CENTER FOR DRUG EVALUATION AND RESEARCH

APPLICATION NUMBER: 21-200

PHARMACOLOGY REVIEW(S)

PHARMACOLOGIST'S REVIEW OF NDA 21,200
(Amendment Dated April 18, 2001)

Reviewer: Ke Zhang, Ph.D.
Pharmacologist

Sponsor & Address: Novartis Pharmaceuticals Corporation
Hanover, New Jersey

Date of HFD-180 Receipt: April 20, 2001

Date of Review: May 23, 2001

DRUG: Tegaserod hydrogen maleate / HTF 919, Tablet

CATEGORY: 5-HT_4 receptor agonist in the treatment of constipation-prone irritable bowel syndrome.

Submission Contents: Requested pharmacology information.

Background:

Sponsor submitted a report of pharmacology study of 5-HT_4-receptor status of human appendix and non-GI abdominal and pelvic organs compared to human intestinal samples on December 15, 2000. This study was reviewed on February 9, 2001. Sponsor stated that the human tissue samples were obtained from ten patients undergoing abdominal surgery in this study. However, sponsor only submitted the data from one patient. Sponsor was asked to submit "(a) 5-HT_4 receptor mRNA data for the tissues collected from all ten patients and (b) numerial data for each patient and group (i.e., mean values with standard deviations or standard errors for each sex and both sexes)" in the Division's letter dated March 29, 2001. In this letter, sponsor was also asked to "define the unit of expression of 1.0 used for 5-HT_4 receptor mRNA in the liver". Sponsor submitted their responses in this submission.

1. The individual human data from all ten patients and mean values with standard deviations or standard errors for each sex and both sexes

Sponsor stated that the availability of normal tissue per GI segment was limited to one or two specimens and the mean values by GI segment and sex were not available.

2. To define the unit of expression of 1.0 used for 5-HT_4 receptor mRNA in the liver.

Sponsor first normalized the 5-HT_4 receptor mRNA expression values of all tissues to a house-keeping/constitutively expressed gene, G3PDH to estimate the relative abundance of mRNAs in different tissues. And then all G3PDH normalized values were compared to that of the liver. Therefore, the relative calculated level of the normalized 5-HT_4 receptor mRNA expression in the liver was 1.0 with no unit.

SUMMARY AND EVALUATION:

In the Division's letter dated March 29, 2001, sponsor was asked to submit the 5-HT_4 receptor mRNA data from all ten patients and mean values with standard deviations or standard errors for each sex and both sexes and to define the unit of expression of 1.0 used for 5-HT_4 receptor mRNA in the liver. In response to these requests, sponsor stated that only a few specimens were collected for each GI segment from all ten patients and thus the mean values by GI segment were not available. Sponsor has defined the unit of expression of 1.0 used for 5-HT_4 receptor mRNA in the liver.

RECOMMENDATION: None.

Ke Zhang, Ph.D. Date
Pharmacologist, HFD-180

Comments:

Jasti B. Choudary, B.V.Sc., Ph.D. Date
Supervisory Pharmacologist, HFD-180

cc:
NDA
HFD-180
HFD-181/CSO
HFD-180/Dr. Choudary
HFD-180/Dr. Zhang

R/D Init.: JChoudary 5/23/01

KZ/deg: 5/23/01
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/s/

Ke Zhang
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PHARMACOLOGIST

Jasti Choudary
5/23/01 02:19:03 PM
PHARMACOLOGIST

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PHARMACOLOGIST'S REVIEW OF NDA 21,200
(Amendment Dated December 15, 2000)

Reviewer: Ke Zhang, Ph.D.
Pharmacologist

Sponsor & Address: Novartis Pharmaceuticals Corporation
Hanover, New Jersey

Date of HFD-180 Receipt: December 18, 2000

Date of Review: February 9, 2001

DRUG: Tegaserod hydrogen maleate / HTF 919, Tablet

CATEGORY: 5-HT_4 receptor agonist in the treatment of constipation-prone irritable bowel syndrome.

Submission Contents: (1) a pharmacology study and (2) a draft labeling.

PHARMACOLOGY:

Investigations on the 5-HT_4 receptor status of human appendix and non-GI abdominal and pelvic organs compared to human intestinal samples
(RD-2000-02503)

To examine the presence of 5-HT_4 receptors in human and non-human primate tissues, 5-HT_4 receptor mRNA level was determined using [redacted] technique in human appendix, gastrointestinal (GI) tract, non-GI abdominal and pelvic organs including ovary, uterus, fat, lung, and liver. The human tissue samples were obtained from ten patients undergoing abdominal surgery at the [redacted] 5-HT_4 receptor mRNA level in various tissues from non-human primates (4 cynomolgus monkeys, *macaca fascicularis*) was also determined. The results from one patient were presented in Figure 1A. The results from four cynomolgus monkeys were depicted in Figure 2A. These figures are attached below.

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Figure 1A

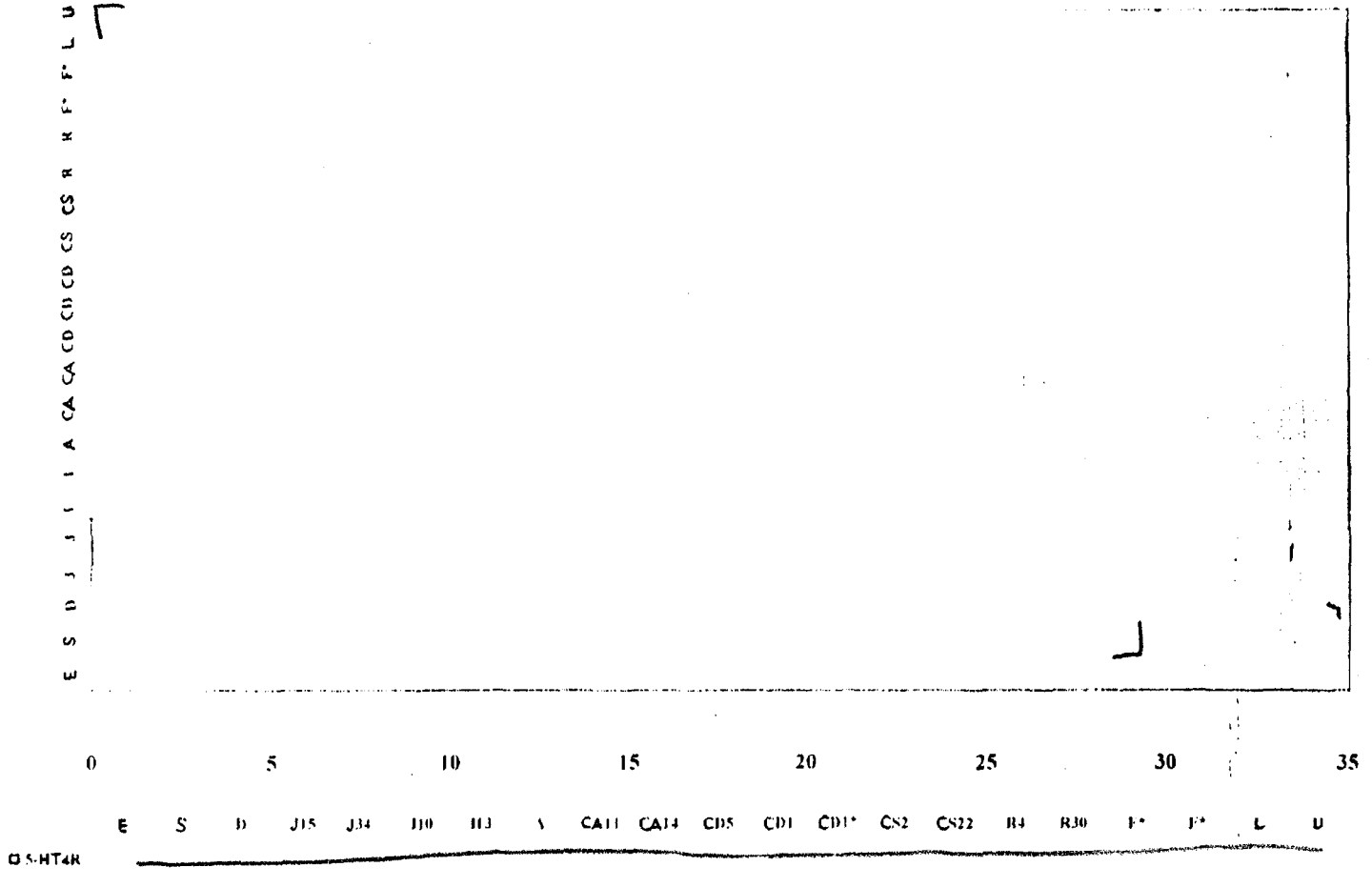

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Figure 1A: The graph illustrates the relative abundance of 5-HT_4 receptor mRNA in human gastrointestinal (E: esophagus. S: stomach. D: duodenum. J: jejunum, I: ileum. A: appendix. CA: colon ascend.. CD: colon descend.. CS: colon sigmoid.. R: rectum. and L: liver). fat (F) as well as uterine (U) tissue. Each bar represents the level of human 5-HT_4 receptor mRNA from one tissue specimen (1 patient). The values calculated following normalization to G3PDH (housekeeping gene) are given in the table relative to the amount of 5-HT_4 receptor mRNA in liver (reference organ).
CD1*: Second isolation of mRNA from colon descend. of patient #1.
F*: 5-HT_4 receptor mRNA from fat was not normalized to G3PDH (not constitutively expressed in fat).

Relative levels of 5-HT_4 receptor mRNA in human tissues

Tissues	5-HT_4 receptor mRNA levels
Liver (L)	1.0
Jejunum (J)	28.6, 7.1
Ileum (I)	20, 9.6
Appendix (A)	19.3
Stomach (S)	14.7
Duodenum (D)	12.7
Colon Ascend (CA)	10.3, 9.7
Colon Sigmoid (CS)	6.2, 5.6
Colon Descend (CD)	5.4, 5.2, 5.1
Rectum (R)	14.2, 6.9
Esophagus (E)	4.0
Uterus (U)	3.6
Ovary (O)	1.5, 0.5
Fat (F)	1.5, 1.1

The values are obtained from Figure 1A (see the figure legend above).
Liver is as reference organ and the value in the liver is set to 1.0.

The human tissue samples were obtained from ten patients undergoing abdominal surgery. However, sponsor only submitted the data from one patient. Sponsor should be asked to submit the individual human data from all ten patients and mean values with standard deviations or standard errors for each sex separately and together and to define the unit of 1.0 for the liver.

Figure 2A

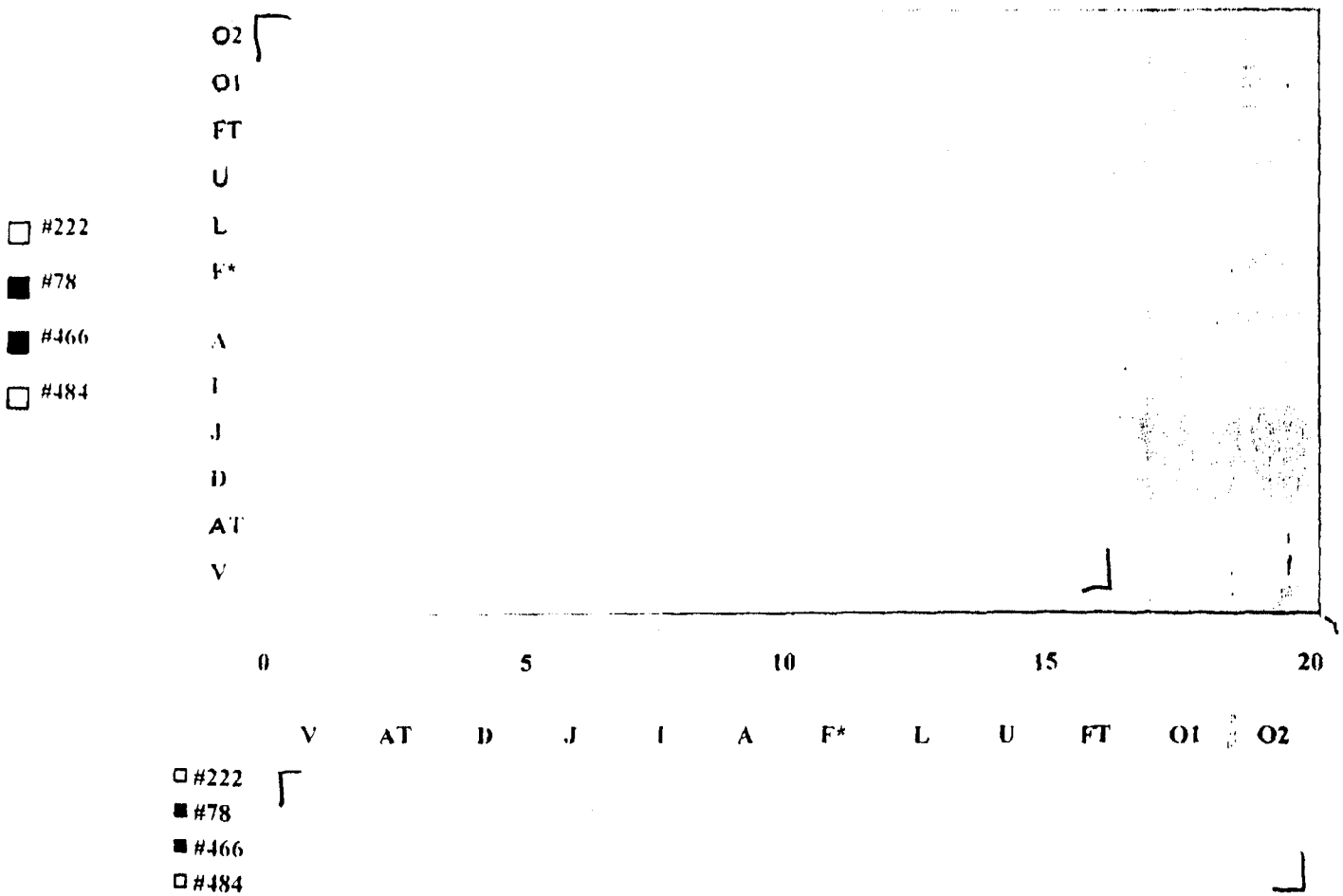
O2
O1
FT
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Figure 24: The graph illustrates the relative abundance of 5-HT_4 receptor mRNA in non-human primate gastrointestinal (D: duodenum, J: jejunum, I: ileum, A: appendix, and L: liver), fat (F), heart (A: atrium, V: ventricle), uterus (U), fallopian tube (FT) as well as ovary (O) tissue. Each bar represents the level of non-human primate 5-HT_4 receptor mRNA from one tissue specimen (1 monkey). The values calculated following normalization to G3PDH (housekeeping gene) are given in the table relative to the amount of 5-HT_4 receptor mRNA in liver.

Relative levels of 5-HT_4 receptor mRNA in non-human primate tissues (cynomolgus monkeys)

	Mean		
	Females (n=2)	Males (n=2)	Total mean (n=4)
Ileum (I)	2.4	12.5	7.4
Jejunum (J)	1.9	7.0	4.4
Appendix (A)	2.7	7.4	5.0
Duodenum (D)	1.7	8.5	5.1
Atrium (AT)	0.7	2.8	1.7
Ventricle (V)	0.5	1.2	0.9
Uterus (U)	1.8		
Fallopian tube (FT)	1.6		
Ovary (O) O1 O2	 0.7 1.35		
Fat (F)	0 (0, 0)	1.8	

The values are obtained from Figure 2A (see the figure legend above).
Liver is as reference organ and the value in the liver is set to 1.0.
* = Individual values in parenthesis.

The results indicated that 5-HT_4 receptor mRNA are present throughout both human and non-human primate GI tract. The highest level of 5-HT_4 receptor mRNA was found in the small intestine (jejunum and ileum) followed by the appendix. The levels of 5-HT_4 receptor mRNA in the human small intestine and appendix are up to 19-29 times that in the reference organ (liver). It appears that the levels of 5-HT_4 receptor mRNA in male monkeys are higher than those in female monkeys. Sponsor stated that "clinical data on tegaserod do not indicate an increased risk for appendicitis". The levels of 5-HT_4 receptor mRNA in non-GI tissues were much lower than those in the GI-tissues. For examples, detectable amount of 5-HT_4 receptor mRNA was found in the human uterus (3.6 times that in the liver) and ovaries (comparable to that in the liver). The physiological functions and clinical significance of the presence of 5-HT_4 receptors in these non-GI tissues are not known.

5-HT_4 receptor mRNA was also detected in non-human primate heart (comparable to that in the liver). This is consistent with the findings of presence of 5-HT_4 receptors in the human atrium in published reports (Naunyn-Schmiedeberg's Arch Pharmacol. 1991,

334:150-159 and 1996, 353:592-595). No safety cardiovascular pharmacology studies were conducted in monkeys. The pharmacology studies in dogs submitted in the original NDA 21,200 on February 11, 2000 demonstrated that HTF 919 had no effects on heart rate, blood pressure, and ECG following single oral doses up to 10 mg/kg. The $AUC_{0\text{-}24\ hours}$ value at this dose was 924-1110 ng.h/ml, which is about 46-55 times the human exposure (20.1 ng.h/ml) at the recommended human dose (12 mg/day).

LABELING:

The labeling is according to 21 CFR, Subpart B. The following revisions in the labeling are recommended.

1. Carcinogenesis, Mutagenesis, Impairment of Fertility

Sponsor's Version:

[

]

Evaluation: Systemic exposure data were not included.

Suggested Version:

[

]

2. Pregnancy

Sponsor's Version:

Pregnancy

Pregnancy Category B

[

]

[ ] Because animal reproduction studies are not always predictive of human response, this drug should be used during pregnancy only if clearly needed.

[ ]

Evaluation: Systemic exposure data were not included. The statement of ——— should be removed entirely since Tegaserod hydrogen maleate is not intended to be used ———

Suggested Version:

Pregnancy

Teratogenic Effects. Pregnancy Category B: ——— studies have been performed in rats at oral doses up to 100 mg/kg/day [ ] and rabbits at oral doses up to 120 mg/kg/day [ ] and have revealed no evidence of impaired fertility or harm to the fetus due to tegaserod [ ] Because animal reproduction studies are not always predictive of human response, this drug should be used during pregnancy only if clearly needed.

3. Nursing Mothers

Sponsor's Version:

Tegaserod ——— is excreted in the milk of lactating rats with a high milk to plasma ratio.

[ ]

Evaluation: Statements of the potential for serious adverse reactions in nursing infants and tumorigenic potential should be included.

Suggested Version: Tegaserod ——— and its metabolites are excreted in the milk of lactating rats with a high milk to

plasma ratio. It is not known whether [redacted] is excreted in human milk. [redacted] many drugs are excreted in human milk, [redacted] the potential for serious adverse reactions in nursing infants [redacted] potential for tumorigenicity shown for Tegaserod [redacted] in the mouse carcinogenicity study, a decision should be made whether to discontinue nursing or to discontinue the drug, taking into account the importance of the drug to the mother.

SUMMARY AND EVALUATION:

The new drug application for tegaserod maleate (NDA 21,200) was approvable based on the approvable letter dated on August 11, 2000. In this letter, it was recommended that sponsor submit the results of the pharmacology study of 5-HT_4-receptor status of human appendix and non-gastrointestinal abdominal and pelvic organs compared to human intestinal samples. The study report is submitted in this submission. In this study, the human tissue samples were obtained from ten patients undergoing abdominal surgery. However, sponsor only submitted the data from one patient. Sponsor should be asked to submit the individual human data from all ten patients and mean values with standard deviations or standard errors for each sex separately and together. In this report, sponsor used the liver as reference organ and set the value of 5-HT_4 receptor mRNA in the liver to 1.0. Sponsor should also be asked to define the unit of 1.0 for the liver and to provide the actual values of 5-HT_4 receptor mRNA for all tissues. Based in the results provided, 5-HT_4 receptor mRNA are present throughout both human and non-human primate GI tract. The highest level of 5-HT_4 receptor mRNA was found in the small intestine (jejunum and ileum) followed by the appendix. The levels of 5-HT_4 receptor mRNA in non-GI tissues (uterus, ovary, liver, and fat) were much lower than those in the GI-tissues. The physiological functions and clinical significance of the presence of 5-HT_4 receptors in these non-GI tissues are not fully understood.

Sponsor also submitted a draft labeling in this submission. The preclinical portion of the draft labeling was not different from the labeling submitted in the original NDA. The labeling was originally reviewed in the pharmacology review of this NDA on July 14, 2000. The recommendation remains the same. Sponsor should be asked to revise the labeling as recommended.

RECOMMENDATION:

1. Sponsor should be asked to submit the individual human data from all ten patients and mean values with standard deviations or standard errors for each sex separately and together in the pharmacology study entitled "Investigations on the 5-HT_4 receptor status of human appendix and non-GI abdominal and pelvic organs compared to human intestinal samples".

2. Sponsor should be asked to define the unit of 1.0 for the liver and to provide the actual values of 5-HT_4 receptor mRNA for all tissues.

3. Sponsor should be asked to revise the labeling as recommended.

Ke Zhang, Ph.D. Date
Pharmacologist, HFD-180

Comments:

Jasti B. Choudary, B.V.Sc., Ph.D. Date
Supervisory Pharmacologist, HFD-180

cc:
NDA
HFD-180
HFD-181/CSO
HFD-180/Dr. Choudary
HFD-180/Dr. Zhang

R/D Init.: J Choudary 1/22/01

KZ/deg: 1/29/01, 2/9/01
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/s/

Ke Zhang
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PHARMACOLOGIST

Jasti Choudary
2/14/01 01:18:58 PM
PHARMACOLOGIST

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Levine

JUL 14 2000

NDA 21,200 Review #1

Sponsor & Address: Novartis Pharmaceuticals Corporation
East Honover, NJ

Reviewer: Ke Zhang, Ph.D.
Pharmacologist

Date of Submission: Original - February 11, 2000
Amendment - May 25, 2000

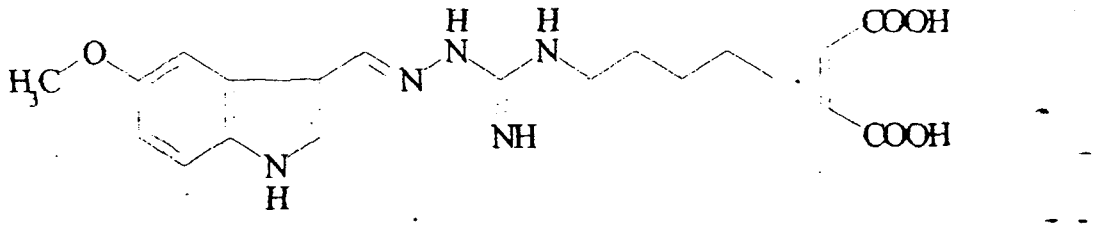
Date of HFD-180 Receipt: Original - February 14, 2000
Amendment - May 30, 2000

Date of Review: July 11, 2000

REVIEW AND EVALUATION OF PHARMACOLOGY AND TOXICOLOGY DATA
Original Summary

DRUG: Zelmac / Tegaserod hydrogen maleate / HTF 919, Tablet

3-(5-Methoxy-1H-indol-3-ylmethylene)-N-pentylcarbazimidamide hydrogen maleate



Molecular Formula: $C_{20}H_{27}N_5O_5$ MW: 417.5

CATEGORY: 5-HT_4 receptor agonist in the treatment of constipation-prone irritable bowel syndrome

Related INDs: IND [redacted]

Marketing Indications and Dose: Zelmac is indicated for treatment of [redacted] patients with constipation-prone irritable bowel syndrome. The recommended oral dose is 6 mg b.i.d.

PRECLINICAL STUDIES AND TESTING LABORATORIES:

Type of Study	Study #	Lot #	lab	Page #
Pharmacology				4-11
Absorption, Distribution, Metabolism and Excretion (ADME):				11-32
Pharmacokinetic study in mice				
Metabolism in mice				
Pharmacokinetic studies in rats				
Tissue distribution studies in rats				
Pharmacokinetic study in dogs				
Protein binding studies				
Metabolism study in human liver and intestine				
In vitro study on metabolic pathway				
O-demethylation in rat and human liver				
Effects of HTF 919 on human P450				
Acute Toxicity:				32-33
Acute oral toxicity study in mice	321952	91903	1	
Acute oral toxicity study in rats	321963	91903	1	
Acute i.v. toxicity study in mice	321974	91903	1	
Acute oral toxicity study in rats	321985	91903	1	
Subacute/subchronic/chronic Toxicity:				
13-week oral dosing ranging study in mice	177DFM	94904	2	53-57
13-week oral exploratory study in mice	971076	94906	2	57-61
17-day i.v. toxicity study in rats	087929	Y197 0992	3,4	33-35
26-week oral toxicity study in rats	395R	91903	2,3	36-40
2-week i.v. toxicity study in dogs	338422	Y196 0992	1	40-43
26-week oral toxicity study in dogs	SANDOZ 395D	Y197 0992 91903	2,3,5	43-46
52-week oral toxicity study in dogs	95/019/0592	94904	6	46-51

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Type of Study	Study #	Lot #	lab	Page #
Carcinogenicity:				
Oral carcinogenicity study in mice	034/970331	94906	6	62-79
Oral carcinogenicity study in rats	029/970357	94905	6	80-95
Special Toxicity:				
Local i.v. tolerance study in rabbits	73LT/RB	Y197 0992	2	51
Skin irritation study in rabbits	232796	97003	7	52
Assessment of hypersensitivity in guinea pigs	232807	97003	7	52-53
Effects on progesterone, estradiol, corticosterone, and prolactin levels in female rats	7041R	91903	2	100
Effects on progesterone, estradiol, corticosterone, and prolactin levels in female rats	7047R	91903	2	100
Reproductive Toxicity:				
An oral Segment I study in rats	SANDOZ 3021R	91903	2	96-100
An oral (in feed) Segment I study in rat	3043R	94905	2	101-105
An oral Segment II study in rats	SANDOZ 4001R	91903	2	106-117
An oral Segment II study in rabbits	SANDOZ 4007K	91903	2	117-125
An oral (in feed) Segment III study in rats	578/972144	94906	6	125-127
Mutagenicity:				
Ames tests	Mut.Bakt.51/91 Mut.Bakt.4/93 Mut.Bakt.11/94 Mut.Bakt.22/94 Mut.Bakt.53/94	91903 91902 94904 94905 94906	2 2 2 2 2	127-130
In vitro chromosomal aberration test in Chinese hamster V79 cells	Z.29	91903	2	130-131
A forward mutation assay at HGPRT locus in V79 Chinese hamster cells	HV 5	91903	2	131-132
Unscheduled DNA synthesis test in rat hepatocyte primary cultures	UDS 1	91903	2	132
Mouse bone marrow micronucleus test	MK 23	91903	2	133

1 = [redacted]
2 = sponsor's laboratory, 3 = [redacted]
4 = [redacted]
6 = [redacted], 7 = [redacted]

Following studies were previously submitted to IND [redacted] (1) pharmacology, (2) ADME, (3) acute toxicity studies in mice and rats, (4) 2-week oral dose ranging study in mice, (5) 13-week oral dose ranging study in mice, (6) 17-day i.v. toxicity study in rats, (7) 4-week oral dose ranging study in rats, (8) 26-week oral toxicity studies in rats, (9) 2-week i.v. toxicity study in dogs,

(10) 26-week oral toxicity study in dog, (11) 52-week oral toxicity study in dogs, (12) carcinogenicity studies in mice and rats, (13) oral Segment I study in rats, oral Segment II embryo-fetal development studies in rats and rabbits, (14) Ames tests, (15) in vitro chromosome aberration tests in Chinese hamster V79 cells, (16) a forward mutation assay at HGPRT locus ion V79 Chinese hamster cells, (17) Unscheduled DNA synthesis test, and (18) mouse bone marrow micronucleus test. These studies were previously reviewed on September 26, 1995 (initial submission) and on July 2, 1998 (amendment # 084). These reviews are incorporated in the appropriate portion of the present review.

PHARMACOLOGY:

Predominant symptoms of constipation-prone irritable bowel syndrome (IBS) include abdominal pain and discomfort, and constipation. 5-hydroxytryptamine (5-HT, serotonin) is believed to play a major role in the etiology of IBS. HTF 919 is a partial serotonin type-4 (5-HT_4) receptor agonist and can regulate gastrointestinal (GI) motility, intestinal secretion, and possesses an antinoceptive effect on pain threshold. This would be therapeutically useful in patients with IBS. The pharmacological activities of HTF 919 were characterized in both *in vitro* and *in vivo* preparations.

Primary Activity

1. *In Vitro* Studies:

GI transit depends upon the peristaltic reflex. To study the pharmacological activities of HTF 919 as a prokinetic agent, its effects on the peristaltic reflex were examined in the isolated guinea pig ileum using the method of Trendelenburg. Both circular and longitudinal muscles can be simultaneously investigated in this preparation. The results indicated that HTF 919 produced a dose dependent stimulation of peristaltic movements of the longitudinal muscle layer (pEC_{50} = 7.69) and a parallel inhibition of the circular muscle activity (pIC_{50} = 7.84). The results were compared with known 5-HT_4 agonists including the O-desmethyl analogue of HTF 919 (216-454), two substituted benzamide analogues, cisapride and [redacted], and metoclopramide. For example, 216-454 was about 20-fold (pEC_{50} = 9.02) more potent than HTF 919 for stimulating the longitudinal muscle movement and the relative maximum efficacy of HTF 919 and 216-454 was 51% and 44% when

comparing with metoclopramide (100%). In comparison with ——— (pEC_{50} = 8.25), HTF 919 was slightly less potent for stimulating the longitudinal muscle movement. Cisapride only produced a little stimulatory effect on the longitudinal muscle and the maximum peristaltic stimulation was only 24% at 10^{-7} M. The inhibitory effect of HTF 919 (maximum inhibition = 66.6% at 10^{-5} M) on the circular muscle was more pronounced than that of 216-454 (maximum inhibition = 31.1% at 10^{-9} M). To further characterize its 5-HT_4 receptor stimulatory activities, HTF 919 was studied using a field-stimulated guinea-pig ileum model and its effects were compared with 5-hydroxytryptamine (5-HT, serotonin), 216-454, cisapride and ———. In this preparation, HTF 919 increased the electrically-induced twitch contractions with pD_2 = 6.97. It was ~10 times less potent than 5-HT (pD_2 = 7.92). The efficacy (intrinsic activity = 0.22) of HTF 919 was much less than that of 5-HT (intrinsic activity = 1). In contrast, 216-454 and cisapride were also partial agonists with intrinsic activities of 0.6 and 0.65, respectively. However, 216-454 was the most potent agonist (pD_2 = 9.3) among the tested compounds and cisapride was the least potent with pD_2 = 5.98. ——— showed full agonist activity (intrinsic activity = 1.2) and was as potent as HTF 919 (pD_2 = 7.11 for ——— and pD_2 = 6.97 for HTF 919).

In another *in vitro* study, the isolated colonic segments from rats and guinea pigs were placed in a bathed chamber with Krebs-bicarbonate medium. The bathed chamber was separated into 3 compartments (central, orad, and caudad) and the contraction or relaxation of the circular muscles was measured in the orad and caudad compartments using force-displacement transducer. The bathing medium from these compartments were collected for measurement of calcitonin gene-related peptide (CGRP), vasoactive intestinal peptide (VIP), and substance P. The results indicated that addition of HTF 919 in the central compartment produced a concentration-related release of CGRP into the central compartment with ED_{50} = 8.6 nmol/l in guinea pigs and 12.3 nmol/l in rats. HTF 919 also increased the release of VIP into orad compartment and substance P into caudad compartment. The ED_{50} values were summarized in Table 1 on page 222 in volume 1.10. This table is attached below.

Table 1. EC_{50} Values for Release of CGRP, SP, and VIP Induced by the 5-HT_4 Agonist HTF 919

	EC_{50} (nmol/L)	
	Rat colon	Guinea pig colon
SP release	13.1 ± 2.5	5.2 ± 4.5
CGRP release	12.3 ± 1.8	8.6 ± 3.4
VIP release	4.5 ± 2.6	6.8 ± 3.2

HTF 919 also produced ascending contractions (orad compartment) and descending relaxation (caudad compartment) with ED_{50} of 1.6-4.8 nmol/l in both rats and guinea pigs. This peristaltic reflex was inhibited by GR 113808A, a 5-TH_4 antagonist but not LY 278584, a 5-TH_3 antagonist, suggesting that the HTF 919-induced peristaltic reflex is mediated by 5-TH_4. The HTF 919-stimulated release of CGRP, VIP, and SP and peristaltic reflex were also observed in human jejunum preparation but no ED_{50} values were provided. These *in vitro* functional studies indicated that HTF 919 can stimulate the peristaltic reflex.

The affinity of HTF 919 was determined in calf and human caudate for 5-HT_4 receptors. The results indicated that HTF 919 had a high affinity for 5-HT_4 receptors in both calf and human caudate with pK_D of 7.84±01 (calf) and 7.71±0.07 (human). The affinity of cisapride and ——— for 5-HT_4 receptors are similar to that of HTF 919 in both species with pK_D of cisapride of 7.37 (calf) and 7.22 (human) and pK_D of ——— of 6.7 (calf) and 6.38 (human). However, the major human metabolite of HTF 919, SDZ 244-120 (M29.0), had very low affinity for 5-HT_4 receptors with pK_D of 3.72 (calf) and 4.11 (human). The ligand binding studies were also conducted in the pig and rat cortex, pig choroid plexus and calf caudate for 5-HT_{1A}, 5-HT_{1C}, 5-HT_3 and 5-HT_2 receptors. The pK_D values for 5-HT, HTF 919, 216-454, cisapride and ——— were determined in these studies. These values were summarized in the following sponsor's table.

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Tab. 3.1. Displacement of various radioligands by 5-HT, SDZ HTF 919, ——— cisapride, and SDZ 216-454, respectively. Affinities for the individual receptors are expressed as pK_D values (n = 3).

Compound	5-HT_{1A}	5-HT_{1C}	5-HT_{1D}	5-HT_2
5-HT	8.51	7.69	8.44	5.53
SDZ HTF 919	6.49	7.17	7.70	5.60
———	4.91	5.38	< 4	4.69
Cisapride	5.72	6.30	5.26	8.14
SDZ 216-454	7.39	7.95	8.13	6.65

In brief, HTF 919 has moderate affinity for 5-HT_{1A}, 5-HT_{1C}, 5-HT_{1D} but not for 5-HT_2 receptors. In contrast, ——— showed negligible affinity for all these receptors. Cisapride has very high affinity for 5-HT_2 receptors and 216-454 displayed substantial affinities for 5-HT_1 receptors. HTF 919 was also demonstrated to be a partial agonist for 5-HT_{1D} receptors in the pig anterior cerebral artery (pD_2 = ~6.3 and intrinsic activity = 0.13) and a competitive antagonist for 5-HT_{1C} receptors (pK_B = ~6.9) in pig choroid plexus cells. However, the physiological or pathological roles of activation of these receptors and their clinical relevance are not clear at present. HTF 919 is almost devoid of affinity for 5-HT_3, histaminergic (H_1), α-adrenergic, dopamine (D_1 and D_2), opiate, and muscarine receptors.

2. *In Vivo* Studies:

The pharmacological activities of HTF 919 were also characterized in the *in vivo* preparations including rats, cats, guinea pigs and dogs. It was demonstrated that intravenous administration of HTF 919 significantly increased the basal lower esophageal sphincter (LES) pressure in anesthetized cats at 0.03 and 0.1 mg/kg. For example, it increased the pressure ~13.3 ± 4.5 Torr at 0.1 mg/kg. In contrast, cisapride and ——— had no effects on the pressure at doses up to 0.32 mg/kg. At 1 mg/kg, ——— moderately increased the pressure, whereas cisapride was inactive. HTF 919 significantly increased the gastric emptying rate of solids following p.o. and i.p. administrations in rats. This significant increase was observed only at 0.1 mg/kg and both higher and lower doses were without any effects. At 0.1 mg/kg,

HTF 919 increased the gastric emptying rate to ~70% (p.o.) or ~90% (i.p.) from the control (~20%). Intraperitoneal application of ——— and 216-454 also significantly increased the rate of gastric emptying and this effect was similar to that observed with HTF 919. The effects of HTF 919 on the gastric emptying of liquids were examined in guinea pigs. HTF 919 significantly increased the gastric emptying of liquids (ED_{50} = 0.1 µg/kg i.p.). In contrast, ——— (ED_{50} = 0.44 mg/kg, i.p.) was ~4000 times less potent than HTF 919. Intraperitoneal administration of HTF 919 significantly stimulated the small intestinal motility in guinea-pigs with ED_{50} = 0.1 µg/kg. This was ~130 times more potent than ——— (ED_{50} = 440 µg/kg). HTF 919 also significantly increased the small intestinal motility in dogs at concentrations > 0.1 mg/kg s.c. or 0.3 mg/kg p.o. and the stimulatory effect lasted for 2-5 hours after administration of HTF 919. The effects of HTF 919 on the large intestinal motility were investigated in an experimental model of the impaired colonic motor activity (treated with lidamidine, an α_2 receptor agonist) in mice. Intraperitoneal administration of HTF 919 at 0.01 mg/kg significantly reduced the transit time from 183 minutes to 135 minutes in this model. In contrast, 216-454 showed very similar effects, whereas cisapride and ——— were inactive. The results suggest that HTF 919 can improve the impaired colonic motor activity in this model.

Colonic transit of ^{111}In-labeled pellets was determined using ——— following intravenous administration of HTF 919 in conscious dogs. The results indicated that HTF 919 significantly increased the colonic transit at dose of 0.03 mg/kg as early as the first hour of dosing.

HTF 919 at doses up to 0.1 mg/kg i.p. had no effect on basal gastric acid secretion in rats and at doses up to 0.1 mg/kg s.c. or 3 mg/kg p.o. had no effect on the bethanechol-stimulated gastric acid secretion in dogs.

The mechanism responsible for the prokinetic effect of HTF 919 is not clear at present. It has been proposed that as a 5-HT_4 receptor partial agonist, HTF 919 can stimulate release of acetylcholine via activation of 5-HT_4 receptors located in the nerve terminals on both cholinergic interneurones and motor neurones in GI tract and subsequently induce peristalsis.

Peristaltic reflex of the colon is mediated by intrinsic pathway (via 5-HT_4 receptor within the gut) and

extrinsic pathway. The extrinsic pathway is mediated by sensory neurons projecting through spinal cords responding to stretch (e.g. gut distension) which elicits sensations such as fullness, urge to defecate, discomfort, and pain. To investigate the effects of HTF 919 on the extrinsic pathway, the firing rate of spinal rectal afferents was measured following intravenous administration of HTF 919 in decerebrated cats. HTF 919 significantly reduced the balloon pressure-increased firing rate with ED_{50} of 0.7 mg/kg. Effects of HTF 919 on the colo-rectal sensitivity were also determined in rats. In this study, colo-rectal distension was produced by increasing rectal pressure by insertion of balloon into the rectum and the number of abdominal contractions (a criterion of pain) were recorded following intraperitoneal (i.p.) administration of HTF 919. The results indicated that HTF 919 at i.p. doses of 0.1 and 0.3 mg/kg significantly reduced the abdominal contractions (11-12 contractions/5 min) induced by low pressure of colorectal distension of 15 mmHg as compared to the control (16-17 contractions/5 min), suggesting that HTF 919 possesses an antinoceptive effect on pain threshold. These actions would be therapeutically useful in the alleviation of the symptoms of constipation-prone irritable bowel syndrome such as abdominal pain and discomfort, and constipation.

Secondary Activity

1. Central Nervous System (CNS): The effects of HTF 919 on CNS were tested in mice and rats. Oral administration of HTF 919 at 32 mg/kg slightly increased the activity and reduced the abdominal tonus in mice. In the acute toxicity studies in mice and rats (see below), HTF 919 at ≥ 100 mg/kg p.o. or 5 mg/kg i.v. produced sedation.

2. Cardiovascular System: An *in vitro* study was conducted to assess the effects of HTF 919 on cloned HERG channels expressed in HEK293 cells (mammalian cells). These cells (HEK293) were treated with HTF 919 at 1, 5, 10, and 50 μM. The results indicated that HTF 919 inhibited the HERG channel with IC_{50} of 13 μM. In contrast, cisapride produced almost complete inhibition at 2 μM with IC_{50} of 44 nM. HTF 919 had no significant effects on action potential duration, amplitude and maximum rate of depolarization, and diastolic membrane potential recorded from isolated guinea pig ventricular papillary muscle at 10, 100 and 1000 nM. In another *in vitro* study, rabbit hearts were removed

and placed in a Langendorff apparatus and ECG was recorded. The perfused isolated hearts were then treated with HTF 919 at 0, 0.5, 1, 5, 10, and 50 μM. QT intervals measured from ECG recordings were 230±4, 226±2, 233±4, 233±7, 233±8, and 259±19 ms at 0, 0.5, 1, 5, 10, and 50 μM, respectively. HTF 919 had no effects on QT interval at concentrations up to 10 μM and prolonged QT interval by ~12% at concentration of 50 μM (1 μM = 417.5 ng/ml). The concentration of HTF 919 at 50 μM (~21 μg/ml) is much higher than the therapeutic plasma level (~6 ng/ml) following oral dose of 12 mg/day. The main human metabolite (5-methoxyindole-3-carboxylic acid glucuronide) had no effects on QT interval at concentrations up to 50 μM. In contrast, cisapride increased QT interval by 15% at concentration of 0.1 μM and erythromycin prolonged QT interval by 15% at 100 μM.

In an *in vivo* study in rats, intravenous administration of HTF 919 at doses of 0.01 and 0.1 mg/kg had no effects on blood pressures, heart rate, left ventricular dp/dt, cardiac output and total peripheral resistance in anesthetized rats. ECGs were not affected at these doses. However, HTF 919 at 1 mg/kg decreased systolic (~9-15%), diastolic (~13-20%) and mean arterial blood pressures (~10-17%) and total peripheral resistance (~16-19%), and produced negative dp/dt (~6-19%). Further investigation on vascular tone of rat thoracic aorta indicated that HTF 919 had no effects on basal tone of the rat thoracic aorta at concentrations up to 10 μM. However, it inhibited the contraction induced by phenylephrine (0.5 μM) or by norepinephrine (0.5 μM) in a dose dependent manner with IC_{50} of 62.1±7.2 nM (phenylephrine) or 640.5±23.1 nM (norepinephrine). In anesthetized dogs, intraduodenal administration of HTF 919 had no effects on blood pressure, respiration rate, heart rate, femoral arterial blood flow, and ECG at 0.1, 1, and 10 mg/kg. In conscious dogs, single oral doses of HTF 919 had no clear treatment related effects on heart rate, blood pressure, and ECG at 0.3, 4, and 10 mg/kg. Plasma levels of HTF 919 were determined in this study. Maximum plasma levels of HTF 919 following 0.3, 4, and 10 mg/kg were 16.9, 138, and 401 ng/ml (males) or 3.62, 104, and 277 ng/ml (females), respectively. AUC_{0-24hr} values of HTF 919 following 0.3, 4, and 10 mg/kg were 49.9, 374, 1110 ng.h/ml (males) or 6.71, 345, and 924 ng.h/ml (females), respectively.

3. Endocrine System: Subcutaneous injection of HTF 919 at doses of 0.01, 0.1, 1 and 10 mg/kg did not have any effect on luteinizing hormone (LH), growth hormone (GH), testosterone and

glucose levels in rats. HTF 919 had no effects on corticosterone level at doses < 10 mg/kg and on prolactin secretion at doses up to 0.1 mg/kg. However, HTF 919 increased corticosterone level ~100% at 10 mg/kg and inhibited prolactin secretion with an ID_{50} ≈ 10 mg/kg.

In summary, the preclinical pharmacological studies demonstrated that HTF 919 is a 5-HT_4 receptor partial agonist with pD_2 = 6.97 and intrinsic activity = 0.22 (5-HT's intrinsic activity = 1) in guinea pig ileum. The *in vitro* studies showed that HTF 919 stimulated peristaltic movements of the longitudinal muscle layer and inhibited the circular muscle activity in isolated guinea pig ileum and colon. The *in vivo* studies revealed that HTF 919 stimulated gastric empty of solids in rats and liquids in guinea pigs and accelerated the small intestinal transit and motility in guinea pigs and dogs and the large intestinal motility in mice. It was also demonstrated that HTF 919 significantly reduced the balloon pressure-increased firing rate of spinal rectal afferents with ED_{50} of 0.7 mg/kg (i.v.) in decerebrated cats and the abdominal contractions (11-12 contractions/5 min) induced by low pressure of colorectal distension of 15 mmHg at i.p. doses of 0.1 and 0.3 mg/kg in rats, suggesting that HTF 919 possesses an antinoceptive effect on pain threshold. These actions would be therapeutically useful in the alleviation of the symptoms of constipation-prone irritable bowel syndrome such as abdominal pain and discomfort, and constipation.

ABSORPTION, DISTRIBUTION, METABOLISM AND EXCRETION (ADME):

MOUSE:

Absorption and disposition of [^{14}C] SDZ HTF 919 after single oral and intravenous doses in male mice
(R98-1266-02)

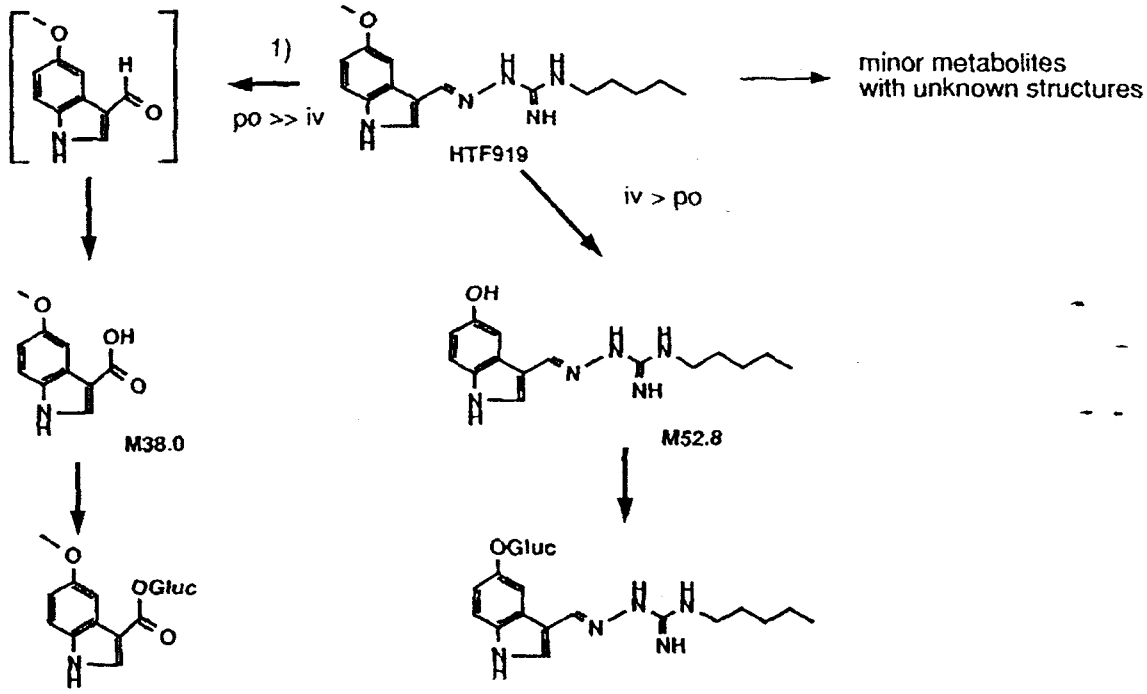
Methods: To study the absorption and disposition of HTF 919 in mice, single oral and i.v. doses of ^{14}C-HTF 919 were given to male mice. The oral doses were 20 mg/kg (by gavage). The intravenous dose was 2 mg/kg. Specific activity was 5.2 MBq/mg (i.v.) or 0.73 MBq/mg (oral). The radioactivity was measured by [redacted] The parent drug and its metabolites were determined using [redacted]

Results:

Absorption: The absorption of HTF 919 following oral dose was relatively quick (time to peak concentration, T_{max}, = 0.5-3 hours as measured by radioactivity). The percentage of absorption was 20-30% and the oral bioavailability was ~8%. Maximal plasma level of the parent drug was 356 pmol/ml (107 ng/ml) detected at 1 hour after oral dose. The plasma level of the parent drug was 2545 pmol/ml detected at 5 minutes following i.v. dose and the parent drug declined quickly with half life of 2.15 hours.

Tissue distribution: Following i.v. dose, volume of distribution and clearance were 12.9 l/kg and 0.12 l/hr, respectively.

Metabolism: The metabolic pathways following both oral and i.v. routes were depicted in a figure on page 191 in volume 1.46 and this figure is attached below.



1) mainly acid-catalyzed hydrolysis in the stomach (po)

Following oral dose, the metabolism of HTF 919 mainly involved an imine cleavage to an intermediary aldehyde which was catalyzed by gastric acid in stomach, and oxidized to the acid M38.0 and partially glucuronidated to M29.0. The metabolites M29.0 and M38.0 accounted for 57% of the AUC_{0-8hr} of radioactivity in plasma. Following i.v. dose, however, the major metabolic pathway was demethylation of HTF 919 to M52.8 and subsequent glucuronidation to M40.6. The metabolites M40.6 and M52.8 accounted for ~21% of the AUC_{0-8hr} of radioactivity in plasma. The plasma levels of parent compound and metabolites following oral and i.v. doses of HTF 919 were presented in Tables 5 and 6 on pages 207 and 208 in volume 1.46. These tables are attached below.

Table 5: Concentrations of metabolites and HTF919 in plasma after po dose

Plasma concentrations of metabolites and HTF919 (expressed as pmol/ml) in male mice following a 20 mg/kg po dose of [^{14}C]HTF919-hml; pools of three animals

Sample collection time [h]	0.5	1	3	8	0-8h
Compound/Peak	Concentration [pmol/ml]				AUC_{0-8h}[a] [pmol*h/ml]
Front peak	22	15	75	86	507
M29.0	665	684	1162	815	7291
M38.0	45	82	34	42	349
M40.6	788	642	275	51	2286
HTF919 (parent compound)	249	356	155	20	1161
Sum of not assigned or trace peaks	404	291	273	109	1792
Total RA-concentration[b]	2173	2070	1973	1123	13387

[a] calculated using trapezoidal rule up to last time point measured; concentrations C_o at time zero (t_o) were taken as zero.

[b] Recovery from sample preparation and ——— were ~100%.

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Table 6: Concentrations of metabolites and HTF919 in plasma after iv dose

Plasma concentrations of metabolites and HTF919 (expressed as pmol/ml) in male mice following a 2 mg/kg iv dose of [^{14}C]HTF919-hml; pools of three animals

Sample collection time [h]	0.083[a]	1	3	8	0-8h
Compound/Peak	Concentration [pmol/ml]				AUC_{0-8h}[b] [pmol*h/ml]
Front peak	n.d.	38	9	30	160
M29.0	1407	205	20	7	1089
M38.0	337	22	19	9	289
M40.6	1051	216	43	12	1020
M52.8	n.d.	49	9	5	117
HTF919 (parent compound)	2545	70	30	7	1601
Sum of not assigned and trace peaks	924	264	51	27	1092
Total RA-concentration[c]	6263	863	180	97	5523

a) M29.0 and M38.0 are mainly due to ——— present as an impurity in the formulation ——of dose by mol for 0.083 h group, ___for the other groups).

b) calculated using trapezoidal rule up to last time point measured; concentrations C_0 at time zero (t_0) were taken equal to conentrations at time 0.083 min (for HTF919 and total RA) or taken as zero (for metabolites).

c) Recovery from sample preparation and —— were ~100%.

n.d.: not detected

Excretion: The total radioactivity recovered in feces and urine accounted for 94.2% (oral) or 87.2% (i.v.) of the dose administered. Following oral dose 70.3% of the dose given was recovered in feces and 24% in urine. Following i.v. dose 65% of the dose given was recovered in feces and 22.2% in urine.- -

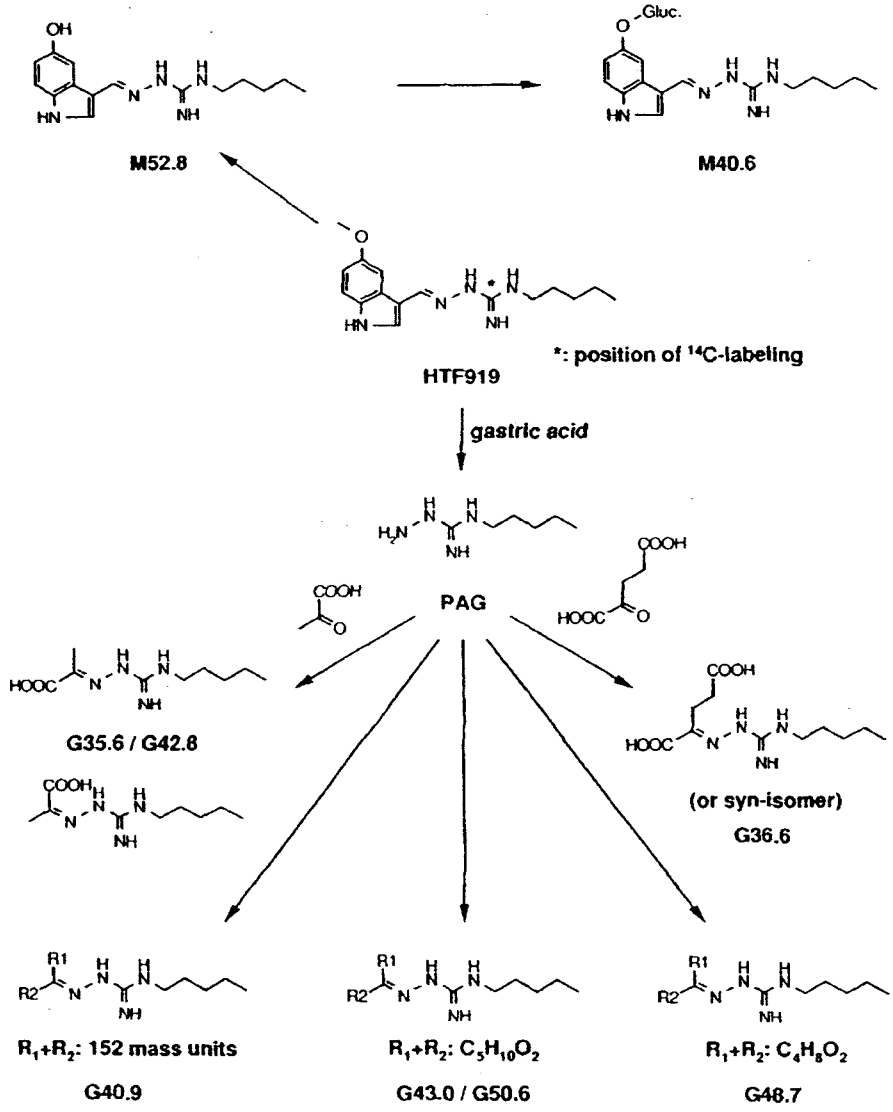
Metabolism of HTF 919 in male mice after oral dose of [^{14}C] SDZ HTF 919
(R98-2187)

Methods: To study the metabolism of HTF 919 in mice, single oral dose of ^{14}C-HTF 919 were given to male mice at 20 mg/kg (by gavage). HTF 919 was labeled at the guanidine carbon atom. Specific activity was 4.12 MBq/mg. The radioactivity was measured by ——— The parent drug and its metabolites were determined using ———

Results: The metabolites M52.8 and M40.6 by demethylation of HTF 919 were also found in this study. In addition, a new metabolic pathway was identified which was depicted in Figure 6 on page 44 in volume 1.46. This table is attached below.

Figure 6: Proposed metabolite structures and biotransformation pathways

Shown are only metabolites which appeared in the ——— (i.e. which contain the labeled carbon atom). PAG: pentylaminoguanidine (N'-amino-N-pentylguanidine); Gluc.: glucuronyl



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In the new metabolic pathway, pentylaminoguanidine (PAG) was formed by hydrolytic cleavage of HTF 919 in stomach. A number of the condensation products of PAG were identified (G35.6, G42.8, G40.9, G43.0, G50.6, G48.7, and G36.6). G35.6 and G42.8 were isomeric condensation products of PAG with pyruvate.

RAT:

Absorption and disposition of [^{14}C] SDZ HTF 919-hml after single and multiple oral and single intravenous doses in rats
(303-120)

Methods: To study the absorption and disposition of HTF 919 in rats, single and multiple oral doses (gastric intubation and in feed) and a single i.v. dose of HTF 919 were given to rats. The oral doses were ~60 mg/kg (by gastric intubation) and 46 mg/kg (achieved dose in feed). The intravenous dose was 3 mg/kg. The drug was labeled with ^{14}C at indole-3-carboxaldehyde with specific activity of 136.5 or 138.3 μCi/mg. Following administration of the test compound, blood, bile, urine and feces were collected at various time intervals. The radioactivity was measured by [redacted] The parent drug and its metabolite (SDZ 216-454) were determined using [redacted] method. The tissue distribution was determined using [redacted] The percentage of absorption (f_a) and bioavailability (f) of HTF 919 were calculated using the following equation:

$$f_a \text{ or } f = \frac{AUC_{p.o.}}{AUC_{i.v.}} \times \frac{dose_{i.v.}}{dose_{p.o.}}$$

The radioactivity and parent drug data were used to determine f_a and f, respectively. Other parameters were calculated from the parent drug data using the following equations:

(1). Total clearance (Cl_T) = $dose_{i.v.}/AUC_{i.v.}$
(2). Apparent volume of distribution (V_z) = Cl_T/λ_z
(3). First-pass effect (f_m) = $(f_a - f)/f_a$

λ_z is the rate constant of the terminal elimination.

Results:

Absorption: HTF 919 was slowly absorbed (time to peak concentration, T_{max}, ≈ 10 hours as measured by radioactivity). The percentage of absorption was ~32% and the oral bioavailability was ~8%. Elimination of the drug measured by its radioactivity (distribution phase $T_{1/2}$= 0.9 hours, elimination phase $T_{1/2}$= 30 hours) was longer than that of the parent drug (distribution phase $T_{1/2}$= 0.1 hours, elimination phase $T_{1/2}$= 2 hours). The maximum plasma level (C_{max}) of radioactivity was 603 ± 187 and 524 ± 24 ng equiv./ml following oral (60 mg/kg) and i.v. (3 mg/kg) administrations, respectively.

Tissue distribution: The apparent volume of distribution (V_z = Cl_T/λ_z) was 17.5 ± 1.3 l/kg and the tissue to plasma ratio of parent drug and radioactivity were >20:1 and >2:1, respectively, suggesting that the parent drug and its metabolites are extensively distributed extravascularly. The tissue distribution patterns are similar in the male and female rats and the highest radioactivity was found in the kidney, intestine and stomach (the presumed site of the pharmacological action of the drug) and blood vessels. The total body clearance of HTF 919 was 1.8 ± 0.2 l/h (~5.5 l/kg/h).

Metabolism: HTF 919 was rapidly and extensively metabolized and its unconjugated 5-O-demethyl derivative (SDZ 216-454 or M52.8) was identified and it is only a minor biotransformation product. SDZ 216-454 is also a potent 5-HT_4 receptor partial agonist with intrinsic activity of 0.6 (intrinsic activity of 5-HT and HTF 919 = 1 and 0.22, respectively).

Excretion: The radioactive metabolites of HTF 919 were excreted mainly via the bile into the feces. About 70-82% of the administered radioactive dose was recovered in the feces and ~12-20% in urine.

The summary data were presented in the sponsor's table on page 8-2918 in Volume 1.9. This table is attached below.

ADMINISTRATION		i.v.		oral suspension	
DOSE (mg-hml/kg)		3		60	
PARAMETERS		Mean ±	SD	Mean ±	SD
Plasma radioactivity					
C(0), C_{max}	[ng-eq/ml]	524	24	603	187
t_{max}	[h]			10	10
AUC	[ng-eq.h/ml]	2481	739		
AUC (0-24h)	[ng-eq.h/ml]	1280	227	8102	1312
$t_{\frac{1}{2}\lambda 1}$	[h]	0.9	0.6		
$t_{\frac{1}{2}\lambda z}$	[h]	30	14		
Plasma parent drug					
C(0)	[ng/ml]	509	107		
AUC	[ng.h/ml]	377	33		
AUC (0-7h)	[ng.h/ml]	259	20	392	
$t_{\frac{1}{2}\lambda 1}$	[h]	0.1	0		
$t_{\frac{1}{2}\lambda z}$	[h]	2.1	0.1		
Disposition parameters					
Absorption	(blood) %			24	
	(plasma) %			32	
	f_a %			28	
Bioavailability (f)	%			≤8	
First-pass effect (f_g)	%			≥71	
CL	[l/h]	1.8	0.2		
V_z	[l/kg]	17.5	1.3		
Excretion (radioactivity)					
Urine (0-24h)	%	16.6	1.2	7.3	3.0
(0-72h)	%	19.7	1.8	12.3	0.6
Feces (0-24h)	%	30.3	19.6	18.7	23.9
(0-72h)	%	70.2	9.0	82.1	11.1

Absorption and disposition of [^{14}C] SDZ HTF 919-hml after single oral and intravenous doses in rats
(1997/077)

Methods: To study the absorption and disposition of HTF 919 in rats, single oral and i.v doses of ^{14}C-HTF 919 were given to rats at 15 mg/kg and 3 mg/kg, respectively. The drug was labeled with

^{14}C at indole-3-carboxaldehyde with specific activity of 140.4 μCi/mg. Following administration of the test compound, blood, bile, urine and feces were collected at various time intervals. The radioactivity was measured by [redacted] The parent drug and its metabolites were determined using [redacted] method. The tissue distribution was determined using [redacted]

Results:

Absorption: HTF 919 was absorbed with T_{max}, time to peak concentration, ≈ 1-2 hours. The percentage of absorption was ~19-28% (radioactivity). The oral bioavailability of HTF 919 was low (2-22%). The maximum plasma level (C_{max}) of radioactivity was 181 ± 68 ng equiv./ml following oral and C_{max} for HTF 919 was 64 ± 31 ng/ml. The radioactivity declined bi-phasically with half life of 2.6 hours (distribution phase) or 15.3 hours (termination phase).

Tissue distribution: The highest level of radioactivity was found in the kidney, adrenals, liver, and stomach. At ~48 and 72 hours after dosing, radioactivity was still detected in the liver, kidney, skin, and stomach.

Metabolism: The metabolic pathway was depicted in a figure on page 153 in volume 1.46 and this figure is attached below.

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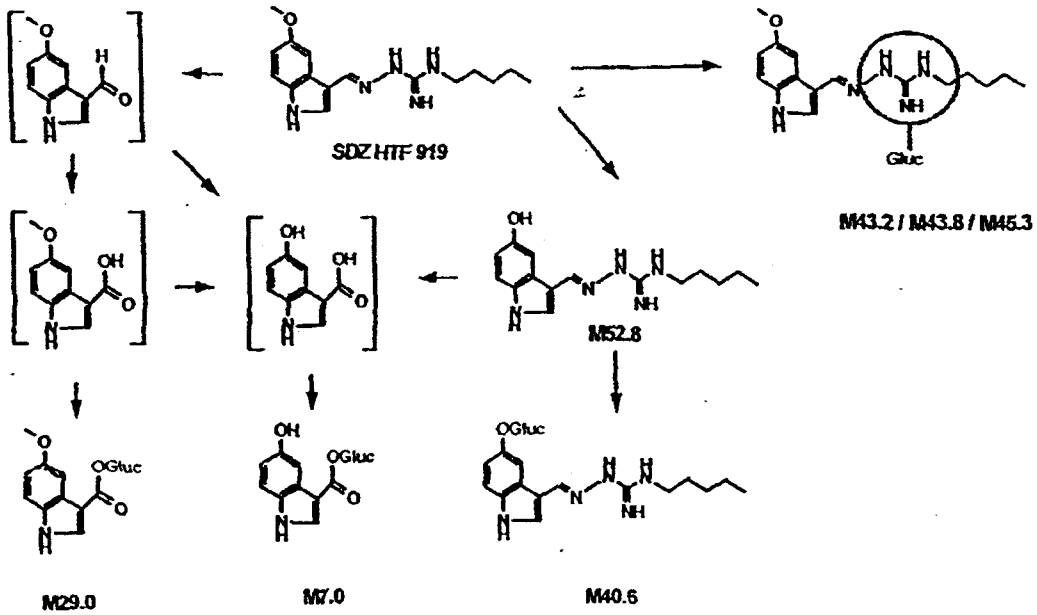

The metabolism of HTF 919 was characterized by an imine cleavage by gastric acid in stomach to subsequently formation of M29.0 and M7.0. The major systemic metabolic pathway was O-demethylation with glucuronidation to M52.8 and to M40.6. A further systemic metabolism included a direct N-glucuronidation of the parent compound leading to metabolites M43.2, M43.8, and M45.3.

Excretion: About 10% and 95% of the administered radioactive dose was recovered in the urine and in feces, respectively, following oral dose. After i.v. dose, majority of the radioactivity was detected in the bile (70.5%).

Tissue Distribution in Rats Following a Single Intravenous Doses of [^{14}C] SDZ HTF 919-hml
(303-209)

Methods: To study the tissue distribution of HTF 919 in rats, a single i.v dose of ^{14}C-HTF 919 was given to rats at 3 mg/kg. The drug was labeled with ^{14}C at indole-3-carboxaldehyde with specific activity of 140.4 µCi/mg. Following administration of the test compound, the radioactivity in the blood and tissue were determined using [redacted] method.

Results: The highest level of radioactivity was detected at 1 hour after dosing in the thyroid gland, followed by renal cortex,

adrenal, brown fat, renal medulla, heart, and liver. The radioactivity declined quickly below the quantification limit ——— in most tissue at 72 hours after dosing except in the adrenal, liver, renal medulla and cortex, pituitary, and lacrimal gland. The radioactivity level in the blood was much lower than those detected in the tissues.

Tissue Distribution Study in Eyes (melanin) after oral and intravenous doses of [^{14}C] SDZ HTF 919-hml (hydroxymaleate salt) in albino and pigmented rats
(303-245)

Methods: To study the affinity of HTF 919 to melanin, single oral and i.v doses of ^{14}C-HTF 919 were given to pigmented and albino rats at 15 mg/kg and 3 mg/kg, respectively. The drug was labeled with ^{14}C at indole-3-carboxaldehyde with specific activity of 1.36 MBq/mg. Following administration of the test compound, the radioactivity in the whole eyes was measured by ——— method. Tissue distribution of the test drug was also determined using ———

Results: The results indicated that the radioactivity was detected in the ocular membranes in both pigmented and albino rats at 5 minutes after dosing. The level of radioactivity in the ocular membranes was similar to that in the blood.

Passage of [^{14}C] SDZ HTF 919 into Rat Milk after Single Oral Administration
(Report # ——— R98-1298)

Methods: To study passage of [^{14}C] SDZ HTF 919-hml into the milk, lactating rats were given a single oral dose (gavage) of [^{14}C] SDZ HTF 919 (specific activity = 101.7 KBq/mg) at 60 mg/kg. Milk and blood samples were collected at 1, 2, 4, 6, 8, and 24 hours after dosing (4 rats/sampling time). Oxytocin was injected intravenously (1 IU/rat) 5 minutes before each collection of milk. Approximately 1 ml milk per rat was collected from each collection. Right after milking, blood was collected from vena cava. The radioactivity was determined using ——— The parent drug and its metabolites (glucuronide) in time pooled milk and plasma samples were analyzed using ———

Results: The results indicated that the peak level of radioactivity was detected at 6 hours after dosing in both plasma and milk and the ratio of milk to plasma $AUC_{0-24hours}$ (radioactivity) was 3, suggesting that a substantial amount of HTF 919 passed into milk in the lactating rats. The parent drug and its glucuronide represented 14% and 63% of the total radioactivity in the plasma. In contrast, the parent drug accounted for 2/3 of the total radioactivity and only small amount of glucuronide was detected in the milk. The level of the parent drug in the milk was 129, 1843, and 2194 ng.equ./ml at 2, 4, and 8 hours, respectively. The level of the parent drug in the plasma was 71, 114, 66 ng.equ./ml at 2, 4, and 8 hours, respectively. The ratio of milk to plasma concentration of parent drug was 1.8, 16, 33 at 2, 4, and 8 hours, respectively.

A study on permeability of [^{14}C] SDZ HTF 919-hml via blood-brain barrier was conducted in rats following a bolus injection in carotid artery (303-205). The results indicated that the percentage of HTF 919 extracted by the brain was very low (~2%).

DOG:

Absorption and disposition of [^{14}C]SDZ HTF 919 after single oral and intravenous doses in dogs
(303-151)

Methods: To study the absorption and disposition of HTF 919 in dogs, single oral (by ——— or gavage) and i.v. doses of ^{14}C-HTF 919 were given to dogs. The oral doses were ~15 mg/kg (by ———) or 8.6 and 9.9 mg/kg (by gavage). The intravenous dose was ~1.0 mg/kg. The drug was labeled with ^{14}C at indole-3-carboxaldehyde with specific activity of 136.5 or 138.3 µCi/mg. The radioactivities in the blood, plasma, urine and feces were measured by ——— The parent drug and its metabolite in plasma and urine were measured using ——— ——— method.

Results:

Absorption: Following a single oral administration of HTF 919 in dogs, the peak concentrations of the drug (radioactivity) were reached within 2 hours. The percentage of absorption of HTF 919 was ~52-62% and its oral bioavailability was 22-27%. Following oral and i.v. administrations of HTF 919, the plasma or blood

concentrations of the parent drug and radioactivity decreased bi- or triphasically. Its terminal half live measured by its radioactivity in plasma ($T_{1/2} \approx$ ——— hours) was longer than that of the parent drug ($T_{1/2} \approx$ ——— hours), suggesting that metabolites have longer elimination half life. C_{max} of parent drug were 416 and 1946 ng equiv./ml following oral (15 mg/kg) and i.v. (1.0 mg/kg) administrations of HTF 919, respectively.

Distribution: HTF 919 and its metabolites were moderately associated with the blood cells (~34%). The tissue distribution was extensive as indicated by its mean apparent volume of distribution (12.4 ± 2.7 l/kg). The total body clearance of HTF 919 was 13.6 ± 1.1 l/h (~ 1.0 l/kg/h).

Metabolism: 5-O-demethyl derivative (SDZ 216-454) was only a minor biotransformation product.

Excretion: The parent drug and its metabolites were excreted mainly via the bile into the feces. About 62-75% of the administered radioactive dose was recovered in the feces and ~21-25% in urine.

The summary data were presented in the sponsor tables on pages 8-2965 and 8-2965 in Volume 1.9. These tables are attached on the following pages.

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	oral	oral (sol.)	i.v.
Dose (mg/kg)	15.0	8.6-9.9	1.0
PARAMETERS	**MEAN**	**MEAN**	**MEAN ± SD**
Blood radioactivity			
C_{max}; C(0) [ng-eq.ml^{-1}]	1795	766	2250 ± 213
nC_{max};nC(0)* [ng-eq.ml^{-1}]	166	115	3124 ± 296
t_{max} [h]	2.0	2.0	
AUC [ng-eq.h.ml^{-1}]	28395	24713	4253 ± 302
nAUC* [ng-eq.h.ml^{-1}]	2622	3716	5907 ± 419
$t_{½a}$ [h]	0.3	0.5	
$t_{½λ1}$ [h]	1.1		0.1 ± 0
$t_{½λ2}$ [h]	6.5	3.4	9.3 ± 5.2
$t_{½}$ [h]	75	71	74 ± 6
Plasma radioactivity			
C_{max}; C(0) [ng-eq.ml^{-1}]	2927	1000	1834 ± 264
nC_{max};nC(0)* [ng-eq.ml^{-1}]	270	151.1	2547 ± 366
t_{max} [h]	1.5	2.0	
AUC [ng-eq.h.ml^{-1}]	39041	27991	5058 ± 425
nAUC* [ng-eq.h.ml^{-1}]	3605	4209	7025 ± 591
$t_{½a}$ [h]	0.4	0.7	
$t_{½λ1}$ [h]	0.9		0.1 ± 0
$t_{½λ2}$ [h]	6.5	3.0	7.6 ± 0.6
$t_{½}$ [h]	88	77	75 ± 4
Plasma parent drug			
C_{max}; C(0) [ng.ml^{-1}]	416	248	1946 ± 314
nC_{max}; nC(0)* [ng.ml$_{.}$]	38	36.0	2702 ± 435
t_{max} [h]	2.0	0.5	
AUC [ng.h.ml^{-1}]	2787	1419	730 ± 54
nAUC* [ng.h.ml^{-1}]	258	213	1014 ± 75
$t_{½a}$ [h]	0.7	0.5	
$t_{½λ1}$ [h]			0.08 ± 0.02
$t_{½λ2}$ [h]	0.9	1.1	0.6 ± 0.2
$t_{½}$ [h]	5.7	6.7	8.6 ± 1.5

* nC_{max}, nC(0), nAUC = C_{max}, C(0), AUC normalized to 1 mg/kg dose
Note: only mean values are shown when n = 2

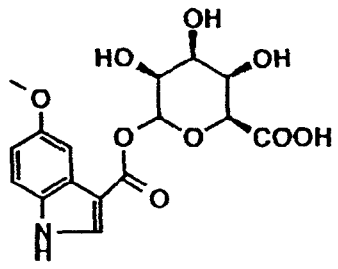
	oral	oral (sol.)	i.v.
Dose (mg/kg)	15.0	8.6-9.9	1.0
PARAMETERS	MEAN	MEAN	MEAN ± SD
Disposition parameters			
Absorption [%]	52	62	
Bioavailability f [%]	27	22	
First-pass f_m [%]	49	63	
Clearance Cl [l/h]			13.6 ± 1.1
Volume distr. V_z [l/kg]			12.4 ± 2.7

Excretion of radioactivity (% of dose)

	oral	oral (sol.)	i.v.
urine 0-24 h	18.2	19.6	16.6 ± 2.3
urine 0-96 h	20.7	24.6	23.6 ± 0.8
feces 0-96 h	75.1	62.0	69.0 ± 2.8
Total 0-96 h	95.7	86.6	92.6 ± 3.4

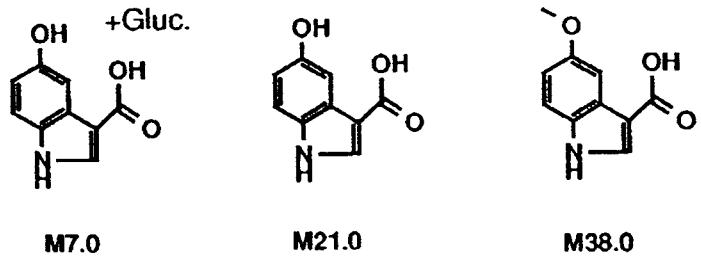
Note: only mean values are shown when n = 2

Addendum: In a separate report (report #R98-1438-01), sponsor identified metabolites (M29.0, M7.0, and M38.0) in the urine obtained from the above pharmacokinetic study in dogs. Metabolite M29.0 identified in both dog and human urine was acid glucuronide of 5-methoxy-indole-3-carbocylic acid (see below).



M29.0

Metabolite M7.0 was the demethylated analogue of M29.0 and M38.0 was the corresponding aglycon of M29.0. A fraction containing M7.0 was treated with glusulase and M21.0 (aglycon of M7.0) was formed. The structures of M7.0, M21.0, and M38.0 were depicted on page 5-63 in volume 1.48 and this figure is attached below.



M7.0 M21.0 M38.0

Blood Distribution and Plasma Protein Binding of HTF 919 in human, dog, and rat blood
(303-214)

Methods: To determine the blood distribution and plasma protein binding of HTF 919 in human, rat, and dog blood, heparinized blood samples obtained from healthy volunteers, rats, and dogs were spiked with ^{14}C-HTF 919 at various concentrations (20, 200, 2000, and 20000 ng/ml). For blood distribution study, tubes with 2 ml blood containing ^{14}C-HTF 919 were incubated for 30 minutes at 37° C and then centrifuged for 15 minutes at 1600 g to separate plasma. The radioactivity was determined in both plasma and blood samples and the fraction of HTF 919 present in plasma was calculated. For protein binding study, 4 ml plasma samples containing ^{14}C-HTF 919 were incubated for 30 minutes at 37° C and then fraction of 1 ml plasma samples was used for equilibrium dialysis for 5 hours at 37° C. The radioactivity was then determined and the free drug in the plasma was calculated.

Results: The results indicated that ~66% of HTF 919 was found in the human blood cells (74% in rat blood cells and 57% in dog blood cells) at concentration of 20 ng/ml. The plasma protein binding of HTF 919 was high in all species (98.2% in humans, 97.8% in rats, and 97.5% in dogs).

Blood Distribution and Plasma Protein Binding of ^{14}C-HTF 919 in mice
(R99-2601)

Methods: To determine the plasma protein binding of HTF 919 in mouse blood, heparinized blood samples obtained from male mice were spiked with ^{14}C-HTF 919 at various concentrations (20, 200, and 2000 ng/ml). The spiked plasma samples were centrifuged at 200,000 g at 37° C and then the radioactivity was determined in the supernatant and in the samples before ultracentrifugation. The protein binding of the test drug was calculated using the following equations:

Bound (%) = 100 - Free

Free(%) = (Csu/Ci) x 100

Csu = radioactivity level in the supernatant
Ci = radioactivity level in the plasma sample

Results: The results indicated that the plasma protein binding of HTF 919 was high (96%) in mice.

Metabolism of HTF 919 by Human Liver and Intestine Slices
(303-226)

vol48,p105

Methods: To evaluate the metabolism of HTF 919 by the human liver and intestine, the liver and intestine slices were obtained from human tissue users and placed in culture media. Following an incubation period of 90 minutes, media containing 1 or 5 µM of ^{14}C-HTF 919 was added. The tissue slices were homogenated for determination of radioactivity using [redacted]

Results: The results indicated that in the liver slice the initial rate of ^{14}C-HTF 919 total metabolite formation was [redacted] pmol/hr/mg protein (2 slices). HTF 919-glucuronides (M43.2, M43.8, and M45.3) accounted for 32% of the total metabolites at 1 hour, 46% at 4 hours, and 50% at 18-24 hours of culture. In the intestine slices, HTF 919-glucuronides (M43.8 and M43.2) accounted for 43% of the total metabolites at 1 hour and 57% at 24 hour. The results suggested that HTF 919 was metabolized primarily to N-glucuronides in the human liver and intestine.

In Vitro Study on Metabolic Pathway of HTF 919 in Human Liver Microsomes
(303-225)

Methods: To investigate the oxidative and conjugative enzymes and/or degradative pathways for HTF 919 metabolism, human liver microsomes were prepared from frozen liver samples and incubated in incubation buffer with NADPH regenerating system and ^{14}C-HTF 919. The radioactivity was determined using

Results: The results indicated that HTF 919 was slowly metabolized by human liver microsomes (HL3). Approximately 20-30% of parent compound was converted to metabolite peaks. One of these peaks had the same retention time as O-demethyl standard (SDZ 216 454) and formation of this peak was decreased by ~75% in the presence of quinidine, a specific inhibitor of P450 CYP2D6, suggesting that CYP2D6 is responsible for the formation of this peak. Glucuronidation of SDZ 216-454 (O-demethyl) was demonstrated in the human liver microsomes. Direct glucuronidation of HTF 919 by UDP-glucuronyltransferase was also demonstrated.

Low pH (2-2.5) degraded HTF 919 and the degraded product was an aldehyde. The aldehyde cleavage product was oxidized by liver cytosolic aldeyde oxidase to carboxylic acid which was subsequently metabolized by UDP-glucuronyltransferase. Human leukocyte myeloperoxidase also converted HTF 919 to SDZ 216-454 and aldehyde, suggesting that the peroxidative pathway overlaps with low-pH pathway. The metabolic pathways demonstrated above were predicted in Figure 1 on page 143 in volume 1.48 and this figure is attached below.

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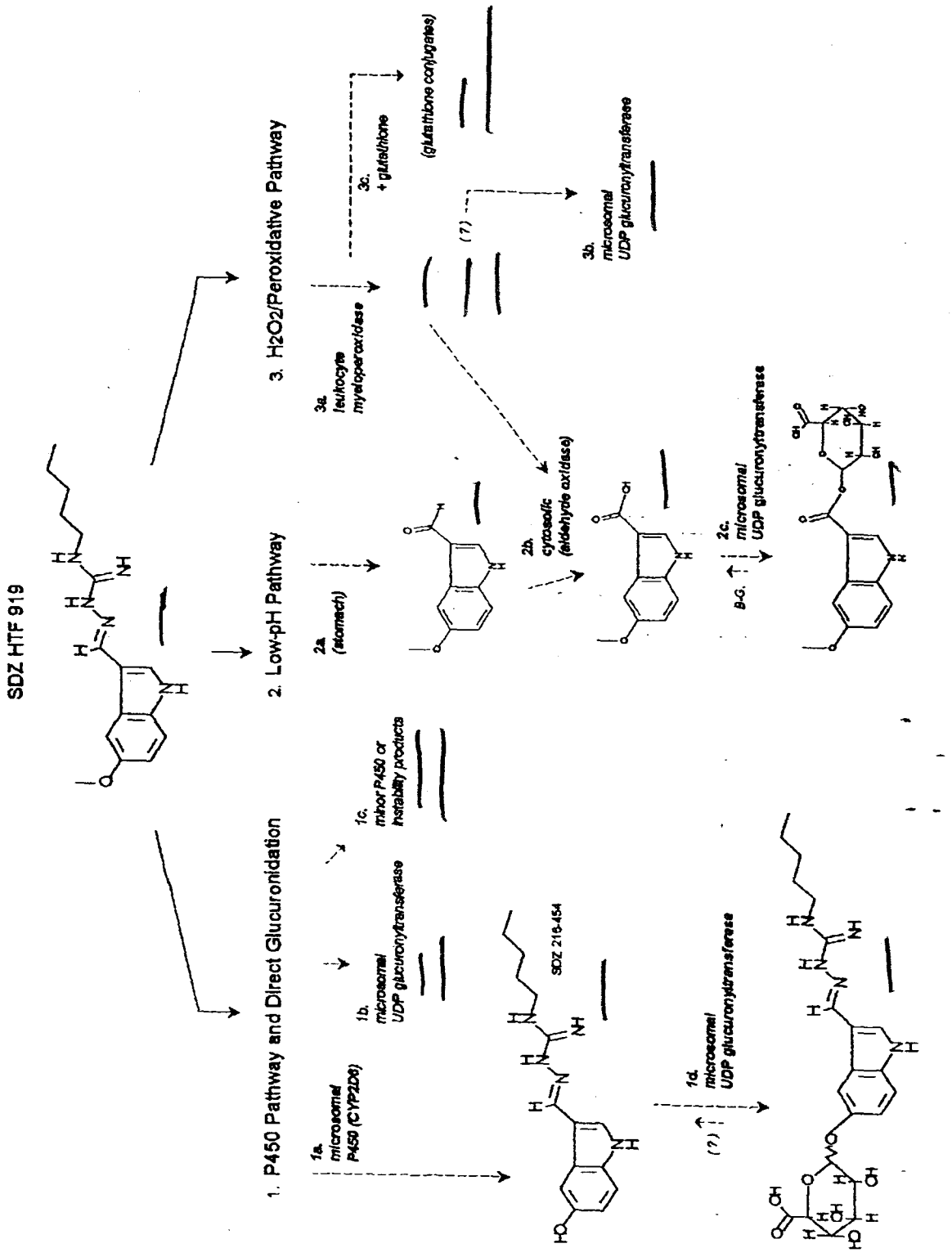
ON ORIGINAL

FIGURE 1: **A summary of metabolic pathways 1-3 observed for SDZ HTF 919 using microsomes and S9 enzyme fractions and myeloperoxidase.**

Abbreviations used: ~~~ with retention time in min. — — — method of ——— B-G, refers to B-glucuronidase enzyme.



O-Demethylation of HTF 919 in Rat and Human Liver Microsomal Incubations
(303-189)

Methods: To investigate the metabolism of HTF 919 by demethylation, rat and human liver microsomes were incubated at 37° C in incubation buffer with NADPH regenerating system and HTF 919 or SDZ 216-454 (50 μM) for up to 120 minutes. The levels of HTF 919 and its metabolites were determined using

Results: The results indicated that the metabolism of HTF 919 was slow and two metabolites were identified (O-demethylated SDZ 216-454 and an unknown metabolite). The initial rate of SDZ 216-454 formation in the rat liver microsomal incubations was 10.6 pmoles/min/mg protein or 20 pmoles/min/nmoles P-450. The SDZ 216-454 formation rate in the liver microsomes obtained from rats treated with phenobarbital (10 pmoles/min/mg protein) was similar to that in untreated rats (10.6 pmoles/min/mg protein), suggesting that phenobarbital inducible CYP-450 was not involved in the O-demethylation of HTF 919. In the human liver microsomal incubations, SDZ 216-454 formation rate was 5.4 pmoles/min/mg protein or 17.4 pmoles/min/nmoles p-450. The results suggested that HTF 919 is metabolized to its O-demethylated metabolite SDZ 216-454 in both rats and humans.

Effects of HTF 919 and its Main Metabolites, the glucuronide of 5-methoxyindole-3-carboxylic acid, on Human CYP450
(303-239)

Methods: To evaluate the effects of HTF 919 on human CYP450, human liver microsomes were incubated with NADPH regenerating system in the presence and absence of HTF 919 or HTF 919 acid glucuronide at 37° C. The CYP450 substrates and their metabolites were determined using

Results: The results indicated that HTF 919 had little effects on CYP2C8, 2C9, 2C19, 2E1, and 3A. HTF 919 inhibited CYP1A2 and CYP2D6 with Ki of <1 μM for phenacetin and bufuralol.

To compare the similarities and differences among the species including mice, rats, dogs and humans, the results of pharmacokinetic studies were summarized in the following table.

	Mouse	Rat	Dog	Human
Dosing, mg/kg	20, oral	60,gast. intub.	15, —	0.24[d], tablet
% absorption	20-30	32	52-62	-----
Bioavailability,%	8	8	22-27	11-12
T_{max}, hr	—	10[a]	1.5[a]	1.0[b]
C_{max}, ng equiv./ml	107 ng/ml[b]	603 ± 187[a]	416 ng/ml[b]	6.3±2.7 ng/ml[b]
Terminal $T_{1/2}$, hr	2.15 (i.v.[c])	30[a], 2[b] (i.v.[c])	88[a], 5.7[b]	7.7±4.5
Volume of distribution, l/kg	12.9	17.5±1.3 (i.v.[c])	12.4 ± 2.7	7.36[ce]
Clearance[g], l/h/kg	0.48	7.2 (i.v.[c])	1.36	1.54 (i.v.[c])
%[a] in feces	70.3	82.1 ± 11.1	75.1	58
%[a] in urine	24	12.3 ± 0.6	20.7	27

a = radioactivity, b = parent drug, c = i.v. dose at 2 mg/kg in mice, 3 mg/kg in rats, 3 mg in humans, d = 12 mg dose divided by 50 kg body weight (12/50 = 0.24 mg/kg)
e = Volume of distribution = 368 L divided by 50 kg body weight (368/50 = 7.36 l/kg)
g = Clearance (l/h) was normalized by body weight of 0.025 kg (mouse), 0.25 kg (rat), 10 kg (dog), and 50 kg (human).

In brief, HTF 919 was moderately absorbed in mice (20-30%), rats (32%) and dogs (52-62%) and its oral bioavailability was lower in mice and rats (8%) than in dogs (~22-27%). The oral bioavailability of HTF 919 in humans was 11-12%. HTF 919 was slowly eliminated in both rats and dogs as indicated by its long terminal half lives of plasma radioactivity (30 hours in rats and 88 hours in dogs). The terminal half lives of the parent drug were ~2, ——— and 7.7 hours in rats, dogs, and humans, respectively. There are four metabolic pathways identified. The first pathway involves hydrolytic cleavage in stomach after oral dose followed by oxidation and conjugation of the resulting aldehyde to formation of M29.0 (5-methoxyindole-3-carboxylic acid glucuronide), M7.0 (demethylated analogue of M29.0), and M38.0 (aglycon of M29). M29.0 (SDZ 244-120) has been identified in all species including mice, rats, dogs, and humans. M29.0 had very low affinity for 5-HT_4 receptors in calf (pK_D = 3.7, pK_D for HTF 919 = 7.8) and human (pK_D = 4.1, pK_D for HTF 919 = 7.7). The second pathway is the systemic metabolic pathway including a direct glucuronidation of HTF 919 to three isomeric metabolites (M43.2, M43.8, and M45.3) in both rats and humans. The third pathway involves O-demethylation of HTF 919 and the 5-O-demethyl

derivatives includes M52.8 (unconjugated 5-O-demethyl derivative) and M40.6 (conjugated 5-O-demethyl derivative) identified in mice, rats and dogs but not in humans. M52.8 (SDZ 216-454) is a potent 5-HT_4 receptor partial agonist with intrinsic activity of 0.6 (intrinsic activity of 5-HT and HTF 919 = 1 and 0.22, respectively). M52.8 is also more potent than HTF 919 for stimulating the longitudinal muscle movement in isolated guinea pig ileum (pEC_{50} = 9.02 for SDZ 216-454 and 7.69 for HTF 919). The fourth pathway involves oxidation in the indole substructure followed by conjugation identified in rats only. Protein binding of HTF 919 was very high in all species tested including mice (96%), rats (97.8%), dogs (97.5%), and humans (98.2%). HTF 919 was extensively distributed in the extravascular compartments with volume of distribution of ~13 l/kg in mice, 17.5 l/kg in rats, 12.4 l/kg in dogs, and 7.4 l/kg in humans. HTF 919 was mainly excreted via the bile into feces in mice, rats, and dogs. A substantial amount of HTF 919 is excreted into the milk in the lactating rats.

TOXICOLOGY:

ACUTE TOXICITY:

Testing Laboratories:
[ ]

Study Start and Completion Dates: March 18, 1992 and May 12, 1992 (— 321952), March 10, 1992 and May 13, 1992 — 321974), March 20, 1992 and May 15, 1992 — 321963), and March 17, 1992 and May 13, 1992 (— 321985).

GLP and QAU compliance statement: Sponsor included a statement of compliance with GLP regulation and a quality assurance statement in each study.

Methods: To evaluate the acute toxicity of HTF 919, HanIbm:NMRI (SPF) mice at ages of 6-7 weeks at start and HanIbm:WIST rats at ages of 8-10 weeks at start were employed in the acute toxicity studies. In the acute toxicity studies in mice, a single dose of HTF 919 was given to mice at 100, 200, 500 and 1000 mg/kg by oral gavage — 321952) or at 1, 5 and 10 mg/kg intravenously (— 321974). In the acute toxicity study in rats, a single dose of HTF 919 was given to rats at 100, 500, 1000 and 2000 mg/kg by oral gavage (— 321963) or at 1, 5, 10 and 100 mg/kg intravenously — 321985). The animals were fasted for ~16-17

hours (mice) or 16-22 hours (rats) before receiving the oral doses. There were no control groups. These animals were observed for mortality/viability and signs of toxicity four times during the first day and daily thereafter for total 15 days. Body weights were determined before and on days 8 and 15 after dosing. At termination, all animals (both mice and rats) were necropsied and the observations were recorded.

Results: In mice, clinical signs of toxicity were sedation, dyspnea and rales, muscular spasms, ventral recumbence, ruffled fur observed at all dose (p.o. and i.v.) levels except at 1 mg/kg i.v. dose at which no clinical signs were observed. After oral administration of this drug, the minimal lethal dose was 100 mg/kg (one male mouse died) and all animals died at 1000 mg/kg. After intravenous injection of this drug, the minimal lethal dose was 5 mg/kg and all animals died at 10 mg/kg.

In rats, clinical signs of toxicity were sedation, uncoordinated movement, ruffled fur, dyspnea (i.v. only), muscular spasms (i.v. only) and ventral recumbence (i.v. only) observed at all dose (p.o. and i.v.) levels except at 500 mg/kg oral dose and 1 mg/kg i.v. dose at which no clinical signs were observed. After oral administration of this drug, no rats died and thus the minimal lethal dose should be greater than 2000 mg/kg. After intravenous injection of this drug, the minimal lethal dose was 5 mg/kg and all animals died at 10 and 100 mg/kg.

SUBACUTE/SUBCHRONIC/CHRONIC TOXICITY:

RAT:

17-day intravenous infusion study with SDZ HTF 919 in the rat
087929)

Testing Laboratories: [ ]

Study Start and Completion Dates: December 24, 1992 and April 22, 1993

GLP and QAU Compliance Statement: Sponsor included a statement of compliance with GLP regulation and a quality assurance statement.

Animals: Males (364-377 g, 10 weeks)

Females (227-251 g, 10 weeks)
Wistar rats, outbred from [redacted]

Methods: To evaluate the toxicity of HTF 919 in rats, intravenous infusion of HTF 919 was given to rats for a period of 1 hour per day at 0 (0.9% NaCl), 0.2, 2.0 mg/kg/day, and a placebo group for 17 days. Clinical signs of toxicity were observed daily. Mortality/viability were observed at least twice daily. Body weights (daily) and food consumption (weekly) were determined. Ophthalmoscopic examinations were conducted before and during week 2 of the treatment. Hematology, clinical chemistry and urinalysis were determined at termination. All animals were necropsied at termination and the organs were weighed. The toxicokinetic profile was determined 24 hours after the 16th dosing and 5 minutes, 1 and 2 hours after the 17th dosing.

Results:

1. Clinical Signs: There were no clinical signs of toxicity seen during the study except the adverse reaction to the treatment at the injection site (tail) observed in the 2.0 mg/kg group. These reactions included swelling, redness and necrosis.

2. Mortality: Two animals (one from placebo group and one from the 0.2 mg/kg group) died and sponsor stated that the deaths were not related to the treatment.

3. Body Weight: Body weight was reduced (~7-15% compared day 18 to day 1) in all groups including the control and placebo groups. The reduction in the 2.0 mg/kg group (male) was ~15%.

4. Food Consumption: There were no treatment related changes in the food consumption.

5. Ophthalmoscopy: There were no treatment related alterations observed during the study.

6. Hematology: Hemoglobin (12-20%), hematocrit (14% in males), mean cell hemoglobin concentration (~6-7%) were significantly reduced in the 2.0 mg/kg group. RBC distribution width (31-33%) and platelet counts (38-40%) were significantly increased in the 2.0 mg/kg group. Prothrombin time was also increased 8.6% in the 2.0 mg/kg female group.

7. Clinical Chemistry: There were significant increases in the urea (33%) in the 2.0 mg/kg male group and alkaline phosphatase (39%) in the 0.2 mg/kg male group. Albumin was significantly reduced ~19% in the 2.0 mg/kg female group.

8. Urinalysis: There were no treatment related changes observed during the study.

9. Organ Weights: Ratio of spleen to body weight was significantly increased in the 2.0 mg/kg group by ~34-35% compared to the control group and this ratio was also increased in the placebo group (males, 22.5%). Ratio of testes to body weight was significantly increased in the placebo group by ~15% compared to the control.

10. Gross pathology: There were no treatment related changes observed during the study except the local adverse reactions at the injection site (tail) including swelling, redness and necrosis.

11. Microscopic pathology: Histopathological examination revealed that there were partial to complete necrotizing inflammation of the epidermis and dermis with thickening of the epidermis at the injection site (tail) in the 2.0 mg/kg group. The changes in the 0.2 mg/kg group were comparable to those in control and placebo groups. Histopathological examination also revealed that there was an increase in the hemopoietic activity in the red pulp in the spleen in the 2.0 mg/kg group.

12. Toxicokinetics: The plasma concentrations were proportional to the administered doses. C_{max}s were ~15.3-16.5 and ~69 ng/ml for the 0.2 and 2.0 mg/kg/day groups, respectively. The plasma concentrations were not markedly different between males and females. Area under the curve (AUC, 0-3 hours) was ~122 ng.h/ml in the 2.0 mg/kg/day group at termination. AUC was not determined in the 0.2 mg/kg/day group since most of the plasma concentrations were below the limit of detection.

In summary, in the 17-day toxicity study in rats, intravenous infusion of HTF 919 was given to rats for a period of 1 hour per day at 0.2 and 2.0 mg/kg/day for 17 days. There were no clinical signs of toxicity observed during the study except swelling, redness and necrosis seen at injection site (tail) in the 2.0 mg/kg group. Histopathological examination revealed that there was an increase in the hemopoietic activity in the red pulp in the spleen in the 2.0 mg/kg group.

26-Week Oral (in feed) Toxicity Study in Rats
(395R)

Testing Laboratories: Sponsor's laboratory and [redacted]

Study Start and Completion Dates: September 23, 1991 and April 30, 1993

GLP and QAU Compliance Statement: Sponsor included a statement of compliance with GLP regulation and a quality assurance statement.

Animals: Males (210-276 g, 8 weeks)
Females (152-203 g, 8 weeks)
SPF HanIbm:WIST, BRL rats

Methods: To determine the potential toxicity of SDZ HTF 919 in rats, SDZ HTF 919 was given to rats (14/sex/group) in feed at 0, 15, 60 and 240 mg/kg/day for 26 weeks. The dose selection was based on the results of the 4-week pilot study in rats (110DFR). In this study, body weight was decreased by 55% (females) and 90% (males) at high dose of 300 mg/kg/day and by 40% (males) at mid dose of 100 mg/kg/day. There were no other treatment related changes. Therefore, the dose selection appears reasonable. There was another 4-week oral (in feed) dose ranging study in rats (129DFR). In this study, the toxicity profile of a new batch of HTF 919 (batch # 91903) was compared with that of batch #906/502. Similar toxicity profile was identified between both batches. In the current study, clinical signs of toxicity and mortality were observed daily. Body weights and food consumption were determined weekly. Hematology and clinical chemistry were determined at weeks 7, 14, 26 and 31 (4/sex/group for week 31). Urinalysis was conducted at weeks 7, 14 and 26. Ophthalmoscopy was performed at weeks 15 and 26. All animals were necropsied at termination and the organs were weighed. Gross and microscopic examinations were then conducted and the following organs were examined histopathologically: liver, thymus, heart, kidney, spleen, pancreas, lung, trachea, esophagus, salivary glands, lymph nodes (mandibular and mesenteric), thyroid gland, parathyroid gland, tongue, skeletal muscle, stomach, duodenum, jejunum, ileum, colon, skin with mammary gland, right eye, sciatic nerve, aorta, cerebrum, cerebellum, spinal cord, pituitary gland, testes/ovaries, epididymides, prostate, uterus, seminal vesicles, urinary bladder, adrenals, bone and bone marrow (sternum) and any tissue found to be abnormal on macroscopic

examination. The plasma level of test drug was determined on termination day between 7:30 and 8:30 am just prior to sacrifice.

Results:

1. Clinical Signs: Hair loss was observed in all groups (1 control, 2 low dose, 2 mid dose and 14 high dose).

2. Mortality: There were no deaths.

3. Body Weight: The initial and final body weights in the control group were 250 ± 19 and 473 ± 50 g (males) and 175 ± 14 and 282 ± 23 g (Females), respectively. Terminal body weight gains were decreased by 36% (males) or 22.4% (females) in the high dose group. The information is summarized in the following table.

Mean body weights (g)

	0 mg/kg/day	15 mg/kg/day	60 mg/kg/day	240 mg/kg/day
Males				
Initial	250	249	251	250
13 weeks	426	434	444	350
26 weeks	473	484	491	392
Females				
Initial	175	173	173	175
13 weeks	264	258	260	236
26 weeks	282	278	277	258

The results were also depicted in figures 1 and 2 on pages 32 and 33 in volume 18.3. These figures are attached below.

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SDZ HTF 919 (Study No.:395R)
26-Week Oral (In Feed) Toxicity Study in Rats
Mean Values: Body Weight (grams)

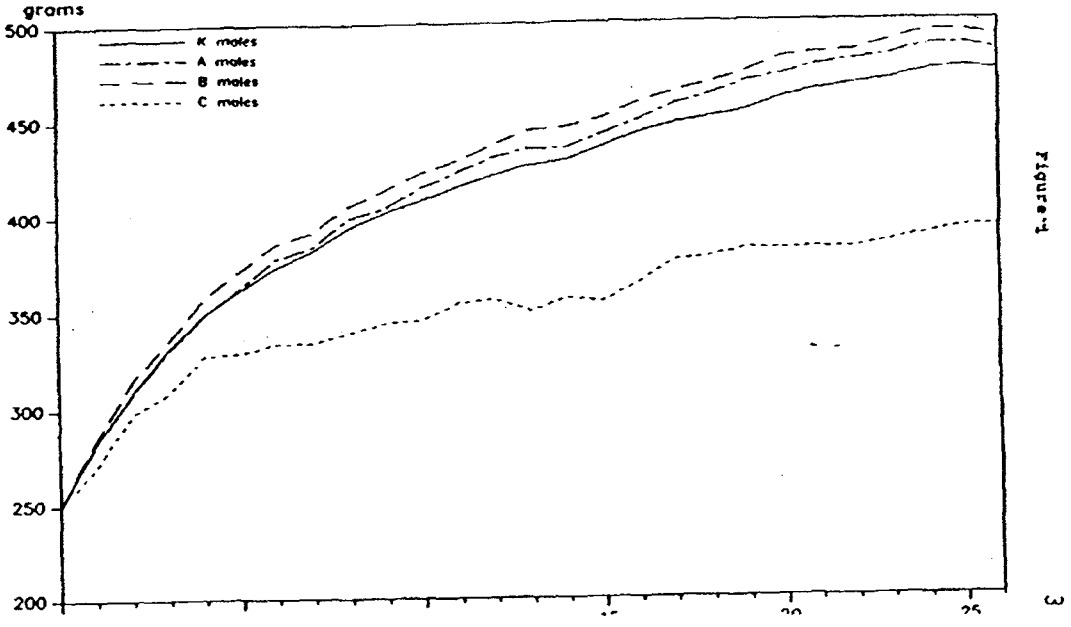

Figure-1

SDZ HTF 919 (Study No.:395R)
26-Week Oral (In Feed) Toxicity Study in Rats
Mean Values: Body Weight (grams)

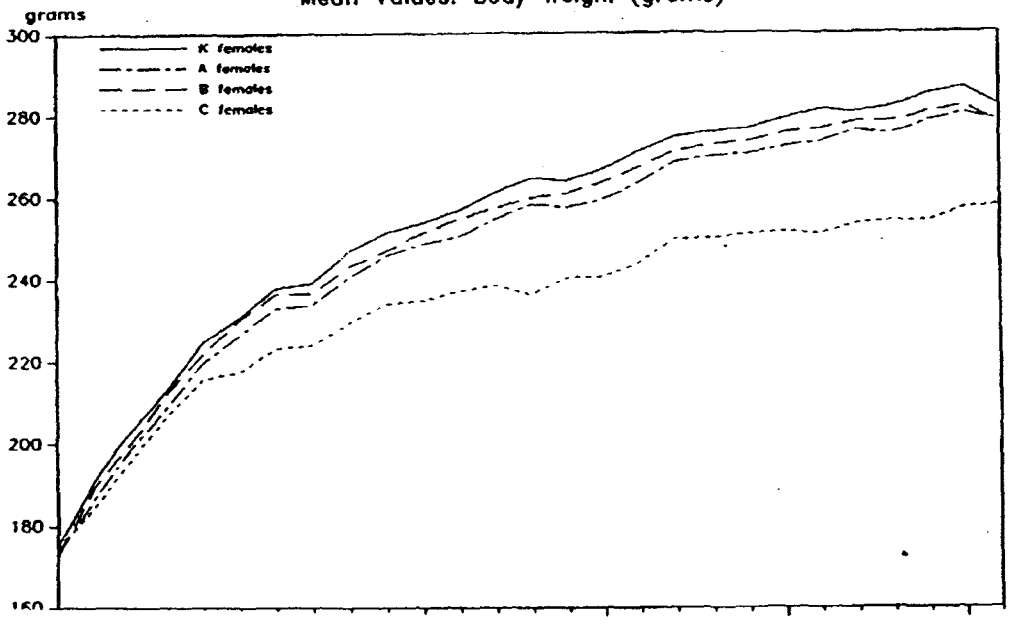

Figure-2

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4. Food Consumption: The food consumption for control males and females were 21-24.4 and 16-21 g/animal/day, respectively. The food consumption was significantly reduced during the entire treatment period in the high dose group (11-24% in males and 13-19% in females) as compared to the control. The drug intake was presented in a table on page 11 in volume 18.3 and this table is attached below.

Mean cumulative drug intake (mg/kg/day) in week 26:

	Males	Females
Controls	0	0
Low Dose	15	15
Mid Dose	60	60
High Dose	242	248

5. Hematology: The platelet count was significantly increased in the high dose males (20-23%) than that in control animals during weeks 7, 14 and 26. Reticulocytes were significantly decreased by 28-30% in the high dose males during weeks 14 and 26. These changes returned to normal at the end of recovery period (week 31). Monocytes were decreased by 37% at week 26 in the high dose males.

6. Clinical Chemistry: Significant decrease in total protein (5-8%) was seen in the high dose males during weeks 7, 14 and 26.

7. Urinalysis: There were no treatment related changes.

8. Ophthalmoscopic Examination: There were no treatment related changes.

9. Organ Weights: The relative organ weights to body weight of brain (17%) and testes (20%) were increased in high dose males. The relative pituitary gland weight was increased by 18% in high dose females.

10. Gross Pathology: There were no treatment related changes.