CENTER FOR DRUG EVALUATION AND RESEARCH

APPLICATION NUMBER: NDA 21029

PHARMACOLOGY REVIEW(S)

Division of Oncology Drug Products, HFD-150

REVIEW AND EVALUATION OF PHARMACOLOGY AND TOXICOLOGY DATA

Original, Review No. 1

NDA No. 21-029

Serial No(s).: 000 **Type:** NDA **Date of Submission** 8/13/98 **CDR stamp date**: 8/13/98

Information to be Conveyed to Sponsor: Yes (X), No ( )

Reviewer: Wendelyn J. Schmidt, Ph.D.

Date Review Completed: 2/8/99

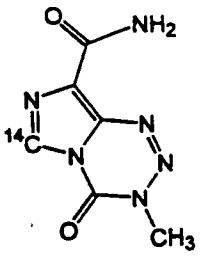
Sponsor: Schering Corporation

Manufacturer (if different): Drug substance:
Drug Product:

Drug Name: Primary: temozolomide Other Names: Temodal, SCH52365

Chemical Name: 8-carbamoyl-3-methylimidazol [5,1-d]-1,2,3,5 tetrazine-4 (3H)-one

Structure:



CAS Number: 85622-93-1

Molecular Weight (and Formula optional): $C_6H_6N_6O_2$; mw=194.15

Related INDs/NDAs/DMFs: IND IND

Class: antineoplastic (alkylating agent)

Indication: "...for the treatment of adult patients with malignant glioma (glioblastoma multiforme and anaplastic astrocytoma) at first relapse and as first line treatment for patients with advanced metastatic malignant melanoma."

Clinical Formulation: 5, 20, 100, or 250 mg capsules

	mg ingredients/capsule			
	5 mg	20 mg	100 mg	250 mg
Temozolomide				
Lactose Anhydrous, NF				
Sodium starch glycolate NF				
Colloidal silicon dioxide NF				
Tartaric Acid NF				
Stearic acid NF				
TOTAL				

Route of Administration: oral

Proposed Dose and Schedule:
Chemotherapy-naïve patients: 200 mg/m^2 DX5 q 28 days
Previously treated patients: 150 mg/m^2 DX5 q 28 days

Previous Review(s), Date(s) and Reviewer(s): IND and IND
W. Schmidt

Studies Reviewed in this NDA: (note: volume #'s or CANDA cited in individual reviews)

Pharmacology:

1. Plowman, J., Waud, W.R., Koutsoukos, A.D., Rubinstein, L.V., Moore, T.D. and Grever, M.R. (1994) Preclinical antitumor activity of temozolomide in mice: efficacy against human brain tumor xenografts and synergism with 1,3-bis(2-chloroethyl)-1-nitrosourea. Cancer Res. 54: 3793-3799.
2. Mitchell, R.B. and Dolan, M.E. (1993) Effect of temozolomide and dacarbazine on O 6 - alkylguanine-DNA alkyltransferase activity and sensitivity of human tumor cells and xenografts to 1,3-bis(2-chloroethyl)-1-nitrosourea. Cancer Chemother. Pharmacol. 32: 59-63.

Pharmacokinetics

1. P-6059. SCH 52365: Biliary excretion and enterohepatic circulation of radioactivity following administration of a single oral dose of 14 C-SCH 52365 suspension to male rats. Kenilworth (NJ): Schering-Plough Research Institute; 1996 Nov.
2. P-6097. SCH 52365: Absorption, metabolism, excretion, and pharmacokinetics of 14 C-SCH 52365 following a single oral or intravenous dose in male rat. Kenilworth (NJ): Schering-Plough Research Institute; 1996 Dec.
3. P-6371. SCH 52365: Tissue distribution of radioactivity by whole body autoradiography following a single oral administration of 14 C-SCH 52365 suspension to male rats. Kenilworth (NJ): Schering-Plough Research Institute; 1996 Dec.
4. P-5949. SCH 52365: Absorption, distribution, and metabolism of 14 C-SCH 52365 following a single oral dose in the male rat. Kenilworth (NJ): Schering-Plough Research Institute; 1996 Dec.
5. P-6263. SCH 52365: Tissue distribution of 14 C-SCH 52365 following a single oral dose in the female pigmented rat. Kenilworth (NJ): Schering-Plough Research Institute; 1996 Dec.
6. P-6098. SCH 52365: Absorption, metabolism, excretion, and pharmacokinetics of 14 C-SCH 52365 following a single oral or intravenous dose in the male dog. Kenilworth (NJ): Schering-Plough Research Institute; 1997 Jan.
7. P-6072. SCH 52365: Single-dose two-way crossover comparative bioavailability study of formulated versus unformulated SCH 52365 capsules in dogs. Kenilworth (NJ): Schering-Plough Research Institute; 1996 Dec.
8. P-6478. SCH 52365: Pharmacokinetics of SCH 52365 and its metabolite MTIC following a single oral suspension administration of SCH 52365 to male and female rats. Kenilwortn (NJ): Schering-Plough Research Institute; 1998 Mar.
9. P-6468. SCH 52365: Pharmacokinetics of SCH 52365 and its metabolite MTIC following a single oral suspension administration of SCH 52365 to male and female beagle dogs. Kenilworth (NJ): Schering-Plough Research Institute; 1998 Mar.
10. D-26672. SCH 52365: Stability of temozolomide and MTIC (active metabolite), quantitation of MTIC in human plasma. Kenilworth (NJ): Schering-Plough Research Institute; 1993 Jun.
11. Tsang LLH, Farmer PB, Gescher A, Slack JA. Characterization of urinary metabolites of temozolomide in humans and mice and evaluation of their cytotoxicity. Cancer Chemother. Pharmacol. 1990;26:429-436.

12 Slack JA, Goddard C, Stevens MFG, Baig GU, Griffin MJ. The analysis and murine pharmacokinetics of a new antitumor agent: CCRG 81045. J. of Pharm. and Pharmacology 1986;38:63P.

Toxicology

1. P-5987. Three-cycle oral toxicity study of SCH 52365 in rats.

2. P-6054. Six-cycle oral toxicity study of SCH 52365 in rats.

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

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

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

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

Special Toxicity

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.
2. P-6490. Effect of temozolomide on gastrointestinal function in rats. Kenilworth (NJ): Schering-Plough Research Institute; 1993 Sep.
3. P-6280. Dermal sensitization study in guinea pigs (Buehler's Technique Modified) with SCH 52365 (temozolomide).

Reproductive Toxicology

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

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

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

Genetic Toxicology

1. P-6495. Salmonella-Escherichia coli/Mammalian microsome reverse mutation assay of SCH 52365 (temozolomide).
2. P-6454. Chromosome aberration study of SCH 52365 in human peripheral blood lymphocytes.
3. P-5866. M&B, 39,831 (R.P. 46,161) Ames Test.

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

Studies not reviewed:

Pharmacodynamics:

1. Shealy YF, Krauth CA. Imidazoles. II. 5(or 4)-(Monosubstituted triazeno) imidazole-4(or 5)-carboxamides. J. Med. Chem. 1966;9:34-38.

2. Catapano CV, Broggini M, Erba E, Ponti M. Mariani L, Citti L, D'Incalci M (1987). In vitro and in vivo methazolastone-induced DNA damage and repair in L-1210 leukemia sensitive and resistant to chloroethylnitrosoureas. Cancer Research 47, 4884-4889.
3. D'Incalci M, Citti L, Taverna P, Catapano CV (1988). Importance of the DNA repair enzyme O 6 -alkyl guanine alkyltransferase (AT) in cancer chemotherapy. Cancer Treatment Reviews 15, 279-292.
4. Pegg AE (1990). Mammalian O 6 -alkylguanine-DNA alkyltransferase: Regulation and importance in response to alkylating carcinogenic and therapeutic agents. Cancer Research 50, 6119-6129.
5. Lee SM, Thatcher N, Crowther D, Margison GP (1994). Inactivation of O 6 -alkylguanine-DNA alkyltransferase in human peripheral blood mononuclear cells by temozolomide. British Journal of Cancer 69, 452-456.
6. Gerson SL, Trey JE, Miller K, Berger NA (1986). Comparison of O 6 -alkylguanine-DNA alkyltransferase activity based on cellular DNA content in human, rat and mouse tissues. Carcinogenesis 7, 745-749.
7. Jun GJ, Ro JJ, Kim MH, Park GH, Paik WK, Magee PN, Sangduk K (1986). Studies on the distribution of O 6 -methylguanine-DNA methyltransferase in rat. Biochemical Pharmacology 35, 377-384.
8. Fong LYY, Jensen DE, Magee PN (1990). DNA methyl-adduct dosimetry and O 6 -alkylguanine-DNA alkyl transferase activity determinations in rat mammary carcinogenesis by procarbazine and N-methylnitrosourea. Carcinogenesis 11, 411-417.
9. Schmähl D, Habs M (1979). Carcinogenic action of low dose cyclophosphamide given orally to Sprague-Dawley rats in a lifetime experiment. International Journal of Cancer 23:706-712.
10. Singer B (1979). N-Nitroso alkylating agents: Formation and persistence of alkyl derivatives in mammalian nucleic acids as contributing factors in carcinogenesis. Journal of the National Cancer Institute 62, 1329.
11. Wheelhouse RT, Stevens MFG. Decomposition of the antitumor drug temozolomide in deuteriated phosphate buffer: methyl group transfer is accompanied by deuterium exchange. J. Chem. Soc. Chem. Commun. 1993;15:1177-1178.
12. Denny BJ, Wheelhouse RT, Stevens MFG, Tsang LLH, Slack JA. NMR and molecular modeling investigation of the mechanism of activation of the antitumor drug temozolomide and its interaction with DNA. Biochemistry 1994;33:9045-9051.
13. Baer, J.C., Freeman, A.A., Newlands, E.S., Watson, A.J., Rafferty, J.A. and Margison, G.P. (1993) Depletion of O 6 -alkylguanine-DNA alkyltransferase correlates with potentiation of temozolomide and CCNU toxicity in human tumor cells. Br. J. Cancer 67: 1299-1302.
14. Wedge, S.R., Porteous, J.K., May, B.L. and Newlands, E.S. (1996a) Potentiation of temozolomide and BCNU cytotoxicity by O 6 -benzylguanine: a comparative study in vitro. Br. J. Cancer 73: 482-490.
15. Hartley, J.A., Mattes, W.B., Vaughan, K. and Gibson, N.W. (1988) DNA sequence specificity of guanine-N7 alkylations for a series of structurally related triazenes. Carcinogenesis 9: 669-674.
17. Bobola, M.S., Tseng, S.H., Blank, A., Berger, M.S. and Silber, J.R. (1996) Role of O 6 -methylguanine-DNA methyltransferase in resistance of human brain tumor cell lines to the clinically relevant methylating agents temozolomide and streptozotocin. Clin. Cancer Res. 2: 735-741.
18. Wedge, S.R., Porteous, J.K. and Newlands, E.S. (1997) Effect of single and multiple administration of an O 6 -benzylguanine/temozolomide combination: An evaluation in a human melanoma xenograft model. Cancer. Chemother. Pharmacol. 40: 266-272.

9. Clin Doc 97009275. Absorption, metabolism and excretion of 14 C-SCH 52365 in patients with advanced cancer [study report for Protocol C95-006-01]. Kenilworth (NJ): Schering-Plough Research Institute; 1997 Jan.
10. Clin Doc 96358306. SCH 52365: A phase I study of SCH 52365 in pediatric patients with advanced cancer [study report for Protocol I93-125-01]. Kenilworth (NJ): Schering-Plough Research Institute; 1997 Jan.
11. I95-018. A randomized, Phase III study of temozolomide (SCH 52365) versus dacarbazine in the treatment of patients with advanced, metastatic malignant melanoma. Kenilworth (NJ): Schering-Plough Research Institute; 1998.
12. D-28505. SCH 52365: Temozolomide in the treatment of metastatic malignant melanoma with brain metastases (memo). Kenilworth (NJ): Schering-Plough Research Institute; 1998 Apr.
13. 28656. A Phase II/pharmacokinetic study of temozolomide (SCH 52365) in the treatment of patients with advanced hepatocellular carcinoma. Pharmacokinetics of SCH 52365 and MTIC (memo). Kenilworth (NJ): Schering-Plough Research Institute; 1998 Jun.
14. Reid JM, Stevens DC, Rubin J, Ames MM. Pharmacokinetics of 3-methyl-(triazen-1-yl)imidazole-4-carboximide following administration of temozolomide to patients with advanced cancer. Clin. Canc. Res. 1997; 3:2393-2398.
15. Clin Doc 97206350. Population pharmacokinetic analyses of SCH 52365 in adult patients with anaplastic astrocytoma or glioblastoma multiforme [study report for Protocols I93-114, I93-114A, C93-169, C94-022, I94-122, I94-123, C94-123 and C94-091]. Kenilworth (NJ): Schering-Plough Research Institute; 1997 Oct.

Pharmacokinetics and ADME:

1. D-26394. SCH 52365: Validation of a assay for SCH 52365 (temozolomide) in rat plasma.
2. D-26614. Determination of SCH 52365 in rat plasma by

3. P-6474. SCH 52365: An method for quantitation of MTIC (a bioconversion product of temozolomide) in rat plasma. Kenilworth (NJ): Schering-Plough Research Institute; 1997 Apr.
4. D-26886. Determination of SCH 52365 in rat urine by

5. D-26509. SCH 52365: Validation of assay for SCH 52365 (temozolomide) in rat brain.
6. D-26395. SCH 52365: Validation of assay for SCH 52365 (temozolomide) in dog plasma.
7. D-26615. Determination of SCH 52365 in dog plasma by

8. P-6462. SCH 52365: An method for quantitation of MTIC (metabolite of temozolomide) in dog plasma. Kenilworth (NJ): Schering-Plough Research Institute; 1997 May.
9. Welch RM, Brown A, Ravitch J, Dahl R. The in vitro degradation of cisatracurium the R, cis-R-isomer of atracurium, in human and rat plasma. Clinical Pharmacology & Therapeutics 1995;58(2):132-142.
10. Schulman MP, Buchanan JM. Biosynthesis of the Purines II. Metabolism of 4-amino-5-imidazolecarboxamide in pigeon liver. Journal of Biological Chemistry 1952;196:513-526.
11. Miller CS, Gurin S, Wilson DW. 14 C Labeled 4(5)-amino-5(4)-imidazolecarboxamide in the biosynthesis of purines. Science 1950;112:654-655.
12. Flaks JG, Erwin MJ, Buchanan JM. Biosynthesis of the Purines XVI. The synthesis of adenosine 5'-phosphate and 5-amino-4-imidazolecarboxamide ribotide by a nucleotide pyrophosphorylase. Journal of Biological Chemistry 1957;228:201-213.
13. Krenitsky TA, Neil SM, Elion GB, Hitchings GH. Adenine phosphoribosyltransferase from monkey liver. Specificity and properties. Journal of Biological Chemistry 1969;17:4779-4784.

14. Thomas CB, Arnold WJ, Kelley WN. Human adenine phosphoribosyltransferase. Purification, subunit structure and substrate specificity. Journal of Biological Chemistry 1973;248:2529-2535.
15. Herbert V, Streiff RR, Sulivan LW, McGreer PL. Deranged purine metabolism manifested by aminoimidazolecarboxamide excretion in megaloblastic anemias, hemolytic anemia, and liver disease. The Lancet 1964;45-46.
16. Conzelman GM, Mandel HG, Smith PK. The incorporation of radiocarbon from 4-amino-5-imidazolecarboxamide into the purines of tumor-bearing mice. Journal of Biological Chemistry 1953;201:329-334.
17. Horgan CMT, Tisdale MJ. Antitumor Imidazotetrazines VIII. Uptake and decomposition of a novel antitumor agent Mitozolomide (CCRG 81010; M and B 39565; NSC 353451) in TLX5 mouse lymphoma in vitro. Biochemical Pharmacology 1985;34:217-221.
18. Wyngaarden JB, Seegmiller JE, Laster L, Blair AE. Utilization of hypoxanthine, adenine and 4-amino-5-imidazolcarboxamide for uric acid synthesis in man. Metabolism, Clin. and Exptl. 1959;8:455-464.
19. Deodhar SD, Pittman G. A study of the metabolism of 4-amino-5-imidazolecarboxamide (AIC) in folic acid deficiency in rats. Cleveland Clinic Quarterly
20. McGeer PL, McGreer EG, Hasselback R. Preliminary survey of 4-amino-5-imidazolecarboxamide excretion in leukemia and other illnesses. Canad. M.A.J. 1961;85:437-439.
21. Segmiller JE, Laster L, Stetten DeWitt. Incorporation of 4-amino-5-imidazolecarboxamide-4-C13 into uric acid in the normal human. Journal of Biological Chemistry 1955;216:653-662.
22. Zimmerman TP, Deeprose RD. Metabolism of 5-amino-1-_-D-ribofuranosylimidiazole -4-carboxamide and related five-membered heterocycles to 5'-triphosphates in human blood and L5178Y cells. Biochemical Pharmacology 1978;27:709-716.
23. D-26687. Determination of SCH 52365 in dog urine by

Toxicology:
1. Graziano MJ, Courtney CL, Meierhenry EF, Kheoh T, Pegg DG, Gaugh AW (1996). Carcinogenicity of the anticancer topoisomerase inhibitor, amsacrine, in Wistar rats. Fundamental and Applied Toxicology 32, 53-65.
2. [PDR] Physician's Desk Reference (1996). 50th edition, Medical Economics Company Inc. Montvale, NJ.
3. McMartin DN, Sahota PS, Gunson DE, Hsu HH, Spaet RH (1992). Neoplasms and related proliferative lesions in control Sprague-Dawley rats from carcinogenicity studies. Historical data and diagnostic considerations. Toxicologic Pathology 20, 212-225.
4. P-5876. Single-cycle oral (capsule) toxicity study of SCH 52365 in dogs using SCH 52365 manufactured at
5. P-5861. A single-dose study in intravenous injection in mice of compound CRC 84/07. Surrey (UK): British Industrial Biological Research Association; 1988 Jul.
6. P-5860. A single-dose study by intraperitoneal injection in mice of compound CRC 84/07. Surrey (UK): British Industrial Biological Research Association; 1988 Apr.
7. P-5863. A repeat-dose study by intraperitoneal injection in mice of compound CRC 84/07. Surrey (UK): British Industrial Biological Research Association; 1988 Oct.
8. P-5862. A repeated-dose study by intraperitoneal injection in rats of compound CRC 84/07. Surrey (UK): British Industrial Biological Research Association; 1988 Mar.
9. P-5879. Three-cycle oral toxicity study of SCH 52365 in rats. Lafayette (NJ): Schering-Plough Research Institute; 1993 Dec. Interim report.

Studies Previously Reviewed:

Pharmacology: (reviewed in IND review #1)

1. Stevens MFG, Hickman JA, Langdon SP, Chubb D, Vickers L, Stone R, Baig G. Goddard C, Gibson NW, Slack JA, Newton C, Lunt E. Antitumor activity and pharmacokinetics in mice of 8-carbamoyl-3-methyl-imidazo[5,1-d]-1,2,3,5-tetrazin-4(3H)-one (CCRG 81045; M & B 39831), a novel drug with potential as an alternative to dacarbazine. Cancer Research. 1987;47:5846-5852.
2. Tsang LLH, Quarterman CP, Gescher A, Slack JA. Comparison of the cytotoxicity in vitro of temozolomide and decarbazine, prodrugs of 3-methyl-(triazen-1-yl)imidazole-4-carboxamide. Cancer Chemother. Pharmacol. 1991;27:342-346.
3. Tisdale, M.J. (1987) Antitumor imidazotetrazinones-XV. Role of guanine O 6 alkylation in the mechanism of cytotoxicity of imidazotetrazinones. Biochem. Pharmacol. 36: 457-462.
4. D'Incalci, M., Taverna, P., Erba, E., Filippeschi, S., Potenza, D., Mariani, L., Citti, L. and Catapano, C.V. (1991) O 6 -Methylguanine and temozolomide can reverse the resistance to chloroethylnitrosoureas of a mouse L1210 leukemia. Anticancer Res. 11: 115-122.
5. D-26535. In vitro myelotoxicity of temozolomide to human CFU-GM. Kenilworth (NJ): Schering-Plough Research Institute; 1993 Aug.

Pharmacokinetics: (IND review #1)

1. P-5864. SCH52365: Pharmacokinetics of SCH 52365 in rats following a single oral gavage and intravenous dose. Kenilworth (NJ): Schering-Plough Research Institute; 1996 Nov.
2. P-5919. SCH 52365: Mass balance and excretion of 14 C-SCH 52365 following a single intravenous and oral dose in the male rat. Kenilworth (NJ): Schering-Plough Research Institute; 1994 May.
3. P-5892. SCH 52365: Pharmacokinetics of SCH 52365 in beagle dogs following a single oral gavage or a single intravenous crossover dose. Kenilworth (NJ): Schering-Plough Research Institute; 1993 Oct.

Toxicology: (IND review #1)

1. P-5867. Single-dose oral toxicity study of SCH 52365 in mice.
2. P-5868. Single-dose intraperitoneal study of SCH 52365 in mice.
3. P-5869. Single-dose oral toxicity study of SCH 52365 in rats.
4. P-5871. Single-dose oral toxicity study with lower doses of SCH 52365 in rats.
5. P-5870. Single-dose intraperitoneal toxicity study of SCH 52365 in rats.
6. P-5872. Single-dose oral toxicity study of SCH 52365 in beagle dogs.
7. P-5873. Single-dose oral toxicity study with lower doses of SCH 52365 in beagle dogs.
8. P-5874. Single-cycle oral toxicity study of SCH 52365 in rats.
9. P-5875. Single-cycle oral toxicity study with lower doses of SCH 52365 in rats.

Note: Portions of this review were excerpted directly from the sponsor's submission.

INTRODUCTION/ DRUG HISTORY

The original IND for temozolomide (TEM) was submitted by the NCI in May, 1993. Schering began development of TEM in Dec. 1993. TEM, a pro-drug for MTIC; is converted without metabolic activation to MTIC at physiologic pH. Dacarbazine (DTIC), an approved drug, is also a pro-drug for MTIC. Temozolomide is orally bioavailable (approximately 100%) and crosses the blood brain barrier. Half-life ranges around 1 hour in most species. The proposed mechanism of action is cross-linkage of DNA.

The toxicologic profile of temozolomide includes marrow toxicity, g.i. damage, liver and kidney necrosis (possibly secondary to bacterial infiltration), and at high doses in mice only, a bleomycin-like damage to the lung. In a multiple cycle rat study, mammary carcinomas were found in females beginning at 3 months. No mammary carcinomas were observed in a 6 cycle dog study. Damage to reproductive organs was also noted. Hepatic damage in the mouse and rat correlated with elevated liver enzymes.

PREVIOUS CLINICAL EXPERIENCE

Temozolomide has been investigated in Phase I, and Phase II malignant melanoma, high grade glioma and low grade non-Hodgkin's lymphoma. Doses in phase II trials ranged from 750-1200 mg/m^2 over 5 days (150-250 mg/m^2/day). Toxicities were primarily myelosuppression and nausea/vomiting. Hepatic damage, and, in glioma patients, cerebral edema/increased cranial pressure (may be disease related) were noted in rare instances.

I. PHARMACOLOGY

1. Plowman, J., Waud, W.R., Koutsoukos, A.D., Rubinstein, L.V., Moore, T.D. and Grever, M.R. (1994) Preclinical antitumor activity of temozolomide in mice: efficacy against human brain tumor xenografts and synergism with 1,3-bis(2-chloroethyl)-1-nitrosourea. Cancer Res. 54: 3793-3799.

Athymic NCR-nu/nu mice bearing human tumor xenografts implanted either intracranially or sc were treated with gavage TEM at doses of 180-600 mg/kg (the LD30) and the tumor size measured. Even if treatment of U251 glioblastoma with 270, 400 or 600 mg/kg TEM was delayed until tumor had reached 300 mg, 8-10/10 mice were tumor-free on day 71. No schedule dependence of activity was noted. When the combination of BCNU and TEM (BCNU administered before TEM) was used in the less sensitive SF-295 cell line, tumor growth delays of up to 400% were seen at doses which did not result in death (doses of >40 mg/kg BCNU + >180 mg/kg TEM were lethal). Statistical analysis of the data suggested synergistic effects with the two drugs.

Table 2 *Activity of p.o. temozolomide and i.v. BCNU against staged s.c. implanted SF-295 and U251 glioblastoma xenografts*

Approximately 30-mg tumor fragments were implanted s.c. into the axillary region of groups of 10 (20 controls) athymic NCr-nu/nu mice on Day 0. Treatment was initiated when tumor sizes ranged from 63 to 294 mg (SF-295 model) or from 63 to 108 mg (U251 model). All vehicle-treated control tumors grew well with median doubling times of 1.4 and 2.5 days for the SF-295 and U251 experiments, respectively. Median time to 4 doublings was 7.8 days for SF-295 control tumors. Median time to 2 doublings was 8.3 days for U251 control tumors.

Compound	Route and treatment schedule	Dose (mg/kg/day)	No. of drug deaths	No. of complete regressions	No. tumor free[a]	Growth delay[b] (%)	Net $\log_{10}$ cell kill[b]
SF-295 glioblastoma experiment							
Temozolomide	p.o. Day 6	600	2/10	3/10	2/10	>315	>5.3
		400	0/10	0/10	1/10	237	4.0
	p.o. Days 6, 10, 14	200	1/10	0/10	2/10	295	3.2
		133	0/10	1/10	1/10	232	2.2
	p.o. Days 6–10	120	0/10	1/10	0/10	>336	>4.8
		80	1/10	0/10	0/10	182	2.2
BCNU	i.v. Day 6	40	0/10	3/10	1/10	301	5.1
		27	0/10	0/10	1/10	121	2.0
	i.v. Days 6, 10, 14	27	2/10	0/10	7/10	>336	>3.9
		18	0/10	1/10	0/10	276	2.9
U251 glioblastoma experiment							
Temozolomide	p.o. Day 7	600	0/10	1/10	9/10	675	6.7
		400	0/10	4/10	6/10	478	4.8
	p.o. Days 7, 11, 15	200	2/10	3/10	5/10	640	5.4
		133	0/10	8/10	2/10	548	4.5
	p.o. Days 7–11	120	0/10	3/10	7/10	694	6.5
		80	1/10	6/10	3/10	624	5.8
BCNU	i.v. Day 7	40	0/10	10/10	0/10	283	2.8
		27	0/10	9/10	0/10	196	2.0
	i.v. Days 7, 11, 15	27	1/10	9/10	0/10	460	3.6
		18	0/10	8/10	0/10	281	1.8

[a] Day 40 for SF-295, Day 86 for U251.
[b] Excludes tumor-free mice.

Table 4 *Median percentage of tumor growth delay attained following treatment of advanced stage s.c. human SF-295 glioblastoma xenografts with p.o. temozolomide and/or i.v. BCNU: BCNU/temozolomide sequence*

Treatment was initiated on Day 9 when individual tumor weights ranged from 100 to 343 mg. Temozolomide was administered 2 h after BCNU. Median doubling time and median time to 3 doublings for control tumors were 1.7 and 7.1 days, respectively. Growth delays include tumor-free survivors on Day 51 but exclude drug-related deaths. Except where noted, all treatments were equal to, or below, maximally tolerated doses.

BCNU (mg/kg)	Temozolomide (mg/kg)				
	0	180	270	400	600
0		1	39	28	190
18	17	244	228	380	NT[a]
27	59	401	444	>492	NT
40	242	>492	443[b]	>492[c]	NT
60	258	NT	NT	NT	NT

[a] NT, not tested.
[b] Toxic treatment; 4 of 10 apparent drug-related deaths.
[c] Toxic treatment, 3 of 10 apparent drug-related deaths.

2. Mitchell, R.B. and Dolan, M.E. (1993) Effect of temozolomide and dacarbazine on O 6 - alkylguanine-DNA alkyltransferase activity and sensitivity of human tumor cells and xenografts to 1,3-bis(2-chloroethyl)-1-nitrosourea. Cancer Chemother. Pharmacol. 32: 59-63.

HT29 human colon adenocarcinoma cells (both in culture and injected sc in NIH Swiss female nude mice) were used to determine the effects of TEM, DTIC and BCNU on alkylguanine alkyltranferase (AGT) levels and cytotoxicity. Growth inhibition *in vitro* was measured by MTT assay. To measure in vivo AGT levels, mice were treated with drug when tumor reach 400-600 mm^3, then killed 4 or 16 hours after drug treatment. In vivo efficacy was monitored by measuring tumor volume.

In culture, exposing cells to TEM (500 uM for 4 hours) prior to BCNU resulted in a log greater cell kill; however, adding TEM after BCNU or at concentrations of 200 uM had almost no effect. AGT activity was decreased by 31% and 74% at 200 and 500 uM TEM for 4 hours. DTIC in vivo was less effective than TEM even in combination with BCNU. The sponsor suggested that the increased efficacy with TEM was due to depletion of AGT by TEM to allow greater DNA adducting with BCNU. Toxicity was also increased with the combinations.

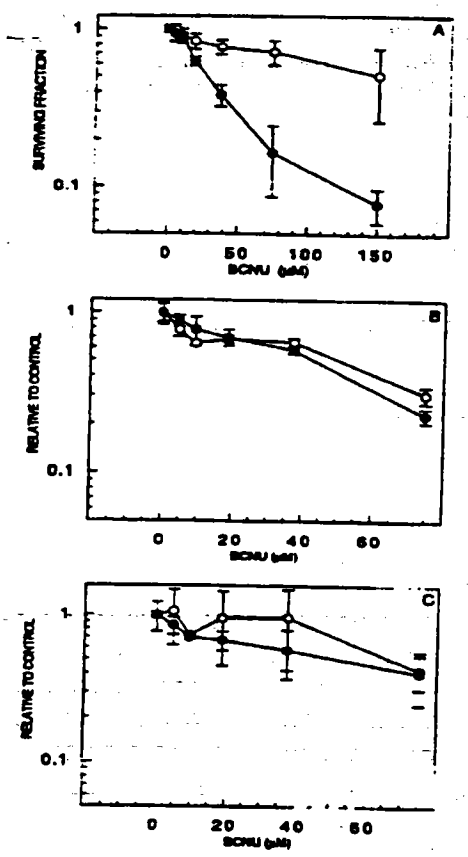


Fig. 1 A–C. HT29 cells (500 per well) were allowed to adhere to 96-well plates for 24 h. After cells were treated with temozolomide for 2 h, BCNU was added at the indicated concentrations for an additional 2 h. Fresh medium was added and cells were incubated for 5 days at 37°C. Growth inhibition was measured by the tetrazolium salt (MTT) assay. A Cells were treated without (○) or with (●) 500 μM temozolomide prior to BCNU exposure. B Cells were treated without (○) or with (●) 200 μM temozolomide prior to BCNU exposure. C Cells were treated without (○) or with (●) 500 μM temozolomide for 4 h after BCNU exposure. Each point represents the mean of three determinations (± SD). Each experiment was repeated with similar results

3. Friedman, H.S., Dolan, M.E., Pegg, A.E., Marcelli, S., Keir, S. Catino, J.J., Bigner, D.D. and Schold, S.C. (1995) Activity of temozolomide in the treatment of central nervous system tumor xenografts. Cancer Res. 55: 2853-2857.

Balb/c mice with sc human tumor xenografts (mer$^+$ repair proficient tumors) were administered ip TEM at the LD10 (411 mg/m^2/day for 5 consecutive days or 1200 mg/m^2 as a single dose) and their survival observed. On the DX5 schedule, 12/127 mice died. With the addition of O6-benzylguanine (an inhibitor of O6-alkylguainine-alkyltransferase, a DNA repair enzyme) the LD10 was decreased to 750 mg/m^2 as a single dose. Significant increases in lifespan and complete remissions were seen at these doses in a series of CNS tumor xenografts (see table below). Activity in these cell lines with TEM was greater than that of BCNU or procarbazine by 4-15 fold.

Table 2 *Activity of temozolomide against s.c. CNS tumor xenografts*

Xenograft	Derivation	Experiment	Regimen[a]	Median time to 5 times initial tumor volume of control tumors (days)	T-C[b]	Regressions[c]
D-341 Med	Medulloblastoma	1	411 mg/m² × 5 days	30.3	3.5	2/9[d]
		2	411 mg/m² × 5 days	31.4	0.83[d]	0/10[d]
		3	1200 mg/m² × 1 day	28.9	10.9	1/8[d]
		4	1025 mg/m² × 1 day	29.3	8.6	5/9
D528 EP	Ependymoma	1	411 mg/m² × 5 days	75.2	68.3	8/2 (2)
		2	411 mg/m² × 5 days	43.3	90+	9/9 (7)
D612 EP	Ependymoma	1	411 mg/m² × 5 days	40.8	72.8	8/8
		2	411 mg/m² × 5 days	40.2	86.1	10/10
D-456 MG	Childhood GBM[e]	1	411 mg/m² × 5 days	44.5	120+	7/7 (7)
		2	411 mg/m² × 5 days	28.1	120+	8/8 (8)
D-212 MG	Childhood GBM	1	411 mg/m² × 5 days	39.5	56.2	9/9
		2	411 mg/m² × 5 days	37.4	47.4	10/10
D-54 MG	Adult AA	1	411 mg/m² × 5 days	7.5	40.8	10/10 (1)
D-245 MG	Adult GBM	1	411 mg/m² × 5 days	20.5	108.3	8/8 (1)
		2	411 mg/m² × 5 days	25.6	111.9	9/9

[a] Temozolomide was given by i.p. injection at a volume of 90 ml/m².
[b] Growth delay in days, defined as the difference between the median time for tumors in treated (T) and control (C) animals to reach five times the volume recorded at initiation of treatment.
[c] Defined as a decrease in tumor volume over two successive measurements, number of regressions/number treated. Numbers in parentheses, number of complete regressions.
[d] Values not statistically significant ($P < 0.01$).
[e] Abbreviations are as used in Table 1.

Table 3 *Treatment of CNS tumor xenografts growing s.c. in athymic nude mice with BCNU, procarbazine, or temozolomide*

Xenograft	Derivation	BCNU[a]		Procarbazine[b]		Temozolomide[c]	
		T-C (days)[d]	Regressions[e]	T-C (days)[d]	Regressions[e]	T-C (days)[d]	Regressions[e]
D-212 MG	Childhood GBM[f]	4.1[g]	0/10[g]	7.5	2/10[g]	56	9/9
D-456 MG	Childhood GBM	6.1	1/10[g]	47.2	10/10 (2)	116.4	8/8 (8)
D612 EP	Ependymoma	18.3	9/10	48.9	10/10	86	10/10
D528 EP	Ependymoma	10.9	1/10	23.2	9/9	68.3	8/8 (2)

Abbreviations: GMB, glioblastoma multiforme.
[a] BCNU was given by single i.p. injection at a dose of 100 mg/m² in 30% ethanol at a volume of 90 ml/m².
[b] Procabazine was given by i.p. injection at a dose of 700 mg/m² in 0.9% saline at a volume of 90 ml/m² daily for 5 consecutive days.
[c] Temozolomide was given by i.p. injection at a dose of 411 mg/m² in 10% DMSO in 0.9% saline at a volume of 90 ml/m² daily for 5 consecutive days.
[d] Growth delay in days, defined as the difference between the median time for tumors in treated (T) and control (C) animals to reach five times the volume recorded at initiation of treatment.
[e] Defined as a decrease in tumor volume over two successive measurements, number of regressions/number treated. Numbers in parentheses, number of complete regressions.
[f] Abbreviations as used in Table 1.
[g] Values not statistically significant ($P < 0.01$).

Summary of Pharmacology

Temozolomide is an imidazotetrazinone similar in structure to both dacarbazine (DTIC) and mitozolomide. The putative mechanism of action is by degradation to MTIC and alkylation of DNA. TEM has both in vitro and in vivo activity against a number of tumor lines, including human CNS tumors implanted intracranially. Several of the metabolites of TEM also have activity: Tsang et al. have demonstrated activity in the TLX5 cell line of both temozolomide acid (approximately equivalent to parent drug) and MTIC (approximately twice as active as parent). Doses with activity in vitro were in the ug/mL range, while efficacy was seen in vivo at doses less than or equal to the LD10.

The activity of TEM has been shown in some models to increase when the levels of O6-alkylguanine-DNA-alkyltransferase (AGT), which removes alkyl groups from DNA (DNA repair), were diminished. This was demonstrated in several ways: greater activity in tumor lines were AGT activity was inherently low (e.g. brain tumors), and increased activity with the inhibitor of AGT, O6-benzylguanine; however, the correlation was not consistent among all tumor lines. Several papers have shown additive or synergistic activity with a variety of other chemotherapeutic agents including BCNU and cisplatin. The increase in activity of combinations of drugs with TEM may be due to irreversible inhibition (by alkylation) of AGT by TEM.

II. PHARMACOKINETICS AND TOXICOKINETICS

1. P-6059. SCH 52365: Biliary excretion and enterohepatic circulation of radioactivity following administration of a single oral dose of 14 C-SCH 52365 suspension to male rats. Kenilworth (NJ): Schering-Plough Research Institute; 1996 Nov. Vol. 1.59

Conducted: at Schering Plough Research Institute, Kenilworth, NJ in Sept 1994.
Drug used: ^{14}C-TEM, batch 31760-58-7
Vehicle: 0.4% methylcellulose for oral, 20% DMSO in saline for iv
Dose: 200 mg/m^2 (approximately 88-91 uCi/rat)
Route: comparison of intravenous vs oral gavage
Animals: adult male Sprague-Dawley rats, 5/dose; oral group: 320-354 g, iv group: 362-380 g, bile duct cannulated >1 hour pre-drug.
Sample collection: bile: 0-2, 2-4, 4-6, 6-8, 8-25, 24-48 h.
urine: 0-2, 2-4, 4-6, 6-8, 8-25, 24-48 h
feces: 0-8, 8-24, 24-48 h
Method of analysis:

Results:

The quantities of drug in bile were too low to allow for analysis of metabolites. The metabolites in the urine were not analyzed in this study. Metabolites in the feces included parent drug, temozolomide acid, and AIC, and at 48 hours, an unidentified product. The proportion of parent drug was highest between 0-8 hours and declined steady thereafter with greater quantities of the metabolites. Absolute values were not given.

Total recovery by the oral route ranged from 77% to 94%; with iv administration, total recovery range from 69% to 83%. The majority of drug was excreted in the urine, with >25% of the administered dose remaining in the carcass. Minimal differences were seen in the excretion patterns by oral and iv routes.

Table 6 Mean Total Excretion of Radioactivity After Oral or Intravenous Administration of ^{14}C-SCH 52365 to Rats.

Group	Mean Percent of Dose (%CV)											
	Bile		Urine		Feces		GI Contents		Carcass		Total	
Oral (n=5)	1.35	(41)	41.2	(45)	1.68	(100)	16.0	(116)	27.0	(14)	87.2	(7)
Intravenous (n=4)	1.57	(50)	34.4	(44)	1.07	(104)	3.27	(57)	36.0	(39)	76.3	(8)

2. P-6097. SCH 52365: Absorption, metabolism, excretion, and pharmacokinetics of 14 C-SCH 52365 following a single oral or intravenous dose in male rat. Kenilworth (NJ): Schering-Plough Research Institute; 1996 Dec. Vol. 1.60.

Conducted:
Drug used: ^{14}C-TEM, batch 31760-122-8
Vehicle: 0.4% methylcellulose for oral, 20% DMSO in saline for iv
Dose: 200 mg/m^2; group 1 and 3: single iv dose, group 2, 4, 5 oral gavage
Route: comparison of intravenous vs oral gavage
Animals: male albino rats, 68/group 1, 64/group 2, 5/group 3, 4, 16/group 5; 4 rats/ time point in groups 1 and 2. Weights were 200-255 g.
Sample collection:
Blood and plasma: 0.08, 0.25, 0.50, 0.75, 1, 1.5, 2, 3, 4, 6, 8, 12, 24, 48, 72, 120h
Urine: 0-4, 4-8, 8-24, 24-48, 48-72, 72-96, 96-120, 120-144, 144-168 h.
Feces: 0-8, 8-24, 24-48, 48-72, 72-96, 96-120, 120-144, 144-168 h.
CSF: 0.25, 1, 3, 6 h

Results:

With the exception of some "blood in the plasma" at 0-4 hours (attributed to hemolysis by DMSO vehicle), there were no remarkable signs in the treated rats. Plasma levels of both TEM and total radioactivity were followed. Parent drug was no longer detectable by 12 and 24 hours by iv and oral administration; however, metabolites were still present in plasma at 168 hours. The half life was slightly longer with oral administration. Approximately 3/4 of the total radioactivity AUC was attributable to parent drug. In comparing plasma levels of TEM to CSF levels, CSF levels of TEM were approximately 40%, and of the CSF fraction, >95% of which is parent drug at up to 6 hours. Twenty to 27% of the drug was protein bound.

The majority of drug was excreted within the first 24 hours after administration. Urinary excretion was the primary route. No significant differences were seen between iv and oral administration indicating near total bioavailability.

Route	% dose in urine	% dose in feces	total % dose recovered
oral	78.1	2.80	82.8
Intravenous	82.6	2.19	86.2

The metabolites in plasma and urine were analyzed by Although the plasma profile varied greatly with route and time, the primary form present was parent drug. During the first 4 hours, urine profile of TEM metabolites differed only slightly with iv and oral routes (percentages of each type of metabolite were similar, although the traces appeared to have more peaks with oral administration). The radioactivity recovered as parent drug in urine was 31% of the total dose by iv and 26% by po at 0-4 hours. The percentage of metabolites in urine vs. total urine levels of radioactivity are shown in the following table.

Pharmacokinetic Parameters of Radioactivity and SCH 52365 in Plasma Following Intravenous and Oral Administration of 200 mg of ^{14}C-SCH 52365/m^2 to Male Rats

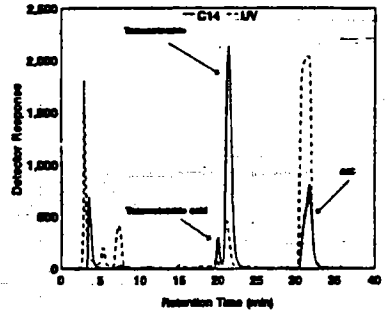
Parameter	Unit	IV	PO
		Total Radioactivity	
Cmax	µg equiv/g	42.8	38.6
Tmax[a]	hr	0.08	0.25
AUC (tf)	µg equiv-hr/g	102	120
% Absorbed	%	NA[b]	118
		SCH 52365	
Cmax	µg equiv/g	44.0	42.7
Tmax[a]	hr	0.08	0.5
AUC (tf)	µg equiv-hr/g	78.0	87.5
AUC (t)	µg equiv-hr/g	78.6	87.6
t½	hr	1.15	1.25
CL/F	mL/min	1.42	1.35
CL/F (/kg)	mL/min/kg	8.44	5.95
Vdarea/F	L	0.14	0.15
Vdarea/F (/kg)	L/kg	0.84	0.65
Bioavailability	%	NA[b]	111

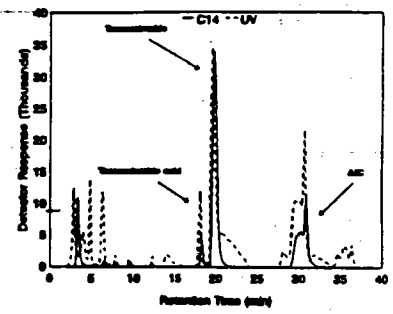
a: Occurred at first evaluated time point
b: Not applicable for this dosing form.

Table 1 Urinary Metabolite Profiles of Radioactivity After IV and PO Administration to Male Rats.

Time (hr)	M1	M2	TMA	TMZ	AIC
	Intravenous: Percent of Total ^{14}C Analyzed				
	12	1	3	60	24
	25	1	4	39	11
	69	6[a]	2	16	16
	83	2	1	3	4
	91	1	0	1	0
	93	0	0	0	0
	Oral: Percent of Total ^{14}C Analyzed				
	10	1	5	64	7
	27	1	4	38	23
	51	1	2	18	17
	82	1	1	4	2
	89	2	0	1	0
	89	0	0	0	0

a: Radioactivity less than 1 percent of the run was reported as zero.





3. P-6371. SCH 52365: Tissue distribution of radioactivity by whole body autoradiography following a single oral administration of 14 C-SCH 52365 suspension to male rats. Kenilworth (NJ): Schering-Plough Research Institute; 1996 Dec. vol. 1.60

Conducted: Schering Plough Research Institute, Kenilworth NJ, March 1996.
Drug used: 14C-TEM, batch 31760-58-7
Vehicle: 0.4% methylcellulose
Dose: approximately 160 mg/m^2
Route: oral gavage
Animals: male Sprague Dawley rats, 12 weeks old, 116-140 g; 2/ time point
Sample collection: 0.25, 1, 3, 6, 24, 168 hours post-dose
Method of analysis:

Results:

Although the reproductions were of relatively poor quality, it was obvious that the radioactivity was widespread including the brain by 15 minutes after administration. At 6 hours, radioactivity was seen primarily in the spleen, liver, thymus, kidney, bone marrow and intestinal wall. At 24 hours, radioactivity was till present in the gi tract, liver, kidney, and testes and had faded still further in these organs by 168 hours.

4. P-5949. SCH 52365: Absorption, distribution, and metabolism of 14 C-SCH 52365 following a single oral dose in the male rat. Kenilworth (NJ): Schering-Plough Research Institute; 1996 Dec. vol. 1.61

Conducted:
Drug used: 14C-TEM, batch 31760-58-7; "cold" drug: batch # 28395-129
Vehicle: 0.4% methylcellulose
Dose: 200 mg/m^2
Route: oral gavage
Animals: male Crl:(LE)BR: Long-Evans rats,5 rats/timepoint, Weights were 200-229 g.
Sample collection: 0.25, 2, 6, 12, 24, 72, 168 h: organs, blood/plasma
0-4, 4-8, 8-24, 24-48, 48-72, 72-96, 96-120, 120-144, 144-168 h: urine
0-8, 8-24, 24-48, 48-72, 72-96, 96-120, 120-144, 144-168 h: feces
Method of analysis:

Results:

At 15 minutes, the majority of the radioactivity is in the stomach and small intestine. With time, radiolabel is distributed primarily to the kidneys, liver, marrow and gi tract. Brain levels of radiolabel are higher than those in the plasma from 6 to 168 hours (3 fold higher at 6 hours to 35

fold at 168 hours, calculated half-life 122 hours although the "eyeball method" suggests a half-life closer to 70 hours). Significant and persistent quantities of radiolabel were also found in the adrenal glands, Harderian gland, thyroid, eyes, muscle, and testes. When the radioactivity is calculated as the percentage of the dose, the majority is found in the carcass. Even at 168 hours, approximately 15% of the dose is still in the animals. Between 0.10 and 0.23% of the total dose is present in the brain during the interval of observation.

Similar pharmacokinetics and excretion profiles to those in the previous experiments were obtained in this investigation.

Mean Concentrations of Radioactivity in Plasma and Tissues Following Oral Administration of a 200 mg ^{14}C-SCH 52365/m^2 Dose to Male Rats

Tissue	Mean µg Equivalents ^{14}C-SCH 52365/g[a]						
	0.25 hr	2.0 hr	6.0 hr	12.0 hr	24.0 hr	72.0 hr	168.0 hr
Adrenal glands	22.0	31.8	33.1	32.3	28.8	21.2	12.7
Bone marrow (femur)	21.8	28.3	25.8	23.8	18.8	6.09	1.91
Brain	5.29	8.29	6.02	5.75	5.44	4.35	3.40
Carcass (residual)	17.8	19.3	9.85	7.54	6.67	5.01	3.05
Eyes	14.1	21.9	14.5	10.4	10.2	8.82	6.65
Fat (reproductive)	3.85	4.48	4.17	3.85	3.71	2.81	1.36
Harderian glands	17.8	23.1	18.7	17.8	17.9	14.8	8.76
Heart	18.8	17.8	12.8	10.2	8.73	6.88	4.72
Kidneys	31.0	61.5	60.7	56.8	53.1	38.8	22.1
Large intestine with contents	18.4	32.8	31.8	12.1	6.88	2.88	1.16
Liver	31.2	40.8	60.3	38.3	34.4	28.3	13.8
Lungs	20.4	33.8	38.4	38.3	38.8	23.9	13.8
Lymph nodes (mesenteric)	18.7	22.4	31.8	34.6	28.8	17.8	8.23
Muscle (thigh)	18.6	17.2	8.70	4.84	4.04	3.34	2.30
Pancreas	17.1	16.1	13.9	11.4	12.8	8.03	6.31
Plasma	31.8	22.8	2.82	0.488	0.298	0.127	0.016
Skin (pigmented area/dorsal/shaved)	20.3	22.8	12.8	9.47	6.13	7.07	1.32
Small intestine with contents	64.9	53.1	31.8	19.8	18.8	8.16	2.38
Spleen	18.8	28.5	38.9	33.8	32.7	34.8	11.0
Stomach with contents	700	88.7	11.8	3.88	4.14	2.73	2.00
Submaxillary glands	18.9	28.8	38.8	34.2	28.1	18.4	11.5
Testes	7.28	30.8	11.4	8.38	8.38	7.88	5.88
Thymus	18.0	28.8	27.9	28.2	34.8	17.8	4.84
Thyroid	17.1	23.2	27.1	21.4	18.6	16.1	10.3

a Mean concentrations determined from 5 rats per time point (Groups 1 and 2).

Pharmacokinetic Parameters[a] of Total Radioactivity Following Oral Administration of a 200 mg ^{14}C-SCH 52365/m^2 Dose to Male Rats

Tissue	C_{max} (µg equivalents/g)	T_{max} (hr)	β (hr^{-1})	$t_{1/2}$ (hr)[b]	$AUC_{(tf)}$ (µg equivalents·hr/g)	$AUC_{(I)}$ (µg equivalents·hr/g)
Plasma	31.8	0.25	0.0188	36.8	136	136
Adrenal glands	33.1	6	0.0059	117	3,880	5,740
Bone marrow (femur)	28.3	2	0.0186	37.2	1,870	1,870
Brain	8.29	2	0.0031	221	748	1,870
Carcass (residual)	19.3	2	0.0051	136	880	1,400
Eyes	21.9	2	0.0025	276	1,630	4,280
Fat (reproductive)	4.48	2	0.0051	136	470	882
Harderian glands	23.1	2	0.0046	153	2,410	4,380
Heart	18.8	0.25	0.0047	148	1,248	2,240
Kidneys	61.5	2	0.0063	111	6,380	8,840
Large intestine with contents	32.8	2	0.0084	82.8	848	882
Liver	60.3	6	0.0072	96.1	4,570	6,180
Lungs	38.1	24	0.0050	139	4,880	6,480
Lymph nodes (mesenteric)	31.8	6	0.0077	90.4	2,830	3,880
Muscle (thigh)	18.6	0.25	0.0037	186	888	1,330
Pancreas	17.1	0.25	0.0043	166	1,800	2,880
Skin (pigmented area/dorsal/shaved)	22.8	2	0.0110	63.8	1,080	1,170
Small intestine with contents	64.9	0.25	0.0095	73.3	1,880	1,740
Spleen	38.9	6	0.0076	92.8	3,880	5,380
Stomach with contents	700	0.25	0.0080	117	1,480	1,830
Submaxillary glands	38.8	2	0.0053	131	3,190	5,380
Testes	30.8	2	0.0030	230	1,340	3,380
Thymus	27.9	6	0.0115	60.2	2,880	3,010
Thyroid	27.1	6	0.0051	136	2,880	4,880

a Pharmacokinetic parameters determined from mean plasma concentration data of 5 rats per time point (Groups 1 and 2).
b The radioactivity half-life values for individual tissues were calculated for the sole purpose of determining potential radioactivity exposure in humans. The tissue half-life values in this table should be interpreted with caution.

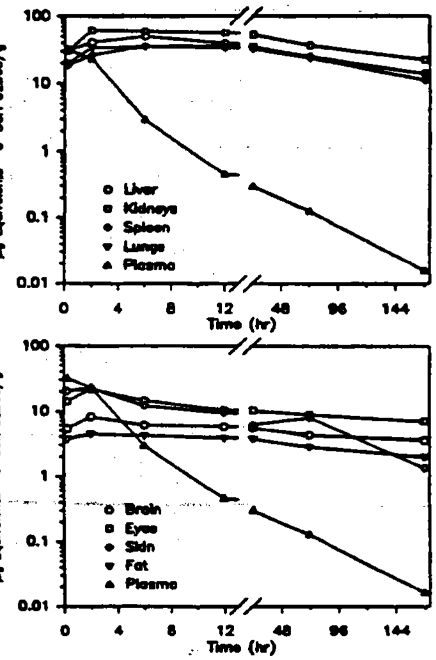
C_{max} µg equivalents/g maximum concentration
T_{max} hr time of maximum concentration
β hr^{-1} terminal phase elimination rate constant
$t_{1/2}$ hr terminal phase half-life
$AUC_{(tf)}$ µg equivalents·hr/g area under the plasma or tissue concentration-time curve from time zero to the last measurable time point
$AUC_{(I)}$ µg equivalents·hr/g area under the plasma or tissue concentration-time curve from time zero to infinity.

Mean Amounts of Radioactivity in Plasma and Tissues Following Oral Administration of a 200 mg ^{14}C-SCH 52365/m^2 Dose to Male Rats

Tissue	Mean Percent of Radioactive Dose[a]						
	0.25 hr	2.0 hr	6.0 hr	12.0 hr	24.0 hr	72.0 hr	168.0 hr
Adrenal glands	0.01	0.02	0.03	0.02	0.02	0.02	<0.01
Bone marrow (femur)	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Brain	0.15	0.23	0.17	0.16	0.16	0.12	0.10
Carcass (residual)	40.9	53.4	26.3	20.8	18.7	14.8	9.88
Eyes	0.06	0.09	0.05	0.04	0.04	0.03	0.03
Fat (reproductive)	0.07	0.08	0.08	0.08	0.07	0.06	0.05
Harderian glands	0.06	0.08	0.07	0.08	0.08	0.07	0.05
Heart	0.26	0.23	0.15	0.12	0.12	0.10	0.08
Kidneys	0.83	1.88	1.54	1.38	1.38	0.98	0.72
Large intestine with contents	1.03	1.98	2.91	1.92	1.88	0.51	0.21
Liver	3.86	4.35	6.10	6.15	5.83	4.80	2.82
Lungs	0.37	0.58	0.54	0.58	0.57	0.42	0.28
Lymph nodes (mesenteric)	0.02	0.05	0.03	0.03	0.04	0.03	<0.01
Muscle (thigh)	0.33	0.44	0.17	0.13	0.13	0.11	0.08
Pancreas	0.21	0.14	0.17	0.17	0.20	0.15	0.11
Plasma	1.75	1.12	0.14	0.02	0.02	<0.01	<0.01
Skin (pigmented area/dorsal/shaved)	0.29	0.88	0.36	0.28	0.17	0.31	0.05
Small intestine with contents	6.06	4.43	3.77	2.88	2.13	0.70	0.36
Spleen	0.15	0.22	0.27	0.28	0.22	0.19	0.09
Stomach with contents	20.3	2.47	1.37	0.80	0.40	0.27	0.15
Submaxillary glands	0.12	0.18	0.17	0.16	0.18	0.14	0.09
Testes	0.36	0.89	0.47	0.41	0.38	0.36	0.29
Thymus	0.14	0.18	0.21	0.13	0.13	0.07	0.02
Thyroid	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Total	91.0	73.4	46.8	38.4	31.7	23.9	15.2

a Mean percent of radioactive dose determined from data of 5 rats per time point (Groups 1 and 2).

Mean Concentration of Radioactivity in Representative Tissues Following Oral Administration of a 200 mg ^{14}C-SCH 52365/m^2 Dose to Male Rats (Groups 1 and 2)



5. P-6263. SCH 52365: Tissue distribution of 14 C-SCH 52365 following a single oral dose in the female pigmented rat. Kenilworth (NJ): Schering-Plough Research Institute; 1996 Dec. vol. 1.62.

Conducted:

Drug used: 14C-TEM, batch 31760-58-7; "cold" drug: batch # 28395-129

Vehicle: 0.4% methylcellulose

Dose: 200 mg/m^2

Route: oral gavage

Animals: female Crl:(LE)BR: Long-Evans rats,5 rats/timepoint, 183-207 g.

Sample collection: 0.25, 2, 6, 12, 24, 72, 168 h: organs, blood/plasma

0-4, 4-8, 8-24, 24-48, 48-72, 72-96, 96-120, 120-144, 144-168 h: urine

0-8, 8-24, 24-48, 48-72, 72-96, 96-120, 120-144, 144-168 h: feces

Method of analysis:

Results:

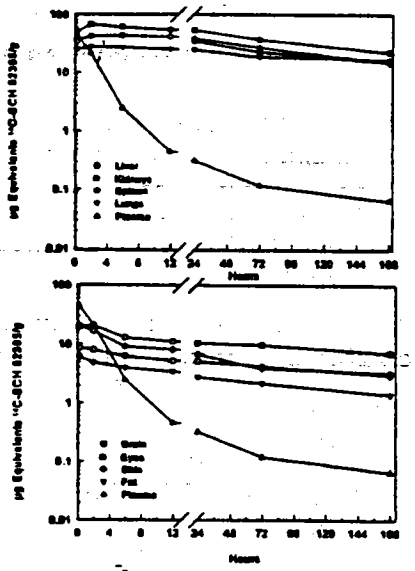
Within 15 minutes, high levels of label were found in the upper gi tract, bone marrow, and kidneys, with lesser amounts in the liver, thymus, lymph nodes, adrenals, heart, ovaries, and spleen. Levels in the brain were higher than those in plasma by 6 hours and ranged from approximately 3 to 40 fold between 6 and 168 hours. The majority of the total dose was found in the carcass, with high levels remaining in the kidney, and liver even at 168 hours. Percentage of dose in the brain ranged from 0.48 to 0.16% of the total radioactivity and a rough approximation of the half-life is 70 hours. Excretion profiles were a total of 2.85% of the dose in feces, 76.5% in urine and a total of 84% of the dose recovered by 168 hours. The largest percentage of label was excreted in the urine between 4 and 8 hours post dose.

Mean Concentrations of SCH 52365 and Radioactivity in Plasma Following Oral Administration of a 200 mg ^{14}C-SCH 52365/m^2 Dose to Female Rats

Time (hr)	Mean SCH 52365 Concentration in Plasma (µg/mL)[a,b]	Mean Radioactivity Concentration in Plasma (µg equiv/mL)[b,c]
0.25	40.9	45.9
2.0	16.6	22.7
6.0	1.49	2.63
12.0	ND	0.481
24.0	ND	0.335
72.0	ND	0.126
168.0	ND	0.088
Cmax	40.9 µg/mL	45.9 µg equiv/mL
Tmax	0.25 hr	0.25 hr
AUC(tf)	91.6 µg-hr/mL	151 µg equiv-hr/mL

a: Concentration of 12- through 168-hr samples were 0 µg/mL.
b: Mean concentrations determined from 5 rats per time point.
c: Before pharmacokinetic parameters were calculated, plasma concentrations in µg equiv/mL were converted to µg equiv/mL by multiplying by the plasma density of 1 mL = 1.033 g.

Figure 2. Mean Concentration of Radioactivity in Representative Tissues Following Oral Administration of a 200 mg ^{14}C-SCH 52365/m^2 Dose to Female Rats (Groups 1 and 2).



Mean (n=5) Concentrations of Radioactivity in Tissues and Residual Carcass Following Oral Administration of a 200 mg ^{14}C-SCH 52365/m^2 Dose to Female Rats

Tissue	Mean µg Equivalents/g*						
	0.25 hr	2.0 hr	6.0 hr	12.0 hr	24.0 hr	72.0 hr	168.0 hr
Adrenal glands	27.5	32.5	28.5	27.8	24.2	17.8	12.7
Bone marrow (femur)	35.5	33.4	28.4	25.3	19.3	9.89	3.90
Brain	8.83	8.17	6.35	6.33	5.21	4.33	3.80
Carcass (residual)	25.3	18.2	9.09	6.87	5.27	4.85	3.17
Eyes	19.8	21.3	13.1	11.3	10.6	8.86	7.28
Fat (reproductive)	6.18	4.86	4.03	3.48	2.81	2.20	1.42
Harderian glands	26.8	23.1	19.2	17.8	16.7	15.5	11.8
Heart	32.7	20.8	11.1	8.41	6.91	5.97	5.75
Kidneys	96.6	89.5	91.8	86.1	84.4	37.7	22.7
Large intestine	24.8	24.0	25.2	18.3	14.4	10.7	8.28
Large intestine contents	2.03	1.85	5.84	3.75	0.884	0.337	0.099
Liver	37.2	43.1	46.0	42.8	36.0	23.0	15.1
Lungs	25.4	28.1	27.4	25.4	25.2	16.7	16.7
Lymph nodes	30.0	33.1	28.3	28.7	27.8	19.1	11.1
Muscle (thigh)	25.0	16.8	8.57	3.90	3.80	2.77	2.23
Ovaries	29.5	27.1	23.1	22.1	26.3	12.8	7.39
Pancreas	29.5	21.7	16.1	15.3	14.5	14.0	10.3
Skin (dorsal, pigmented, shaved)	20.8	17.0	8.33	8.27	6.94	3.88	3.31
Small intestine	36.6	33.4	32.6	28.8	17.8	7.17	4.34
Small intestine contents	28.0	8.95	8.12	5.84	2.53	0.448	0.160
Spleen	34.5	43.2	44.8	42.3	38.1	27.4	14.9
Stomach	62.0	24.5	20.6	17.1	16.7	12.4	8.84
Stomach contents	21.4	1.23	1.78	1.12	0.114	0.084	0.021
Submaxillary glands	29.2	32.1	28.1	28.0	25.2	16.4	13.5
Thymus	30.0	24.2	20.4	25.1	21.5	15.4	8.16
Thyroid	30.0	27.3	26.6	24.8	23.2	13.6	11.8
Uterus	28.8	23.0	19.7	13.5	15.5	11.6	4.31
Blood	45.9	32.7	3.63	0.49	0.24	0.13	0.07

* Mean concentrations determined from 5 rats per time point (Groups 1 and 2).

Mean (n=5) Amounts of Radioactivity in Plasma, Tissues, and Residual Carcass Following Oral Administration of a 200 mg ^{14}C-SCH 52365/m^2 Dose to Female Rats

Tissue	Mean Percent of Radioactive Dose*						
	0.25 hr	2.0 hr	6.0 hr	12.0 hr	24.0 hr	72.0 hr	168.0 hr
Adrenal glands	0.03	0.04	0.03	0.03	0.03	0.02	0.02
Bone marrow (femur)	0.02	0.02	0.01	0.01	0.01	<0.005	<0.005
Brain	0.40	0.46	0.36	0.31	0.28	0.24	0.16
Carcass (residual)	75.7	53.1	27.0	20.5	17.8	14.1	8.83
Eyes	0.05	0.09	0.05	0.04	0.04	0.03	0.03
Fat (reproductive)	0.31	0.30	0.23	0.20	0.17	0.13	0.09
Harderian glands	0.09	0.10	0.06	0.07	0.05	0.05	0.05
Heart	0.20	0.23	0.13	0.10	0.11	0.07	0.06
Kidneys	2.79	3.72	3.41	3.13	2.67	2.08	1.22
Large intestine	1.38	1.91	1.91	1.37	1.09	0.72	0.34
Large intestine contents	0.79	1.22	1.97	1.84	0.54	0.16	0.06
Liver	7.89	8.62	9.10	8.89	6.27	5.08	3.71
Lungs	1.19	1.16	1.17	1.09	1.03	0.62	0.79
Lymph nodes (mesenteric)	0.12	0.10	0.12	0.09	0.07	0.06	0.02
Muscle (thigh)	1.99	2.05	0.43	0.33	0.41	0.23	0.25
Ovaries	0.07	0.06	0.06	0.05	0.04	0.03	0.02
Pancreas	0.43	0.34	0.21	0.18	0.20	0.24	0.11
Pituitary	1.71	1.02	0.10	0.02	0.01	0.01	<0.005
Skin (dorsal, shaved)	1.38	1.27	0.73	0.76	0.53	0.39	0.33
Small intestine	3.81	3.45	3.51	3.86	1.39	0.93	0.47
Small intestine contents	0.57	2.71	1.89	1.44	0.07	0.15	0.07
Spleen	0.34	0.27	0.28	0.27	0.23	0.16	0.09
Stomach	4.08	1.13	0.84	0.80	0.73	0.60	0.39
Stomach contents	4.74	0.38	0.83	0.27	0.05	0.02	<0.005
Submaxillary glands	0.16	0.17	0.10	0.10	0.14	0.12	0.09
Thymus	0.21	0.13	0.17	0.15	0.12	0.08	0.02
Thyroid	0.01	0.01	0.01	0.01	0.01	<0.005	<0.005
Uterus	0.19	0.14	0.12	0.08	0.07	0.07	0.02
Total	115	83.8	54.6	46.0	37.4	27.2	18.2

* Mean percent of radioactive dose determined from data of 5 rats per time point (Groups 1 and 2).

6. P-6098. SCH 52365: Absorption, metabolism, excretion, and pharmacokinetics of 14 C-SCH 52365 following a single oral or intravenous dose in the male dog. Kenilworth (NJ): Schering-Plough Research Institute; 1997 Jan. Vol. 1.63

Conducted:

GLP: Yes

Drug used: 14C-TEM, batch 31760-122-8; "cold" drug: batch # 28395-129

Vehicle: 0.4% methylcellulose for oral; 20% DMSO in 0.9% NaCl for iv

Dose: 200 mg/m^2

Route: oral gavage or intravenous

Animals: Male beagle dogs, 4 dogs/group, 9-12 months old, 11.3-13.2 kg.

Study design: po/iv crossover after 1 week: G1: iv then po, G2: po then iv; G3: po w/ CSF collection

Sample collection:

0.08, 0.25, 0.5, 0.75, 1, 1.5, 2, 3, 4, 6, 8, 12, 24, 48 72, 120 h: blood/plasma

0-4, 4-8, 8-24, 24-48, 48-72, 72-96, 96-120, 120-144, 144-168 h: urine

0-8, 8-24, 24-48, 48-72, 72-96, 96-120, 120-144, 144-168 h: feces

0.25, 1, 3, 6 h: CSF, blood

Method of analysis:

Results:

All dogs vomited between 1.5 and 6 hours of dosing. Soft/reduced feces were also observed in treated dogs. There was no remarkable differences in the AUC between oral and intravenous administration of TEM in dogs (bioavailability calculated at 110%). While total radioactivity in the CSF was 54% of that in plasma, parent drug only accounted for 31% of the drug as compared to plasma. Protein binding is shown in the following table.

Total recovery of radiolabel was lower than that in the rat (65-69% of dose recovered). Again, the majority of label was excreted in the urine, with 2-3% in the feces. Excretion in urine was maximal in the first 24 hours.

Pharmacokinetic Parameters of Radioactivity and SCH 52365 in Plasma Following Intravenous and Oral Administration of 200 mg of ^{14}C-SCH 52365/m^2 to Male Dogs

Parameter	Unit	IV	PO
		Total Radioactivity	
Cmax	µg equiv/g	22.0	18.0
Tmax[a]	hr	0.08	0.38
AUC (t/)	µg equiv-hr/g	74.7	73.6
% Absorbed	%	–	101
		SCH 52365	
Cmax	µg/mL	23.0	17.7
Tmax[a]	hr	0.10	0.41
AUC (t/)	µg-hr/mL	36.3	38.5
AUC (I)	µg-hr/mL	37.1	39.7
t½	hr	1.43	1.65
CL/F	mL/min	54.3	49.8
CL/F (/kg)	mL/min/kg	4.47	4.12
Vdarea/F	L	6.75	7.07
Vdarea/F (/kg)	L/kg	0.56	0.59
Bioavailability	%	–	110

a: First evaluated time point.

All pharmacokinetic parameters are based on mean concentration-time data of 8 dogs in a two way cross-over design.

Mean Concentration of Radioactivity and SCH 52365 in Plasma and CSF and Pharmacokinetic Parameters Following Oral Administration of 200 mg ^{14}C-SCH 52365/m^2 Dose to Male Dogs

Hr	Mean Concentration			
	Total Radioactivity[b]		SCH 52365[c]	
	Plasma[d]	CSF[e]	Plasma	CSF
0.25[f]	4.12	0.346	5.08	0.36
1	8.84	2.86	15.1	2.75
3	9.13	6.02	11.1	3.46
6	4.53	3.04	4.28	1.48

Parameter	Unit			Unit		
Cmax	µg equiv/g	10.9[g]	6.02[g]	µg/mL	11.8[f,g]	3.58[g]
Tmax	hr	2.0	3.0	hr	2.3[g]	2.0
AUC (t/)	µg equiv-hr/g	43.8	23.8	µg-hr/mL	47.2[g]	14.8
CSF/Plasma	%	–	54.3	%	–	31.4

a: First evaluated timepoint.

b: Units of total radioactivity (µg equiv/g).

c: Units of SCH 52365 (µg/mL).

d: Plasma data are a mean from 4 dogs per timepoint in Group 3.

e: CSF samples are a mean from 4 dogs per timepoint in Group 3

f: Mean of individual Cmax values.

g: Mean of 3 dogs only. Dog No. H06529 appeared to be an outlier and was excluded from the mean.

Mean Percent of Drug-Derived Radioactivity Bound to Dog Plasma Proteins Following Oral Administration of 200 mg ^{14}C-SCH 52365/m^2 Dose to Male Dogs

Hr	Mean Percent Bound (%CV)
0.25	45.9 (8.34)[a]
1	31.8 (12.4)[b]
3	24.2 (21.3)[b]
6	14.0 (26.5)[b]

a: n=3.

b: n=4.

Mean Recovery of Radioactive Dose (%CV)

Matrix	IV[a]	(%CV)	PO[a]	(%CV)
Plasma	0.22	8.06	0.23	2.57
Cage wash/wipe	0.46	34.3	0.96	63.1
Cage rinse	2.83	28.7	3.80	30.9
Emesis	0.29	61.1	1.74	54.8
Feces	2.35	28.5	2.57	48.0
Urine	58.5	3.70	60.5	6.56
Total	64.6	4.26	69.8	3.03

a: Mean Data from 4 dogs in Group 1, Phase 1.

b: Mean Data from 4 dogs in Group 2, Phase 1.

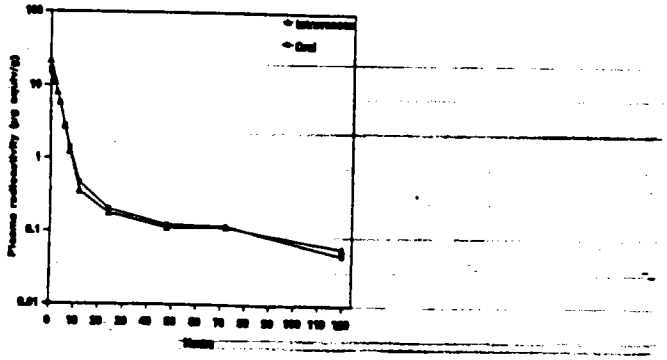
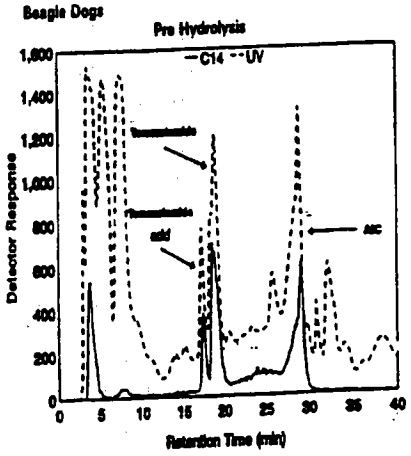


FIGURE 2. Mean Concentration of Radioactivity in Plasma Following Intravenous and Oral Administration of 200 mg ^{14}C-SCH 52365/m^2 to Male Dogs.

Table 2 Urinary Metabolite Profiles of Radioactivity After IV and PO Administration of ^{14}C-SCH 52365 to Male Dogs					
	Intravenous: Percent of Total ^{14}C Analyzed				
Time (hr)	M1[a]	M2[b]	TMA	TMZ	AIC
	15	1	7	25	50
	50	2	7	9	33
	52	1	4	3	23
	97	0	0	0	1
	96	0	0	0	2
	99	1	0	0	0
	Oral: Percent of Total ^{14}C Analyzed				
	18	1	9	32	34
	37	2	6	12	8
	39	5	0	5	27
	80	3	0	0	6
	96	1	0	0	0
	37	0	0	0	0

a: Retention time = 3.6-3.8 min
b: Retention time = 6.8-7.0 min

Figure 22. Metabolite Profile of Drug-Derived Radioactivity in Urine (0-4 hr) Following Intravenous Administration of ^{14}C-SCH 52365 Solution to Beagle Dogs



7. P-6072. SCH 52365: Single-dose two-way crossover comparative bioavailability study of formulated versus unformulated SCH 52365 capsules in dogs. Kenilworth (NJ): Schering-Plough Research Institute; 1996 Dec. vol. 1.64.

The AUC_{0-24} for the two formulations were not statistically different (18.3 vs 17.9 ug•hr/mL).

8. P-6478. SCH 52365: Pharmacokinetics of SCH 52365 and its metabolite MTIC following a single oral suspension administration of SCH 52365 to male and female rats. Kenilworth (NJ): Schering-Plough Research Institute; 1998 Mar. vol. 1.65

Conducted: Schering Plough Research Institute, Kenilworth, NJ in Nov. 1996.
Drug used: batch # 36438-023
Vehicle: 0.4% methylcellulose
Dose: 200 mg/m^2 (actual dose 255 mg/m^2)
Route: oral gavage
Animals: Sprague Dawley rats, 36/sex (3/timepoint), M: 231 g, F: 193 g
Sample collection: 0.25, 0.5, 0.75, 1, 1.5, 2, 3, 4, 6, 8, 12, 24 h: blood/plasma
Method of analysis:

Results:

The plasma kinetics of both TEM and MTIC are shown in the following tables and graphs. Both MTIC and TEM have similar Tmax and half lives and kinetics were similar in both genders. MTIC AUC levels are approximately 2% of the parent drug levels. Urinary MTIC was not measured.

Table 2 Pooled Plasma Concentrations and Pharmacokinetic Parameters of SCH 52365 Following A Single Oral Suspension Administration of SCH 52365 to Male and Female Rats.

Hour	Unit	SCH 52365 Plasma Concentration[a] (µg/mL)	
		Male	Female
Cmax	µg/mL	21.5	31.4
Tmax	hr	0.75	0.25
t1/2	hr	1.17	1.22
tf	hr	8	8
AUC(tf)	µg·hr/mL	54.4	55.7
AUC(I)	µg·hr/mL	55.2	56.4

a: n=3 rats/gender/time point

Table 3 Pooled Plasma Concentrations and Pharmacokinetic Parameters of MTIC Following A Single Oral Suspension Administration of SCH 52365 to Male and Female Rats.

Hour	Unit	MTIC Plasma Concentration[a] (µg/mL)	
		Male	Female
Cmax	µg/mL	0.329	0.385
Tmax	hr	0.50	1.00
t1/2	hr	1.64	1.35
tf	hr	8	6
AUC(tf)	µg·hr/mL	1.00	1.11
AUC(I)	µg·hr/mL	1.04	1.17
Ratio[b]	%	1.88	2.07

a: n=3 rats/gender/timepoint
b: $AUC(I)_{MTIC}/AUC(I)_{SCH\ 52365}$

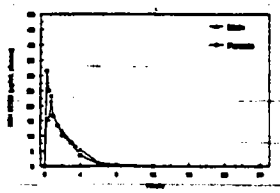


Figure 1 Pooled Plasma Concentrations of SCH 52365 Following A Single Oral Suspension Administration of SCH 52365 to Male and Female Rats.

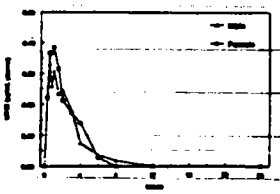


Figure 2 Pooled Plasma Concentrations of MTIC Following A Single Oral Suspension Administration of SCH 52365 to Male and Female Rats.

9. P-6468. SCH 52365: Pharmacokinetics of SCH 52365 and its metabolite MTIC following a single oral suspension administration of SCH 52365 to male and female beagle dogs. Kenilworth (NJ): Schering-Plough Research Institute; 1998 Mar. vol 1.66.

Conducted: Schering Plough Research Institute, Kenilworth, NJ in Nov. 1996.
Drug used: batch # 36438-023
Vehicle: 0.4% methylcellulose
Dose: 200 mg/m^2 (actual dose 249-250 mg/m^2)
Route: oral gavage
Animals: Beagle dogs, (3/sex), 2 years old, 11.2-12.9 kg
Sample collection: 0.25, 0.5, 0.75, 1, 1.5, 2, 3, 4, 6, 8, 12, 24 h: blood/plasma
Method of analysis:

Results:

All dogs vomited between 1 and 3 hours post-dose. Minimal differences were noted between gender in kinetic parameters. Both MTIC and TEM reached their Tmax within 1 hour; both had half-lives of about 1.5 hours. Details are shown in the following tables and graphs. Urinary MTIC was not measured.

Table 2 Mean Plasma Concentrations and Pharmacokinetic Parameters of SCH 52365 Following a Single Oral Suspension Administration of SCH 52365 to Beagle Dogs.

		SCH 52365 (µg/mL plasma)									
		Males					Females				
Hours	Units	Dog 1	Dog 2	Dog 3	Mean	%CV	Dog 4	Dog 5	Dog 6	Mean	%CV
0		0	0	0	0	ND	0	0	0	0	ND
0.25		12.0	10.2	3.53	8.58	52	7.60	7.06	8.40	7.69	9
0.5		7.39	10.6	4.02	7.34	45	6.24	7.33	10.2	7.92	26
0.75		6.46	9.57	4.69	6.91	36	6.08	7.70	10.1	7.96	25
1		5.91	8.32	5.16	6.46	26	6.84	5.17	6.55	6.19	14
1.5		4.20	6.95	4.66	5.27	28	6.38	3.78	6.99	5.72	30
2		3.60	5.8	3.79	4.40	28	5.03	2.82	3.57	3.64	28
3		2.21	2.95	2.31	2.49	16	1.89	1.43	2.30	1.84	27
4		1.24	2.04	1.54	1.61	25	1.71	1.76	2.38	1.95	19
6		0.656	0.836	0.619	0.704	16	0.811	0.759	0.728	0.766	5
8		0.243	0.366	0.281	0.297	21	0.228	0.239	0.277	0.248	10
12		0	0	0	0	ND	0	0	0	0	ND
24		0	0	0	0	ND	0	0	0	0	ND
Cmax	µg/mL	12.0	10.6	5.16	9.25	39	7.60	7.70	10.2	8.50	17
Tmax	hr	0.25	0.50	1.0	0.58	66	0.25	0.75	0.50	0.50	50
t1/2	hr	1.59	1.55	1.60	1.58	2	1.68	1.77	1.60	1.68	5
tf	hr	8	8	8	8	0	8	8	8	8	0
AUC(tf)	µg hr/mL	19.1	26.6	16.3	20.7	26	20.6	17.4	23.5	20.5	15
AUC(I)	µg hr/mL	19.7	27.4	16.8	21.3	25	21.3	18.1	24.2	21.2	14

ND = Not determined

Table 3 Mean Plasma Concentrations and Pharmacokinetic Parameters of MTIC Following a Single Oral Suspension Administration of SCH 52365 to Beagle Dogs.

		Plasma MTIC (ng/mL)									
		Males					Females				
Hours	Units	Dog 1	Dog 2	Dog 3	Mean	%CV	Dog 4	Dog 5	Dog 6	Mean	%CV
0		0	0	0	0	ND	0	0	0	0	ND
0.25		217	122	116	152	37	288	126	196	203	40
0.5		216	159	138	171	23	301	147	221	223	35
0.75		193	147	188	176	14	226	139	217	194	25
1		181	129	170	160	17	240	127	209	192	30
1.5		136	118	143	132	10	195	124	130	150	26
2		109	99.5	118	109	9	146	102	116	121	19
3		74.1	80.2	86.7	80.3	8	95.6	66.5	78.5	80.2	18
4		39.9	39.5	59.6	46.3	25	67.2	44.3	50.4	54.0	18
6		16.4	20.2	23.9	20.2	19	25.3	18.2	21.6	21.7	16
8		0	0	0	0	ND	0	0	10.3	3.43	173
12		0	0	0	0	ND	0	0	0	0	ND
24		0	0	0	0	ND	0	0	0	0	ND
Cmax	ng/mL	217	159	188	188	16	301	147	221	223	35
Tmax	hr	0.25	0.50	0.75	0.50	50	0.50	0.50	0.50	0.50	0
t1/2	hr	1.40	1.71	1.60	1.57	10	1.59	1.60	1.64	1.61	[illegible]
tf	hr	6	6	6	6	0	6	6	8	6.7	17
AUC(tf)	ng·hr/mL	525	449	533	502	9	707	440	610	586	23
AUC(I)	ng·hr/mL	557	496	586	546	8	764	482	634	627	22
Ratio[a]	%	2.83	1.82	3.49	2.71	31	3.09	2.96	2.82	2.96	10

a: AUC(I)mtic/AUC(I)msz
ND = Not determined

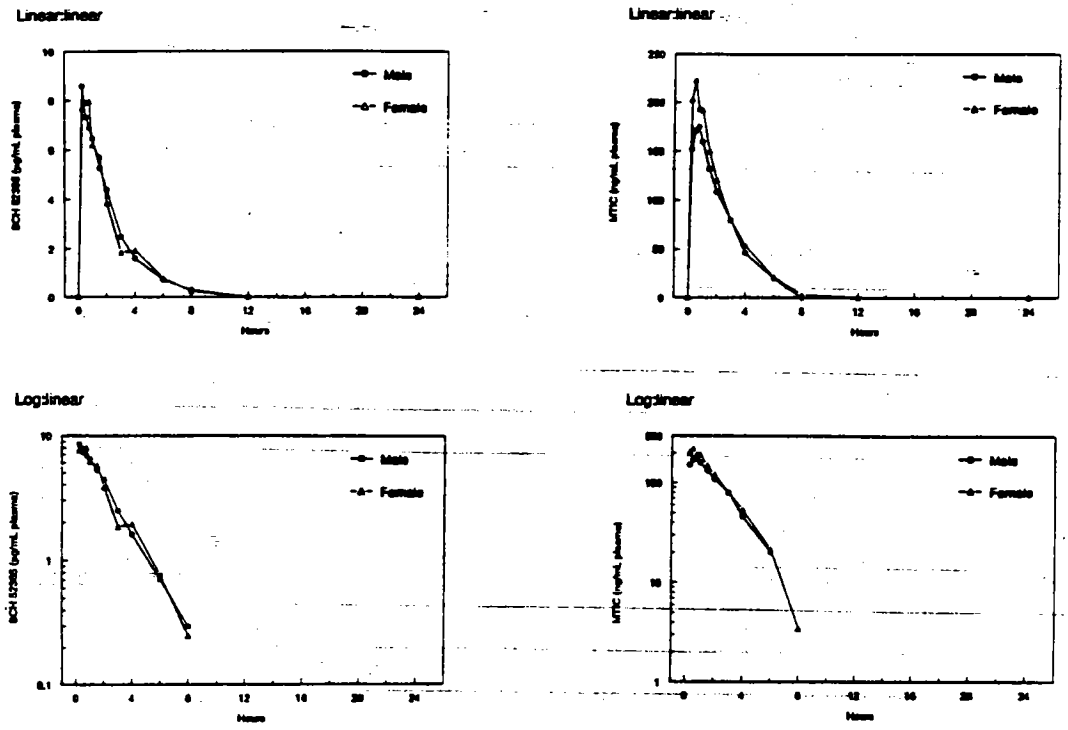


Figure 1 Mean Plasma Concentrations of SCH 52365 Following a Single Oral Administration of SCH 52365 Suspension to Male and Female Dogs.

Figure 2 Mean Plasma Concentrations of MTIC Following a Single Oral Administration of SCH 52365 Suspension to Male and Female Dogs.

10. D-26672. SCH 52365: Stability of temozolomide and MTIC (active metabolite), and HPLC quantitation of MTIC in human plasma. Kenilworth (NJ): Schering-Plough Research Institute; 1993 Jun.

By [redacted] at room temperature, TEM was stable for about 15 minutes, while MTIC was stable for approximately 30 minutes. TEM degraded to trace amounts of MTIC and a majority of AIC. MTIC degraded to AIC. At 4°C, TEM was stable for 30 minutes and MTIC for 1 hour. TEM was more stable under acidic conditions where MTIC rapidly degraded, while MTIC was more stable under basic conditions, where TEM degraded.

11. Tsang LLH, Farmer PB, Gescher A, Slack JA. Characterization of urinary metabolites of temozolomide in humans and mice and evaluation of their cytotoxicity. Cancer Chemother. Pharmacol. 1990;26:429-436. Vol. 1.67.

Drug used: 14C-TEM from [redacted]
Vehicle: 10% DMSO in saline
Dose: 40 mg/kg (120 mg/m^2—noted as being non-toxic, but efficacious)
Route: ip
Animals: Balb/c mice; urine obtained from patients treated with 200-920 mg/m^2
Sample collection: 8 hour intervals for 72 hours: urine, respired air
Method of analysis: [redacted]

Results:

In the mouse, up to 52% of the radiolabel was excreted in the urine while an additional 30% of the radiolabel was excreted in respired air. In the urine, 39% of the dose was excreted

unchanged. An additional 3% of drug was excreted in the feces, while 8% was associated with the carcass (total recovery 93%). Three peaks were found in the human patient urine: parent drug, temozolomide acid (3-methyl-2,3-dihydro-4-oxoimidazo[5,1-d]-tetrazine-8-carbxoylic acid, also found in mouse) and a second unidentified metabolite. The cytotoxicity of the temozolomide acid was almost equivalent to that of temozolomide in TLX5 lymphoma cells (approximately 5 mg/L).

12 Slack JA, Goddard C, Stevens MFG, Baig GU, Griffin MJ. The analysis and murine pharmacokinetics of a new antitumor agent: CCRG 81045. J. of Pharm. and Pharmacology 1986;38:63P. Vol. 1.58.

An system with an internal standard, detection at 325 nm, and limit of detection of 10 ng/mL was described. At 37°C, the half life of TEM in phosphate buffer was 89.3 minutes. No decomposition in DMSO over 14 weeks was observed. Pharmacokinetics in the mouse are shown in the table below (dose, 20 mg/kg, in male Balb/c).

Route	Cmax (mg/L)	Elimination $t_{1/2}$ (h)	AUC_{0-8}(mg•hr/L)
IP	25.8	1.13	36.71
PO	34.8	1.29	36.56

Summary of Pharmacokinetics

The pharmacokinetics and excretion of TEM has been investigated in the mouse, rat, dog and human. With the exception of the mouse, the dosing was administered as a body surface area dose (not back calculated from a body weight normalization). Due to the instability of TEM in plasma at room temperature (t1/2 = 15 minutes), samples were acidified at collection. MTIC, a major metabolite and the putative active moiety, in unstable in acid or plasma (t1/2 = 5.5 minutes). Thus, the two components could not be analyzed concurrently.

AUC and Cmax for rat, dog and human were within a factor of 2 of each other (mouse was closer to 3 fold difference). Little inter-experimental variability was noted. In the rat and dog, the Cmax levels were similar by measurement. However, the AUC values measured by the two techniques differed greatly. This is not unexpected with an extensively metabolized compound. Bioavailability based on plasma levels was near 100% in each species. Little difference in ADME parameters were noted with gender in any species tested.

There was also good agreement between the toxicokinetic data from multiple day dosing and that in the single dose experiments. Only TEM itself was measured. AUC was linear with dose in both rat and dog, as well as being linear over a range from 25 to 1000 mg/m^2. Human data correlated well with other species both in Cmax and AUC levels. No significant accumulation of parent drug was seen between day 1 and day 5.

TEM/ MTIC	Ref #	Dose mg/m^2	Species	Sex	Route	Detection method	Cmax ug/mL	AUC ug•hr/ml	Half-life hours	Bioavail-ability
TEM	12	60	MOUSE	M	IP		25.8	36.7	1.13	—
TEM	12	60	MOUSE	M	PO		34.8	36.6	1.29	100%
TEM	2	200	RAT	M	IV		42.8	102	—	—
TEM	2	200	RAT	M	IV		44.0	78	1.15	—
TEM	2	200	RAT	M	PO		36.6	120	—	118%
TEM	2	200	RAT	M	PO		42.7	87.5	1.25	111%
TEM	4	200	RAT	M	PO		31.8	135	35	—
TEM	5	200	RAT	F	PO		45.9	151	—	—
TEM	5	200	RAT	F	PO		40.9	91.6	—	—
TEM	8	255	RAT	M	PO		21.5	54.4	1.17	—
MTIC	8	255	RAT	M	PO		0.329	1.00	1.64	—
TEM	8	255	RAT	F	PO		31.4	55.7	1.22	—
MTIC	8	255	RAT	F	PO		0.385	1.11	1.35	—
TEM	6	200	DOG	M	IV		22.0	74.7	—	—
TEM	6	200	DOG	M	IV		23.0	36.3	1.43	—
TEM	6	200	DOG	M	PO		18.0	73.6	—	101%
TEM	6	200	DOG	M	PO		17.7	38.5	1.65	110%
TEM	9	250	DOG	M	PO		9.2	16.3	1.58	—
MTIC	9	250	DOG	M	PO		0.186	0.502	1.57	—
TEM	9	250	DOG	F	PO		8.5	20.5	1.68	—
MTIC	9	250	DOG	F	PO		0.223	0.586	1.61	—

Species	Experiment	dose (mg/m^2)	AUC (ug•hr/mL)
Rat	3 cycle*	0	0
		25	5.4
		50	9.1
		200	29.2
	6 cycle	0	0
		25	5.8
		50	9.6
		125	22.3
Dog	1 cycle HD	0	0
		200	31.2
		500	69.4
		1000	109.1
	1 cycle LD	25	2.5
		50	6.1
		125	16.5
	3 cycle	25	3.2
		50	6.4
		125	16.5
	6 cycle	25	3.9
		50	7.4
		125	18.8
Human	1 cycle	100	15.5
		150	17
		200	33.2
		250	43

The metabolic fate of TEM is shown in the following figure. It is interesting to note that in both rat and dog, the putative active metabolite, MTIC, accounted for 2-3% of the total plasma drug level (based on AUC). Elimination half lives were between 1.1 and 1.7 hours by the method for both TEM and MTIC. Overall label half-life was approximately 30 fold longer which would be logical if a final metabolite, AIC, is incorporated in biochemical pathways. Metabolism, based on the profiles in rat and dog was slightly more extensive by the oral route (greater number of peaks).

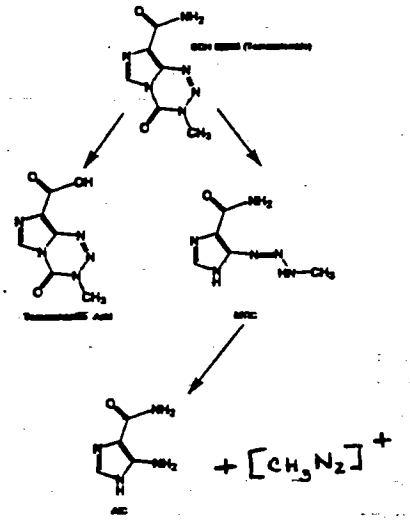


Figure 12. Proposed Metabolism and/or Degradation Products of SCH 52000.

Although the actual percentage varies, the majority of drug is excreted in the urine by rat, dog and mouse. Total recovery is rarely greater than 90% even with 7 days collection; again suggesting incorporation into normal biochemical processes.

Excretion of ^{14}C-TEM following a single dose (% of total dose excreted by each route)							
Species	Sex	Route	% other	% urine	% feces	% carcass	% total recovery*
Mouse	M	IP	30% respiratory	52%	3%	8%	93%
Rat	M	PO	1.4% bile	41%	2%	27%	87%
	M	IV	1.6% bile	34%	1%	36%	76%
	M	PO	—	78%	3%	—	83%
	M	IV	—	83%	2%	—	86%
	F	PO	—	76%	3%	—	84%
Dog	M	PO	—	60%	3%	—	70%
	M	IV	—	58%	2%	—	65%

* % total recovery also includes cage washings

TEM showed relatively broad distribution outside the blood stream. Initially, the ^{14}C-label was primarily found in the gut and kidney. A comparison of brain vs plasma AUCs shows that the exposure of TEM in the brain is actually 5.5 fold higher than that in plasma in the male rat. Half-life of radiolabel in the brain is around 70 hours. Radiolabel persisted in most tissues for up to 7 days at levels greater than those seen in the plasma. These tissues included liver, kidney, spleen, lungs, brain, eyes, skin, and fat.

III. TOXICOLOGY

1. P-5987. Three-cycle oral toxicity study of SCH 52365 in rats

Conducted at:
When conducted: June-Sept 1993
GLP: YES
Drug Lot #: INV # 920236001, BA # 28396-103 (AJ-A8/2), RIC # 17505912
Formulation: 0.4% methylcellulose
Doses: 0, 25, 50, 200 mg/m^2
Schedule: DX5 q 28 days X 3 cycles
Species used: Sprague Dawley plus rats, 6 weeks old, M: 208-256 g, F: 167-216 g
#/sex/dose: 30/sex/dose for toxicity study; 60/sex/dose for plasma analysis
Last day of observation: 85

Measurements and Observations:
Twice daily: mortality and clinical signs
Weekly: body weight
Days 6, 34, 62; days 12, 40, 68; days 28, 56, 84: hematology, serum chemistry in 10/sex/dose (corresponds to day 6, 12, 28 for each cycle)
Days 27, 55, 83; urinalysis (4 and 24 hours after dosing)
Plasma PK: 5/sex/group at predose, 15, 30 minutes, 1, 2, 4 hours first and last days of dosing.
Termination: 10/sex/dose on day 62, 20/sex/dose on day 85: gross pathology, organ weights, histopathology

Mortality and clinical signs:
All rats survived to scheduled sacrifice. Clinical signs included alopecia, masses (2/30 HD M, 18 HD females), rough haircoat. Masses in the inguinal and thoracic regions were apparent as early as day 57 in females, day 78 in the 2 males (thoracic only).

Body weight and food consumption:
Only HD males showed statistically and biologically relevant decreases in body weight. Weight losses in the males did not recover prior to the next cycle of TEM and are shown in the following table. Food consumption dropped by approximately 10% during the dosing period.

Cycle	Day	% decrease in body weight vs. controls	
		HD Males	HD Females
Cycle 1	Day 1	—	—
	Day 8	10%	4%
Cycle 2	Day 1	8%	2%
	Day 8	13%	6%
Cycle 3	Day 1	10%	4%
	Day 8	14%	5%
Final Sac	after fast	14%	3%
body weight gain	Day 1-85	26%	14%

Hematology:

Changes in hematologic parameters are shown in the following table. Platelet and WBC reductions were relatively similar with multiple cycles of drug. Reductions in WBC # reflected reductions in segmented neutrophils, monocytes and lymphocytes. Reductions in platelet number were less severe with repeated exposure to TEM.

% change as compared to controls							
	Cycle #	Males			Females		
		Day 6	Day 12	Day 28	Day 6	Day 12	Day 28
RBC #	1	↓6% H	—	—	↓7% H	—	—
	2	↓7% H	—	—	↓11% H	—	—
	3	↓10% H	—	—	↓10% H	—	↓15% H
Retic #	1	↓89% H	↑34% H	—	↓93% H	↑43% H	—
	2	↓>90% H	—	—	↓>95% H	—	—
	3	↓20% L/M, ↓67% H	—	—	↓31% L,M; ↓84% H	—	—
Platelet #	1	—	↓19% L, M; ↓49% H	—	—	↓11%L, ↓18% M, ↓45% H	—
	2	↑16% H	↓15% H	—	—	↓13% M, ↓34% H	—
	3	↑23% H	↓10% H	—	—	↓12% M, ↓19% H	—
WBC #	1	↓27% L,M; ↓60%H	—	—	↓15% L, ↓47% H	—	—
	2	↓22% L, M; ↓52% H	↓26% H	↓20% H	↓27% L, M; ↓50% H	↓32% H	↓23% M, ↓23% H
	3	↓12% M, ↓43% H	↓25% H	↓25% H	↓12% L, ↓27% M, ↓50% H	↓10% M, ↓42% H	—

Serum Chemistry:

Changes in serum chemistry parameters were minimal and occurred mostly in the HD rats during the first cycle. Glucose was increased on day 6 in the males and females by 10-25%. Globulin was decreased by 20% of less during all three cycles on day 6 or 12.

Gross Pathology:

There were more observations in the females than in the males. The majority of changes were seen only at HD and are summarized in the following table.

Incidence of macroscopic observations in the 3 cycle rat study				
	Males		Females	
	Interim	Final	Interim	Final
Mammary gland-mass	—	—	2/10 H	17/20 H
Thymus-small	3/10 M 6/10 H	—	1/10 M 1/10 H	
Thoracic cavity- adhesion	1/10 H	—	—	—
Abdominal cavity-adhesion/mass	—	1/20 M	—	—
Subcutaneous tissue mass	—	1/20 H	—	—
Skin-mass	—	1/20 H	—	—
Uterus-mass	—	—	—	1/20 H
Uterus-lumen fluid	—	—	1/10 C 2/10 L, M 3/10 H	1/20 L
Spleen-enlarged	—	—	—	1/20 H
Kidney-depressed area	—	—	—	1/20 M,H
Stomach-thickened mucosa/wall	—	—	—	1/20 H

Organ weights:

Thymic weight was significantly reduced in all dose groups immediately following dosing for the third cycle (day 62), but mostly resolved by the end of the third 28 day course (day 85). Changes in organ weights are shown in the following table.

% change in organ weight as compared to controls								
	Males				Females			
	Interim		Final		Interim		Final	
	Abs	Rel	Abs	Rel	Abs	Rel	Abs	Rel
Thymus	↓18% L ↓37% M ↓53% H	↓14% L ↓32% M ↓45% H	— — ↓14% H	—	↓20% L ↓46% M ↓65% H	↓19% L ↓47%M ↓60% H	—	—
Spleen	↓18% H	—	↓18% H	—	↓26% H	↓19% H	↑30% H	↑33% H
Kidney	—	↑12% H	—	—	—	↑12% H	—	↑11% H
Liver	—	↑17% H	—	—	—	—	↑29% H	↑33% H
Testes	↓23% H	↓10% H	↓19% H	—	—	—	—	—
Prostate	—	—	↓14% H	—	—	—	—	—
Epididymis	↓13% H	—	↓18% H	—	—	—	—	—
Ovary	—	—	—	—	—	—	↑17% H	↑20% H

Histopathology:

The most relevant changes at the microscopic level were thymic lymphoid depletion at interim sacrifice (recovered by the end of the cycle), syncytial cells in the testes/epididymis, marrow hypocellularity and mammary carcinoma (either in situ or as invasive). Some gi toxicity was seen in females and consisted of crypt epithelial necrosis in the intestines and non-dose

dependent ulceration/edema/necrosis in the stomach. Incidences of these findings, as well as other observations that may increase in incidence with longer exposure are summarized in the following table.

Incidence of microscopic observations				
Observation	Males		Females	
	Interim Sac n=10	Final Sac n=20	Interim Sac n=10	Final Sac N=20
Spleen—capsule fibrosis	1 H	—	—	—
Spleen—lymphoid depletion	—	—	4 H	—
Liver--chr. inflammation	1 H	3 C, 1 M	—	—
Liver—necrosis	1 L, 1 H	3 C, 2 M	—	—
Testes—syncytial cells	10 H	20 H	—	—
Epididymis--syncytial cells	10 H	2 H	—	—
Thymus—lymphoid depletion	6 L, 10 M, 10 H	—	6 L, 10 M, 10 H	—
Marrow-hypocellular	4 H	—	8 H	—
Marrow—hemorrhage	—	—	10 H	—
Skin--chr. active inflammation	1 M, 1 H	1 L	1 H	1 M
Skin-necrosis	1 H			
Thoracic cavity--adhesion	1 H	—	—	—
Abdominal cavity—adhesion	—	1 M	—	—
Duodenum--crypt epithelial necrosis	–	—	8 H	—
Jejunum—crypt epithelial necrosis	—	—	5 H	—
Ileum—crypt epithelial necrosis	—	—	2 H	—
Cecum –crypt epithelial necrosis	—	—	6 H	—
Colon—crypt epithelial necrosis	—	—	7 H	—
Mammary--carcinoma in situ	—	—	5 H	6 H
Mammary--carcinoma	—	4 H	2 H	18 H*
Mammary--benign fibroma	—	—	—	1 H
Lung--alv./bronc. epithelial hyperplasia	—	1 H	—	—
Stomach—ulcer	—	—	—	1 L
Stomach-edema/necrosis/suppurative inflammation	—	—	—	2 H
Uterus—deciduoma	—	—	—	1 H
Eye—suppurative inflammation	—	—	—	1 H
Mand. LN--lymphoid hyperplasia	—	—	—	1 M
Ovary—cyst	—	—	—	1 M , 1 H

* of the 18 females with mammary carcinoma, 15 had carcinomas at multiple sites

Plasma levels of TEM were only measured out to 4 hours for pharmacokinetic determinations. The % CV was usually around 30%, although it did rise as high as 42%. No significant changes were noted in AUC between genders or over time (suggesting no accumulation of drug). Tmax ranged between 30 minutes and 1 hour. AUC was close to linear with dose.

Dose (mg/m^2)	Day	Cmax[a]	Tmax[a]	AUC(tf)[a]
25	1	3.28	0.50	7.06
	5	2.06	0.50	4.82
	29	2.14	0.50	4.87
	33	2.49	0.50	5.07
	57	2.59	0.50	5.77
	61	2.45	0.50	4.79
50	1	4.84	0.50	10.0
	5	3.88	0.50	9.23
	29	3.63	0.50	8.57
	33	4.21	0.50	8.67
	57	4.39	0.25	9.38
	61	4.33	0.50	8.62
200	1	17.4	0.50	37.5
	5	15.4	0.50	32.2
	29	11.1	1.00	25.8
	33	13.1	0.50	26.5
	57	13.3	0.50	27.9
	61	15.0	0.50	25.6

a Pharmacokinetic parameters were determined from mean plasma concentration-time data from all rats.

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

2. P-6054. Six-cycle oral toxicity study of SCH 52365 in rats.

Conducted at:
When conducted: March-September, 1994
GLP: Yes
Route: oral gavage
Vehicle: 0.4% aqueous methylcellulose
Drug Lot #: RMA #A-930250, BA #28395-103,[AJ-A8.2], RIC # 17505912
Dosing: 0, 25, 50, 125 mg/m^2/day
Schedule: DX5 Q 28 days X 6 cycles (i.e. D1-5, 29-33, 57-61, 85-89, 113-117, 141-145)
Species: Sprague Dawley /plus rats; 7 weeks old, M: 199-254 g; F: 145-192 g
#/sex/dose: 35/sex/dose for toxicity; 60/sex/dose for PK determination
Duration of observation: D146 (interim sac), D169 (final sac)

Measurements and Observations:
Twice daily: mortality and clinical signs
Pretest, weekly: body weight, food consumption
Pretest, D5, 33, 61, 89, 117, 145, 166: ophthalmoscopy

Day 6, 12, 27/28 for each cycle (15, 10, 10 rats/timepoint) serum chemistry, hematology, urinalysis
Day 1, 5 of 1st, 3rd, and 6th cycle: blood for PK determinations
Termination: gross pathology, organ weights, histopathology

Mortality and clinical signs:

Deaths are summarized in the following table.

	Males		Females	
	# Dead	Day(s) of death (week #)	# dead	Day(s) of death (week #)
Control	0	—	0	
LD	1	161 (23)	0	
MD	1	144 (21)	0	
HD	8	44(7), 90(13), 120(28), 127(19), 2@140(20), 158(23) 162(24)	10	56(8), 124(18), 130(19), 138(20), 140(20), 143(21), 146(21), 2@158(23), 161(23)

With the exceptions of hypoactivity/hunched posture, convulsion and respiratory difficulties in a few of the early death rats, there did not appear to be a good correlation between incidence of masses, swollen thoracic/inguinal area or other damage between survivors and early death animals. In males the following signs occurred dose dependently: cold to touch, hunched posture, limited use of hind limbs, swollen thoracic/cervical/abdominal/inguinal region (HD only), thin appearance, convulsion (1 HD only), red urine, mucoid feces, corneal abrasions, exophthalmus, eye ulceration, hair loss, and tissue masses (mostly cervical, abdominal, seen in 1 MD, and 6 HD males). Signs were similar in the females with the addition of head tilt, ulceration of the eye (not dose dependent), and an increase in the number of animals with small and/or large masses (2 MD and 31 HD).

Ophthalmoscopy:

Changes were seen at very low incidence (1-2/35) and included congestion of the iris/anterior chamber, and cornea opacity/neovascularization/scarring/perforation.

Body weight and food consumption:

Body weight changes are summarized in the following table. Body weight decrements in the HD groups were increased with each cycle of dosing. Food consumption dipped during the first week of each cycle by <10% in the MD and HD groups.

% decrease in body weight at HD as compared to controls				
	Males		Females	
	Day 1	Day 8	Day 1	Day 8
Cycle 1	—	7%	—	7%
Cycle 2	4%	8%	5%	8%
Cycle 3	8%	10%	7%	10%
Cycle 4	8%	10%	9%	10%
Cycle 5	9%	11%	9%	10%
Cycle 6	9%	12%	7%	13%
Overall wt gain	↓8% @ MD, 28% @ HD		↓16% @ MD, 21% @ HD	

Hematology:

Cycle #	Day of cycle	Males			Females		
		RBC #	PLT #	WBC #	RBC #	PLT #	WBC #
1	6	—	↑20%	↓17%L,M ↓44% H	—	↑18% M ↑11% H	↓18% L ↓24% M ↓36% H
	12	—	↓14%L ↓19%M ↓38% H	↓39% H	—	↓11% M ↓17% H	↓12% H
	28	—	—	—	—	—	—
2	6	—	—	↓25% M ↓34% H	—	↑13% H	↓26% L,M ↓40% H
	12	—	↓11% M ↓19% H	—	—	↓31% H	↓22% H
	28	—	—	↓17% H	—	—	↓19% H
3	6	—	↑15% H	↓16% M ↓34% H	—	—	↓13% L ↓16% M ↓40% H
	12	—	↓22% H	↓23% H	—	↓12% M ↓21% h	↓23% H
	28	—	—	↓20% H	—	—	↓12% H
4	6	—	↑10% H	↓16% M ↓36% H	—	—	↓16% M ↓33% H
	12	—	↓22% H	↓32% H	—	↓20% H	—
	28	—	—	↓18% H	—	—	—
5	6	↓10%	↑16% H	↓13% M ↓33% H	↓11% H	—	↓10% M,H
	12	—	↓21% H	↓19% H	↓32% H	↓31% H	↑109% H
	28	↓16%	—	—	↓22% H	↓28% H	↑80% H
6	6	—	↑11% H	↓25% H	↓37% H	—	↑26% H
	12	—	↓20% H	↓11% H	↓18% H	↓27% H	↑118% H
	28	—	↑18% H	↓14% H	↓25% H	↓35% H	↑118% H

Serum Chemistry:

With the exception of the rats that were about to die, changes in serum chemistry parameters were minimal, particularly in the males. In the HD rats about to die, BUN was elevated <10 fold, while AST/ALT/ALP were elevated by up to 50 fold (calcium was increased in 2 HD females by 40%). In the surviving rats, changes of 10-20% as compared to controls were seen in globulin (decreases cycles 2-4 males, 5-6 females), glucose (increases in cycle 2-3 only, both sexes), inorganic phosphate (increases in females cycles 3, 5, 6) and potassium (decreases in females cycle 3-6).

Urinalysis: No significant changes in urinary parameters were seen with treatment.

Gross Pathology:

The observations in early death, interim sacrifice and final sacrifice are shown in the following table.

Macroscopic Observations in 6 cycle rat study

Description	Males			Females		
	Early death	Interim Sac	Final Sac	Early death	Interim Sac	Final Sac
Abdominal cavity-fluid/adhesions	2/8 H			—		
Abdominal cavity—mass	—	—	1/13 H	—	—	—
Adrenal-mass	2/8 H			—		
Adrenal-enlarged	1/1 L, M	—		—	1/14 H	
Brain-ventral surface indented	2/8 H	—		—	1/14 H	
Brain-raised area	2/8 H			—		
Brain-dark area	1/1 L			—		
Eye-exophthalmus	—	—		—	1/14 H	
Eye-internal opacity	—	1/14 H	—	—	2/14 H	1/20 M, 1/11 H
Eye-ulceration	2/8 H	—	1/13 H	—	—	—
Harderian gland-mass	—	—		—	1/14 H	
Head-coronal area-mass	1/8 H			—		
Kidney-pelvis dilated	1/1 M, 2/8 H			—		
Kidney-enlarged	1/1 M, 1/8 H	—	1/13 H	—	—	—
Kidney-dilated, enlarged, irregular, pale, mottled	1/1 M			—		
Kidney-pale	—	—	—	—	—	2/11 H
Liver-dark area	1/1 L, 2/8 H	—	1/19 M	—	—	—
Liver-enlarged	1/1 L, 1/1 M, 1/8 H	—	1/20 C, 2/19 M, 2/13 H	2/10 H		
Liver-friable/mottled	—	—	—	—	—	1/11 H
Liver-mass	—	1/15 M		—	—	
Liver-pale	2/8 H					
Lung—mottled/dark area	1/1 L			1/10 H		
Lung—pale	—	—	1/13 H	—	—	—
Lymph node—dark enlarged	1/1 L	—	—	2/10 H	—	1/11 H
Mammary area-mass	2/8 H	1/14 H	7/13 H	9/10 H	14/14 H	2/20 L, 2/20 M, 11/11
Ovary-small	—	—	—	—	1/14 H	
Pancreas-mass	—	—	1/13 H	—	—	—
Prostate—enlarged/firm	—	—	1/13 H	—	—	—
Seminal vesicle-enlarged	1/8 H			—		
Seminal vesicle—mass	—	—	2/13 H	—	—	—
Skin-mass	—			1/10 H		
Spleen-small	2/8 H	—		—	1/14 H	
Spleen-enlarged	1/1L, 1/8 H	—	—	3/10 H	4/14 H	3/11 H
Stomach-dark area	1/8 H	—	—	2/10 H	1/14 H	1/20 M
Stomach—thickened mucosa	—	—	4/13 H	—	—	1/11 H
Subcutaneous tissue-mass	—	4/14 H	4/13 H	—	—	—
Thoracic cavity—mass/fluid/adhesion	1/8 H			—		
Uterus-small	—	—	—	—	2/14 H	
Uterus—mass	—	—	—	—	—	1/11 H
Urinary bladder-distended	1/1 L, 1/1 M, 2/8 H					

Organ weights:

Organ weights at the interim and final sacrifices are shown in the following table. Changes in spleen weights in the HD females correlated with myeloid hyperplasia. Thymus weight changes were resolving by the final sacrifice, while spleen, liver and adrenal weights worsened with time.

% change in organ weights as compared to controls								
Organ	Males				Females			
	Interim Sac		Final Sac		Interim Sac		Final Sac	
	Abs.	Rel.	Abs.	Rel.	Abs.	Rel.	Abs.	Rel.
Thymus	↓59% H	↓53% H	↓24% H	↓9% H	↓25% M, ↓50% H	↓18% L, ↓25% M, ↓45% H	↓29% H	↓18% H
Spleen	↓16% H	↓6% H	—	↑17% H	↑100% H	↑109% H	↑161% H	↑201% H
Liver	—	↑10%M,H	—	↑14% H	↑28% H	↑36% H	↑26% H	↑45% H
Testes	↓13% H	—	↓18% H	—	—	—	—	—
Epididymis	↓19% H	↓10% H	↓12% H	—	—	—	—	—
Adrenals	—	—	↑23% H	↑50% H	↑21% H	↑31% H	↑10% H	↑26% H
Sal. gland	—	—	—	↑20% H	↓16% H	↓12% H	↓10% H	—
Kidney	—	—	—	↑25% H	↑10% H	↑19% H	—	↑12% H

Histopathology:

The LD early death male had malignant lymphocytic lymphoma in the kidney, liver, lymph node, marrow, and spleen. The MD rat had suppurative inflammation of the kidney; cause of death was attributed to genitourinary disease. In the HD rats, 2/8 had no determined cause of death, 2/8 had genitourinary disease, 3/8 had fibrosarcomas, and 1/8 had malignant fibrous histiocytoma (lumbar cord, adrenal, spleen, stomach, liver pancreas and marrow). In the 10 HD females that died prior to schedule, 7/8 had mammary carcinomas (1-6 masses/rat, mean=3.9 masses/rat); only one had an undetermined cause of death.

Microscopic observations are summarized in the following table. Mammary carcinomas were more common in the females than in the males; although they did occur in both sexes. Other malignancies observed included carcinoma of the seminal vesicles, fibrosarcoma of the eye, seminal vesicle, subcutaneous tissue, and prostate; sarcoma of the salivary gland and endometrial/stromal uterus; and schwannomas of the heart, harderian gland and cptic nerve. Benign tumors included adenomas of the skin (basal cell), adrenal cortex, lung and pituitary. Hyperplasia was observed in the adrenal (possibly progressing to adenoma), spleen, and marrow. Hypertrophy was also observed in the pituitary. Skin keratoacanthomas were more frequent in males.

Other targets of toxicity included liver and kidney, with minimal damage to the gi tract. Male reproductive organs showed minimal signs of damage (syncytial cells, but no atrophy). Retinal degeneration, suppurative inflammation, and cataracts were observed in the eyes of the HD males and at lower doses in the females; these incidences were similar to those seen in the ophthalmoscopic observations.