD group. The most striking changes are summarized in the following table; absolute weights and weights relative to body weight did not differ to an appreciable extent. Almost all changes resolved by the end of the cycle.

% change in organ weight at HD as compared to controls				
	Males		Females	
	Interim	Final	Interim	Final
Thymus	↓15%	—	↓45%	—
Thyroid	—	—	↓40%	—
Uterus	—	—	↓45%	↓40%

Histopathology:

The most relevant findings in the histopathologic examination were thymic lymphoid depletion (seen only at interim sac) and testicular syncytial cells/epididymal immature/abnormal sperm cells (recovery only) in the HD group. Congestion of the spleen, lymph nodes, liver, and gastrointestinal tract were seen sporadically. Marrow hypocellularity was noted only in the early death dog. Other findings, such as microcysts in the pituitary, thymus, thyroid and parathyroid, also occurred in the control dogs.

PK:

Plasma levels of TEM were measured twice as the first time phosphoric acid was not added to the samples to stabilize the drug. No gender related differences or accumulation of drug were noted. AUC and Cmax were relatively linear with dose. By visual inspection, initial half life was >1 hour.

Mean (%CV) Pharmacokinetic Parameters of SCH 52365 in Dogs Following Oral Administration in a Single-Cycle Oral Toxicity Study with Lower Doses

Dose (mg/m²)	Sex	Day	Cmax	Tmax	AUC(inf)[a]
25	M[b]	1	1.02 (40)	1.00 (50)	2.12 (56)
		5	1.11 (20)	1.29 (58)	2.44 (22)
	F[c]	1	1.45 (34)	1.19 (83)	2.85 (29)
		5	1.06 (39)	1.43 (57)	2.36 (45)
	Combined M & F[d]	1	1.24 (38)	1.09 (61)	2.53 (42)
		5	1.06 (30)	1.36 (57)	2.40 (34)
50	M[b]	1	2.71 (19)	1.21 (47)	5.90 (17)
		5	2.40 (47)	1.18 (53)	5.32 (48)
	F[c]	1	2.94 (14)	1.36 (48)	6.33 (13)
		5	3.06 (34)	0.86 (57)	6.97 (30)
	Combined M & F[d]	1	2.82 (16)	1.29 (48)	6.06 (16)
		5	2.73 (40)	1.07 (54)	6.15 (39)
125	M[b]	1	7.16 (13)	0.88 (58)	15.8 (2)
		5	6.98 (22)	1.04 (69)	16.3 (21)
	F[c]	1	7.86 (13)	0.82 (38)	16.3 (7)
		5	7.69 (19)	1.14 (65)	17.7 (16)
	Combined M & F[d]	1	7.50 (15)	0.75 (47)	16.0 (11)
		5	7.29 (21)	1.09 (59)	16.1 (20)

a n=7
b n=14
c df = 4 for most dose
Cmax: Maximum plasma concentration
Tmax: Time of maximum plasma concentration
AUC(inf): Area under the plasma concentration-time curve from time zero to time of last quantifiable sample
%CV: Coefficient of variation, expressed as percent.

5. P-5988. Three-cycle oral toxicity study of SCH 52365 in dogs.

Conducted at:
When conducted: June-Sept. 1993
GLP: Yes
Route: oral capsule
Drug Lot #: INV #920236001, BA #28395-103,[AJ-A8.2], RIC # 17505912
Dosing: 0, 25, 50, 125 mg/m^2/day
Schedule: DX5 Q 28 days X 3 cycles (i.e. D1-5, 29-33, 57-61)
Species: Beagle dogs; 12-15 months old; M: 9.2-14.4 kg; F: 8.6-12.4 kg
#/sex/dose: 7/sex/dose

Termination: D68 (interim sac, n=3/sex/dose), D85 (final sac, 4/sex/dose)

Measurements and Observations:

Twice daily: mortality and clinical signs
Pretest, weekly: body weight,
Daily: food consumption, rectal temperature
Pretest, D6, 36, 58, 79: ophthalmoscopy
Day 4, 32, 60, 79: blood pressure
Day 3, 31, 59, 78: ECG
Day 6, 10, 12, 28 for each cycle: hematology
Day 10 of each cycle, day 84: serum chemistry, urinalysis
Day 1, 5, 29, 33, 57, 61: blood for PK determinations @ 0.25, 0.5, 1, 2, 4 hr
Termination: gross pathology, organ weights, histopathology

Mortality and clinical signs:

All dogs survived to scheduled sacrifice. In the males, one HD dog exhibited diminished appetite and hypo-activity. More common (and dose dependent) were incidences of emesis, diarrhea, or discolored feces. Pale gums were noted in 5/7 HD males. One HD male had a tissue mass in the scrotum. In the females, similar changes were noted, but no masses were found.

Body weight and food consumption:

There were no statistically significant differences in body weight or body weight gain in the treated dogs. However, there was a slight trend toward decreased weight at the HD. Food consumption did not alter to a statistically significant extent, although there was a trend toward decreased food intake in the HD females.

Body temperature: No treatment related changes were noted.

Ophthalmoscopy: No treatment related changes were noted.

ECG: No treatment related changes were noted.

Hematology:

RBC # was not affected by TEM treatment. Changes in platelet # and WBC # were mostly confined to the HD group and are summarized in the following table. Decrements in platelet and WBC # were slightly greater in females than in males as well as having greater duration. WBC # decreases were reflections of decrements in segmented neutrophil, lymphocyte and monocyte numbers.

% decrease as compared to controls in 3 cycle dogs

Component	Day of cycle	Males			Females		
		Cycle 1	Cycle 2	Cycle 3	Cycle 1	Cycle 2	Cycle 3
Platelet #	6	—	15% H	—	16% H	31% H	20% H
	10	42% H	28% H	29% H	65% H	55% H	58% H
	12	66% H	50% H	46% H	86% H	73% H	76% H
	28	—	—	—	24% H	19% H	—
WBC #	6	30% H	33% H	33% H	49% H	17% M 51%H	58% H
	10	26% H	37% H	35% H	42% H	16% M 48% H	52% H
	12	31% H	28% H	33% H	45% H	15% M 55% H	54% H
	28	—	—	—	17% H	24% H	—

Serum Chemistry:

The changes in serum chemistry were minimal and of questionable significance. Glucose was increased by 10-15% in HD males and females. LDH was decreased dose dependently to up to 70% at the HD in both sexes. Inorganic phosphate was increased by 13% in the HD males at day 84.

Gross Pathology:

Again, there were few observations in the treated groups that differed from control. Changes are summarized in the following table.

Incidence of macroscopic observations in 3 cycle dogs.

	Males		Females	
	Interim	Final	Interim	Final
Kidney—pale area				1/4 H
Liver—enlarged		1/4 H		
Lung—adhesion		1/4 M		
Lung—mottled				1/4 M
Skin—sore/alopecia		1/4 H		1/4 H
Spleen—depressed area		1/4 H		
Spleen—pale area			1/3 H	
Urinary bladder—dark area			2/3 H	1/4 C, 1/4 H

Organ weights:

Thymic weights (absolute/relative) were decreased in the HD females by 33% (LD) to 63% (HD) at interim sacrifice which persisted in the HD at final sacrifice (decreased approximately 20% absolute/relative). Changes in organ weight that are possibly relevant (and should be followed in the 6 cycle study) included decreases in adrenal weight (female, interim), uterus/ovary (final), and prostate (interim).

Histopathology:

The microscopic changes in the 3 cycle dog study are summarized in the following table. Several of the changes are included for their possible value in evaluating the 6 cycle study. The most relevant findings were the thymus lymphoid depletion and testicular/epididymal syncytial cells. Mammary changes in the dog were also seen in controls.

Incidence of microscopic changes in the 3 cycle dog

	Male		Female	
	Interim Sac N=3	Final Sac N=4	Interim Sac N=3	Final Sac N=4
Thyroid—C cell hyperplasia	1 L, 1 M	2 C, 1 L, 1 M; 1 H	1 C, 1 L	1 C, 3 L, 1 M, 3 H
Spleen—siderotic plaque	1 L, 1 M, 2 H	1 L	1 L, 2 H	1 C, 1 L, 1 H
Kidney—mononuclear cell infilt.	1 L, 1 M, 1 H	—	—	—
Pancreas—chronic inflam/atrophy	1 H	—	—	—
Testes—syncytial cells increased	1 H	1 H	—	—
Testes—focal atrophy	1 L, 1 M, 1 H	1 C, 1 H	—	—
Epididymis—spermatic/granulomatous inflam	1 H	1 M	—	—
Epididymis—immature sperm	1 H	1 H	—	—
Prostate—chronic inflammation	1 L, 2 M, 2 H	1 C, 1 M, 1 H	—	—
Thymus—lymphoid depletion	3 H	1 H	2 M, 3 H	1 H
Tongue—chronic inflammation	1 M	2 L, 1 M, 2 H	—	—
Skin—inflammation	1 H	2 H	—	1 H
Esophagus—submucosal glandular inflammation		1 C, 2 M, 2 H	2 H	1 H
Mammary gland—hypertrophy/hyperplasia	—	—	2 C, 2 M, 2 H	2 C, 2 L, 2 M, 2 H
Lung—bronchopneumonia/foreign body pneumonia	—	1 M, 1 H	—	—
Trachea—mononuclear cell infilt.	1 L	2 H	—	—

PK:

Plasma levels of TEM were obtained for up to 4 hours following dosing and analyzed by

Plasma levels were not significantly affected by either gender or multiple days of dosing (accumulation). AUC and Cmax were relatively linear with dose. Actual values are shown in the following table.

Mean (%CV) Pharmacokinetic Parameters of SCH 52365 in Dogs (Males and Females Combined) Following Oral Administration

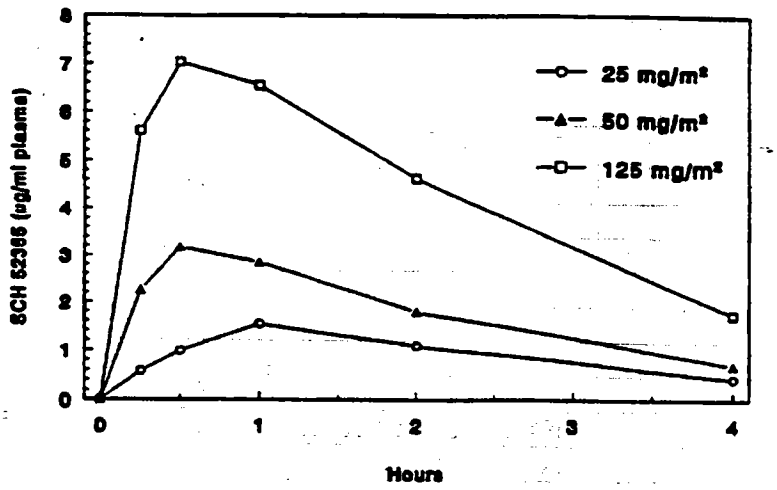
Dose (mg/m^2)	Day	Cmax[a]	Tmax[a]	AUC(tf)[a,b]
25	1	1.81 (29)	0.96 (55)	3.73 (21)
	5	1.56 (25)	1.13 (82)	3.24 (21)
	29	1.58 (30)	0.70 (88)	3.26 (30)
	33	1.35 (14)	1.05 (54)	2.87 (14)
	57	1.54 (24)	1.25 (79)	3.31 (27)
	61	1.62 (48)	1.13 (103)[c]	3.09 (52)
50	1	3.53 (18)	0.59 (57)	7.30 (15)
	5	3.56 (28)	0.83 (69)	7.10 (18)
	29	2.89 (21)	1.04 (55)	6.36 (16)
	33	2.65 (22)	0.88 (49)	5.53 (15)
	57	3.17 (22)	0.84 (122)	6.38 (27)
	61	2.51 (48)	1.32 (76)	5.65 (44)
125	1	8.92 (30)	0.63 (83)	17.6 (20)
	5	9.12 (16)	0.84 (69)	18.1 (11)
	29	8.43 (24)	0.93 (69)	17.2 (17)
	33	5.76 (51)	0.75 (96)[d]	12.0 (51)
	57	8.13 (35)	0.68 (71)	16.9 (32)
	61	8.15 (32)	0.70 (87)	17.4 (31)

a Pharmacokinetic parameters reported as the mean (n = 14) of male (n = 7) and female (n = 7) data combined, unless otherwise noted.
b tf = 4 hr for most dogs
c n = 13, (7 males and 6 females)
d n = 13, (6 males and 7 females)

Cmax	µg/ml	Maximum plasma concentration
Tmax	hr	Time of maximum plasma concentration
AUC(tf)	µg-hr/ml	Area under the plasma concentration-time curve from time zero to time of final quantifiable sample

%CV - Coefficient of variation, expressed as percent

Figure 1. Mean Plasma SCH 52365 Concentrations Following Oral Administration to Dogs (Males and Females Combined) on Day 1



6. P-6055. Six-cycle oral toxicity study of SCH 52365 in dogs.

Conducted at:
When conducted: March-Sept 1994
GLP: YES
Drug Lot #: BA # 28395-103 (AJ-A8/2)
Doses: 0, 25, 50, 125 mg/m^2
Schedule: DX5 q 28 days X 6 cycles
Species used: Beagle dogs, 11-12 months old, M: 9.3-12.0 kg, F: 7.3-9.7 kg
#/sex/dose: 7/sex/dose
Last day of observation: 85

Measurements and Observations:

Twice daily: mortality and clinical signs
Daily: Food consumption, rectal temperature
Weekly: body weight
Pretest, Day 6, 34, 62, 90, 118, 146, 167: ophthalmoscopy
Pretest, day 4, 32, 60, 88, 116, 144, 165: blood pressure, respiratory rate
Day 3, 31, 59, 87, 115, 143, 164; ECG
Days 6, 10, 12, 28 of each cycle: hematology
Day 10 of each cycle: serum chemistry
Day 9 of each cycle: urinalysis
Days 1 and 5 of cycles 1, 3, 6: Predose, 15, 30 minutes, 1, 2, 4 hours post-dose: PK
Termination: 3/sex/dose on day 152, 4/sex/dose on day 169: gross pathology, organ weights, histopathology

Mortality and clinical signs:

All dogs survived to scheduled sacrifice. Abnormal observations were rarely made at the LD. Signs in the MD and HD included pale gums, discolored/mucoid/soft feces and emesis. One HD female looked thin, another HD female had bloody looking feces.

Body weight and food consumption:

There were no remarkable changes in the male body weights. In the females, the HD weights were decreased by 10% at final two weeks. No noteworthy alterations in food consumption were observed with treatment.

Body temperature: No major changes were noted.

Blood pressure and respiratory rates: There were no significant changes with treatment.

Hematology:

Hematology values in females were affected to a slightly greater extent than those in males. Decrements in WBC # were a reflection of decreases in segment neutrophils and lymphocytes. RBC # was not affected in the males, while slight anemia was seen in females after the third cycle. Platelet and WBC #'s were dropped by up to 75% (more severe in females) but recovered, or even overshot, prior to the next cycle. Females were altered at all doses, while males were affected only at the MD and HD.

% change as compared to controls							
Cycle #	Day #	Males			Females		
		RBC #	WBC #	Platelet #	RBC #	WBC #	Platelet #
1	6	↓11%	↓32% H	—	—	↓19% M ↓44% H	↓22% M ↓26% H
	10	—	↓12% M ↓37% H	↓55% H	—	↓18% M ↓44% H	↓25% M ↓65% H
	12	—	↓34% H	↓12% M ↓76% H	—	↓43% H	↓27% M ↓84% H
	28	—	—	—	—	—	↓11% H
2	6	—	↓13% M ↓25% H	—	—	↓18% H	↓11% M ↓23% H
	10	—	↓31% H	↓36% H	–	↓36% H	↓43% H
	12	—	↓31% H	↓60% H	–	↓33% H	↓15% M ↓67% H
	28	—	—	↑17% H	—	—	—
3	6	—	↓16% M ↓25% H	—	—	↓46% H	↓17% H
	10	—	↓34% H	↓29% H	↓10% H	↓19% M ↓52% H	↓10% M ↓44% H
	12	—	↓12% M ↓38% H	↓54% H	↓10% H	↓29% L,M ↓55% H	↓12% M 64% H
	28	—	—	↑28% H	—	—	—
4	6	—	↓15% M ↓40% H	—	↓12% H	↓13% M ↓43% H	↓18% M ↓17% H
	10	—	↓44% H	↓23% H	↓10% H	↓46% H	↓19% L ↓21% M ↓51% H
	12	—	↓18% M ↓52% H	↓55% H	↓14% H	↓53% H	↓18% L ↓22% M ↓79% H
	28	—	—	↑33% H	—	↓19% H	—
5	6	—	↓12% M ↓37% H	—	↓12% H	↓24% M ↓42% H	↓15% L, M, H
	10	—	↓34% H	↓21% H	—	↓27% L ↓38% M ↓53% H	↓12% L ↓14% M ↓37% H
	12	—	↓43% H	↓53% H	—	↓12% L ↓24% M ↓39% H	↓13% L ↓24% M ↓74% H
	28	—	↓11% H	↑33% H	↓12% H	—	—
6	6	—	↓15% M ↓35% H	↑13% H	↓11% M ↓18% H	↓21% M ↓46% H	—
	10	—	↓12% M ↓36% H	↑12% H	↓10% M ↓15% H	↓12% M ↓49% H	↓45% H
	12	—	↓14% M ↓42% H	↓51% H	↓13% H	↓23% M ↓49% H	↓13% M ↓72% H
	28	—	↓13% M ↓19% H	↑42% H	—	—	↓32% H

Serum Chemistry:

Changes in serum chemistry were minimal and occurred only in the HD groups after cycle 3. Cholesterol was decreased by up to 25% in both sexes. LDH was decreased by approximately 75% in the HD females.

Urinalysis: There were no remarkable changes with treatment.

Gross Pathology:

Again, minimal gross changes were observed with treatment. Changes are summarized in the following table.

Incidence of macroscopic findings				
	Males		Females	
	Interim Sac	Final Sac	Interim Sac	Final Sac
Mammary gland—thickened	—	—	2 MD	1C, 4L, 3M
Kidney—pale area	—	—	1 HD	—
Cecum—intusscepted/dk mucosa	—	1 HD	—	—
LN-darkened	—	—	—	1 HD

Organ weights:

Most changes were of minimal biological or statistical significance and are summarized in the following table. Mammary thickening was noted in most of the females, but was attributed to normal hormonal cycling.

% change as compared to controls								
	Males				Females			
	Interim Sac		Final Sac		Interim Sac		Final Sac	
	Abs.	Rel.	Abs.	Rel.	Abs.	Rel.	Abs.	Rel.
Thyroid	↓25% H	↓30% H	↓16% H	↓22% H	—	—	—	—
Thymus	–	–	–	–	↓43% H	↓40% H	—	—

Histopathology:

Observations were all graded as minimal or slight. Relevant notations are summarized in the following table. Almost no damage to the gastrointestinal tract was observed. A small degree of lymphoid or marrow depletion was seen. The other target organs were the testes and epididymis and possibly the uterus. Microcysts were seen in the pituitary, thyroid, parathyroid and thymus, although the frequency in the controls were as high as those in the treated.

Incidence of microscopic observations in the 6 cycle dog				
Description	Males		Females	
	Interim Sac n=3	Final Sac n=4	Interim Sac n=3	Final Sac n=4
Spleen—extramedullary hematopoiesis	1 H	4 H	1 L, 2 H	4 H
Colon—congestion	1 H	—	—	—
Mes. LN—congestion	1 M, 1 H	—	—	3 H
Testes—syncytial cells	1 L, 3 H	1C, 1 M, 3 H	—	—
Epididymis—immature/abnormal sperm	3 H	1 C, 3 H	—	—
Thymus—lymphoid depletion	1 L, 1 M, 3 H	—	2 H	1C, 1 L
Tongue—chronic inflammation	1 H	1 L, 1 M	1 L, 1 H	1 C, 1 L, 2 M
Cecum—congestion	—	1 H	—	—
Uterus—papillary endometrial proliferation	—	—	—	1 M

Plasma levels of TEM were measured during cycles 1, 3, and 6 by with a detection limit of 0.10 ug/mL. No significant accumulation or differences in pharmacokinetics with gender were observed. AUC and Cmax were essentially linear with dose.

Mean (%CV) Pharmacokinetic Parameters of SCH 52365 in Male and Female Dogs Following Oral Administration

Dose, (mg/m²)	Sex	Day	Cmax[a] (ug/ml)	Tmax[a] (hr)	AUC(t)[a,b] (ug-hr/ml)
25	Male	1	2.01 (14)	0.75 (43)	4.21 (11)
		5	2.18 (26)	0.79 (79)	4.82 (21)
		57	1.84 (46)	0.71 (80)[c]	3.27 (47)
		61	1.68 (21)	1.21 (47)	3.80 (11)
		141	1.41 (28)	1.07 (67)	3.24 (19)
		145	2.27 (26)	0.93 (58)	4.89 (20)
	Female	1	2.12 (61)	0.76 (43)	4.57 (60)
		5	2.15 (22)	1.00 (50)	4.56 (22)
		57	1.08 (67)	1.81 (76)	2.24 (73)
		61	1.31 (24)	0.86 (28)	2.63 (25)
		141	2.39 (21)	1.07 (42)	5.54 (22)
		145	1.56 (60)	1.17 (59)[c]	3.59 (58)
50	Male	1	3.45 (14)	1.07 (42)	7.62 (18)
		5	3.86 (46)	0.83 (49)[c]	8.96 (46)
		57	3.52 (32)	0.71 (94)	7.22 (22)
		61	2.49 (71)	0.67 (58)[c]	5.74 (70)
		141	3.00 (57)	1.36 (96)	6.51 (50)
		145	4.03 (47)	0.75 (88)	8.42 (27)
	Female	1	3.70 (14)	1.00 (75)	7.82 (18)
		5	3.98 (10)	0.81 (47)	8.35 (10)
		57	3.38 (21)	0.86 (71)	7.22 (17)
		61	2.86 (59)	0.38 (37)[c]	5.73 (60)
		141	4.02 (29)	0.86 (71)	8.83 (28)
		145	4.04 (47)	1.32 (108)	8.13 (45)
125	Male	1	8.57 (8)	1.07 (63)	18.8 (8)
		5	8.98 (25)	0.96 (81)	18.2 (21)
		57	8.55 (21)	1.64 (78)	17.5 (28)
		61	5.80 (21)	1.93 (82)	12.5 (68)
		141	8.15 (19)	1.39 (57)	20.4 (10)
		145	10.0 (26)	1.57 (79)	22.5 (20)
	Female	1	9.05 (40)	0.71 (51)	18.9 (38)
		5	8.91 (80)	1.07 (63)	18.7 (65)
		57	8.70 (24)	0.86 (86)	19.4 (16)
		61	8.96 (35)	0.50 (71)	17.7 (33)
		141	8.19 (19)	0.96 (57)	19.6 (24)
		145	9.82 (31)	0.71 (87)	19.9 (32)

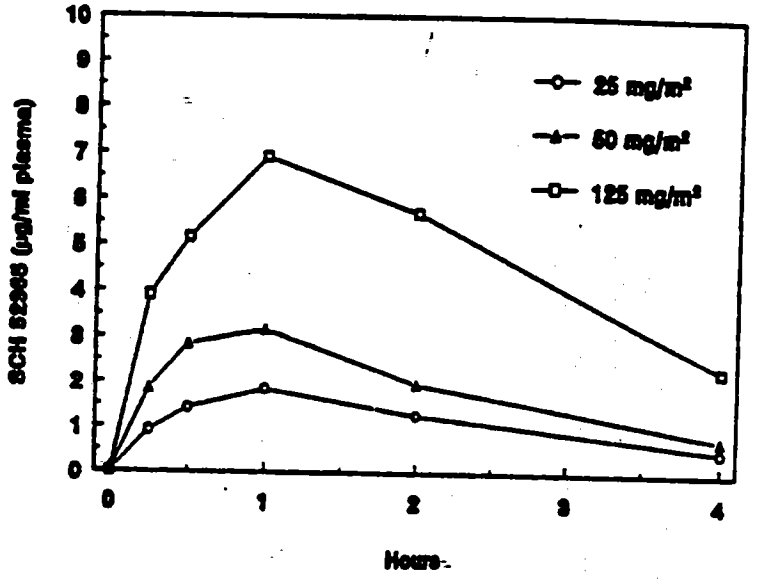
[a] Pharmacokinetic parameters reported as the mean of n=7 male or n=7 female dogs, unless otherwise noted.
[b] t(-4 hr for most dogs
[c] n=6

Mean (%CV) Pharmacokinetic Parameters of SCH 52365 in Dogs (Males and Females Combined) Following Oral Administration

Dose (mg/m^2)	Day	Cmax[a] (µg/ml)	Tmax[a] (hr)	AUC(0)$^{t_{1/2}}$[a,b] (µg·hr/ml)
25	1	2.08 (44)	0.75 (41)	4.39 (43)
	5	2.17 (23)	0.89 (82)	4.59 (21)
	57	1.35 (53)	1.19 (69)[d]	2.75 (53)
	61	1.48 (24)	1.04 (44)	3.12 (23)
	141	1.80 (36)	1.07 (64)	4.39 (34)
	145	1.91 (44)	1.04 (57)[e]	4.29 (40)
50	1	3.58 (14)	1.04 (57)	7.72 (17)
	5	3.82 (31)	0.82 (46)[d]	7.85 (31)
	57	3.48 (28)	0.79 (79)	7.22 (18)
	61	2.57 (62)	0.82 (60)[e]	5.73 (62)
	141	3.51 (47)	1.11 (81)	7.87 (44)
	145	4.04 (43)	1.04 (107)	8.28 (34)
125	1	8.81 (28)	0.89 (62)	18.4 (28)
	5	8.96 (44)	1.02 (98)	18.9 (48)
	57	8.83 (22)	1.25 (83)	18.5 (22)
	61	7.28 (53)	1.21 (109)	15.1 (50)
	141	8.67 (19)	1.18 (58)	20.0 (17)
	145	8.42 (28)	1.14 (81)	21.1 (28)

[a] n=14, (7 males and 7 females)
[b] t$_{1/2}$=4 hr for most dogs
[c] n=13, (7 males and 6 females)
[d] n=13, (6 males and 7 females)
[e] n=12, (6 males and 6 females)

Figure 2. Mean Plasma SCH 52365 Concentrations Following Oral Administration to Dogs (Males and Females Combined) on Day 5



Summary of Toxicology

The NOEL and lowest lethal doses (LLD) from the TEM toxicity experiments are shown in the following table. NOEL's provided were frequently the lowest doses tested in a given experiment. At first glance, the 3 and 6 cycle dog experiments appear to be inadequately dosed; however, based on the lethality seen in single cycle experiments at doses ≥200 mg/m^2, the levels chosen are adequate.

Species	Route	Schedule	NOEL (mg/m^2)	LLD (mg/m^2)
Mouse	PO	Single dose	500	1000
	IP	Single dose	<500	1000
	IF	DX5	—	375 (LD_{10})
	IP	DX5/wk X 4	42	210
Rat	PO	Single dose	<5000	<5000
	PO	Single dose	750	1500
	IP	Single dose	200	2000
	PO	DX5 q 28 days X 1 cycle	<200	200
	PO	DX5 q 28 days X 1 cycle	<25	200
	PO	DX5 q 28 days X 3 cycles	<25	>200
	PO	DX5 q 28 days X 6 cycles	<25	25 in M; 125 in F
Dog	PO	Single dose	<400	600
	PO	DX5 q 28 days X 1 cycle	<200	200
	PO	DX5 q 28 days X 1 cycle	50	125
	PO	DX5 q 28 days X 3 cycles	25	>125
	PO	DX5 q 28 days X 6 cycles	<25	>125

Oral and intraperitoneal dosing yielded relatively similar lethality patterns and exposure levels. In the dog, the dose response curve was relatively steep for lethality: no deaths in most experiments at 125 mg/m^2, and all dogs dead at 200 mg/m^2. Deaths in the dog were from the acute effects of marrow depletion (sepsis, hemorrhage) with liver and kidney damage. In contrast, in the 3 cycle rat study at 200 mg/m^2, all rats survived to the scheduled sacrifice. With an additional 3 months of exposure at 125 mg/m^2, deaths in the rat were attributed to genitourinary disease (males only) and tumors. No significant signs of hyperplasia, adenomas or malignant tumors were seen in the dog over 6 cycles.

With the exception of the tumors, toxicities in rat and dog were quite similar. The target organs of toxicity were hematopoietic organs, male reproductive organs, and at high doses, gastrointestinal tract, liver and kidney. Dogs were susceptible to vomiting and changes in feces. Decreases in RBC's, WBC's and platelets, all of which usually resolved by the next cycle, were noted in both rat and dog. Only in the 6 cycle dog study did a hematologic parameter, RBC #, worsen with successive cycles. In the 6 cycle rat study females, blood elements actually increased in the last cycles, which may be associated with the onset of leukemia. Serum chemistry changes were minimal with the exception of those animals about to die: slight increases in glucose (more severe with increasing dose), questionable changes in electrolytes and cholesterol/triglycerides. In the moribund animals, increases in liver enzyme levels, BUN/creatinine and glucose; and decrements in electrolytes and serum proteins were noted. Organ weight changes, with the exception of decrements in thymic weights, were also of questionable significance.

Pathologic changes in rats and dogs differed with dose and species. Animals dosed with higher levels of TEM (>200 mg/m^2) died with a few weeks of treatment and showed a very different profile from the deaths occurring in the later cycles. The animals dosed with high levels of TEM died with liver and kidney necrosis, gastrointestinal necrosis/atrophy/hemorrhage (from

tongue through colon), and marrow depletion. Hemorrhages were also noted in the brain and lung. Additionally, in the eye, retinal degeneration and necrosis was observed. In contrast, with longer treatment at lower doses, no dogs died at doses of up to 125 mg/m^2 for 6 cycles while the rats died of genitourinary disease (males only) or tumor complications.

Pathologic changes seen in the 3 and 6 cycle studies were similar in rat and dog with the exception of neoplastic changes in the rat. Thymic lymphoid depletion was consistently observed, but resolved by the end of each cycle. Marrow depletion, spleen lymphoid depletion and a low incidence of lymph node hyperplasia were less frequent. Almost no gastrointestinal necrosis or villous atrophy was noted at doses <200 mg/m^2. Syncytial cells were seen in both rats and dogs, while only dogs had frank testicular atrophy; however, testicular atrophy was observed in the rat at higher doses (single cycle study). A low incidence of ocular changes were seen in the 3 and 6 cycle studies, but were more common and severe in single cycle, higher dose studies and included hemorrhage and degeneration of the retina. In the rat, liver necrosis was also noted at the HD in the 6 cycle study. Toxicities in the rat were more prevalent than in dog, but similar in profile.

One of the most noteworthy features of the rat experiment was the prevalence of tumors. In the 3 cycle rat study, immediately following treatment, only 2 HD females had evidence of mammary carcinoma. By the end of the third month, 18/20 HD females and 4/20 HD males had mammary carcinomas. With an additional 3 cycles of treatment (6 cycles total), a veritable laundry list of tumors were observed including Schwannomas of the heart, Harderian gland, and optic nerve; fibrosarcomas of the heart, eye, seminal vesicle, abdominal cavity/subcutaneous tissue, and prostate; sarcoma of the salivary gland and uterus (endometrial stroma); and carcinomas of the mammary gland, and seminal vesicles. Additionally, benign adenomas of the adrenal cortex, pituitary, thyroid, and lung; keratoacanthomas of the skin, ovarian granuloma were also observed. The most prevalent tumors were mammary carcinomas, seen in all three dose groups of the rats (i.e. no NOEL determined). Mammary hyperplasia was also observed in the female dogs, but the incidences were similar to those in control dogs and was attributed in pathology reports to normal menstrual cycling. It is unknown if dogs would also manifest tumors if observed for longer periods of time.

Histopathology Inventory for NDA #21-029

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

Vagina	X	X	X	X	X	X	
Zymbal gland							

V. SPECIAL TOXICITY STUDIES

1. Deleve LD. Dacarbazine toxicity in murine liver cells: a model of hepatic endothelial injury and glutathione defense. The Journal of Pharmacology and Experimental Therapeutics 1994;268(3):1261-1270.

Murine liver hepatocytes and sinusoidal endothelial cells (SEC's) were isolated, exposed to dacarbazine (0, 1.5, 3, 6 mM DTIC) for up to 16 hours. No effect on viability of hepatocytes was seen, while viability in SEC's was decreased by up to 93% with 6 mM DTIC. These cells were previously shown to metabolize DTIC. Neither pulmonary artery endothelial or human umbilical vein endothelial cells were affected significantly by DTIC. The onset of toxicity occurred after 12 hours following drug application and closely correlated with glutathione depletion. BSO depletion of GSH increased the toxicity; similarly, excess glutathione diminished toxic effects of DTIC.

2. P-6490. Effect of temozolomide on gastrointestinal function in rats. Kenilworth (NJ): Schering-Plough Research Institute; 1993 Sep. Vol 1.24.

Conducted at: Schering Plough Research Institute, Kenilworth, NJ
When conducted: 1996
GLP: No
Drug Lot #: INV # 960170001, RIC # 17505912
Formulation: 0.4% methylcellulose
Dose: 200 mg/m^2, oral gavage
Control: vehicle (0.4% methylcellulose, positive control: atropine, 10 mg/kg.
Schedule: DX5 q 28 days X 3 cycles
Species used: rat, further details not specified

Experiments conducted:
1) Gastric emptying study: n=6/group; 30 minutes or 6 hours after drug administration a test meal was administered by oral gavage, 30 minutes later the rats were killed. The stomachs were ligated, weighed, opened and emptied, then re-weighed. Percentage of meal retained was calculated.
2) Intestinal transit study: n=6/group; rats were given oral gavage of vehicle (0.4% carboxymethylcellulose), 200 mg/m^2 TEM or 10 mg/kg atropine. Thirty minutes after drug, a test meal was administered. Finally, 10 minutes after the test meal, the rats were killed, the small intestine removed and opened to determine how far the test meal had moved.
3) Gastric ulcerogenicity study: Rats were administered either vehicle, 200 mg/m^2 TEM or 10 mg/kg indomethacin. Four hours after treatment, the rats were killed, the stomach removed and opened, and the number of lesions counted and measured.

Results:

Gastric emptying time was decreased with TEM by 47% at 30 minutes after drug, while atropine inhibited gastric emptying by 83%. No change from control was noted in intestinal transit time (atropine reduced by 50%). Final no mucosal lesions were noted with TEM, while indomethacin resulted in lesions in all treated rats.

3. P-6280. Dermal sensitization study in guinea pigs (Buehler's Technique Modified) with SCH 52365 (temozolomide).

Conducted at:
When conducted: 5-6/95
GLP: Yes
Drug lot #: not provided
Vehicle: mineral oil
Positive control: 0.10% DNCB (2,4-dinitrochlorobenzene)
Species used: Albino guinea pigs Crl:(HA)BR, M: 298-401g; F: 266-346 g
Screen: 1 guinea pig/dose; 25%, 50%, 75% and moistened w/mineral oil (100%)
6 hours under a patch, then examined @ 24, 48 h post patch removal.
Final study: G1: naïve control (n=20), G2: TEM induction and challenge G3: positive control. TEM just moistened w/mineral oil (100%), 6 hr exposure weekly for 3 weeks, then rechallenge 13 days after last exposure for 4 hrs. Criteria: edema, erythema.

Results: No effects on body weight were seen. No dermal reactions were seen in the screen. No reactions were seen with G1. In G2, 10/20 guinea pigs showed very slight reactions to the application of test article, but only 3/20 showed a very slight reaction at the rechalleng time. Positive controls had severe reactions by the second reapplication of drug.
TEM was deemed not to be a dermal irritant.

Summary of Special Toxicity Studies

Temozolomide had an effect on gastric emptying time, but minimal effect on other gastrointestinal parameters. Based on the effects of dacarbazine (DTIC), temozolomide may also cause hepatic occlusive disease by its effect on liver blood vessel endothelial cells. This can be modified by glutathione stores. Temozolomide was not shown to be a dermal irritant.

Reproductive Toxicity:

1. P-6452. Dose-range finding developmental toxicity study in rats with SCH 52365.

Conducted at:
When conducted: April-May 1996
GLP: Yes
Route: oral gavage
Drug Lot #: batch # 36438-023 in 0.4% methylcellulose 1, 5 mg/mL.
Schedule: daily on days 8-12 of presumed gestation.
Doses: 0, 5, 25, 50, 150 mg/m^2/day
Weight at day 0: 214-260 g
Species: 8/dose Sprague Dawley Crl:CD BR rats, 10 weeks-old
Duration of observation: mating through day 20 of presumed pregnancy

Measurements and Observations
Twice daily: mortality and clinical signs
Days 0,6,8,10, 13, 16, 18, 20: body weight, food consumption
Termination (d20): Maternal: gross pathology, # and placement of implantation sites, live and dead fetuses, early/late resorptions, abnormalities of uterus or embryonic sacs, corpora lutea. Fetal: sex, weight, external abnormalities

Results:

All dams survived to scheduled sacrifice on day 20. The observations are summarized in the following table. Changes in maternal weight were attributable to diminished uterine weight. TEM was fetotoxic at 150 mg/m^2, a dose where no maternal toxicity was observed. Except at HD where 6/8 fetuses were male, no change in the sex ratio was found.

Observation	0	5 mg/m^2	25 mg/m^2	50 mg/m^2	150 mg/m^2
Maternal obs.	—	—	—	—	—
Maternal weight	—	—	—	—	↓20%
Maternal wt. gain	—	—	—	—	↓50%
Maternal wt wo/uterus	—	—	—	—	—
Wt of gravid uterus	—	—	—	↓11%	↓>90%
Food consumption	—	—	—	—	—
Abortions	—	—	—	—	—
Litters with viable fetuses	8 (100%)	8 (100%)	7 (100%)	8 (100%)	2 (25%)
Implantation sites	113	107	129	111	118
Fetal resorptions	4 (3.5%)	9 (8.4%)	1 (0.8%)	4 (3.6%)	110 (93.2%)
Dead fetuses	0	0	0	0	0
Fetal weights	—	—	—	—	↓50%
External fetal anomalies	—	—	—	—	—

2. P-6453. Dose-range finding developmental study in rabbits with SCH 52365

Conducted at:
When conducted: June-July 1996
GLP: no (no QA)
Route: oral gavage
Drug Lot #: batch # 36438-023 in 0.4% methylcellulose
Schedule: daily on days 8-12 of presumed gestation.
Doses: 0, 5, 25, 50, 150 mg/m^2/day
Weight at day 0: 2895-4466 g
Species: 8/dose of rabbits , 5 months old
Duration of observation: mating through day 29 of presumed pregnancy

Measurements and Observations

Twice daily: mortality and clinical signs
Days 0,4,8,10,13,17,21,24,29: body weight, food consumption
Termination (d20): Maternal: gross pathology, uterine weight, # implantation sites, live and dead fetuses, early/late resorptions, abnormalities of uterus or embryonic sacs, corpora lutea. Fetal: sex, weight, external abnormalities

Results:

All rabbits survived to scheduled sacrifice with no abnormal clinical signs. The remaining observations are summarized in the following table. One LD rabbit had fluid filled thoracic cavity.

Fetal variations included foreshortening of the skull (32 fetuses/7 litters), and upper incisors not erupted (2 fetuses/2 litters). All of the HD fetuses were malformed. Malformations

included non-patent nares (17/6), exophthalmos (7/5), malrotated hindlimbs (5/3), brachymelia (37/7), oligodactyly (29/7), flexed hindpaws (22/6), polydactyly (7/4), arthrogryposis (20/6), abnormal position of digits (22/6), apodia (1/1), umbilical hernia (1/1), bilateral hare lip (6/3), cleft palate (19/6), incisors absent (2/2), spina bifida (1/1), acaudate (8/5), and rudimentary tail (33/7).

Observation	0	5 mg/m^2	25 mg/m^2	50 mg/m^2	150 mg/m^2
Maternal weight	—	—	—	—	—
Maternal wt. gain	—	—	—	↓10%	↓14%
Maternal wt wo/uterus	—	—	—	—	—
Wt of gravid uterus	—	—	—	—	↓30%
Food consumption	—	—	—	—	↓D12-15; 40%
# dams pregnant	8	7	8	7	7
Abortions	—	—	—	—	—
Litters with viable fetuses	8 (100%)	7 (100%)	8 (100%)	7 (100%)	7 (100%)
Implantation sites	60	66	66	61	60
Fetal resorptions	1 (1.7%)	3 (4.5%)	1 (1.5%)	0 (0%)	19 (32%)
Dead fetuses	0	0	0	0	0
Fetal weights	—	—	—	↓11%	↓39%
External fetal variations	—	—	—	1/1*	32/7*
External fetal malformations	—	—	—	—	41/7 (100%)

* # fetuses/# litters with observation

In conclusion, at 150 mg/m^2, where no maternal toxicity was observed, all fetuses were malformed. Thus, TEM is teratogenic in rabbits. The NOAEL for maternal effects is 150 mg/m^2. The fetal NOAEL is 25 mg/m^2.

3. P-6547. Rat developmental toxicity study with SCH 52365.

Conducted at:
When conducted: Sept-Oct 1996
GLP: Yes
Route: oral gavage
Drug Lot #: batch # 36438-023 in 0.4% methylcellulose 1, 5 mg/mL.
Schedule: daily on days 8-12 of presumed gestation.
Doses: 0, 25, 50, 75 mg/m^2/day
Weight at day 0: 214-260 g
Species: 25/dose, Sprague Dawley rats, 9 weeks old
Duration of observation: mating through day 20 of presumed pregnancy

Measurements and Observations
Twice daily: mortality and clinical signs
Days 0,6,8,10,13,14,16,18,20: body weight, food consumption
Termination (d20): Maternal: gross pathology, # and placement of implantation sites, live and dead fetuses, early/late resorptions, abnormalities of uterus or embryonic sacs, corpora lutea. Fetal: sex, weight, external, soft tissue and skeletal anomalies (variations/malformations).

Maternal mortality and clinical signs:

All dams survived to scheduled sacrifice. One HD dam had hunched posture, chromodaccryorrhea, red/black vaginal discharge, and pale coloration between day 11 and 16.

Body weight and food consumption:

Body weight changes became apparent as early as day 13. Changes on day 20 are summarized in the following table. Food consumption did not differ to a significant extent. At sacrifice, body weight without uterus did not differ between treated and controls.

% change as compared to controls			
	LD	MD	HD
body weight	↓2%	↓5%	↓8%
body weight gain	↓6%	↓14%	↓22%
Gravid uterus weight	↓3%	↓17%	↓31%

Maternal gross pathology:

One HD female had dilated pelvis of the kidneys.

Litter data:

All dams were pregnant; however, one LD dam had no viable fetuses. There were no differences between treated and controls in corpora lutea, implantation sites, preimplantation loss, or # of live fetuses.

Fetal body weight: Fetal body weights were decreased by 5%, 15%, and 34% in LD, MD, and HD respectively. Body weights in females were slightly lower than those in males.

Fetal variations and malformations

Overall incidences of malformations and variations are shown in the following table. Fetal external malformations observed at HD included malrotated hindlimbs (125 fetus/19 litters), flexed front paws (10 fetuses/5 litters), adactyly (3 fetuses/1 litter), open eyes (1 fetus/1 litter), bracymelia (14 fetuses/6 litters), syndactyly (1 fetus/1 litter), micromelia (2 fetus/2 litters), macromelia (1 fetus/1 litter), brachydactyly (1 fetus/1 litter), polydactyly (1 fetus/1 litter), gastroschisis (1 fetus/1 litter), and rudimentary tail (1 fetus/1 litter). External malformations in the 1 LD fetus included agnathia, astomia and single nostril. Soft tissue malformations were internal hydrocephaly (17 fetuses/10 litters), diaphragmatic hernia (MD: 4 fetus/3 litters; HD: 34 fetuses/15 litters), and small kidneys (HD:1 fetus/1 litter). Skeletal malformations included vertebral anomaly (LD:2 fetuses/2 litters; HD:3 fetuses/3 litters), misshapen scapula (HD: 120 fetuses/24 litters), misshapen clavicle (HD: 1 fetus/1 litter), forked/fused ribs (HD: 3 fetuses/2 litters), tibia displaced/absent/misshapen (HD: 38/44/29 fetuses respectively), misshapen/displace/fibula (HD 11/1 fetuses respectively), misshapen femur (HD: 4 fetuses) and misaligned ilium (HD: 4 fetuses).

Incidence of fetal malformations: #fetus with anomaly/total fetuses (# litters with anomalous fetus/total # litters)				
	Control	LD	MD	HD
External variations	0/351 (0/25)	0/359 (0/24)	0/335 (0/25)	0/341 (0/25)
External malformations	0/351 (0/25)	1/359 (1/24)	0/335 (0/25)	138/341 (22/25)
Soft Tissue Variations	13/175 (8/25)	17/177 (13/24)	31/166 (14/25)	100/171 (25/25)
Soft Tissue malformations	0/175 (0/25)	0/177 (0/24)	4/166 (3/25)	49/171 (19/25)
Skeletal Variations	147/176 (24/25)	146/182 (24/24)	158/169 (25/25)	169/170 (25/25)
Skeletal malformations	0/176 (0/25)	2/182 (2/24)	0/169 (0/25)	134/170 (24/25)

The sponsor concluded that the maternal NOAEL was 75 mg/m^2 (HD), while the fetal NOAEL was 25 mg/m^2 (LD). TEM is teratogenic in rats.

Summary of Reproductive Toxicity:

Temozolomide caused malformations in both the rat and rabbit at doses with no appreciable maternal toxicity; thus, the drug is teratogenic. Additionally, in the rabbit, at non-maternotoxic doses, TEM is embryolethal as shown by increased resorptions. Malformations in the rat were external, internal soft tissue, and skeletal. Dose levels for effects are shown in the following table. Multiple types of malformations were noted in both the rat and rabbit (where only external changes were measured). In all experiments, a daily X 5 schedule on days 8-12 of gestation was used, which does not conform to the usual ICH recommendations.

Species	Maternal NOEL	Fetal NOEL	Dose with Malformations	Malformations	Embryolethality
Rat—dose range	50 mg/m^2	50 mg/m^2	150 mg/m^2	—	Y, 150 mg/m^2
Rat—final	75 mg/m^2	25 mg/m^2	75 mg/m^2	Y	N
Rabbit dose range	150 mg/m^2	25 mg/m^2	150 mg/m^2	Y	Y, 150 mg/m^2

Y= yes, N= no

Genetic Toxicity:

1. P-6495. Salmonella-Escherichia coli/Mammalian microsome reverse mutation assay of SCH 52365 (temozolomide).

Conducted at:
When conducted: 9-12/96
GLP: Yes
Drug batch: #37120-001
Vehicle: DMF
Strains used: *Salmonella typhimurium* TA98, TA100, TA1535, TA97a, TA102 and *Eschericia coli* WP2*uvr*A.
Positive controls: 2-aminoanthracene, 2-nitrofluorene, sodium azide, ICR-191, mitomycin C, 4-nitroquinolone-N-oxide
Plates/dose in final study: 3
Concentrations in final study: 62.5-2500 ug/plate
Concentrations in range-finding assay: 1 plate/dose, 10 doses, 0.100-2500 ug/plate
S9 fraction: purchased from Molecular Toxicology Inc. (from male Sprague Dawley rats induced with arochlor).

Results:

Solubility of the drug limited testing to 2500 ug/plate. Background lawn was reduced slightly at 2500 ug in the absence of S9 fraction. In the presence and absence of S9, greater than 2 fold increases in revertants were seen at doses of 1000 and 2500 ug/plate in TA98, TA100, TA1535, and TA102. TA97a and WPuvrA were more sensitive to the effects of TEM, with >2 fold increases in revertants at 62.5 ug with S9 (WP2uvrA without S9), and TA97a at 125 ug without S9. Similar results were seen in the second assay. Positive controls met the criteria for a positive assay.

Conclusion: TEM was positive with and without metabolic activation.

2. P-6454. Chromosome aberration study of SCH 52365 in human peripheral blood lymphocytes.

Conducted at:
When conducted: 5-9/96
GLP: Yes
Drug batch: #37120-001
Vehicle: deionized water

Cells used: Human lymphocytes from normal male and female; duplicate cultures/dose

Positive controls: mitomycin C, cyclophosphamide.

Harvest times: 24, 48 hours (3 h with drug, then 24 or 48 to harvest with metabolic activation).

Concentrations: 0, 3.93, 7.85, 15.7, 31.3, 62.5, 125, 350, 500, 1000 ug/mL for the 24 h harvest; up to 125 ug/mL in the 48 h harvest. Solubility limited top dose.

S9 fraction: purchased from Molecular Toxicology Inc. (from male Sprague Dawley rats induced with arochlor).

Scoring cells: 1000 cells/culture for mitotic index were counted. Mitotic index must be <40% of solvent controls. 100 mitotic cells were counted for aberrations.

Results:

The sponsor used acceptable criteria for solubility, and reduction in mitotic index. The 2-3 replications differed slightly in outcome, however, TEM was deemed positive both with and without metabolic activation, usually at concentrations less than 125 ug/mL.

3. P-5866. M&B, 39,831 (R.P. 46,161) Ames Test

Conducted at:
When conducted: 5/86
GLP: No
Drug batch: MPW-1368B
Vehicle: DMSO
Strains used: *Salmonella typhimurium* TA98, TA100, TA1535, TA1537, TA1538
Positive controls: 2-nitrofluorene, sodium azide, 9-aminoacridine, 2-anthramine
Plates/dose in final study: 3
Concentrations in final study: 0, 62.5, 125, 250, 500, 1000 ug/plate

Results:

At 2500 ug/plate, survival was 3%; at 5000 ug/plate, TEM was insoluble. Only in TA1538 did TEM fail to raise the number of revertants more than 2 fold above controls in the presence and absence of metabolic activation. Positive controls increased the number of mutants by more than 5 or 10 fold.

4. IARC Monographs on the Evaluation of the Carcinogenic Risk of Chemicals to Humans (1987). Supplement 7, Dacarbazine, pp 184-185; Procarbazine, 327-328.

The IARC committee deemed the evidence for carcinogenicity in humans inadequate. However, in rats, tumors were found at less than 18 weeks in the mammary gland, thymus, spleen, and brain. In offspring of dams treated at the end of pregnancy, neuromas were found. In mice, tumors were found in the lung, hematopoietic system and uterus. Dacarbazine did not cause SCE's in patients, but was positive for SCE and mutagenesis in CHO cells and bacteria.

5. IARC Monographs on the Evaluation of the Carcinogenic Risk of Chemicals to Humans (1981). vol. 26, Dacarbazine, pp. 203-215; Procarbazine HCl, pp. 311-339. Vol. 1.29.

The recommended dose of DTIC is iv at 100-250 mg/m^2 DX5 q 3 weeks. The data discussed was from the Beal and Skibba articles reviewed below. By the ip route in mice (25, 50 mg/kg, 3X/week X 6 months), survival was decreased and the following tumor incidences were noted (combined DTIC groups): in the males, 21/41 lung tumors, 15/41 lymphomas, 10/41 splenic hemangiosarcomas; in the females 16/19 lung tumors, 5/19 uterine tumors. Ip

administration of DTIC resulted in similar types of tumors as dietary admix in the rat. The IARC concluded that DTIC was carcinogenic in mouse and rat, as well as teratogenic and mutagenic; however, the data in humans is insufficient.

Procarbazine had similar results in rodents with the addition of renal tumors. The final evaluation of the IARC was that there was sufficient evidence to call procarbazine a carcinogen in rodents, limited evidence of carcinogenicity in monkeys, and sufficient evidence for carcinogenicity in humans as part of combination chemotherapy but not for procarbazine alone.

6. Beal DD, Skibba JL, Croft WA, Cohen SM, Bryan T (1975). Carcinogenicity of the antineoplastic agent, 5-(3,3-Dimethyl-1-triazeno) -imidazole-4-carboxamide, and its metabolites in rats. Journal of the National Cancer Institute 54, 951-957. (CANDA)

Sprague Dawley (both sexes) or Buffalo (female only) rats were administered DTIC or its metabolites, MTIC, 5-diazoimidazole-4-carboximide (d-ICA), 2-azahypoxanthine (2-AH) and 5-aminoimidazole-4-carboxamide (AIC), as a dietary admix (up to 0.1% of feed) for up to 46 weeks. IP doses of DTIC, d-ICA, MTIC were also investigated. Animals were observed for up to 66 weeks. Tissue distribution of DTIC was also investigated to see if there was a correlation between tumor site and concentration of radiolabeled DTIC.

Primary tumors noted included mammary adenocarcinoma and adenofibroma, thymic and splenic lymphosarcoma, uterine leiomyosarcoma, as well as hemangiomas, cerebral ependymomas, pulmonary alveolar carcinoma, and eye leiomyosarcoma. Tissue distribution of radiolabel did not correlate with tumor incidence (largest concentration of label in bladder, gi tract). Both DTIC and MTIC resulted in significant tumor incidence. Single doses of either drug resulted in tumors. Data are shown below.

TABLE 2.—*Incidence of tumors in female Sprague-Dawley rats after ip injection of DTIC*

Number of rats	Control group	Dose (mg/rat)	Injection schedule	Tumor incidence [a]					
				Mammary		Lymphosarcoma		Leiomyosarcoma of the uterus	Others
				Adenocarcinoma	Adenofibroma	Thymic	Splenic		
16	E	400 [b]	Single	11 (2) [c]	5 (2)	7	9	2	1 cerebral ependymoma.
16	E	250	Single	5 (2–3)	4 (2)		2	1	2 embryonal adenosarcomas.
16	E	100	Single	1 (1)	7 (1)			1	1 ependymoblastoma. 1 adrenal cortical adenoma.
20	E	2.5	3 times/wk (12 wk)		6 (2)		1	7	1 cerebral ependymoma. 3 bronchogenic adenocarcinomas. 1 kidney cortical adenocarcinoma.

[a] After 66 wk.
[b] Dissolved in 1.5 ml citrate-mannitol solution.
[c] Numbers in parentheses indicate average No. of tumors per animal.

TABLE 4.—*Incidence of tumors in female Sprague-Dawley rats administered DTIC metabolite*

Compound	Number of rats	Control group	Route of administration	Dose: Percent of diet	Dose: Weeks at dose	Dose: Cumulative dose (mg/rat)	Tumor Incidence: Mammary: Adenocarcinoma	Tumor Incidence: Mammary: Adenofibroma	Tumor Incidence: Leiomyosarcoma of the uterus	Tumor Incidence: Others
Diaz-ICA	12	C	Oral	0.025	14	425				1 splenic lymphosarcoma.
				0.000	20					1 stomach carcinoma (stage 1).
Diaz-ICA	16	H	Ip	0.67 mg/	10	21	1	4		
				0.000	50					
Diaz-ICA	16	I	Ip	0.22 mg/	10	2.5				
				0.000	56					1 urinary bladder papilloma.
2-AH	20	K	Oral	0.025	21	601	3			
				0.000	45					1 adrenal cortical adenoma.
AIC	20	H	Oral	0.050	21	1,278	2			
				0.000	45					3 spondyroblastomas.
										1 thyroid carcinoma.
										1 kidney cortical adenocarcinoma.
MTIC	16	H	Oral	0.050	14	290		13	3	1 thymic lymphosarcoma.
				0.000	52					
MTIC	16	J	Ip	10 mg/kg	0	1		3	3	
				0.000	06					
MTIC	16	K	Ip	0.50 mg/	10	15		1	2	1 splenic lymphosarcoma.
				0.000	56					

[a] Averaged only rats per animal.
[b] Diluted in 0.5 ml 0.85 w NaCl; administered three times/wk.
[c] Suspended in 1.0 ml CMC solution.
[d] Suspended in 0.5 ml CMC solution; administered three times/wk.

TABLE 1.—*Incidence of tumors in rats fed DTIC*

Strain and No. of rats	Sex	Control group	Dose: Percent of diet	Dose: Weeks fed	Dose: Cumulative dose (mg/rat)	Tumor Incidence: Mammary: Adenocarcinoma	Tumor Incidence: Mammary: Adenofibroma	Tumor Incidence: Lymphosarcoma: Thymic	Tumor Incidence: Lymphosarcoma: Splenic	Tumor Incidence: Leiomyosarcoma of uterus	Tumor Incidence: Others
Sprague-Dawley:											
16	M	A	0.10	1.5	974	8 (1–2)[a]		16	5		1 hemangioma.
			0.05	12.5							
			0.00	4.0							
24	F	B	0.10	1.5	740	24 (6)		24	21		10 cerebral ependymomas. 4 pulmonary alveolar carcinomas.
			0.05	12.5							
			0.00	4.0							
12	F	C	0.05	14.0	638	6 (2–3)		8	3		
16	F	C	0.00	10.0	316	10 (1–2)		1			
			0.05	10.0							
16	F	D	0.01	5.0	570	1 (1)	12 (3)	3	2	2	2 telomyosarcomas (eye, blood vessel).
			0.00	14.0							
Buffalo:											
16	F	L	0.05	11.0	354	18 (4–5)		3			
			0.00	4.0							

[a] Number in parentheses indicates average No. of tumors/animal.

7. Skibba JL, Ertürk E, Bryan GT (1970). Induction of thymic lymphosarcoma and mammary adenocarcinomas in rats by oral administration of the antitumor agent, 4(5)-(3,3-dimethyl-1-triazeno) imidazole-5(4)-carboxamide. Cancer 26, 1000-1005. (located from CANDA)

Female Sprague Dawley rats (4 weeks old, 24/group) were administered either a control diet or 0.1% DIC (dacarbazine, DTIC) as a feed admix for 1 week, then 0.05% DTIC for an additional 13 weeks (dose reduced due to excessive weight loss). Rats were then observed for an additional month. Rats were observed daily for tumor formation, palpated weekly after week 4, then killed and examined microscopically at the end of the experiment.

The dose was calculated from food analysis as 7.3 mg/rat/day (cumulative dose 740 mg/rat). Assuming an average weight of 300 g, the daily dose is approximately 24 mg/kg/day or 146 mg/m^2/day. All of the rats developed multiple tumors of varied types. By week 18, only 3/24 rats in the DIC group were alive as compared to 24/24 controls. No tumors were found in the controls, while mammary adenocarcinoma (24/24), thymic lymphosarcoma (24/24), cerebral ependymoma (9/24) and pulmonary alveolar carcinomas (4/24) were found in the DIC rats (mean 5 tumors/rat). Tumors were palpable by week 10 (both mammary and thymic tumors).

Other observations included splenic enlargement, marrow neoplastic proliferation with erosion of bone (12/24), and widespread leukemic infiltrates.

Summary of Genetic Toxicity:

Temozolomide was mutagenic in bacterial systems (Ames test) and clastogenic in a mammalian test (human peripheral lymphocyte chromosomal aberrations). Administration of MTIC, one of the metabolites of TEM, to Sprague Dawley rats at 890 mg/rat total dose as a dietary admix, or as a single dose ip (10 mg/kg) resulted in a significant incidence of leiomyosarcoma of the uterus and adenofibromas of mammary gland. Dacarbazine, another alkylating agent with common metabolites with TEM, has been deemed a mutagen, clastogen, and rodent carcinogen (variety of tumor types), although there is insufficient evidence to label it as a human carcinogen.

OVERALL SUMMARY AND EVALUATION

Temozolomide is a imidazotetrazinone with metabolites partially identical to dacarbazine. Temozolomide and its metabolites, temozolomide acid and MTIC all have relatively similar levels of activity *in vitro* (IC50's in the ug/mL range). In murine models, significant tumor growth inhibition was observed at doses at or below the LD_{10}, suggesting a low therapeutic index typical of alkylating agents. The proposed mechanism of action of TEM is the methylation of DNA, preferentially at guanine nucleotides. Activity of drug partially correlates with levels of O6-alkylguanine-DNA-alkyltransferase.

TEM is unstable in plasma with a half life of less than 15 minutes at room temperature. MTIC is less stable with a half-life of 5.5 minutes. Acidification of plasma samples in PK studies allowed for stabilization of the TEM, but not the MTIC. In the rat and dog, AUC and Cmax for oral TEM were linear with doses between 25 and 1000 mg/m2. Bioavailability was approximately 100% in each species analyzed. AUC and Cmax levels correlated well in rat, dog and human on a body surface area basis. AUC levels at or above 30 ug•hr/mL were associated with severe toxicity and death in each species. Half life of the drug was between 1 and 1.6 hours in all experiment. The half-life of the presumed active species, MTIC, was similar in rat and dog, as well as being similar to that of the parent compound. MTIC accounted for less than 3% of the total exposure to TEM. TEM was excreted primarily via the urine in mice, rats, dogs, and humans. Metabolites in the urine included unchanged parent drug, temozolomide acid, AIC, and 2 polar metabolites.

The total bioavailability and similar ADME is also demonstrated by the similar degrees of toxicity in the oral and iv/ip routes and across species. Toxicities were remarkably similar across species with the exception of the incidence of tumors seen in the 3 and 6 cycle rat study. Typical toxicities were primarily associated with marrow suppression (decreases in RBC, WBC and platelets, marrow hypocellularity, thymic lymphoid depletion, some splenic hyperplasia), gastrointestinal hemorrhage and atrophy, some liver necrosis, and retinal damage. With higher doses, hepatic, liver, and gastrointestinal necrosis, and multi-organ hemorrhage (including brain) were observed. No new toxicities or increases in the degree of hematologic toxicity were seen with multiple cycles of administration with the exception of tumor formation in the rat.

As TEM is both mutagenic and clastogenic, it is not surprising that the compound was both

teratogenic in rat and rabbit as well as tumor-forming in the rat. What is surprising is the speed of formation of neoplastic lesions in the rat: mammary carcinomas were observed as early as 3 months. By 6 months, a wide variety of tumors were observed in the rat of both epithelial and mesenchymal origin. A similar profile of tumor types with similar onset were observed with dacarbazine administration. However, in an experiment where only MTIC was administered orally to rats, no mammary carcinomas were observed. Data from Beal's group suggests that a single dose of dacarbazine or MTIC is sufficient to result in tumor formation; however, the experiment was not conducted with TEM.

RECOMMENDATION: The NDA is approvable for Pharmacology/Toxicology

Labeling Review:

/S/

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Concurrence: /S/

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Original IND/NDA/DMF
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