CENTER FOR DRUG EVALUATION AND RESEARCH

APPLICATION NUMBER: 020859

PHARMACOLOGY REVIEW(S)

Parlya

MEMORANDUM

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

DATE: December 11, 1998

FROM: Glenna G. Fitzgerald, Ph.D.
Pharmacology Team Leader
Division of Neuropharmacological Drug Products, HFD-120

TO: NDA 20-859
Sonata™ (zaleplon)
Wyeth-Ayerst
5 and 10 mg capsules

SUBJECT: Approvable Action for Pharmacology and Toxicology

Zaleplon is a non-benzodiazepine $GABA_A$ agonist which is indicated for the treatment of insomnia. The pharmacology and toxicology have been reviewed by Dr. Aisar Atrakchi, and there are no outstanding issues.

The pharmacological profile of zaleplon is similar to that of the benzodiazepines. It possesses anxiolytic, anticonvulsant and sedative effects in *in vivo* assays in rodents and primates. It is less potent than benzodiazepines in some respects such as tolerance, interaction with alcohol, amnesia, hangover. However, it has been shown to possess abuse potential in animals, with a dependence-producing profile equivalent to the benzodiazepines and to zolpidem. It was psychoactive in rats, with dose-related discriminative stimulus effects. The potential for abuse has been reviewed by the HFD-170 staff, who have made appropriate labeling recommendations.

The only other preclinical issue worth noting is the finding of a significant increase in liver adenomas in high dose female mice. The sponsor attributed this finding in labeling to increased weight gain, enzyme induction, and hepatomegaly, concluding no relevance to humans. [redacted]

Recommendations:

This NDA is approvable for pharmacology and toxicology. Recommended labeling follows.

Recommended Labeling for NDA 20-859, Sonata (Zaleplon)

CLINICAL PHARMACOLOGY

Pharmacodynamics

[We have extensively modified this subsection. We have deleted the term [redacted] in referring to the structure, since this could be misleading. We have also deleted [redacted]

Sonata (zaleplon) is a hypnotic agent with a chemical structure unrelated to benzodiazepines, barbiturates, or other drugs with known hypnotic properties. Behaviorally, it shows sedative, anxiolytic, muscle relaxant, and anticonvulsive effects in animal models. However, data derived from these animal studies have indicated that zaleplon has reduced potentiating action with alcohol relative to benzodiazepines, does not show rapid tolerance to its sedative effects, and does not produce amnestic effects at therapeutically relevant doses.

Other nonclinical studies have also shown that zaleplon binds selectively to the brain omega-1 receptor situated on the alpha subunit of the $GABA_A$ receptor complex and potentiates t-butyl-bicyclophosphorothionate (TBPS) binding. Studies of binding of zaleplon to purified $GABA_A$ receptors ($\alpha1\beta1\gamma2$ [omega-1] and $\alpha2\beta1\gamma2$ [omega-2]) have shown that zaleplon has a low affinity for these receptors, with preferential binding to the omega-1 receptor.

PRECAUTIONS

Carcinogenesis, Mutagenesis, Impairment of Fertility

Carcinogenesis

[This statement has been substantially rewritten. Consistent with our proposed Dosage and Administration section, we have used 20 mg as the maximum recommended human dose.]

Lifetime carcinogenicity studies of zaleplon were conducted in mice and rats. Mice received doses of 25, 50, 100, and 200mg/kg/day in the diet for two years. These doses resulted in plasma AUC levels of zaleplon that were approximately 6 to 25 times those measured in humans receiving the maximum recommended daily human dose (MRHD) of 20 mg. There was a significant increase in the incidence of hepatocellular adenomas in female mice in the high dose group. Rats received doses of 1, 10, and 20mg/kg/day in the diet for two years. These doses resulted in plasma AUC values of zaleplon that were approximately 2 to 66 times those measured in humans at the MRHD. Zaleplon was not carcinogenic in rats.

Mutagenesis

[We have modified this statement to reflect our somewhat different interpretation of the data]

Zaleplon was clastogenic, both in the presence and absence of metabolic activation, causing structural and numerical aberrations (polyploidy and endoreduplication), when tested for chromosomal aberrations in the *in vitro* Chinese hamster ovary cell assay and the *in vitro* human lymphocyte assay. In other *in vitro assays*, Zaleplon was not mutagenic in the Ames bacterial gene mutation assay or the Chinese hamster ovary HGPRT gene mutation assay; It did not cause DNA damage in an unscheduled DNA synthesis assay in rat hepatocytes. Zaleplon was not clastogenic in two *in vivo* assays: the mouse bone marrow micronucleus assay and the rat bone marrow chromosomal aberration assay.

Impairment of Fertility

[We have slightly modified this statement]

In a fertility and reproductive performance study in rats, fertility was decreased following administration of an oral dose of zaleplon of 100 mg/kg/day to males and females prior to and during mating. The plasma AUC level of parent drug in female rats was approximately 500 times, and in male rats was approximately 425 times, AUCs measured in humans receiving the maximum recommended daily human dose (MRHD) of 20 mg. This dose was associated with mortality. Follow-up studies indicated that impaired fertility was due to an effect on the female.

Pregnancy: Pregnancy Category C:

[We have slightly modified this statement]

In embryofetal development studies in rats and rabbits, oral administration of up to 100

and 50 mg/kg/day, respectively, to pregnant animals throughout organogenesis produced no evidence of teratogenicity. These doses resulted in plasma AUC levels of parent drug, measured in separate studies in pregnant animals, that were 900 times (rat) and 85 times (rabbit) those measured in humans receiving the MRHD of 20 mg. In rats, pre- and postnatal growth was reduced in the offspring of dams receiving 100 mg/kg/day. This dose was also maternally toxic, as evidenced by clinical signs and decreased maternal body weight gain during gestation. The no effect dose for rat offspring growth reduction was 10 mg/kg (corresponding to plasma AUC levels of zaleplon approximately 56 times those measured in humans receiving the MRHD). No adverse effects on embryofetal development were observed in rabbits at the doses examined.

In a pre- and postnatal development study in rats, increased stillbirth and postnatal mortality, and decreased growth and physical development, were observed in the offspring of females treated with doses of 7 mg/kg/day or greater during the latter part of gestation and throughout lactation. There was no evidence of maternal toxicity at this dose. The no-effect dose for offspring development was 1 mg/kg/day (plasma AUC levels approximately twice those measured in humans receiving the MRHD). When the adverse effects on offspring viability and growth were examined in a cross-fostering study, they appeared to result from both *in utero* and lactational exposure to the drug.

There are no studies of zaleplon in pregnant women; therefore, this drug should be used in pregnancy only if the potential benefit outweighs the potential risk to the fetus.

/S/ [redacted]
Glenna G. Fitzgerald, Ph.D.

Attachment
NDA 20-859
c.c. Div. File
Laughren\Katz\Atrakchi\Mille\Fitzgerald

[redacted]APPEARS THIS WAY ON ORIGINAL

NDA# 20-859
Original Pharmacology Review

NDA#	20-859
Drug:	Zaleplon (Sonata)
Sponsor:	Wyeth-Ayerst Labs P.O. Box 8299 Philadelphia, PA 19101
Indication:	Treatment of Insomnia
Category:	Non-benzodiazepine $GABA_A$ agonist
Sub Date:	Dec 30 1997
Rec Date:	Jan 6 1998
Rev Date:	Oct 1998
Reviewer:	Aisar H. Atrakchi, Ph.D. /S/ 11/23/98
Team Leader:	Glenna Fitzgerald, Ph.D. 11/24/98
Related INDNDA(s)	[redacted]

cc.
/Div File/Orig NDA# 20-859
/G. Fitzgerald/A. Atrakchi/T. Wheelous

[redacted] APPEARS THIS WAY ON ORIGINAL

Table of Content

Table of Content (Cont.)

RECOMMENDATION:

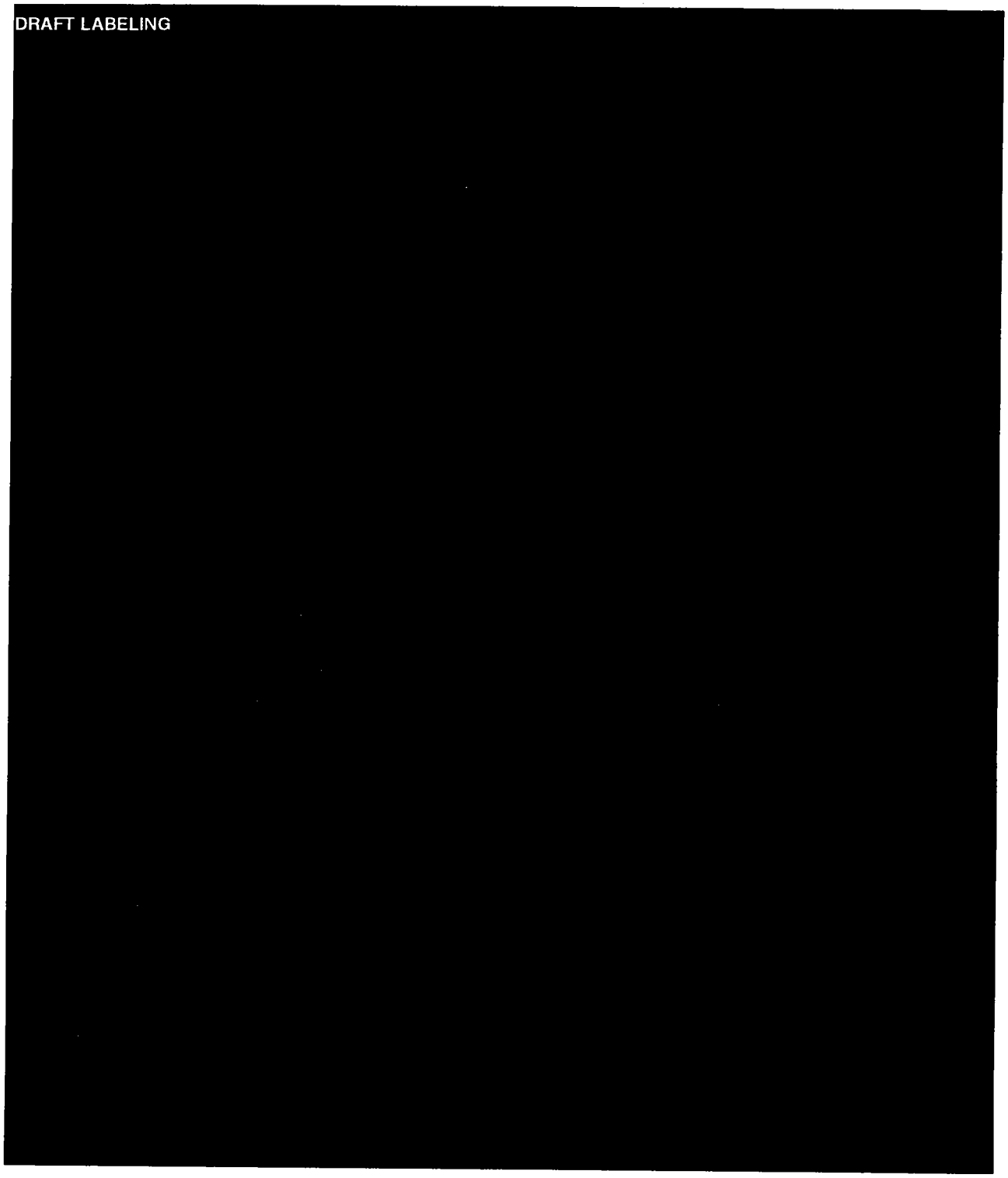
Pre-clinical studies submitted with this NDA, adequately describe and assess the efficacy and safety of zaleplon for the treatment of insomnia at the proposed clinical dose of 10mg/d and upto 20mg/d. There are no additional pre-clinical study requirements.

/S/

Aisar Atrakchi, Ph.D.
Pharm/Tox Reviewer

APPEARS THIS WAY ON ORIGINAL

LABELING:



Pregnancy Category C:

In reproduction studies in rats, oral doses up to 100mg/kg/day and in rabbits, doses up to 50mg/kg/day (49 and 48 times the MRHD on a mg/m^2 basis respectively), administered throughout organogenesis, did not show evidence of teratogenicity. In a rat teratology study in dams dosed 100mg/kg/day, mean fetal body weight was decreased, incidence of delayed ossification was increased, and, offspring postnatal growth was decreased. This dose was maternally toxic as evidenced by clinical signs and decreased maternal weight gain during gestation. Mean C_{max} at 100mg/kg/day dose represents 322 times the mean C_{max} in humans dosed 20mg/day. The NOEL in the rat for maternal toxicity is 1mg/kg/day (0.5 times the MRHD on a mg/m^2 basis) and, for reproductive parameters in males and females, embryofetal development and, teratogenicity, is 100mg/kg/day. In a rabbit teratology study, there were no drug effects on any reproductive parameters up to 50mg/kg/day dose (48 times the MRHD on a mg/m^2 basis). The mean C_{max} after 50mg/kg dose is 51 times the mean C_{max} measured in humans at the maximum recomended dose of 20mg/d. The NOEL for teratogenicity in the rabbit is 50mg/kg/day (48 times the MRHD on a mg/m^2 basis).

When female rats were administered zaleplon during the latter part of gestation and throughout lactation, increased offspring stillbirth, increased postnatal mortality, and decreased offspring growth observed at doses as low as 7mg/kg/day (3 times the MRHD on a mg/m^2 basis). The NOEL for developmental toxicity is 1mg/kg/day (0.5 times the MRHD on a mg/m^2 basis). When the adverse effects on offspring viability and growth were examined in a cross-fostering study, they appeared to result from both pre- and post-natal exposure to the drug.

There are no adequate and well-controlled trials with Zaleplon in pregnant females; therefore, this drug should be used in pregnancy only if the potential benefit justifies the potential risk to the fetus.

Clinical Pharmacology:

The information are technically correct, they could be shortened. My only comment is for the last line/sentences of the 1st paragraph: "and is likely to be devoid of next-day residual effects in human use" I do not think this is neccessary and should be deleted.

PHARMACOLOGY & MECHANISM OF ACTION

Zaleplon, a pyrazolopyrimidine cpd, is a non-benzodiazepine anxiolytic/hypnotic intended to treat insomnia. It is a short acting agent with some advantages over bzd as it is less potent or devoid of some of the adverse effects of bzd such as amnesia, tolerance, interaction with alcohol, and hangover. However, zaleplon was shown to possess abuse potential as rats were able to discriminate it from saline (100% discrimination at 3mg/kg i.p.). The recommended clinical dose is 10mg/d or 0.17mg/kg/d with a maximum daily dose of 20mg or 0.33mg/kg (based on 60kg). In vitro radioreceptor binding studies showed selective binding to brain omega1 site located on the alpha subunit of the bzd-$GABA_A$ receptor complex.

Zaleplon displaced ^{3}H-flunitrazepam with an **IC_{50} 200nM**, compared to 0.5nM triazolam, 17nM diazepam, 114nM flurazepam, and 26nM zolpidem. It also caused a 73% incr in ^{35}S-t-butylbicyclophosphorothionate (TBPS) binding to the Cl channel of the $GABA_A$ - Cl-bzd receptor complex. These findings were similar to those of triazolam and flurazepam. Results from the Nova screen assays, indicated the selective binding of zaleplon to the GABA receptors compared to no binding at >37 other receptors, enzymes, and ion channels. Studies with recombinant $GABA_A$ receptors comparing zaleplon, zolpedim, and zopiclone (non-bzd) binding, zaleplon showed high affinity to $\alpha_1\beta_2\gamma_2$ subunit compared to $\alpha_2\beta_1\gamma_2$ and $\alpha_6\beta_2\gamma_2$ where the K_i at these receptors were **66**, 830, and >100,000nM respectively, the corresponding K_i values for zolpidem were: 14, 131 & >20,000nM respectively, and those for zopiclone were: 19, 33, & >100,000nM respectively (table below from sponsor). This indicated zaleplon selectivity to bzd type1 which is associated with α1 subunit of the $GABA_A$ receptor.

Affinities (k_i) of Recombinant $GABA_A$ Receptors*

Drug	$\alpha_1\beta_2\gamma_2$	$\alpha_2\beta_2\gamma_2$	$\alpha_3\beta_2\gamma_2$	$\alpha_6\beta_2\gamma_2$
zaleplon	66 ± 11	830 ± 149	2550 ± 600	>100,000
zopiclone	19 ± 6	33 ± 9	870 ± 140	>100,000
zolpidem	13.6 ± 0.8	131 ± 54	>20,000	> 20,000

*Data were determined in membranes derived from human embryonic kidney cells (HEK 293) acutely transfected with the rat $GABA_A$ receptor subunit combinations indicated. The data are shown as the k_i (mean ± S.E.M. in nM) of n=3-4 calculated from the respective IC_{50} values using the Cheng-Prusoff equation.

The **sedative** effects of zaleplon were evaluated in rodents using a number of standard tests. When administered orally, it decreased spontaneous motor activity (**ED_{50} 1.4mg/kg** vs. 1.6&25mg/kg for triazolam and flurazepam respectively), decreased muscle tone, and caused motor incoordination (using the inclined screen (IS) and rod walking test, the **ED_{50} was 4&2.7mg/kg** respectively for zaleplon compared to 1.3&4.5mg/kg for triazolam and 18&6.2mg/kg for flurazepam respectively). The effects of zaleplon on motor coordination was blocked by administration of flumazenil, a known bzd antagonist, further supporting the site of

action of zaleplon. These results on motor function and the comparable effect of zaleplon to triazolam at almost equivalent doses, supports the sedative hypnotic effects of this drug. The **hypnotic** effect was also assessed by measuring EEG sleep parameters in rodents and squirrel monkeys (stereotaxic implant of indewlling cortical electrodes). Zaleplon dose-dependently increased sleep duration in rats with oral **ED_{50} of 0.5mg/kg**. In squirrel monkeys it caused a decr and incr in alpha and beta waves (beta shift) similar to that seen with flurazepam and triazolam (all cpds at 4mg/kg i.m.). Another test in squirrel monkeys was the measurement of vigilance or attention by reaction time to respond to a visual stimulus that signaled the limited availability of food reward. Zaleplon caused a decr in both accuracy and latency to respond at oral dose of 4mg/kg. This decr in performance together with the EEG beta shift, is indicative of decrease in attention and/or response to external stimuli.

In general, cpds that interact with $GABA_A$ receptors produce, anxiolytic and anticonvulsant activities in addition to sedation. The **anxiolytic** effect of zaleplon was evaluated in the conflict behavior test using water deprived rodents. Zaleplon inhibited suppressed drinknig in rats at 1mg/kg p.o. dose. Similar study with a different paradigm was used in squirrel monkeys, zaleplon produced anticonflict with an inverted U-shaped dose response curve that peaked at 2mg/kg p.o. dose. Similar results were seen with diazepam.

Amnestic activity was assessed in the passive avoidance test (ability of a cpd to impair acquisition and/or retention of a learned response). Zaleplon showed little effect to induce amnesia at the doses tested (MED (minimum effective dose): 16mg/kg zaleplon, 1mg/kg triazolam, and 8mg/kg flurazepam).

Interaction with alcohol was measured by assessing the ratio of ED_{50} of the drug to decreased motor activity in relation to the minimal effective dose to potentiate the duration of alcohol-induced loss of righting reflex. Zaleplon, triazolam, and flurazepam caused dose-related increase in the duration of loss of righting reflex induced by ethanol within 1hr and 24hr after drug administration. The MED of zaleplon at the 1 & 24hr following 3.2mg/kg ethanol i.p. was: 2.5 & >40mg/kg respectively, and the corresponding values for triazolam: 0.08 & 0.16mg/kg, and for flurazepam: 5 & 10mg/kg respectively. Therefore, it was concluded that zaleplon interacts with ethanol but at reduced ratio compared to triazolam and flurazepam. The doses of zaleplon that interacted with ethanol represent approximately 15->235x higher than the maximum proposed clinical dose on a mg/kg basis.

The **anticonvulsant effect** of zaleplon was compared to that of triazolam and flurazepam and measured against convulsions induced by pentylenetetrazole (ptz)(23mg/kg i.v.), bicuclline (0.125mg/kg i.v.), picrotoxin (3mg/kg s.c.), strychnine (2mg/kg s.c.) and electroconvulsive shock (ECS) applied across the cornea. Convulsions occurred in 99% of control animals. Zaleplon was more effective than flurazepam against convulsions induced by all cpds, it was 2x as protective as triazolam in blocking ptz seizures (0.8 vs 1.5mg/kg p.o. respectively), 1/13 as potent against ECS (19 vs. 1.5mg/kg p.o. respectively), and 1/3-1/9 as potent in protecting against seizures induced by the other cpds.

Tolerance to the drug's effects was tested in rats. Rats were orally dosed with the vehicle or ascending doses of the drug for 4d. Rats were assessed for motor activity and performance on the IS, 1hr after the last treatment. After 4d of daily dosing, zaleplon ED_{50} incr <5x compared to the single dose administration (on 1st day dosing)(ED_{50} 11mg/kg at 4d). In comparison, triazolam ED_{50} for motoar activity increased >20x the ED_{50} after the 1st dose (>100mg/kg d4). The ED_{50} value for the IS for zaleplon was 9mg/kg and that for triazolam >100mg/kg. It was concluded that zaleplon showed a reduced tendency to induce tolerance compared to that developed by triazolam.

Drug Discrimination: sucrose water reinforcement test in rats was the test used to assess the discriminative effects of zaleplon compared to other bzds or non-bzd cpds. Zaleplon was dosed at 3mg/kg i.p. to rats that were trained to discriminate between it and saline. Zaleplon caused a dose dependent response with 100% disrimination at the 3mg/kg dose. Triazolam (0.1-1mg/kg i.p.) and partial bzd agonist, Ro 17-1812 (0.3-3mg/kg i.p.), zolpidem (3-10mg/kg i.p.), and the bzd1 selective agonist CL 218,872 (3mg/kg i.p.) all substituted for zaleplon in most or all rats and the percent of rats in which substitution occurred, incr with incr in dose. The discriminative effect of zaleplon was inhibited by flumazenil (bzd antagonist). It was concluded that the discriminative effects of zaleplon are mediated through bzd receptors but its profile is unique compared to that of the bzd and non-bzd cpds tested in this study.

Abuse Potential: Zaleplon, similar to triazolam and zolpidem, have abuse and dependence liability based on the results obtained in adult male baboons. Zaleplon was administered as a bolus once per day through an indwelling intragastric catheter, the dose increased (0.5 $\log_{10}$) every 17d. Following the 4th increase, the dose was slowly and continuously infused over a 24hr period for 28d. Zaleplon doses were 1, 3.2, 10, and 32mg/kg, those for triazolam were 0.032, 0.1, 0.32, and 1mg/kg. The initial dose was based on lowest discriminable dose determined in a dose discrimination study where lorazepam was the standard discriminative stimulus. Flumazenil was injected i.m. on d14 of each dose condition period to assess withdrawal. Both zaleplon and triazolam produced dose-response increase in ataxia and time to complete fine motor task; locomotor function was not decreased. Both drugs increased lever pressing response that increased food delivery and all baboons gained wt during the study. Flumazenil administration caused precipitation of withdrawal syndrome in both drugs with animals exhibiting nose rubbing, postural changes, and tremor. Severity of withdrwal was increased across baboons but inconsistently during the period of dosing. Sudden discontinuation of the drug led to decline in food intake (for 4wks) and tremors; seizures did not occurr with either drug.

Zaleplon (0.01-0.32mg/kg/injection), similar to triazolam and zolpedim, substituted for cocaine in all baboons that were implanted with indwelling i.v. catheters and trained to self-administer cocaine. This indicated the abuse liability of zaleplon. **These doses represent 0.06-2 times the maximum proposed clinical dose of 10mg/day.**

CVS:

The effects of zaleplon on various parameters of the heart were assessed in conscious normotensive and spontaneously hypertensive rats and in anesthetized dogs. Effects on various parameters of respiration were investigated in rhesus monkeys. Single oral administration of zaleplon to conscious normotensive rats at doses that ranged between 5-200mg/kg caused a statistically sig decrease (19-35% less than the cont), in mean arterial pressure (MAP) at all but 5&30mg/kg doses. The decrease was almost dose dependent (21, 19, & 35% of time 0, in 50, 100, & 200mg/kg dose respectively. The decrease in MAP started from 15min and lasted through the 240min of measurement. Heart rate (HR) was lowered 28% at 3hr post 50mg/kg dose and 17-22% at 3-4hr post 100mg/kg (but not significant), a small but significant increase in HR occurred at 30&120min (20&4.5% of time 0) post 30mg/kg dose; no other effects were recorded. None of the changes in MAP or HR were considered of toxicological significance in normotensive and conscious rats. In male SHR, zaleplon administered as a single oral dose of 25mg/kg, had no effect on urine volume or electrolyte (Na & Cl) excretion upto 5hr postdose. However, male SHR dosed 25mg/kg (n=3) and 100mg/kg (n=1) for 2days showed 15&19% decrease in MAP (21&28mmHg respectively) and 18&38% decrease in mean HR (80&173BPM respectively), relative to the vehicle control; none of these values reached statistical significance (in the reviewer's opinion, statistics are irrelevant for n=1 or 3 animals). These reductions in MAP & HR in SHR maybe reflective of a hypotensive tendency of CL 284-846, the sponsor however, related these findings to sedative effect of the drug and not to a true antihypertensive effect. Intravenous infusion of 5mg/kg dose over 0.5hr to normotensive anesthetized dogs, caused small, transient but significant increase (14-20%) in mean HR at 15-30min of infusion; no effect on MAP or EKG Lead II. All of these effects in rats and dogs were small and of no physiological or toxicological significance. The following respiratory parameters were assessed in rhesus monkeys: ventilatory frequency, tidal volume, and minute volume, effects on chemical control of respiration were determined in air or atmosphere of 5% CO_2 mixed with air. These effects were compared to pentobarb. Doses were cummulative in all monkeys; top doses ranged from 0.3-10mg/kg i.m. Zaleplon decreased respiration at doses that were 3-10 folds higher than those that decreased response to food (lever pressing - sedative effect). Zaleplon had no effect on chemical control of respiration in either air or air + CO_2. At 3 or 10mg/kg cumulative doses, minute volume in all monkeys was markedly reduced.

Pharmacology of metabolites:

Table below from sponsor compares the effects of M1 to those of the parent:

Compd CLNo	3H-Flu IC50 μM	TBPS % Stim mg/kg	TRC MED mg/kg	MTZ ED50 mg/kg	MA ED50 mg/kg	IS ED50 mg/kg
284,846	0.5	62.5	0.80	0.80	1.40	3.90
284,859	27	30	50	>50	80.00	~200

- TBPS a test for sedative effect, (increase binding of TBPS to bzd receptors; TBPS binds to a site near the Cl channel and regulates its function).
- TRC: thirsty rat conflict procedure
- MTZ: metrazol induced convulsions
- MA: motor activity
- IS: inclined screen test for muscle coordination.

M1 & M2 did not bind to bzd receptors. In a drug discrimination test, rats were trained to discriminate zaleplon from saline and a dose response was obtained for zaleplon (see under drug discrimination). Neither metabolite substituted for zaleplon at any dose (3-100mg/kg i.p.), response rates were decreased only at the high dose of 100mg/kg i.p. Triazolam on the other hand, fully substituted for zaleplon at 0.3mg/kg i.p.

It was concluded that neither M1 or M2 contribute to the pharmacologic activity of zaleplon based on the above in vitro and in vivo studies.

Toxicology of Metabolites:

M1 & M2 metabolites were administered to ICR female mice (4-5/dose) i.p. (500mg/kg) or p.o. (500 & 1000mg/kg) as a single dose. Animals were observed for 7 days postdose. There were no deaths in any gr; inactivity was seen in the i.p. group following administration of both metabolites.

M1 & M2 metabolites were administered to SD rats (5/sex/dose) i.p. (10, 100mg/kg) and p.o. (10, 100, 300 & 500mg/kg) as a single dose. TK was determined in 3/sex rats, dosed orally and i.p. at 100mg/kg, pre dose and at postdose upto 24hr. Animals were observed for 14 days postdose. Parameters assessed included: clinical signs, mortality, B.wt., gross exam, and histopathology. There were no deaths, no clinical signs, no gross or histopath findings with either metabolite and with either route. Plasma concentration for M1 after p.o. and i.p. were non-detectable (<0.05ug/ml quantitation limit (ql)) except in 1m/1f p.o. and 1m/2f i.p. Plasma concentration in these rats ranged between <0.05-0.42ug/ml p.o. (at 6hr postdose), and <0.05-68ug/ml i.p. (at 1hr postdose). For M2 metabolite after p.o. dosing, several samples from males

could not be collected due to technical problems (only 1m had 2 values at 5&30min), plasma levels ranged between <0.05-45ug/ml p.o. (at 30min postdose) and, <0.05-207ug/ml i.p. (at 30min postdose). The minimum lethal dose for M1 & M2 was >100mg/kg i.p. and >500mg/kg p.o.

M2 (the main metabolite in humans) was administered orally to beagle dogs (3/sex/dose) at **1, 2, and 4mg/kg/d for 28d.** Control dogs received empty caps. These doses represent 6-24 times the proposed human dose on a mg/kg basis. The following parameters were evaluated: mortality, clinical signs, B.wt, food intake, EKG (prior to- and at end of study), ophthalmology, hematology, clin chem, urinalysis, organ wts, gross, and histopath; plasma levels were determined from blood collected on d14. M2 did not cause death in any group and none of the assessed parameters were affected upto 4mg/kg dose except for adrenal wt and histopath (see below). Therefore, the non toxic effect level (NTEL) is >4mg/kg. Plasma mean steady state $AUC_{0\text{-}24hr}$ were 585±288, 1784±812, and 1967±967ng.hr/ml for 1, 2, and 4mg/kg respectively. M2 exposure in humans after 10mg/d dose is 0.14ug.hr/ml and that for the parent is 0.06ug.hr/ml (based on the sponsor's B.wt of 70kg; we usually use 60kg). In a single oral PK study of M2, 3 dogs were dosed 25mg/kg and the results were: C_{max} 7±1ug/ml (range 6-7.6ug/ml); $AUC_{0\text{-}24hr}$ 19±3ug.hr/ml (range 17-22ug.hr/ml); t_{max} range 0.5-1hr and $T_{1/2}$ range 2-5.6hr. Linear extrapolation of exposure values at 1, 2, and 4mg/kg produces 5, 10, 21 times the human exposure of 0.14ug.hr/ml. In another oral single dose PK study in dogs, M2 was administered at 5mg/kg to 4 dogs with the following results: C_{max} 2±0.6ug/ml (range 1.3-2.7ug/ml), $AUC_{0\text{-}inf}$ 6ug.hr/ml (range 5-7.7ug.hr/ml), t_{max} range 1-2hr and, $T_{1/2}$ range 1.6-7hr. Linear extrapolation of exposure values at 1, 2, and 4mg/kg produces 8, 17, and 34 times the human exposure of 0.14ug.hr/ml.

There was an increased incidence of adrenal cortical vacuolation in all female groups including the control and in males dosed 2&4mg/kg. The severity was slight to mild and the incidence was moderate in the 2 male groups and the incidence was higher in f dosed 1&2mg/kg relative to the concurrent control. Adrenal wt in HDm was decreased (26% of cont) but was unchanged in f. The pathology report (independently peer reviewed) indicated that the morphology of the vacuoles was similar to spontaneous changes seen in control dogs in this and other studies from the sponsor. Therefore, this finding was not considered of toxicological significance.

As of Aug 6 1998 it is our understanding that a report is in preparation to be submitted later, entitled "oral systemic exposure of M2 in rats (LJT2590).

PK

The PK of Zaleplon and its metabolites have been studied via the oral and i.v. routes following single and repeate dosing to a number of species including rat, mouse, pregnant rabbit, dog, and monkey. Therapeutic and toxic doses were tested in these species. The main metabolites studied include M1, M2, and M2 glucuronide.

The following tables from the sponsor presents PK/TK parameters for the mouse, rat, dog, and pregnant rabbit following single and repeate dosing. Some of the values specifically those for the dose range finding and carcinogenicity studies maybe slightly different from those reported by the reviewer in the separate study discussion. This difference is due to the reviewer's rounding of numbers.

TABLE A21. MEAN TOXICOKINETIC PARAMETERS FOR ZALEPLON AND DESETHYL-ZALEPLON IN MICE FROM REPEATED-DOSE STUDIES

Sex	Day of Sampling	N	Dosage (mg/kg/day)	Zaleplon C_{max} (µg/mL)	Zaleplon AUC_{0-24} (µg·hr/mL)	Desethyl-zaleplon C_{max} (µg/mL)	Desethyl-zaleplon AUC_{0-24} (µg·hr/mL)
Oral (Diet) One Week Range Finding Study MIRACL 26067							
M	6-7	3	0.5	0.08	0.67	NA	NA
F	6-7	3	0.5	0.05	0.51	NA	NA
M	6-7	3	25	0.09	0.75	NA	NA
F	6-7	3	25	0.07	0.95	NA	NA
M	6-7	3	100	0.17	1.48	0.18	0.99
F	6-7	3	100	0.29	2.91	0.20	1.84
M	6-7	3	1000	2.18	19.1	5.72	45.0
F	6-7	3	1000	1.99	18.6	4.68	42.8
Two-Week Oral (Diet) Study: GTR 26624							
M	14	4	25	0.057	0.513	0.023	0.182
F	14	4	25	0.120	0.653	0.008	0.141
M	14	4	50	0.082	1.12	0.064	0.853
F	14	4	50	0.060	0.824	0.064	0.785
M	14	4	100	0.423	3.94	0.333	3.01
F	14	4	100	0.090	1.48	0.141	2.12
M	14	4	200	0.962	7.03	1.12	7.72
F	14	4	200	0.381	3.82	0.795	8.17
Oral (Diet and Gavage) Two-Week Range Finding Study: GTR 27217							
Diet							
M	14	4	10	0.013	0.21	ND	ND
F	14	4	10	0.025	0.45	ND	ND
M	14	4	20	0.032	0.47	0.009	0.065
F	14	4	20	0.097	0.92	0.041	0.43
M	14	4	40	0.056	0.49	0.022	0.18
F	14	4	40	0.132	1.08	0.071	0.44
M	14	4	80	0.13	1.66	0.10	1.21
F	14	4	80	0.19	1.93	0.12	1.68
Gavage							
M	14	4	5	0.25	0.22	0.28	0.16
F	14	4	5	0.24	0.21	0.28	0.21

PK Tables (Cont.)

Sex	Day of Sampling	N	Dosage (mg/kg/day)	Zaleplon C_{max} (µg/mL)	Zaleplon AUC_{0-24} (µg*hr/mL)	Desethyl-zaleplon C_{max} (µg/mL)	Desethyl-zaleplon AUC_{0-24} (µg*hr/mL)
			Gavage (continued)				
M	14	4	10	1.42	0.10	0.62	0.59
F	14	4	10	1.03	0.78	1.05	0.90
M	14	4	20	6.57	3.89	1.36	1.77
F	14	4	20	4.39	2.92	1.80	2.24
M	14	4	40	10.6	9.02	3.09	4.57
F	14	4	40	7.84	6.33	4.26	5.90
			Oral (Diet) 3-Month Range Finding Study: MIRACL 26277				
M	7	6	1	0.11	1.86	<0.005	ND
F	7	6	1	0.04	0.78	ND	ND
M	Week 13	6	1	0.03	ND	<0.005	ND
F	Week 13	6	1	0.05	ND	<0.005	ND
M	7	6	40	0.18	2.83	0.08	0.85
F	7	6	40	0.12	1.60	0.04	0.46
M	Week 13	6	40	0.07	1.23	0.04	0.62
F	Week 13	6	40	0.07	0.98	0.05	0.69
M	7	6	240	0.40	5.84	0.98	11.9
F	7	6	240	0.24	3.30	0.63	7.83
M	Week 13	6	240	0.79	9.45	1.05	9.44
F	Week 13	6	240	0.16	3.08	0.64	10.4
			Oral (Gavage) 5-Month Range Finding Study: MIRACL 26929, MIRACL 27039				
M	0	4	20	0.94	1.00	0.72	0.84
F	0	4	20	0.95	1.19	1.35	1.34
M	3-months	4	20	4.09	4.06	2.10	2.17
F	3-months	4	20	2.50	2.20	1.60	1.94
M	0	4	40	3.93	3.80	1.52	1.96
F	0	4	40	4.38	4.89	3.40	4.61
M	3-months	4	40	6.41	6.85	2.60	3.36
F	3-months	4	40	8.73	6.11	3.62	4.71
M	0	4	80	11.3	13.2	3.34	5.88
F	0	4	80	9.76	13.5	7.05	11.8
			Oral (Garage) 42-Weeks Study: GTR 29670				
M	7	4	1	0.042	0.078	NA	NA
F	7	4	1	0.033	0.091	NA	NA
M	7	4	5	0.093	0.19	0.10	0.085
F	7	4	5	0.200	0.28	0.23	0.17
M	7	4	20	3.08	2.61	1.12	1.49
F	7	4	20	3.20	2.61	2.31	2.47

a: Parameter values are obtained from pooled data therefore CV% cannot be calculated

b: AUC_{0-8}

NA = Not available

ND = Not determined

PK tables (Cont.)
Mice (Cont.)

Sex	Day of Sampling	N	Dosage (mg/kg/day)	Zaleplon C_{max} (µg/mL)	Zaleplon AUC_{0-t} (µg·hr/mL)	Desethyl-zaleplon C_{max} (µg/mL)	Desethyl-zaleplon AUC_{0-t} (µg·hr/mL)
Oral (Gavage) 5-Month Range Finding Study: MIRACL 26929, MIRACL 27039							
M	3-months	4	80	19.9	21.4	4.54	8.50
F	3-months	4	80	15.3	15.6	6.45	10.2
M	0	4	160	19.0	39.5	12.0	22.2
F	0	4	160	24.4	34.3	8.79	24.2
M	3-months	4	160	29.6	37.4	7.64	19.6
F	3-months	4	160	35.1	39.0	11.8	22.4
M	0	4	240	23.9	57.7	7.59	25.4
F	0	4	240	29.6	90.7	16.8	61.8
M	3-months	4	240	30.7	53.8	8.40	27.7
F	3-months	4	240	33.0	51.5	10.8	24.5
Oral (Diet) Carcinogenicity Study: GTR 29394[b]							
M	7	4	25	0.07	1.07	0.03	0.28
F	7	4	25	0.42	3.32	0.38	2.44
M	12-months	4	25	0.09	0.97	0.07	0.82
F	12-months	4	25	0.09	1.08	0.07	0.78
M	7	4	50	0.14	1.41	0.07	0.62
F	7	4	50	0.11	1.16	0.06	0.59
M	12-months	4	50	0.21	2.50	0.23	2.73
F	12-months	4	50	0.17	2.36	0.18	2.14
M	7	4	100	0.21	1.69	0.18	1.32
F	7	4	100	0.22	2.05	0.17	1.65
M	12-months	4	100	0.66	5.91	0.77	8.17
F	12-months	4	100	0.48	5.31	0.71	7.35
M	7	4	200	0.28	1.36	0.32	0.88
F	7	4	200	0.35	3.45	0.45	5.74
M	12-months	4	200	1.36	15.3	1.49	18.2
F	12-months	4	200	0.17	4.04	1.51	13.7

PK tables (Cont.)

Mean TK in RATS

Sex	Day of Sampling	N	Dosage (mg/kg/day)	Zaleplon C_{max} (µg/mL)	Zaleplon AUC_{0-24} (µg·hr/mL)	Desethyl-zaleplon C_{max} (µg/mL)	Desethyl-zaleplon AUC_{0-24} (µg·hr/mL)
2-Week Repeated-Dose Study (Diet): GTR 29960							
M	14	4	20	0.67	8.46	0.69	7.67
2-Day Batch Comparison (Gavage): MIRACL 23359							
M	1	3	100 (PC0993)	18.9	93.8	14.8	145
F	1	3	100 (PC0993)	16.8	199	11.7	130
M	1	3	100 (PC1008)	33.3	105	14.3	161
F	1	3	100 (PC1008)	19.9	241	12.7	192
M	1	3	200 (PC0993)	24.2	242	22.8	358
F	1	3	200 (PC0993)	56.1	641	19.1	333
M	1	3	200 (PC1008)	18.5	250	18.9	346
F	1	3	200 (PC1008)	30.1	564	17.9	316
Oral (Gavage) 2-Week Repeated-Dose Toxicity Study: MIRACL 27047, MIRACL 27548							
M	0	5	1	0.126	0.273[b]	0.050	0.120[b]
F	0	4	1	0.281	0.588[b]	0.11	0.296[b]
M	13	5	1	0.073	0.171[b]	0.029	0.082[b]
F	13	5	1	0.216	0.297[b]	0.071	0.128[b]
M	0	5	10	1.34	4.62[b]	1.02	4.13[b]
F	0	5	10	2.61	8.81[b]	1.12	5.24[b]
M	13	4	10	1.47	3.30[b]	1.37	4.31[b]
F	13	5	10	2.82	6.97[b]	1.16	4.15[b]
M	0	5	100	9.44	50.3[b]	10.25	68.9[b]
F	0	5	100	14.9	80.7[b]	6.58	44.0[b]
M	13	5	100	4.31	27.4[b]	7.37	39.1[b]
F	13	5	100	11.7	54.5[b]	7.19	44.0[b]

PK tables (Cont.)
Rats (Cont.)

Sex	Day of Sampling	N	Dosage (mg/kg/day)	Zaleplon C_{max} (µg/mL)	Zaleplon AUC_{0-24} (µg·hr/mL)	Desethyl-zaleplon C_{max} (µg/mL)	Desethyl-zaleplon AUC_{0-24} (µg·hr/mL)
Oral (Gavage) 3-Month Repeated-Dose Toxicity Studies: MIRACL 23438, MIRACL 23441							
M	0	3	5	0.7	2.06	0.4	1.42
F	0	3	5	0.9	1.67	0.4	0.92
M	28/29	3	5	0.8	1.9	0.8	2.56
F	28/29	3	5	0.7	1.5	0.4	1.13
M	91/92	3	5	1.3	4.13	1.2	5.15
F	91/92	3	5	1.3	3.01	0.8	2.24
M	0	3	50	6.8	53.0	7.8	79.5
F	0	3	50	9.7	72.3	5.6	52.9
M	28/29	3	50	10.0	45.2	10.4	84.2
F	28/29	3	50	10.6	61.8	9.4	66.6
M	91/92	3	50	8.9	35.2	11.2	103
F	91/92	3	50	9.1	36.9	10.2	53.5
M	0	3	100	11.5	90.4	11.9	140
F	0	3	100	16.2	125	7.5	92.8
M	28/29	3	100	13.4	76.1	17.7	154
F	28/29	3	100	13.9	101	12.7	122
M	91/92	3	100	12.9	68.3	15.0	153
F	91/92	3	100	11.1	82.1	10.0	109
M	0	3	200	17.6	168	15.4	291
F	0	3	200	18.7	220	12.0	171
M	28/29	3	200	10.3	82.1	19.2	210
F	28/29	3	200	16.0	167	15.6	205
M	91/92	3	200	13.2	131	23.6	316
F	91/92	3	200	30.1	194	32.5	259
Oral (Gavage) 1-Year Repeated-Dose Toxicity Study: MIRACL 25704, MIRACL 24428							
M	0	1-4	5	0.7	1.7	0.4	1.3
F	0	1-4	5	1.0	1.8	0.5	1.1
M	92	1-4	5	2.0	5.1	1.1	5.4
F	92	1-4	5	2.2	4.6	1.0	3.2
M	184	1-4	5	2.1	6.3	1.5	8.4
F	184	1-4	5	3.2	7.1	1.5	5.9
M	366	1-4	5	1.8	6.1	1.3	7.6
F	366	1-4	5	2.6	6.2	1.1	4.2
M	0	1-4	20	4.3	12.1	2.7	14.1
F	0	1-4	20	5.0	22.3	2.5	18.0
M	92	1-4	20	9.0	34.8	6.5	52.3
F	92	1-4	20	12.2	46.2	4.6	32.6
M	184	1-4	20	15.4	66.5	10.3	118
F	184	1-4	20	21.2	109	10.2	99.5
M	366	1-4	20	8.9	46.3	6.1	73.2
F	366	1-4	20	12.0	63.2	6.1	51.1
M	0	1-4	50	6.9	33.3	7.5	76.0
F	0	1-4	50	10.4	51.8	5.6	46.2
M	92	1-4	50	17.5	72.6	15.2	139
F	92	1-4	50	21.7	93.8	9.9	84.0
M	184	1-4	50	23.9	90	19.9	208
F	184	1-4	50	30.7	177	13.9	143
M	366	1-4	50	15.0	68.9	16.5	220
F	366	1-4	50	36.8	144	29.6	163

PK tables (Cont.)

TK Rats

Sex	Day of Sampling	N	Dosage (mg/kg/day)	Zaleplon C_{max} (µg/mL)	Zaleplon AUC_{0-t} (µghr/mL)	Desethyl-zaleplon C_{max} (µg/mL)	Desethyl-zaleplon AUC_{0-t} (µghr/mL)
Oral (Diet) 1-Week Range Finding Study: MIRACL 26068							
M	6-7	3	0.5	0.05	0.43	NA	NA
F	6-7	3	0.5	0.03	0.64	NA	NA
M	6-7	3	25	0.46	6.28	0.37	4.16
F	6-7	3	25	0.74	9.95	0.42	6.36
M	6-7	3	100	2.34	34.9	5.19	75.9
F	6-7	3	100	3.72	56.9	5.20	76.4
M	6-7	3	1000	5.02	116	17.2	390
F	6-7	3	1000	6.55	123	17.5	392
Oral (Diet) 2-Week Range Finding Study: GTR 28266							
M	14	5	1	0.028	0.29	ND	ND
F	14	5	1	0.019	0.31	0.004	ND
M	14	5	10	0.163	2.50	0.128	1.80
F	14	5	10	0.222	3.24	0.118	1.89
M	14	5	20	0.485	6.39	0.482	6.09
F	14	5	20	0.635	9.55	0.387	5.52
Oral (Diet) 3-Month Range Finding Study: MIRACL 26070							
M	7	3	1	0.01	0.13	<0.005	ND
F	7	3	1	0.02	0.20	<0.005	ND
M	86	3	1	0.04	0.39	0.02	0.32
F	86	3	1	0.03	0.44	0.01	0.11
M	7	3	10	0.17	2.37	0.10	1.49
F	7	3	10	0.26	3.24	0.14	1.70
M	86	3	10	0.33	4.81	0.38	5.82
F	86	3	10	0.36	4.78	0.23	3.27
M	7	3	100	3.85	56.1	6.99	100
F	7	3	100	4.11	77.9	4.79	77.6
M	86	3	100	4.57	51.5	10.3	169
F	86	3	100	5.86	90.2	6.72	112

PK tables (Cont.)

Rats (Cont.)

Sex	Day of Sampling	N	Dosage (mg/kg)	Zaleplon C_{max} (µg/mL)	Zaleplon AUC_{0-24} (µg•hr/mL)	Desethyl-zaleplon C_{max} (µg/mL)	Desethyl-zaleplon AUC_{0-24} (µg•hr/mL)
Oral (Diet) Carcinogenicity Study: GTR 29582							
M	7	4	1.0	0.03	0.35	ND	ND
F	7	4	1.0	0.04	0.51	ND	ND
M	180	4	1.0	0.03	0.42	0.02	0.23
F	180	4	1.0	0.02	0.33	0.01	0.10
M	365	4	1.0	0.04	0.44	0.04	0.49
F	365	4	1.0	0.03	0.39	0.01	0.21
M	7	4	10	0.26	3.43	0.16	1.89
F	7	4	10	0.47	4.26	0.18	1.85
M	180	4	10	0.39	5.08	0.52	6.85
F	180	4	10	0.32	3.98	0.24	2.82
M	365	4	10	0.46	6.56	0.93	13.9
F	365	4	10	0.41	5.40	0.32	4.17
M	7	4	20	0.78	9.48	0.54	6.56
F	7	4	20	1.00	10.7	0.46	5.07
M	180	4	20	0.88	10.6	1.62	18.9
F	180	4	20	1.05	12.4	0.75	8.86
M	365	4	20	1.17	15.7	3.18	45.0
F	365	4	20	1.23	16.9	0.89	12.6

a: Parameter values are obtained from pooled data therefore CV% cannot be calculated
b: AUC_{0-t}
c: $AUC_{0-\infty}$
NA = Not available
ND = Not determined

PK tables (Cont.)

Dogs: Mean (CV%) TK parameters

Sex	Day of Sampling	N	Dosage (mg/kg/day)	Zaleplon C_{max} (µg/mL)	Zaleplon AUC_{0-24} (µg·hr/mL)	Desethyl-zaleplon C_{max} (µg/mL)	Desethyl-zaleplon AUC_{0-24} (µg·hr/mL)
Oral 2-Week Repeated-Dose Study: GTR 26892							
M	0	6	40	18.5 (13)	184 (16)	5.5 (15)	65 (19)
M	14	4	40	15.3 (17)	127 (13)	4.0 (16)	43 (17)
M	0	6	200[a]	44.8 (35)	724 (40)	17.0 (24)	283 (23)
M	6	4	200[a]	41.8 (12)	500 (15)	25.8 (16)	314 (14)
Oral 2-Week Repeated-Dose Toxicity Study: MIRACL 23881							
M	0	1	50	12.9	152	ND	ND
F	0	1	50	17.0	214	ND	ND
M	13	1	50	9.6	77.6	ND	ND
F	13	1	50	17.3	105	ND	ND
M	0	1	100	28.0	442	ND	ND
F	0	1	100	10.0	99.8	ND	ND
M	13	1	100	18.4	165	ND	ND
F	13	1	100	11.7	82.0	ND	ND
M	0	1	400	18.6	271	ND	ND
F	0	1	400	45.4	89R	ND	ND
M	13	1	400	ND[b]	ND[b]	ND	ND
F	13	1	400	ND[b]	ND[b]	ND	ND
Oral 3-Month Repeated-Dose Toxicity Study: MIRACL 25439							
M	0	4	5	1.09 (56)	3.28 (63)	0.46 (50)	1.67 (62)
F	0	4	5	0.66 (85)	1.28 (128)	0.28 (50)	0.57 (98)
M	36	4	5	1.89 (37)	6.75 (43)	0.67 (31)	2.95 (39)
F	36	4	5	1.13 (51)	2.83 (32)	0.42 (36)	1.34 (24)
M	86	4	5	2.28 (14)	9.55 (29)	0.62 (11)	3.65 (27)
F	86	4	5	1.94 (8)	6.98 (23)	0.61 (5)	2.80 (15)
M	0	4	50	11.5 (57)	116 (60)	3.76 (50)	44.3 (53)
F	0	4	50	10.5 (43)	111 (49)	3.69 (37)	42.4 (44)
M	36	4	50	14.2 (26)	115 (29)	5.24 (29)	44.9 (29)
F	36	4	50	15.1 (21)	120 (20)	4.93 (23)	43.9 (20)
M	86	3	50	8.60 (5)	92.7 (24)	2.67 (19)	34.8 (35)
F	86	3	50	14.5 (21)	122 (28)	4.43 (31)	42.9 (34)

PK tables (Cont.)
Dogs (Cont.)

Sex	Day of Sampling	N	Dosage (mg/kg)	Zaleplon C_{max} (µg/mL)	Zaleplon AUC_{0-t} (µg•hr/mL)	Desethyl-zaleplon C_{max} (µg/mL)	Desethyl-zaleplon AUC_{0-t} (µg•hr/mL)
Oral 3-Month Repeated-Dose Toxicity Study: MIRACL 23439							
M	0	4	200	32.1 (47)	523 (55)	14.2 (56)	236 (64)
F	0	3	200	38.7 (11)	546 (10)	17.2 (27)	256 (24)
M	36	3	200	43.2 (3)	463 (17)	22.6 (8)	265 (19)
F	36	3	200	27.6 (14)	314 (20)	18.9 (16)	191 (28)
M	86	3	200	32.1 (78)	271 (36)	11.8 (61)	125 (34)
F	86	2	200	44.4 (N=2)	299 (N=2)	21.1 (23)	176 (14)
Oral 3-Month Repeated-Dose Toxicity Study[a]: GTR 28741							
M	92	2	50	11.9	109	ND	ND
F	92	2	50	16.8	152	ND	ND
M	92	2	200	31.3	328	ND	ND
F	92	2	200	40.6	499	ND	ND
Oral 1-Year Repeated-Dose Toxicity Study: MIRACL 25705, MIRACL 26429							
M	0	3	5	1.8 (22)	5.9 (32)	0.7 (14)	2.8 (29)
F	0	3	5	1.7 (35)	6.2 (5)	0.6 (33)	2.9 (7)
M	86	3	5	1.9 (11)	6.5 (6)	0.5 (9)	2.6 (5)
F	86	3	5	2.9 (45)	11.6 (47)	0.9 (44)	4.7 (47)
M	176	3	5	2.3 (7)	8.8 (13)	0.7 (0)	3.6 (8)
F	176	3	5	1.5 (33)	5.3 (42)	0.5 (40)	2.3 (30)
M	356	3	5	2.1 (13)	8.8 (18)	0.6 (5)	3.7 (11)
F	356	3	5	1.9 (35)	6.7 (55)	0.6 (27)	3.0 (50)
M	0	3	20	4.0 (35)	33.3 (50)	1.5 (33)	14.1 (50)
F	0	3	20	3.1 (16)	14.8 (42)	1.1 (18)	6.7 (36)
M	86	3	20	6.0 (75)	43.1 (59)	1.7 (63)	15.5 (48)
F	86	3	20	7.5 (32)	59.7 (9)	2.1 (24)	20.5 (3)
M	176	3	20	9.1 (29)	60.4 (27)	2.2 (14)	19.2 (15)
F	176	3	20	10.2 (18)	68.9 (18)	2.5 (16)	23.1 (6)
M	356	3	20	7.3 (38)	52.1 (20)	2.1 (34)	18.0 (18)
F	356	3	20	8.1 (20)	68.7 (22)	2.2 (25)	21.8 (23)
Oral 1-Year Repeated-Dose Toxicity Study: MIRACL 25705, MIRACL 26429							
M	0	3	40	8.0 (51)	93.9 (60)	2.5 (40)	33.9 (46)
F	0	3	40	9.5 (51)	122 (60)	3.2 (25)	45.0 (25)
M	86	3	40	13.6 (23)	107 (10)	3.8 (21)	40.1 (4)
F	86	3	40	19.2 (8)	132 (1)	4.9 (11)	45.8 (9)
M	176	3	40	13.4 (53)	103 (41)	3.5 (23)	37.4 (40)
F	176	3	40	15.2 (43)	128 (35)	4.3 (37)	43.8 (27)
M	356	3	40	10.5 (55)	87.5 (47)	2.8 (61)	29.9 (46)
F	356	2	40	16.0 (N=2)	159 (N=2)	4.6 (N=2)	53.6 (N=2)

a: Dosed for 6 days only
b: Not determined because dogs died
c: Parameter values are obtained from pooled data therefore CV% cannot be calculated
ND = Not determined

PK (Cont.)
Pregnant rabbit:

Single oral dose of 2, 10, or 50mg/kg CL 284-846 was administered to 4 pregnant rabbits/dose during gd17 by oral gavage. This PK study is a complementary to Seg II rabbit teratology study. Plasma conc and other PK parameters were determined for the parent, des-ethyl zaleplon (CL 284-859), and M2 (CL 345-905, the 5-oxo-zaleplon). Table below from sponsor presents the results values are means (CV%):

ZAL-846 (N=4)

Dose (mg/kg)	C_{max} (µg/mL)	t_{max} (hr)	AUC_{0-inf} (µg*hr/mL)	$t_{1/2}$ (hr)
2	0.29 (145)	1.0 (71)	0.57 (62)[a]	1.5 (84)[a]
10	0.49 (84)	1.8 (29)	2.70 (41)[a]	3.3 (62)[a]
50	2.62 (39)	6.5 (68)	21.1 (31)[a]	3.7 (23)[a]

CL 284,859 (N=4)

Dose (mg/kg)	C_{max} (µg/mL)	t_{max} (hr)	AUC_{0-inf} (µg*hr/mL)	$t_{1/2}$ (hr)
2	0.029 (97)	0.9 (86)	0.074 (ND)[b]	1.4 (ND)[b]
10	0.15 (108)	1.8 (29)	0.79 (ND)[b]	5.5 (ND)[b]
50	0.58 (51)	5.3 (90)	4.6 (79)[a]	3.3 (17)[a]

CL 345,905 (N=4)

Dose (mg/kg)	C_{max} (µg/mL)	t_{max} (hr)	AUC_{0-inf} (µg*hr/mL)	$t_{1/2}$ (hr)
2	0.19 (109)	0.6 (40)	0.54 (27)	2.6 (74)
10	0.28 (76)	1.0 (71)	1.88 (30)[a]	6.2 (98)[a]
50	1.81 (46)	5.6 (95)	14.3 (ND)[b]	6.1 (ND)[b]

[a] N=3
[b] N=2
CV% was not determined (ND) when PK parameters were not estimated in at least 50% of animals

The range of plasma conc at the 3 doses was:

Dose (mg/kg)	Range of plasma conc. (ug/ml) Parent	Des-ethyl	M2
2	<0.005-0.28	<0.005-0.03	<0.005-0.17
10	<0.005-0.5	<0.005-0.15	<0.005-0.25
50	0.8-1.81	0.01-0.45	0.12-1.03

It is noted that at the HD of 50mg/kg, a 2nd peak occurred at 8 or 12hr post dose (mean 5-6.5hr), compared with the 1st max conc recorded at 2hr. This 2nd peak was seen for the parent and the metabolites. The sponsor indicated that the 2nd peak may reflect re-absorption. Also, elimination $t_{1/2}$ was increased with increased dose. This maybe related to re-absorption of the drug or metabolites since plasma levels were still measurable upto 24hr in the higher doses but only till 4-6hr in 2mg/kg dose.

PK (Cont.)

Pregnant rat:

Single oral dose of 1, 10, or 100mg/kg CL 284-846 was administered to 4 pregnant rats/dose during gd15 by oral gavage. This PK study is a complementary to Seg II rat teratology study. Plasma concentrations and other PK parameters were determined for the parent, des-ethyl zaleplon (CL 284-859), M2 (CL 345-905, the 5-oxo-zaleplon), M1 (CL 345-644), & M2 glucuronide. Table below from sponsor presents the results; values are means (CV%):

ZAL-846 (N=4)

Dose (mg/kg)	C_{max} (µg/mL)	T_{max} (hr)	$AUC_{0-\infty}$ (µg•hr/mL)	$t_{1/2}$ (hr)
1	0.195 (15)	0.5 (0)	0.341 (15)	1.07 (14)
10	3.54 (6)	0.8 (38)	12.3 (28)	1.77 (64)
100	18.5 (23)	2.3 (56)	163 (8)	4.26 (43)[a]

CL 284,859 (Des-Ethyl Metabolite) (N=4)

Dose (mg/kg)	C_{max} (µg/mL)	T_{max} (hr)	$AUC_{0-\infty}$ (µg•hr/mL)	$t_{1/2}$ (hr)
1	0.076 (16)	0.8 (38)	0.169 (19)	1.09 (15)
10	1.47 (17)	1.8 (29)	7.28 (26)	1.43 (43)
100	10.2 (21)	5.5 (18)	131 (ND)[b]	1.83 (ND)[b]

CL 345,644 (M1) (N=4)

Dose (mg/kg)	C_{max} (µg/mL)	T_{max} (hr)	AUC_{0-t}* (µg•hr/mL)	$t_{1/2}$ (hr)
1	ND	ND	ND	ND
10	0.032 (20)[a]	2.3 (65)[a]	0.20 (ND)[b]	ND
100	0.10 (52)	5.0 (52)	0.13 - 2.35	9.1[b]

CL 345,905 (M_2) (N=4)

Dose (mg/kg)	C_{max} (µg/mL)	T_{max} (hr)	AUC_{0-t}* (µg•hr/mL)	$t_{1/2}$ (hr)
1	ND	ND	ND	ND
10	ND	ND	ND	ND
100	0.45 (54)[a]	2.0 (0)[a]	1.2 - 4.7	6.6[b]

M_2 Glucuronide (N=4)

Dose (mg/kg)	C_{max} (µg/mL)	T_{max} (hr)	AUC_{0-t}* (µg•hr/mL)	$t_{1/2}$ (hr)
1	ND	ND	ND	ND
10	ND	ND	ND	ND
100	0.27 (58)[a]	4.0 (50)[a]	1.57 (65)[a]	5.1[b]

ND Not determined due to limited concentration data or poor regression fitting

*AUC_{0-t} (t ranged from 6 to 8 hours)

[a]N=3, [b]N=1

CV% was not determined when PK parameters were not estimated in at least 50% of rats

PK (Cont.)
Pregnant rat (Cont):

The range of plasma concentration at the 3 doses was:

Dose (mg/kg)	Range of plasma conc. (ug/ml) Parent	Des-ethyl	M1	M2	M2-glucuronide
1	<0.005-0.20	<0.005-0.07	ND	ND	ND
10	<0.005-3.4	<0.005-1.5	ND	ND	ND
100	<0.005-18	<0.005-10	<0.025-0.11	<0.025-0.45	<0.025-0.26

ND values were BQL or not determined for the 1&10mg/kg doses.

The des-ethyl is the major metabolite in the rat (pregnant and non-pregnant), the other 3 are minor metabolites. The profile in the pregnant rat is similar to that in non-pregnant. Mean plasma t_{max} increased with increase in dose for the parent and metabolites, this was also true for the parent $t_{1/2}$. The desethyl constituted 44 & 80% of the parent C_{max} & AUC respectively, at the highest dose. The other 3 metabolites represent <2% of the parent exposure at the highest dose.

From the above tables, the HIGHEST values for mean C_{max} and AUC_{0-24hr} were for:

Species	C_{max} (ug/ml)	AUC_{0-24hr} (ug.hr/ml)	
Mice	1.5	9.5	@ 200mg/kg p.o. diet for 2yr for conc and, @ 240mg/kg p.o. diet for 3mo.
Rats	6	90	@ 100mg/kg p.o. diet daily for 3mo*
Dogs	45	898	@ 400mg/kg p.o. daily for 2wks
Rabbit/ Pregnant	2.6	21	@50mg/kg p.o. single dose on gd17
Rat/Pregnant	19	163**	@100mg/kg p.o. single dose on gd15

* higher values were seen at 1000mg/kg dosed daily p.o. diet for 1wk: 6.6ug/ml and 123ug.hr/ml for C_{max} & AUC respectively.

** AUC here is determined over 0-24hr.
The above AUC values represent 95-8980 times the maximum mean exposure in humans after 10mg/d clinical dose. The corresponding values for mean C_{max} represent 37.5-1125 times the mean maximum C_{max} in humans after 10mg/d dose.

Absorption & Tissue Distribution:

CL 284-846 was studied in a number of species. Oral bioavailability in CD-1 **mice** after a single p.o. dose of 50mg/kg radioactive drug was 87%; the drug was rapidly absorbed with max concentration reached by 0.5hr postdose; similar values were measured in C57BL/6NCrlBR mice.

Rats administered a single p.o. dose of 5mg/kg ^{14}C-zaleplon, showed an absolute oral bioavailability of 83%. Total body Cl and V_{dss} were 17ml/min/kg and 1.1l/kg respectively. Comparison of plasma radioactivity concentration after p.o. and i.v. dosing, indicates that zaleplon is completely absorbed in the rat. The PK prameters were similar after an i.v. bolus (2.5mg/kg) and 6hr i.v. infusion of 0.4mg/kg/hr. The C_{max}, exposure, Cl, V_{dss}, and bioavailability were not age dependent for zaleplon or its desethyl metabolite as determined following p.o. gavage or i.v. dosing of 4mg/kg to 3, 6, 12, or 18mo old SD rats. The plasma $t_{1/2}$ after the i.v. injection was slightly increased from 0.8hr in 3mo old rats to 1.1hr in the 18mo old rats and the exposure to the desethyl was slightly incr in the 3mo old (2x) relative to the 18mo old rats for both routes. There are no differences in PK parameters of pregnant and non-pregnant rats for the parent and M1, M2, and M2 glucuronide. Zaleplon was administered as a single oral dose to pregnant rats on gd15 at 1, 10, 100mg/kg, the mean max concentration and exposure increased with dose and exposure of the metabolites was <3% of the parent AUC at 100mg/kg. The desethyl metabolite was the main metabolite in the plasma with 40&80% C_{max} and AUC of the parent respectively. Zaleplon is well and rapidly absorbed after repeate dosing in the rat with max concentration reached by 1-1.6hr post dose (5mg/kg p.o. gavage of radioactive zaleplon for 1wk). Comparison of PK parameters of the rat and mouse showed some difference in plasma concentration time profiles between the 2 species with the rat having 6 times higher exposure than the mouse (single p.o. gavage dose of 5mg/kg).

Pregnant **Rabbits** dosed a single p.o. gavage dose of 2, 10, or 50mg/kg on gd17, exposure and concentration increased with dose with mean elimination $t_{1/2}$ of 1.5-3.7hr for the 2-50mg/kg dose and both of these parameters for the desethyl metabolite were 10-30% those measured for the parent.

Following single p.o. dose of zaleplon to **dogs**, plasma $t_{1/2}$ was about 1hr measured following the i.v. injection. Mean absolute bioavailability is 82&87% after oral solution and oral suspension respectively. The V_{dss} on day0 after an i.v. injection of 0.5, 1, and 2mg/kg in a 2wk tox study was 1l/kg and the total Cl ranged between 21-35ml/min/kg; plasma $t_{1/2}$ was 0.5hr and no sex difference in PK parameters was seen. The plasma $t_{1/2}$ after a single p.o. gavage dose of 40mg/kg (max dose used in the 1yr tox study), was 6hr for total radioactivity and the parent and, 5.5hr for the desethyl metabolite. The drug seems to be absorbed slowly in the dog based on comparison of $t_{1/2}$ in i.v. and p.o. dosing (t_{max} 1-4hr). Zaleplon accounted for 72% of C_{max} and 65% of exposure of the total radioactivity. The desethyl metabolite, the main circulating metabolite in the dog, accounted for 21% of total radioactivity, the M2 plasma conc was <0.05ug/ml upto the 8hr sampling time and its exposure represented 0.1% of total radioactivity.

Cynomolgus **Monkeys** were administered a single p.o.(4mg/kg) or i.v. (2mg/kg) dose of radioactive zaleplon in a crossover study. Total absorption of radioactivity was estimated at 56% and mean bioavailability was 23%, elimination $t_{1/2}$ was 1hr after the i.v. dosing. Mean plasma Cl and V_d were 17ml/min/kg and 1.2l/kg respectively.

Tissue Distribution:
In general, Zaleplon tissue distribution was similar in tissues to those measured in plasma with rapid uptake and elimination from all tissues. Distribution of zaleplon was studied using radioactive drug administered orally, autoradiography in pregnant and non-pregnant rats, in placental transfer, and milk excretion studies.

Following single p.o. gavage or i.v. dose administration of radioactive drug (5mg/kg) to SD rats, radioactivity was recorded in all tissues after p.o. with 2.7-4 times higher radioactivity in liver and kidneys than in plasma; other tissues were equivalent to, or less than the radioactivity in plasma. Radioactivity in blood and tissues started decling 8hr after the p.o. dose and was <3% of max concentration by 24hr. Complete elimination of adioactivity was almost complete (0.3% left in few tissues) by 96hr after the p.o. dose. Similar distribution pattern was seen after the i.v. Also, similar distribution pattern was seen after p.o. 5mg/kg to rats assessed till 168hr where concentration were below detection limit in 24 of 30 tissues. When ^{14}C-zaleplon was administered to rats for 7d at daily p.o. gavage dose of 5mg/kg to rats, the radioactive distribution was similar to that after single dose and the radioactive concentration of plasma and tissues at 24hr after 7d dosing were 2x > than those measured at 24hr on d1.

Whole body autoradiography determined in the rat after a single p.o. gavage dose of 5mg/kg showed wide distribution of radioactivity with rapid elimination. Zaleplon distributed rapidly and widely into the various areas of the rat brain with the pineal gland containing slightly higher levels of radioactivity than the rest of the brain areas. By 4hr postdose, no radioactivity was detected in any brain tissue. Melanin binding to zaleplon was assessed by whole body autoradiograohy using male SD and Long-Evans rats following single oral gavage 5mg/kg dose. Concentration of radioactivity were in general, 2-4x > in pigmented than albino rats with some indication of weak and reversible binding to melanin. At the 1hr sampling time, the radioactive concentration in the uveal tract of pigmented rats was >25 times higher than those in albino rats. However, rapid decline was seen by the 2nd hr of sampling at <1.6 times higher values for the pigmented than the albino rats. Small, <1%, of that seen at max concentration, residual concentration were detectable in the pigmented rats at 1-2wk sampling times.

Zaleplon placental transfer and distribution into selective fetal tissues and excretion in milk were studied in rats during gd18 administered single p.o. dose of 5mg/kg of ^{14}C-zaleplon. Fetal liver, brain, and kidney radioactive conc were 20-70% of the corresponding maternal tissue radioactivity. Total radioactivity in the fetus was <25% of maternal plasma conc; elimination of radioactivity from the fetus happened at the same rate as that from maternal plasma. Concentration of zaleplon in milk were 3 times higher than those in plasma for upto 48hr postdose. Rates of radioactive elimination were similar between milk and plasma.

Excretion:

Table below from the sponsor shows the excretion of zaleplon in the rat, dog, and humans over 168hr postdose:

EXCRETION OF RADIOACTIVITY IN RATS, DOGS, AND HUMANS UP TO 168 HOURS FOLLOWING SINGLE- AND REPEATED-DOSING OF ^{14}C-ZALEPLON

Species	Dosage (mg/kg)	Mode of Administration	N	Collection Period (Days)	Percent of Administered Dosage (CV%) Urine	Bile	Feces	Total
Single-Dose Mass Balance in Rats								
Rat	5	IV	6	7	55.0 (14)	ND	34.5 (23)	90.5 (8)[c]
Rat	5	PO (gavage)	6	7	57.2 (13)	ND	31.1 (19)	89.3 (6)[c]
Rat	5	PO (gavage)	4	7	70.3 (7)	ND	28.9 (11)	99.2 (4)
Rat	20	PO (gavage)	4	2	15.5 (26)	69.6 (17)	2.18 (131)	87.2 (17)
Repeated-Dose Mass Balance in Rats								
Rat	5[b]	PO (gavage)	5	7	57.5 (5)	ND	38.6 (6)	96.1 (1)
Single-Dose Mass Balance in Dogs								
Dog	5	IV	4	7	30.4 (33)	ND	59.2 (31)	84.0 (10)[c]
	5	PO (capsule)	3	7	22.8 (17)	ND	60.9 (9)	91.5 (2)[c]
Single-Dose Mass Balance in Humans								
Human	20[d]	PO (solution)	6	5	70.9 (10)	ND	17.0 (54)	87.9 (7)

a: Includes cage wash
b: Samples collected for 168 hours following Day 7 of the multiple-dose study
c: Includes cage wash and emesis
d: Single 20 mg dose
ND = Not determined

Zaleplon elimination is rapid and consistent with its short $t_{1/2}$. In a single dose (i.v. & p.o.) mass balance study in SD rats, zaleplon was excreted rapidly with 65&75% of radioactive dose eliminated within 24hr post the i.v. & p.o. dose respectively. By 168hr postdose, 90&89% of dose was recovered after both routes; 55% of the dose was excreted in urine and 34.5% in feces after the i.v. dose. In bile duct cannulated rats dosed a single 20mg/kg p.o. radioactive zaleplon, excretion was rapid with 87% of dose eliminated within 48hr and 16% appeared in urine, 70% in bile, and 2.2% in feces. With the higher % of drug eliminated in urine of non bile cannulated rats, it is suggested that drug related material eliminated in bile may be reabsorbed and perhaps further metabolized then excreted. In a repeate dose of orally administered ^{14}C-zaleplon (5mg/kg/d for 7d), total recovery of radioactivity in urine and feces was 96% of administered dose within 168hr post the last dose on d7.

In dogs dosed 5mg/kg radioactive zaleplon p.o. or i.v. as a single dose, recovery of radioactivity was similar in urine and feces dosed via i.v. but, in dogs dosed orally, urinary recovery was 21% or half of that seen in feces. Within 24hr, 62&71% of radioactivity was eliminated and a total of 84&92% of dose was recovered over 7d period after i.v. and p.o. dosing.

In healthy men dosed a single p.o. dose of 20mg/d of radioactive zaleplon, the drug was rapidly excreted with 63% of administered radioactivity recovered over the 1st 6hr. Some 88% of dose was recovered in urine (71%) and feces (17%) within 5d of dosing.

Plasma Protein Binding:

The in vitro plasma protein binding for the parent in the mouse, rat, dog, and humans was <70% however, slightly higher binding was measured for the desethyl at <81%. The extent of binding for both cpds was independent on concentration over the plasma concentration range noted in the tox studies and the presence of other cpds had no effect on binding. The in vivo protein binding of the parent ranged from 65% at 0.5hr to 74% at 8hr postdose. Zaleplon had no preferential partitioning into RBCs when determined in the rat and human blood.

Metabolism:

Both in vivo and in vitro studies were done in animals and humans to study the metabolic profile of zaleplon. In the animal species studied and humans, zaleplon was highly metabolized and only little drug is eliminated unchanged. Animals metabolize zaleplon more extensively than humans with the P450 system representing the major pathway in animals whereas, the aldehyde oxidase is the major enzyme responsible for metabolism in humans; both enzymes are found in humans. The P450 system catalyzes N-deethylation and N-deacetylation to form desethyl-zaleplon, desethyl-desacetyl zaleplon, P1, & P2 metabolites. The aldehyde oxidase catalyzes oxidation at the 5-position of the pyrazolopyrimidine ring and forms M1 & M2 metabolites both in humans and animals. In humans, the cytochrome P450 system is the minor pathway of metabolism and clearance of zaleplon whereas, the aldehyde oxidase that forms the M2 metabolite, is the main pathway.

The following figure from the sponsor shows the major metabolic pathways for zaleplon:

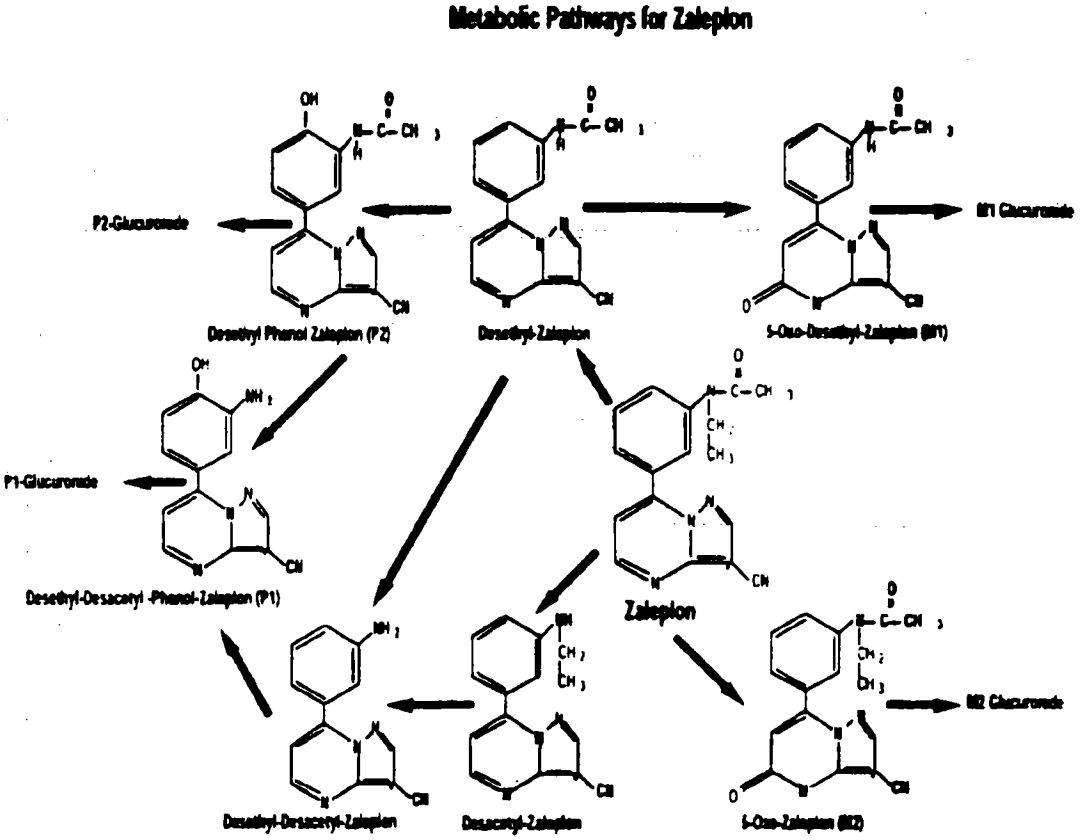


Table below from the sponsor, shows quantitative comparison of zaleplon and its metabolites in humans and animals after p.o. dosing.

MEAN ZALEPLON AND METABOLITE AUC[a] IN HUMAN, MOUSE, RAT, RABBIT AND DOG PLASMA FOLLOWING ORAL DOSAGES

Compound	Human 10 mg[b]	Mouse 50 mg/kg[c]	Rat 50 mg/kg/day	Pregnant Rabbit 50 mg/kg[d]	Dog 40 mg/kg[e]
Zaleplon	0.068	3.5	1.34	21.1	208
Desethyl-zaleplon	0.001	4.2	175	4.6	78
M1	0.02	0.20	0.5	NA	0
M2	0.198	0.76	4.8	14.3	<0.4
M2 Glucuronide	0.84	0	2.4	NA	0

a: μg•hr/mL
b: Data for zaleplon, M2 and M2 glucuronide were from GMR 29033 and GMR 26418 and for desethyl and M1 from GMR 29033
c: Pooled male and female data from a single-dose study[127]
d: Data from a single-dose study in pregnant rabbits[148]
e: Data from a single-dose study in dogs[148]
NA = Not available

The human values were derived from one study at 20mg/d dose normalized to 10mg/d and from a 2nd single dose study in the elderly dosed 10mg/d. The dose in the rat is from a 1yr oral gavage study (6mo samples) at 50mg/kg/d, the mouse and the dog data are from single oral PK studies of 50&40mg/kg respectively. Because of the short $t_{1/2}$ of zaleplon, the steady state dosing should not be different from a single dose profile.

Enzyme Induction:

The potential of zaleplon to induce liver enzymes was assessed by investigating microsomal protein, cytochrom P450 content and reductase activity, and isozyme activity in 2wk studies in mice and rats as well as by microscopic exam of livers in rodent tox studies. Zaleplon is an enzyme inducer particularly at high doses but less or no effect seen at low doses (<100mg/kg/d). Enzyme induction was accompanied by increase in liver wt and liver (centrilobular) hypertrophy as well as proliferation of SER. These findings are consistent with P450 induction. The sponsor indicated that zaleplon induction of hepatic enzymes is an adaptive response to metabolize high levels of zaleplon at the higher doses.

TOXICOLOGY

Acute Tox: [for more detail, see Dr. A. Wilk review dated Apr 16, 1991]:

Single dose acute tox studies with zaleplon were conducted in mice, rats, and dogs. In **mice**, adverse effects were observed at ≥500mg/kg p.o. dose and death occurred at ≥700mg/kg. Adverse effects in mice at ≥ 500mg/kg included clinical signs of CNS depression, decr or absence of feces, wet perianal area, decr B.wt & food intake, red-brown content in stomach & s.intestine, and distended urinary bladder. Similar findings were seen after i.p. dosing in mice with death at ≥500mg/kg and dilated renal pelvis at 700mg/kg dose. In **rats,** death occurred at ≥500mg/kg p.o. and ≥300mg/kg i.p., adverse effects were similar to those in mice in addition to incr in serum Na, Cl, & ALT and decr in serum total proteins and cholesterol at ≥100mg/kg. In **dogs,** no death upto 400mg/kg p.o. gavage dose, adverse effects were seen at ≥10mg/kg with incr in severity with incr dose they included, signs of CNS depression, decr B.wt & food intake at ≥100mg/kg p.o. gavage and, incr in serum ALP and glucose at 400mg/kg.

Subchronic Tox:

Several studies were conducted with zaleplon in rats, and dogs. The following is a list of all studies:

Species	Duration	Route	Dose Range (mg/kg/d)
Mouse	1wk	p.o. diet	0.5-1000
	2wk*	diet & gavage	10-300
	3mo**	diet & gavage	1-1500
	5mo	p.o. gavage	20-240
Rat	1wk	p.o. diet	0.5-1000
	2wk	i.v.	0.5-2
	2wk***	p.o. gavage	0.1-1000
	1mo	p.o. gavage	0.1-100
	3mo$	p.o. diet & gavage	1-200
Dog	2wk$$	p.o. cap	5-400
	2wk	i.v.	0.5-2
	1mo	p.o. cap	1-4 (metabolite M2)
	3mo$$	p.o. cap	5-200

* 5 studies total
** 2 studies total
*** 4 studies total
$ 3 studies total for gavage & 1 diet
$$ 2 studies total

The following is a brief summary of 2wk studies in rats and dogs [see Dr. A. Wilk review for more detail]:

Rat:

≥10mg/kg p.o.	Clinical signs of CNS depression, ⇓ in B.wt & food intake.
≥100mg/kg p.o.	⇑ WBC, ⇓ serum K, Ca, Pi, ALP, & AST.
≥200mg/kg p.o.	⇑ serum ALT, ⇑ liver wt, gastric distension, centrilobular swelling and vacuolation of peripheral hepatocytes.
≥300mg/kg p.o.	death, decreased respiration, ⇓ serum K & TG, ⇑ serum glucose, ⇓ prostate, thymus, & spleen wt, acute lymphoid necrosis, dilatation & vacuolation of renal tubules, and gastric erosion.
≥600mg/kg p.o.	⇓ B.wt & food intake, ⇓ Hb & lymphocytes, hepatocyte vacuolation.
1000mg/kg p.o.	labored breathing.

Dog:

400mg/kg p.o. (cap or gavage)	death, hemorrhage in heart, lung & thymus, vacuolation of hepatocytes & renal tubular epithelium, & lymphoid depletion of spleen.
≥50mg/kg p.o. (cap or gavage)	CNS depression, ⇓ food intake.
200mg/kg p.o. (cap)	drooping eyelids, salivation, emesis, ⇓ spleen wt (absol & rel), ⇑ liver wt (absol & rel).

	C_{max} (ug/ml)	AUC (ug.hr/ml)
50mg/kg	11	91
100mg/kg	15	124

Detailed review and discussion of the 3&5mo tox studies in mice and rats are found in the carcinogenicity section.

3mo dog tox studies: 2 studies were done, one at 5, 50. & 200mg/kg and a 2nd study repeated at 50 & 200mg/kg with 1mo recovery. In both studies, death or moribund kill occurred at ≥50mg/kg dose, clinical signs included CNS depression (hypo- or in-activity, ataxia, etc.), and salivation, emesis, and loose stool at ≥5mg/kg with more frequency at the higher doses. Mean wt and food intake were decr in the 2nd study at both doses (50&200mg/kg) whereas, only random decreases in food intake without an effect on mean wt were observed in the 1st study at 200mg/kg dose. Red blood parameters were decr at ≥50mg/kg in the 1st study and at 200mg/kg in the 2nd study. Mean WBC count increased at ≥50mg/kg in m and at 200mg/kg in f and in both sexes at 200mg/kg in the 2nd study. Platelet count was also increased at ≥50mg/kg. Only in the 2nd study, mean total protein, albumin, and A/G ratio were decr at ≥50mg/kg and, BUN and ALP levels were incr at 200mg/kg dose. In both studies, the mean wt of the adrenals incr at ≥ 50mg/kg in addition, in the 1st study, liver wt was also incr and in the 2nd study, thymus wt was decr. Increased erythropoeisis of bone marrow was seen only in the 1st study with depletion

of mature granulocytes at ≥50mg/kg. In the 2nd study, testes wt was decr and atrophy of seminiferous tubules noted at 200mg/kg, and mean wt of prostate was decr as well as the # of sperm and, atrophy of prostate and epididymal ducts occurred at ≥50mg/kg. During recovery period, 1m of 5 dosed 200mg/kg died, convulsions, incr muscle tone, restlessness, and decr B.wt, were seen during the 1st 4d and, the effects on the male repro organs was still evident but less severe.

The male repro organ changes and the erythropoeisis of bone marrow were not seen in the 1yr dog study (see below), most likely because the highest dose tested was 40mg/kg.

Chronic Tox:

Rat: 1yr ~~Doses: 5, 20, 50mg/kg/d~~

Dog: 1yr Doses: 5, 20, 40mg/kg/d

Rat: 1-yr oral gavage tox study of CL 284,846 in rats/Study# 91027/91029/Lederle, NY/1993

Drug Batch# PC 1008 and 1025/purity 96.9% for PC 1008 and 99.5% for PC 1025. Homogeneity, potency, and stability of the dosing formulation were validated.
Route/Dose/Duration: oral **gavage/5, 20, 50mg/kg/d** administered as a suspension in 0.5% MC + 0.1% polysorbate 80 (10ml/kg)/1-yr (370-372 days)/control group was administered the vehicle in the same manner as the drug groups.
Species/strain/No./sex/gr: rat SD/15/sex/gr; 18/sex/gr for the TK/B.wt at study initiation: 182-248g males and 151-182g females.

The following parameters were evaluated: general clinical observation, behavior, body wt, food intake, clinical chemistry*, hematology*, urinalysis**, ophthalmoscopy (by indirect and focal illumination), organ wt, gross morphology, and microscopical examination (on all control and HD, all dead and sacrificed-moribund rats of LD and MD, all tissues from LDm, and liver, kidneys, adrenals, and gross lesions of LDf and MD rats). Toxicokinetics was also done using additional 18/sex/dose rats that were dosed in the same manner and killed at specified times on days 0, 92, 184, and 366 of treatment. Blood was collected from 1-4rats/sex/gr/timepoint upto 24hr postdose
The rats used for the TK study, were examined only for moribundity/mortality, body wt and food intake. * blood sampled from the orbital sinus during 3, 6, and 12months of treatment.

** urine collected at 6 and 12months

Basis for Dose-selection: 3-month rat tox study (# 89291/89324/yr.1991) the following was observed: mortality in 200mg/kg gr and, 50mg/kg gr, mild reversible wt changes and elevated liver wts. The 5mg/kg was selected as LD providing 10-12x the max anticipated human dose and the 20mg/kg as a middle dose providing 48x margin of safety.