CENTER FOR DRUG EVALUATION AND RESEARCH

APPROVAL PACKAGE FOR:

APPLICATION NUMBER

21-476

Pharmacology Review(s)

Memo

NDA:	21-476
Drug:	Estorra®, Eszopiclone
Sponsor:	Sepracor
Indication:	Hypnotic
Date:	Nov 1st 2004
Reviewer:	Aisar Atrakchi, Ph.D.
Supervisor:	Barry Rosloff, Ph.D.

Re. Submission dated September 30th 2004
Labeling Comments:

Under the Clinical Pharmacology/Pharmacodynamics section of the label the last sentence states:

Reviewing the referenced publications provided by the sponsor, zopiclone showed mean binding affinity of 28, 64, and 29nM to alpha 1, 2, and 3 respectively, in addition it had high affinity at alpha 5 of 46nM (high compared to many other cpd tested (). Therefore, there does not seem to be any significant differential preference of zopiclone to alpha 1 & 3 over alpha 2 & 5 (give ref) it almost had equal affinity to all these receptors.

This reviewer does not believe the finding of this study adds any extra points to zopiclone compared to other hypnotics and therefore, this sentence should be deleted from the label. Moreover, a sentence to this effect is already stated earlier in this section.

This was the only change in this submission made to the non-clinical information in the label, all other pertinent sections were the same as appeared in the February 24th 2004 final draft approvable label.

This is a representation of an electronic record that was signed electronically and this page is the manifestation of the electronic signature.

/s/

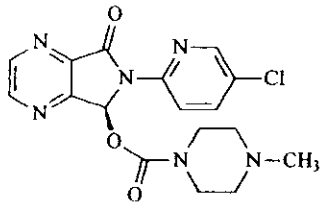
Aisar Atrakchi
11/15/04 11:03:41 AM
PHARMACOLOGIST

Barry Rosloff
11/16/04 12:25:46 PM
PHARMACOLOGIST

PHARMACOLOGY/TOXICOLOGY REVIEW

NDA number: 21-476
Review number: 1
Sequence number/date/type of submission: 000/January 30th 2003/original NDA
Information to sponsor: Yes (x) No ( )
Sponsor and/or agent: Sepracor Inc.,
111 Locke Dr.
Marlborough, MA 01752
Manufacturer for drug substance: Sepracor Canada Ltd.
Windsor, Nova Scotia

Reviewer name: **Aisar Atrakchi, Ph.D.**
Division name: **Neuropharmacological Drug Products**
HFD# **120**
Review completion date: **December 4th 2003**
Drug:
Trade name: Estorra®
Generic name: NA
Code name: Esopiclone/S-isomer of RS-zopiclone
Chemical name: (S)-4-methyl-1-piperazinecarboxylic 5-chloro-2-pyridinyl)-6,7-dihydro-7-oxo-5H pyrrolo[3,4-b]pyrazin-5-yl ester.
Molecular Formula/ Molecular Weight: C17H17ClN6O3/388.81
CAS Registry # 138729-47-2
Structure:



Relevant INDs/NDAs/DMFs: , I58647 (Sepracor)

Drug class: Central Non-benzodiazepine $GABA_A$ agonist.
Indication: Sedative/Hypnotic
Clinical formulation: tablets
Route of administration: oral

Proposed use: Hypnotic

Disclaimer: Tabular and graphical information are constructed by the reviewer unless cited otherwise.

Studies reviewed within this submission: all reports submitted with the NDA and any supplements.

PHARMACOLOGY/TOXICOLOGY REVIEW

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EXECUTIVE SUMMARY

Eszopiclone is the S-enantiomer of RS-zopiclone (RS-zop) developed by Sepracor as a short acting hypnotic. It is a pyrolopyrazine derivative of the cyclopyrolone non-benzodiazepine class of cpds. Although structurally unrelated to benzodiazepines, like them it binds to the $GABA_A$ receptors and therefore shares many of their pharmacological effects. RS-zop was originally developed by Rhone Poulence Rorer in 1981 — . RS-zop at the highest dose tested caused 4 different tumor types in 2 rodent species in the 2 year bioassays these were: mammary carcinomas in female rat, thyroid follicular cell carcinomas in male rat, lung carcinoma in female mice and skin sarcomas in male mice. In addition, incidence of liver neoplastic nodules was increased in 18 month chronic toxicity study in the rat. — the drug is marketed in more than 80 countries world wide since 1987. Sepracor, is developing the S-zop because it believes it to be the active isomer with similar pharmacological and toxicological profile to the racemate but effective at lower doses than the racemate. In meetings with Sepracor, the Division has expressed concerns regarding the tumor findings with the RS-zop, the sponsor was committed to address these tumors and provide support of their irrelevance to human safety. Over the past 20+ years since drug application submission, RPR and currently Sepracor conducted >14 mechanistic studies and consulted experts in the field and provided their written opinions and conclusions, as well as referenced the published literature. Collectively the conclusions can be summarized as follows for each tumor type:

1. Male mouse Skin tumors: RS-zop markedly exaggerated the aggressiveness of group-housed mice ? increase fighting ? wound formation and encrustations ? tumor induction (specifically skin sarcomas). The incidence of sarcomas far exceeded historical control incidence from 12 carcinogenicity studies. The proposed mechanism of drug-increased fighting in group housed mice was supported by a 2nd study where no skin sarcomas were observed in male mice housed individually (100mg/kg/d RS-zop and a control group), compared to tumors observed in another set of mice housed 4/cage at the same dose. Although this mechanism seems to provide a logical conclusion, the incidence of sarcoma in the 2nd set of group housed mice remained much higher in drug group compared to the control mice. Additionally, the incidence and duration of dorsal encrustations in both studies was much higher in drug than in control groups and exceeded historical range from the same 12 carcinogenicity studies and the severity of encrustations shifted to more severe in drug than in control group of group housed mice. Therefore, a drug effect of some kind such as tumor promotion, can not be ruled out. It is of note that perhaps the finding itself may not be relevant to human safety since aggressiveness has not been reported in clinical studies but a drug promoter effect remains a concern.

2. Female mice Lung tumors: the carcinoma incidence exceeded the concurrent control and 4-5 historical data bases. Pathology Working Group (PWG), re-examined the slides and came up with not 2 (as in the original), but 11 different tumor types based on current classification reducing the number of malignant tumors to half that reported originally (2 vs. 4 out of 52). In addition, tumor statistics were re-analyzed twice by 2 different statistical firms with the results still significant using the pair wise but not trend test for combined tumors (adenomas + carcinomas). Nevertheless, though the incidence remained statistically significant (2 HD vs. 0 in control), these tumors may not be of toxicological significance because of absence of metastasis, similar morphology to spontaneous tumors seen in single sex, and re-classification by the PWG made the carcinoma incidence fall within historical range.

3. Male rat Thyroid tumors: follicular cell carcinoma is proposed to be secondary to RS-zop liver enzyme induction, a mechanism irrelevant to human safety since the drug is not a liver inducer in humans and thyroid physiology and clearance is different between rodents and humans. Although not all results from the number of studies conducted were reproducible nor were the changes remarkable, there seem to be some evidence in support of a secondary effect of RS-zop on the thyroids.

4. Female rat Mammary tumors: 2 mechanisms have been proposed, the 1st was originally proposed in the early 1980s and suggested that these tumors are the result of prolonged elevation in 17ß estradiol known to cause mammary tumors in rodents. However, this mechanism could not be supported by the results from the several studies conducted. The 2nd mechanism of early onset of reproductive senescence similar to that of the herbicide atrazine, was later proposed. According to this mechanism, atrazine accelerated the onset of senescence in SD female rats by putting these rats in a state of constant estrus as a result of inadequate estrogen necessary to induce LH surge and ovulation. Serum levels of estrogen are either the same as basal levels or slightly elevated but persistent over a prolonged period therefore, leaving the animal in constant state of estrus. It has been shown that early onset of reproductive senesecne with the persistent elevation in estradiol over prolonged period increased the incidence and/or early onset of mammary tumors in the rat. This mechanism in atrazine and other triazine herbicides was shown after years of investigation and over 40 long term studies with the conclusion that this has been seen *only* in this strain of rats known to have large background spontaneous frequency of mammary tumors (atrazine-induced mammary tumors have not been observed in any other rat strain or in female mice). Sepracor has conducted several mechanistic studies in support of this mechanism and data has been inconsistent and not reproducible. The most recent of these is a long-term study in female rats intended to show via examination of vaginal cytology that eszopiclone and RS-zop cause early onset of senescence. Results from the 6 month interim were submitted on the 28th of August 2003 and showed both RS-zop and eszopiclone induced early senescence in >90% of rats with stronger signal in the latter nevertheless, the positive control atrazine failed to produce its expected response. Although the objectives of the study were met at the 6 month (vaginal cytology indirectly reflective of rats in constant estrus), early onset and/or increased incidence of mammary tumors have not been shown in this study and the protocol is not designed to address these parameters. Moreover, though the 6 month signal for early senescence was stronger with eszopiclone, mammary gland tumor incidence was not increased in the 2 year rat oral gavage carcinogenicity bioassay. It is noted that drug effects on female reproductive parameters the so called endocrine disruption, is common with benzodiazepines and other GABA agonists in absence of tumor induction i.e. such changes not necessarily lead to mammary tumor formation. Therefore, this recently completed study (terminated at 10 month this November, 2003), did not provide satisfactory or convincing explanation for the mechanism of RS-zop induced mammary tumors.

Looking at the overall drug toxicity profile, target organs of toxicity for RS-zop and S-zop based on results from single and repeat dose toxicity studies, are the liver, thyroid, and male reproductive organs. The 2 cpds also affected male and female fertility and caused fetal toxicity, behavioral effects in progeny, and some maternal toxicities as observed in reproductive and developmental studies in rats and rabbits. The RS-zop, S-zop and the biologically active metabolite S-desmethyl were clastogenic in *in vitro* mammalian cytogenetic toxicology assay in presence and/or absence of liver S9 metabolic activation system. It is of note that all consultant opinions regarding tumor findings with the Rs-zop as well as Sepracor's conclusions were reached based on RS-zop being a non-mutagenic cpd. This seemed to also be the justification used in the Canadian label for RS-zop where these 4 tumors were considered irrelevant to humans because the drug was not mutagenic. The latter was based on negative findings in the mutagenicity assays done with RS-zop in the early 1980s. Sepracor had recently conducted the

standard genetic toxicology battery using the RS-zop, S-zop, R-zop as well as S-desmethyl metabolite and 1 or more of these cpds were clearly positive clastogens in 1 assay and equivocal in another (some responses stronger than others). More recently S-zop was tested in rat and mouse 2 year bioassays with negative tumor findings up to the doses tested. However, exposure in the rat study was below the levels generated with the RS-zop at the doses that caused tumors with the RS-zop disqualifying the adequacy of this study. In the mouse carcinogenicity study, although MTD was not achieved, exposure was several folds higher than those observed with the RS-zop when the lung tumors were observed. This provided some level of comfort to the irrelevancy of these tumors to human safety in addition, results of the alternative p53 mouse assay were negative.

In conclusion, this reviewer does not recommend this drug for approval. This is based on the inadequate justification and explanation of the mechanism of RS-zop-induced mammary tumors in female rats and the possibility of a promoter effect in the skin tumors observed in male mice. The proposed mechanism of early senescence that can lead to formation of mammary tumors was not adequately verified and validated. Moreover, marketed benzodiazepine hypnotics acting through the GABA receptors such as zaleplon and zolpidem are not tumorigenic but cause "endocrine disruption" similar to S-zop. Moreover, neither of these drugs was positive in any of the genetic toxicity assays. Also, if this is the mechanism responsible for these tumors, mammary tumors would have been observed early or occurred at higher incidence in the 2 year bioassay conductd with S-zop. Absence of tumors in the S-zop 2 year rat bioassay occurred at exposures much lower than those observed following administration of RS-zop at the dose that caused the mammary tumors, therefore, this study did not adequately assess the drug's potential carcinogenicity in the rat.

RECOMMENDATION ON LABELLING:

1 Page(s) Withheld

_____ § 552(b)(4) Trade Secret / Confidential

_____ § 552(b)(5) Deliberative Process

✓ § 552(b)(5) Draft Labeling

Studies not reviewed within this submission: some pharmacology/mechanism of action and safety pharmacology reports and all studies with S-desmethyl metabolite (refer to IND# — for review).

3.1 INTRODUCTION AND DRUG HISTORY

Zopiclone induced 4 different types of tumors in rats and mice in 2 year dietary bioassays in addition to increased incidence of liver neoplastic nodules in rats dosed orally daily for 18months relative to control groups. Target organs/tissues for toxicity included the thyroid (follicular cell carcinomas in male rats), mammary gland (carcinoma in female rats), lung (carcinoma in female mice), and skin (sarcomas in male mice). In some of these tumors, incidence exceeded historical range of several data bases. Although RS-zop is not approved in the US, it has been marketed as a hypnotic in several countries worldwide.

Sepracor intends to develop Eszopiclone, the ***S-isomer of RS-zop,*** as a short acting hypnotic with the proposal that the main pharmacological activity is due to this enantiomer. The proposed clinical dose of Eszopiclone is — which is less than the marketed RS-zop range of 3.75-7.5mg/d.

3.2 PHARMACOLOGY:

3.2.1. BRIEF SUMMARY: see below

3.2.2 PRIMARY PHARMACODYNAMICS:

RS-zop was as effective as nitrazepam in causing *sedation* demonstrated as reduced motor activity in rats with ED_{50} of **5.2mg/kg p.o**. vs. 4mg/kg for nitrazepam and more potent than chlordiazepoxide or phenobarb at $ED_{50s}$of 17.5&65mg/kg p.o. respectively. Motor activity in a separate experiment in the rat, was significantly reduced by 10mg/kg p.o. dose of RS-zop lasting 3hr. The sedative effect of RS-zop in this study was similar to that of nitrazepam but greater than that of flurazepam. In mice, RS-zop reduced expolratory activity as measured by reduced motor function with an ED_{50} of **11.5mg/kg i.p.** whereas, it was less effective in causing **ataxia** in the rotarod with an ED_{50} of **25mg/kg i.p.**; very limited information is available on the effects of stereoisomers. In an in vivo observational study in mice by Sepracor, the **sedative effects were almost exclusively attributed to the S-form** with the R-form being inactive. *This indicated that the sedation seen with the racemic is due to the S-form.*

In drug *discrimination* study, rats were trained to discriminate between zolpidem (an imidazopyridine non-benzo hypnotic), and saline. Injection of RS-zop and a number of bzd all in a dose related manner, generalized to zolpidem. Similarly, squirrel monkeys trained to discriminate between midazolam and saline; RS-zop, zaleplon and a number of bzd cpd generalized to midazolam.

Effect on *sleep*, RS-zop at 2.5-10mg/kg i.p doses, shortened wake periods in freely moving rats and increased duration of slow wave sleep without affecting quantity or timing of REM. No rebound of activity in wakefulness or REM sleep following drug washout. EEG in various regions of rat and rabbit brains showed that RS-zop at doses between 0.3-3mg/kg induced neocortical spindle activity as well as some beta like activity (15-30Hz) both are properties of all GABAa agonists. In a more recent study in rats, the hypnotic effect of zopiclone was confirmed as the drug induced sleep without modifying the normal sleep architecture.

Mechanism of Action:

Most of the information presented here are for the RS-zop from data in the published literature as well as some conducted by Sepracor.

RS-zop binds non-selectively but with high affinity to <u>*central*</u> benzodiazepine receptors causing inhibition of neurotransmission. The pharmacological profile of zopiclone is qualitatively similar to that of benzodiazepines. In in vitro rat preperation, RS-zop had high affinity binding to hippocampal and cerebellar sites with K_d of 16nM. It also inhibited the in vitro binding of ^{3}H-diazepam in rat cerebellar preperation with the same potency as diazepam and nitrazepam at IC_{50s} of 60, 35, and 70nM respectively. In vivo, ID_{50} for RS-zop of 14mg/kg p.o. inhibited ^{3}H-flunitrazepam binding in mouse brain with almost equal maximum activity as chlordiazepoxide (ID_{50} 17.5mg/kg) but 7x less than that for diazepam (ID_{50} 2.2mg/kg) and 11x less than nitrazepam (ID_{50} 1.25mg/kg). RS-zop did not bind other central neurotransmitters such as GABA $_b$, 5HT, or NE. RS-zop showed some differential preferrence to brain areas of benzodiazepine (bzd) receptor sites such as cerebral cortex (Ki against flunitrazepam was 24nM), cerebellum (Ki 31nM), and hippocampus (Ki 36nM).

RS-zop is similar to clonazepam but unlike diazepam, it does not seem to bind peripheral bzd receptors. Effects of RS-zop are antagonized by flumazenil (the specific competitive antagonist) as well as by reverse agonists.

Noted that little information were found on binding properties and mechanism of action of RS-zop enantiomers or their metabolism (see below). The **S-form had about 50x greater potency** in inhibiting the binding of ^{3}H-flunitrazepam than that of the R-form but only **0.5x that of diazepam.** In a study by Sepracor, neither isomer nor the racemic had any significant affinity to GABA, strychnine-sensitive or insensitive glycine, muscarinic, phencyclidine, MK801, sigma, or peripheral bzd receptors. A slight affinity of 7uM of S-zopiclone to M3 subtype was reported (see below).

Similar to the racemic, the S-zop metabolites (the N-desmethyl- and N-oxide zopiclone), bind to bzd but with very weak binding and neither metabolite has any affinity to muscarinic receptors (see below).

3.2.3 SECONDARY PHARMACODYNAMICS

Sedative Effect:

The sedative properties of the racemate, S-, and R- forms of zopiclone were investigated in CD-1 male mice (n=10 per group). Doses selected were **3, 10, 30mg/kg** for the racemate, S- and R-isomers; saline 0.9% was the vehicle control. Following i.p. injection of the appropriate solution, mice were placed individually in observation cages and were observed every 10min for 15sec upto 1hr postdose. Behavior was rated according to an activity rating scale that was previously developed using racemate zopiclone (3&30mg/kg) and chlordiazepoxide (30mg/kg). 3-Way ANOVA (drug x dose x time) was used to assess the 6 observation times. The study was blinded. A significant dose response was seen indicative of the sensitivity of the rating scale to the different doses of the drugs. The **S-zopiclone was much more sedative than the R- and similar to, or sometimes more sedative than the racemate**. Responses were most evident in the early periods of the 60min i.e. 1st 20min. As time went by, scores of the groups converged because the sedative effect started to wane, the investigator then droped the last 2 readings when scores were equivalent among all groups even at the 30mg/kg, and the seperation/differences between the cpds became more obvious and more significant i.e. the S-form being the more sedative cpd.

Anti-anxiety Effect:

The plus-maze (also referred to X-maze) and the light-dark box paradigms are commonly used methods to assess the potential anxiolytic effect of a drug. The former was used to test the anxiolytic potential of the racemate, R- and S-forms of RS-zop in mice. The drugs were prepared in 0.9% saline which was the vehicle control and chlordiazepoxide was used as the positive control, at 10, 30 or 50mg/kg. RS-zop was administered i.p. at **3, 10, 30mg/kg** the high dose was found to produce maximum *sedation* of the racemate in a previously conducted study (see above). The behavioral measures were done blindly 30min post injection. There were 7-8 mice for zopiclone and vehicle and 4 for the positive control. The latency to move into the dark compartment, number of transitions between compartments, and the time spent in the light compartment, were recorded during the 5min observation period; each parameter was assessed by one-way ANOVA.

Results & Conclusion:

There was a significant effect on all 3 parameters: latency, transitions, and time spent in the light compartment. The S-isomer produced a more significant effect than the R- or the racemate on all 3

parameters indicative that it has a more anxiolytic effect than the other 2 forms. Similar finding was reported in previous study where the S-form was found to be more sedative than the R- and the racemate.

Anti-cholinergic Effect:

Zopiclone racemate, S-, and R-forms were tested to determine their ability to antagonize oxotremorine-induced salivation in mice. Atropine at 5mg/kg was the positive control and 0.25% MC was the vehicle control. There were 10 male mice per group and there were 17 groups which included **3, 10, 30, 100, and 300mg/kg** for each racemate, S-, R- zopiclone, and the controls. Drugs were administered orally 1hr after i.p. injection of 0.5mg/kg oxotremorine. Atropine produced 90% protection whereas, S- and R-zopiclone at 300mg/kg produced only 10% pretection against oxotremorine-induced salivation; all results compared to vehicle control. Oxotremorine-induced salivation was not antagonized at all of the other doses of all 3 cpds. Therefore, zopiclone and its isomers do not seem to possess anticholinergic effect.

Binding to Muscarinic M1-M5 subtypes:

Racemate, S-, and R-zopiclone were tested for their ability to bind to muscarinic receptors using CHO cells. The latter cells were transfected with cDNA encoding the specific receptor. Inhibition of binding of ^{3}H-QNB to each of the recptor subtypes by zopiclone isomers and IC_{50} values were determined (cpm counts). The results showed no or very little binding to muscarinic receptors, no stereoselectivity for binding, and the IC_{50} value ranges were as follows:

S-	$1.3\text{-}3.3\times10^{-4}$ M
R-	8×10^{-5} to 2.5×10^{-4} M
Racemate	7.2×10^{-5} to 1.7×10^{-4} M

It is noted however, that in another study, S-zopiclone showed ≥50% inhibition of binding when neither the R- nor the racemate showed any inhibition. This inhibition of binding was samll compared with that of 4-DAMP as seen from the sponsor's table below:

	Binding IC_{50} and (K_i) Values (nM)	
Zopiclone	**Benzodiazepine**	**M_3**
(+)	26 (22)	13,800 (6,900)
(-)	--- ---	--- ---
(±)	50 (42)	--- ---
Diazepam	13 (11)	--- ---
4-DAMP	--- ---	1.3 (0.65)

Metabolites of Isomers:

Binding of desmethyl- and N-oxide metabolites of the racemate-, R-, and S- zopiclone were assessed at the central bzd receptor sites and at the non-selective muscarinic sites both isolated from rat cerebral

Zopiclone N-Oxide	**Benzodiazepine** [IC_{50} & (K_i) Values, nM]	**Muscarinic** (% Inhibition)
(±)	8,260 (6,940)	17
(+)	7,520 (6,320)	---
(-)	---	12
Diazepam	17 (14)	

Desmethyl Zopiclone	**Benzodiazepine** [IC_{50} & (K_i) Values, nM]	**Muscarinic** (% Inhibition)
(±)	1,100 (924)	10
(+)	545 (458)	19
(-)	7,090 (5,960)	24
Diazepam	12 (10)	

cortex. It was found that the S-zopiclone desmethyl had the highest affinity for the bzd site among the 3 cpds; there was no activity at the muscarininc site. The N-oxide on the other hand, had very weak if at all, affinity to the bzd and muscarinic sites compared with diazepam (tables below from the sponsor):

3.2.4 SAFETY PHARMACOLOGY:

Cardiovascular effects:

Studies in the dog were done to assess CVS (one with the RS- and the 2nd with S-zop).

The CVS and respiratory parameters were studied in conscious male beagle dogs following i.v. bolus administation. Each of the 3 zopiclone forms were administered to 5 males per drug group in an ascending dosing regimen. The doses were as follows: **3, 5, 12mg/kg;** each of the 5 dogs in each of the drug groups received the vehicle therefore, each dog acted as its own control; the vehicle was not reported. The following observations were made upto 2.5-3hrs postdose: MAP, DBP, SBP, HR, 6 Lead EKG (pre-dose baseline and at 5, 10, 15, 20, 25, 30, 60, 90, 120, and 150min postdose), blood gases (pH, pCO2, pO2, HcT, and lactate), clinical signs (upto 24hr postdose), and body wt.

The sponsor made an argument that the findings in this study should be considered in light of the technical aspects of the study such as animal variability since each dog responded differently to the restrain. Also stated, was that the observations were made when the dogs were freely moving (released from slings not restrained). Therefore, as the sponsor proposes, these variations could have had an effect on the hemodynamic and clinical signs caused by zopiclone. Although this may have been the case, control measurements (predose and vehicle) were taken and should account for any variability. It is therefore, the opinion of the reviewer that the findings observed with zopiclone are likely to be drug related and not secondary to these technical issues particularly since the dogs have been acclimated to the sling prior to testing.

Results:

Clinical signs: none in vehicle groups.

3mg/kg racemate and S-zopiclone: all dogs in racemate and S-zop were agitated/excited with vocalization including gnawing & biting and had excessive salivation. These signs reversed by the 3hrs observation period and dogs were normal by the next day. Signs of excitement were mixed with signs of sedation noted at all doses with increased intensity as doses increased. Sedation was observed as eyelid closure, head drop, ataxia, and loss of righting reflex.

R-zopiclone: unlike the racemate and S-form, the R-form did not produce any of the above reported signs.

5&12mg/kg racemate and S-zopiclone: same as those in low dose but with increased intensity/severity and were seen within minutes of dosing. Similar to low dose, dogs were reported normal by end of observation period. The sedative effect was more pronounced in the S-form at these 2 higher doses relative to that seen at the low dose of 3mg/kg and more frequent.

R-zopiclone 5&12mg/kg: signs at the mid dose were similar to those seen with the racemate and S-form at the *low* dose of 3mg/kg; some agitation was seen at 5mg/kg but considered less severe than that seen at that dose with the racemate and S-form. At both 5&12mg/kg doses, R-form produced repetitive and transient sedation in most dogs. The clinical signs were similar to those in the racemate and S-form but as the sponsor put it "smoother" sedation with less adverse effects in some dogs even at the 12mg/kg dose. Again, all dogs recovered and were normal by end of 3hr observation period.

Hemodynamic Effects:

Racemate and S-zopiclone at *3mg/kg* caused a small and transient decrease in MAP in 2-3 of the 5dogs. As a compensatory mechanism, HR was increased; both parameters were normal/baseline by 1hr postdose. No significant effect seen with the R-form. At *5mg/kg* racemate and S-form, findings were more consistent than those at the low dose and included: hypotension in 4/4 racemate group and 4/5 S-zop group. Maximum decrease in MAP of 45mmHg in the racemate was seen within 10min of injection. This decrease was normalized in all but one animal that maintained its depressed pressure of 80-90mmHg through the remainder of the 3hr observation period (relative to baseline of 115mmHg). For both the racemate and S-form a compensatory increase in HR occurred immediately after injection and normalized to baseline within 30min of dosing in the racemate (no mention for the S-form). Only 1 of 5 dogs in the R-form exhibited the reduced MAP that was quickly normalized to baseline. At the *12mg/kg* rapid and consistent decrease in MAP that was 30-40% from baseline immediately post injection in the racemate and S-zopiclone in all dogs. Pressure began returning to baseline but remained below predose level in almost all dogs for the remainder of observation period. Heart rate was also increased as a compensatory mechanism in the racemate group (no mention of response in the S-form). There was no drug effect on EKG.

Abuse liability: A single study using the racemate, assessed drug discrimination compared with zolpidem. For complete evaluation see review by the Abuse liability staff members.

3.2.5 PHAMRMACODYNAMIC DRUG INTERACTIONS:

No studies done.

3.3 PHARMACOKINETICS/TOXICOKINETICS:

3.3.1 BRIEF SUMMARY:

PK and metabolism studies were done previously for RS-zop by RPR [redacted], and are accepted to represent S-zop data. In addition few studies on metabolism and exposure were done with the S-zop by Sepracor. TK parameters for RS-zop were measured in repeat dose toxicity studies in the mouse, rat, rabbit and dog and data compared to those from humans.

RS-zopiclone is rapidly absorbed with an oral bioavailability of 40-46% in the rat and 100-115% in dogs (no 1st pass effect in dogs). The drug is also widely distributed throughout the body following single/repeat oral and/or i.v. administrations to rats and dogs. RS-zop also crossed the BBB in the rat with maximum plasma and brain levels reached at 0.1-0.5hr postdose. The drug was equally distributed between plasma and brain when administered at 0.2mg/kg ^{14}C-zop with brain bioavailability of 61&79% in m and f rats respectively. In mice and rats most of radioactivity was detected in the liver, GI, muscle, and skin. Similarly in the dog, radioactivity was widely distributed but some retention was recorded in the thyroids and pigmented areas of skin and eyes. Plasma kinetics is a two-compartment open model with terminal half life of 4-5hr in humans, but steady state after repeated oral dosing was only reached after the 4th and 17th daily doses in rats and dogs respectively. Repeated dosing in the rat did not affect plasma kinetics with the rapid phase of elimination at $t_{1/2}$ of 0.4-0.8hr followed by the slow phase of $t_{1/2}$ 11-13hr (Sepracor#190-546) but $t_{1/2}$ of 50hr was recorded in Sepracor#190-547. The difference in plasma $t_{1/2}$ between the 2 studies was related to kinetics of elimination of metabolites or other radioactive cpds. Blood consistently had higher levels of radioactivity than plasma. Though repeated dosing had no effect on elimination kinetics ($t_{1/2}$), total radioactivity of plasma and blood were higher after repeat than after

single dose of RS-zop by a factor of 2 for plasma and 5 for blood as well as tissue concentration was 4-10x higher after repeat dosing. Similar to single dose, very little unchanged drug was eliminated in urine and feces after 24hr of dose. Bile duct cannulated rats had much less total plasma radioactivity than un-cannulated rats indicative of enterohepatic recirculation. Most of radioactivity in the rat was cleared by 48hr postdose via lungs (47-55% of radioactive dose), kidneys, and liver/feces (7-17% of radioactive dose), following single oral or i.v. administration of RS-zop. It should be noted that radioactivity remained relatively high in the thyroids by 96hr postdose in the rat. In the dog, urinary elimination accounted for 30% and fecal to 10% over 72hr in both sexes following oral and i.v. administration of RS-zop; pulmonary excretion as CO^2 accounted for 63% of radioactivity in 1f dog following i.v. injection. Plasma elimination $t_{1/2}$ of unchanged drug in the dog was 3-3.6hr.

In the pregnant rabbit, single oral dose of 125mg/kg RS-zop was well absorbed with serum levels of **1.5-3ug/ml** reached between 4-6hr postdose and similar to the rat, very low levels remained detectable by 24hr postdose. Also similar to the rat, urinary elimination was low at 2-7% and up to 0.7% in feces; the N-oxide and N-desmethyl were detected in urine. **S-zop** administered orally to pregnant rabbits at 4&24mg/kg/d during gd6-18 had detectable levels of S-desmethyl and N-oxide (only at the high dose), however, R-zop and R-desmethyl were below detection limit. Levels for all 3 cpds increased more than proportional to dose and drug accumulated with repeated dosing. Mean plasma exposure to **S-zop after 24mg/kg/d S-zop in pregnant rabbits was 16,874ng.hr/ml and C_{max} 3203ng/ml and S-DMZ exposure was 20,090ng.hr/ml and a C_{max} of 2487ng/ml**. RS-zop distributes to milk in nursing mothers with milk to plasma ratio of 0.8.

RS-zop as stated earlier, is extensively metabolized in animals and humans by liver P450 (specifically CYP3A4; also CYP2E1 in humans), through oxidation with only 10% eliminated as unchanged drug. Total of 14 metabolites have been identified and isolated in both animals and humans. The major metabolites in humans are the N-oxide and N-desmethyl accounting for >30% of dose in urine and, in rats and dogs the major metabolites are those of decarboxylation accounting for >50% of dose. RS-zop is eliminated in urine and feces in all species tested with total excretion over 48hr as 34% urinary, 14% fecal and 44% pulmonary of total radioactivity in rats and 31%, 6%, and 53% respectively in the dog. Total Cl is large at 300ml/min compared to the small renal Cl of 10ml/min.

Plasma protein binding of RS-zop in humans is around 45% and up to 60% in animals. *In vitro* plasma protein binding of **S-zop** ranged between 29% in the rat to 59% in humans. S-zop did not inhibit CYP2A6, 2C19, 2E1, or 3A4 and had small but dose dependent inhibition of CYP1A2, 2C9, and 2D6 when tested using human hepatocytes.
Studies were conducted to determine TK parameters of S-zop and metabolites following RS-zop administration to rat and mouse. These studies were done at the top dose tested in the 2yr carcinogenicity studies in these species and exposure compared to humans to assess risk. Study duration ranged between 7d to 3months and included gavage and dietary administrations. The following table summarizes mean plasma data from these studies:

MICE: male and female B6C3F1; Dose: 100mg/kg/d RS-zop; Route: Oral Dietary

Cpd	AUC_{0-24hr} (ng.hr/ml)		
Duration	7 day	90 day	90 day*

S-zop	5573m/4819f	3911m/1450f	5810m/7070f
S-DMZ	637m/960f	1147m/790f	1090m/2310f
R-zop	8809m/6893f	5951m/1698f	ND
R-DMZ	10,045m/11,856f	17,645m/11,083f	ND
N-oxide	6096m/4476f	6834m/2137f	ND

ND = not done * Sepracor# 190-530

MICE: male and female C57BL; Dose: 100mg/kg/d S-zop and 100mg/kg/d S-DMZ Route: Oral Gavage. 100mg/kg/d exceeded the MTD due to deaths at this dose (though sponsor did not consider these deaths drug related):

Dose (mg/kg/d)	$AUC_{0\text{-}24hr}$ (ng.hr/ml) S-zop	S-DMZ
S-zop		
100	12,400m/17,200f	2290m/5780f
200	24,000m/24,700f	6090m/7900f
300	50,000m/42,500f	9170m/11,100f
S-DMZ		
100	-	15,900m/23,800f
200	-	49,700m/66,600f
300	-	111,000m/131,000f

Based on human exposure of 191ng.hr/ml for S-zop and 39ng.hr/ml for S-DMZ measured after 3mg/d dose of S-zop for 7d, the conservative **mouse** exposures to S-zop and S-DMZ after 100mg/kg/d RS-zop dose are 3911m/1450f ng.hr/ml and 1147m/790f ng.hr/ml respectively, representing mouse to human ratios of **20x (m) and 8x (f) for S-zop and 29x (m) and 20x (f) for S-DMZ.**

Mean exposure data in the **RAT** are summarized in the table below following **100mg/kg/d dose of RS-zop** in the **diet** administered for 90d (Sepracor#190-510):

	$AUC_{0\text{-}24hr}$ (mg/kg/d)
S-zop	12,884m/29,465f
S-DMZ	13,099m/8093f
R-zop	3960m/6338f
R-DMZ	14,878m/6939f
N-oxide	8124m/8285f

Based on human exposure of 191 and 39ng.hr/ml for S-zop and S-DMZ respectively, following 3mg/d S-zop for 7d, the rat to human ratios are **67x (m) and 154x (f) for S-zop and 336x (m) and 21x (f) for S-DMZ.**

The following is detailed review of the above and other studies with RS-zop and S-zop.

3.3.2 AND 3.3.3 ABSORPTION & DISTRIBUTION:

Mouse (Sepracor#190-545): study conducted by RPR in 1978. This was a tissue distribution and placental transfer study. Female *OFA pregnant mice* (gd16), were injected i.v. with 2mg/kg ^{14}C-RS-zop. Two mice were killed by immersion in liquid nitrogen, at 2min, and 1, 6, and 24hr postdose, whole body

sections were taken and slices processed and mounted on a radiographic film which was stored in a freezer for 7d. Samples were then removed and film developed and examined semi-quantitatively using autoradiography.

Results:

Highest radioactivity at 2min, was in the **liver**>salivary glands>kidney>spleen>bladder>lachrymal glands>intestinal and stomach walls>lungs>striated muscle>brown fat>brain (highest radioactivity was in the pituitary and cortex), very small label was detected in the fetus. After 1&6hr radioactivity was also detected in urinary bladder and bile duct and by 24hr postdose, trace amounts of label remained in bile duct and intestinal contents, with very little in bladder.

Rat (Sepracor#190-546): study was conducted by RPR in 1977. Male and female — rats administered ^{14}C-RS-zop either via single oral or i.v. dose or repeated (7d), oral dose at 0.2mg/kg/d. Blood, bile, urine, feces, and tissues were collected at specified times as follows (table from sponsor):

Route	Dosage	Sample Collection
Oral, IV	0.2 mg/kg [^{14}C]-zopiclone, single dose	Blood and brain: 0.1, 0.25, 0.5, 1, 3, 6, 12, 24 and 48 h after oral and IV dosing Urine, feces and expired air ($^{14}CO_2$): 0-6, 6-12, 12-24 and 24-48 h after oral and IV dosing Bile: 0-0.5, 0.5-1, 1-3 and 3-6 h after oral dosing Tissues[a]: 6, 24 and 48 h after oral dosing; and 0.15, 0.5 and 1h after IV dosing
Oral	0.2 mg/kg/day (zopiclone, 1st to 5th dose; [^{14}C]-zopiclone, 6th dose)	Blood and brain: 0.1, 0.25, 0.5, 1, 3, 6, 12, 24 and 48 h after oral and IV dosing Urine, feces and expired air ($^{14}CO_2$): 0-6, 6-12, 12-24, and 24-48 h Tissues[a]: 6, 24 and 48 h after oral dosing
Oral	0.2 mg/kg/day [^{14}C]-zopiclone 7 repeated doses	Blood: 1 and 24 h after each of the oral dosing, and 1, 3, 6 and 24 h after the 7th dose

[a]Tissues collected including plasma, brain, heart, lungs, stomach, spleen, liver, kidneys, genital track, intestines, intestinal contents, skin and carcass

Radioactivity was determined by liquid scintillation counting or direct combustion, TLC was used to separate parent from metabolites and radiochromatograms were assessed by a scanner and radioactivity determined by liquid scintillation counting. *In vitro* protein binding was determined from rats dosed *i.v.* and biliary elimination as well as enterohepatic circling were assessed by collecting bile and blood from biliary fistula and total radioactivity determined in bile and plasma of rats with fistula at 0.5, 1, 3, and 6hr post *oral* dosing.

Results:

RS-zop was rapidly absorbed and crossed the BBB with maximum plasma and brain levels of total radioactivity seen at 0.5 (male) and 0.1hr (female) and steady state reached after the 4th oral dose. Absolute bioavailability in plasma was 42&46% in m & f respectively, and in the brain 61 & 79% in m & f respectively (table from sponsor). The drug seemed to distribute equally between plasma and brain since exposure profiles in these 2 organs following either route and in both sexes, were comparable.

PK/Rat, Sepracor#190-546 (Cont.)

Plasma and Brain Pharmacokinetics after Single Oral or IV Dosing

	Male		Female	
Plasma	Oral	IV	Oral	IV
Total radioactivity				
C_{max} (µg Eq/L)				
T_{max} (h)	0.5	NA	0.1	NA
Zopiclone				
C_{max} (µg Eq/L)				
T_{max} (h)	0.5	NA	0.1	NA
$T_{1/2}$ (h)	0.8, 13	0.69, 12	0.37, 2.5, 10.9	0.67, 11.8
Volume of distribution (L)	NA	0.51	NA	0.27
Body clearance (L/h)	1.3	0.5	0.67	0.26
AUC_{48h} (µg•h/L)	49	118	92	200
Bioavailability (AUC_{oral}/AUC_{IV})	42	NA	46	NA
Brain				
Total radioactivity				
C_{max} (µg Eq/kg)				
T_{max} (h)	0.5	NA	0.1	NA
Zopiclone				
C_{max} (µg Eq/kg)				
T_{max} (h)	0.5	NA	0.1	NA
$T_{1/2}$ (h)	0.57, 17.4	0.28, 1.6, 13.3	0.83, 12.6	0.21, 0.94, 8.8
AUC_{48h} (µg•h/kg)	28.8	47.3	72.3	91.3
Bioavailability (AUC_{oral}/AUC_{IV})	61	NA	79	NA

Animals were treated with 0.2 mg/kg [^{14}C]-zopiclone; NA = not applicable

Plasma and Brain Pharmacokinetics after Repeated Oral Dosing

	Plasma		Brain	
	Male	Female	Male	Female
Total radioactivity				
C_{max} (µg Eq/L or µg Eq kg)				
T_{max} (h)	0.1	0.1	0.25	0.5
Zopiclone				
C_{max} (µg Eq/L or µg Eq/kg)				
T_{max} (h)	0.1	0.1	0.25	0.1
$T_{1/2}$ (h)	0.82, 10.2	0.26, 1.7, 13.6	0.95, 10.8	1.1, 7.7
AUC_{48h} (µg•h/L)	63	97	34	61

Animals were treated with 0.2 mg/kg/day of zopiclone (1st to 5th dose)/^{14}C-zopiclone (6th dose) or 0.2 mg/kg/day [^{14}C]-zopiclone 7 repeated dose.

Almost all of the radioactivity was eliminated by 48hr via *lungs, renal, and hepatic* routes at 96% in males and 104% in females after oral and 100 and 93% in males and females respectively, after i.v.

Following oral repeat dosing, total radioactivity accounted for 90&96% in males and females respectively. Following single oral administration, elimination occurred mainly via the lungs at 47 and 55% of radioactivity administered orally or i.v. within 48hr postdose and of this amount, 35-40% occurred within 6hr postdose. Elimination via *feces accounted for 7-8%* of radioactivity administered *i.v. and 11-17% following p.o.* RS-zop similar to mice, was widely distributed with major radioactivity in GI, muscle, liver and skin and only 2% of radioactivity remained after 48hr of dose. Following i.v. most of radioactivity was detected in *muscle, liver, skin, and intestines* within 1hr postdose. Drug kinetics in plasma was unaffected by repeat dosing and steady state was achieved after the 4th dose. kinetics were characterized by biphasic elimination with rapid phase at $t_{1/2}$ of 0.37 to 0.8hr followed by a

PK/rat Sepracor#190-546 (Cont.)

slow phase with $t_{1/2}$ of 11-13hr. Bile duct cannulated rats had much lower total plasma radioactivity (42-84%) than un-cannulated rats between 0.5-6hr postdose indicative of enterohepatic recycling. *In vivo* plasma protein binding was 55% and comparable to that *in vitro* of 50-55%.

A 2nd rat study (Sepracor#190-547; conducted by RPR in 1981), male rats (5/group), received **single** oral dose of ^{14}C-RS-zop **(labeled at the carbonyloxy carbon)**, at 0.2mg/kg and elimination of radioactivity and tissue distribution were similar to the above study.

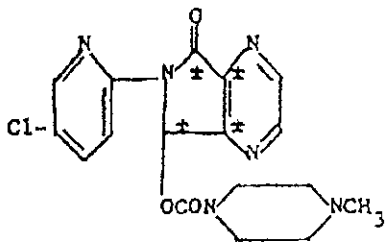
Table 5.D.3-4 Plasma, Blood and Liver Pharmacokinetics of Total Radioactivity in Rats after Oral Dosing

Parameter	Blood	Plasma	Liver
C_{max} (ng Eq/mL)	85.4	79.4	1.25
t_{max} (h)	0.5	0.5	0.5
$t_{1/2}$ elimination (h)	145	50	10

Each animal received 0.2 mg/kg of [^{14}C]-(RS)-zopiclone.

Noted that position of the label did not affect peak levels (0.5hr) but, increased late plasma levels. Blood elimination kinetics (of total radioactivity) declined with 2 $t_{1/2}$ (biexponentially), of 1 and 145hr and triexponentially in plasma with 3 $t_{1/2}$ of 0.4, 4.3, and 50hr (table above). Blood levels always being higher than plasma. The increase in plasma elimination $t_{1/2}$ in this study to 50hr as opposed to 13hr in the above study, stated by the sponsor to be caused by the kinetics of excretion of metabolites or other radioactive cpds.

A 3rd PK study in male rat was done by RPR in 1981 (Sepracor#190-548), where ^{14}C-RS-zop was labeled on the **4a, 5, 7, and 7a carbons**.



Five male rats per group were dosed orally with 0.2mg/kg/d ^{14}C-RS-zop administered for 10d. Blood, urine, and feces were collected at specific times as seen in sponsor table below:

PK/Rat Sepracor#190-548 (Cont.)

Data from the single PK study above (Sepracor#190-547), were compared to the current data following repeat dosing. Plasma and blood levels of total radioactivity were **higher after repeat** than after single administration by a factor of **2 for plasma and 5 for blood**. Terminal elimination $t_{1/2}$ for blood and plasma were similar following single and repeat dosing (49 & 155hr following repeat dosing compared with 50 & 145hr after single administration for plasma and blood respectively). Peak radiolabel occurred at 0.17hr postdose for blood and plasma after repeat dosing and 0.5hr after single dose. Between 24-96hr

Dosage	Sample Collection
0.2 mg/kg/day [^{14}C]-zopiclone, 10 repeated oral dose [each animal (5/group) received 50.62 µg (1.25 mL) of [^{14}C]-zopiclone (50.62 µg corresponding to 7.682 µCi)]	Blood and plasma: 0.5 and 24 h postdose on after 1st, 2nd, 5th, 7th and 9th dose; and 0.17, 0.5, 1, 2, 3, 6, 12, 24, 48, 72, and 96 after the 10th dose. Urine and feces: 0-24 after the 1st dose; and 0-6, 6-12, 12-24, 24-48, 48-72, 72-96 h after the 10th dose Pulmonary excretion (expired $^{14}CO_2$): 0-24, 24-48, 48-72, and 72-96 h after the 10th dose Tissues[a]: 0.5, 3, 6, 24, 48, 72, and 96 h after the 10th dose

[a] Tissues collected in the selected time points including plasma, brain, heart, lungs, stomach, spleen, liver, kidneys, genital track, intestines, intestinal contents, skin and carcass.

postdose, total radioactivity was highest in blood than in tissues. Peak radioactivity in tissues was reached at 0.5hr postdose except for the large intestine at 3hr. Half of radioactivity at 6hr postdose was measured in **liver, kidneys, and GI and the other half in blood, carcass** and other organs. Amount of radioactivity in the brain after 0.5hr was comparable to that after a single dose. Similar to the single administration, very little unchanged drug was eliminated in urine and feces after 24hr of dosing (0.5-3% of dose) also pulmonary excretion after 96hr was small at <1% after the last dose. Similar to blood and plasma, tissue concentration was higher by 4-10x after repeat than after single administration. **[it should be noted that the the radioactivity was high in the thyroids and present at moderate levels by 96hr postdose].**

Rabbit: (Sepracor#190-549), this study was conducted by RPR in 1978. Only 2 female New Zealand white rabbits were tested, RS-zop was administered as a **single oral dose of 125mg/kg** (this was the highest dose tested in the rabbit teratogenicity study). Blood was collected between 1 and 24hr postdose and urine/feces between 0-72hr. Results showed that RS-zop was well absorbed with peak serum levels of **1.5-3mg/l (ug/ml)**, recorded at 4-6hr postdose and by 24hr levels declined to 0.04 and 0.5ug/ml in the 2 rabbits. Similar to the rat, urinary elimination of the parent was low at 2&7% of administered dose and at 0.3&0.7% for fecal elimination. TLC analysis of urine showed N-oxide and N-desmethyl metabolites plus other unidentified metabolites.

Dog: (Sepracor#190-550), this study was conducted by RPR in 1978 using ^{14}C-RS-zop to assess the drug PK in dogs. Beagle dogs (2/sex) were injected i.v. 0.2mg/kg ^{14}C-RS-zop and 6wks later, administered a single oral dose of 0.2mg/kg ^{14}C-RS-zop. Blood was collected between 0.08-48hr for i.v. and 0.5-48hr post oral dosing. Urine and fecal samples collected between 0-6, 6-12, 12-24, 24-48, and 48-72hr

postdose. Total radioactivity in these samples was determined by liquid scintillation counting or direct combustion and amount of unchanged drug was determined by TLC.

Results:
RS-zop was well absorbed after oral dosing with peak plasma levels of total radioactivity of 0.5-1hr postdose in both sexes. Levels declined biexponentially following both routes. Bioavailability in both sexes was high at 108-114% indicative of little or no 1st pass effect (table from sponsor):

Pharmacokinetics of Total Radioactivity and Zopiclone after Oral and IV Dosing

	Male		Female	
Parameters	Oral	IV	Oral	IV
Total radioactivity				
C_{max} (µg Eq/L)	92	121.5 (0.08h)	154	165 (0.08h)
t_{max} (h)	1	NA	1	NA
AUC_{48h} (µg Eq•h/L)	550	441.5	770	585
Zopiclone				
C_{max} (µg Eq/L)	55.9	89.5 (0.08h)	69.3	110.5 (0.08h)
t_{max} (h)	0.5	NA	1	NA
AUC_{48h} (µg•h/L)	136.5	126.5	183.5	153
$t_{1/2}$ (h)	0.85, 2.8	0.68, 3.9	0.59, 3	0.58, 2.8
Bioavailability (AUC_{oral}/AUC_{IV})	108	NA	115	NA
Volume of distribution (l kg of body mass)	NA	2.2	NA	1.6
Body clearance (mL/h per kg of body mass)	1.47	1.53	1.1	1.26
Renal clerance (mL/min per kg body mass)	0.355	0.52	0.27	0.59

Values presented in the table are the average of two animals; NA = not applicable.

PK/Dog, Sepracor#190-550 (Cont.)

Similar to the rat and mouse, RS-zop was extensively metabolized with only 2-4% of dose remaining by 48hr postdose after both oral and i.v. dosing. Urinary elimination over 72hr in both sexes accounted for 30% after both routes and fecal excretion accounted for 10% in both sexes after oral and both routes. Pulmonary elimination expressed as $^{14}CO_2$ was 63% of radioactivity over 12hr in 1f dosed i.v.

Another mass balance study in the dog examined the repeated oral administration of ^{14}C-RS-zop to 7 male beagle dogs at 0.2mg/kg/d for **3wks** (Sepracor#190-551). This study was conducted by RPR in 1982. The carbon label was placed on **4a, 5, 7, and 7a carbons (#190-550, label was placed on the carbonyloxy carbon)** . Blood was collected in 2 phases: immediately before and 1hr postdose and, between 1 and 288 and/or 336hr after the last dose. Tissue and excreta samples were collected up to 336hr postdose. Total radioactivity was determined by liquid scintillation counting or — and, separation of ^{14}C-RS-zop and its metabolites was done by TLC and quantified by liquid scintillation counting.

Results:
1hr after the last dose, high levels of radioactivity were detected in blood and plasma at 95 & 130ng Eq/ml respectively, with steady state reached after the 17th dose at 194 and 295ng Eq/ml respectively, 1hr after the 17th dose. The blood and plasma $t_{1/2}$ of total radioactivity was estimated at 232 and 177hr respectively, and **plasma elimination $t_{1/2}$ for unchanged drug was 3-3.6hr.** Radioactivity was widely distributed after dose, there was some retention of radioactivity in thyroids and pigmented areas of skin and eyes. About 12-15% of the last dose was measured in the bile from dogs killed 4hr postdose but negligible amounts measured at other time points. The drug seemed to be extensively metabolized following repeated dosing as TLC detected a number of unidentified metabolites.

A dietary 7d PK study of RS-zop was done in male and female **B6C3F1 mice** (Sepracor#190-539) to verify exposure following 100mg/kg/d dose used in the 2yr mouse carcinogenicity study (Sepracor#190-834). RS-zop 100mg/kg was admixed in the diet and blood was collected on days 6&7 from 3/sex at 11 time points, animals were allowed access to food up to time of bleed. Mice were killed without further exam. Plasma levels of R-, S- zop, R- and S-desmethyl zop and N-oxide were analyzed using a validated

— There were no deaths or clinical signs. Results (table from sponsor) showed adequate exposure to all 4 cpds following RS-zop administration. **Highest levels of concentration and exposure in both sexes were reached by R-desmethyl followed by R-zop; the N-oxide levels were comparable to those of S-zop.**

Table 5.D.3-10 Plasma Exposures of Zopiclone and its Metabolites in Mice after 7 Days of Dietary Administration of (RS)-Zopiclone

Analyte	Males		Females	
	AUC_{0-22} (ng•h/mL)	C_{max} (ng/mL)	AUC_{0-22} (ng•h/mL)	C_{max} (ng/mL)
(S)-Zopiclone	5573		4819	
(R)-Zopiclone	8809		6893	
(S)-DMZ	637		960	
(R)-DMZ	10045		11856	
Zopiclone N-Oxide	6096		4476	

Based on **human steady state exposure of 191ng.hr/ml for S-zop and 35ng.hr/ml for S-desmethyl zop following 3mg/d dose for 7d**, the mouse to human ratios are 29 and 25 in males and females for S-zop and 18 and 27 in males and females for S-desmethyl zop respectively.

A study comparable in design to the above except it was carried out for 90days was done (Sepracor#190-530). RS-zop was admixed in the diet to provide 100mg/kg/d dose administered to male and female B6C3F1 mice daily for 3months. Clinical signs, B.wt, and food intake were monitored. On days 90&91 blood was collected from 10/sex mice at 11 time points and plasma levels of the 5 cpds (R- & S- zop and desmethyl zop and N-oxide), were determined. All mice tolerated the drug without mortality, 30-36% of male and female mice had alopecia, a transient >10% decrease in B.wt occurred after 1wk of dosing but adopted well thereafter with wt increasing till end of study; food intake was unaffected by treatment.

Table 5.D.3-12 Plasma Exposures After 90 Days of Dietary Administration of (RS)-Zopiclone to Mice Relative to Maximum Anticipated Steady-State Human Exposure*

Analyte	Males		Females	
	AUC_{0-24} (ng•h/mL)	Mouse:Human Ratio	AUC_{0-24} (ng•h/mL)	Mouse:Human Ratio
(S)-Zopiclone	5810	30	7070	37
(S)-DMZ	1090	28	2310	60

*Plasma AUC_{0-24} values were 191 ng•h/mL [(S)-zopiclone] and 38.7 ng•h/mL [(S)-DMZ] after 3 mg/day for 7 days (Study 190-002)

Analyte	Male		Female	
	$AUC_{0-24\,h}$	C_{max}	$AUC_{0-24\,h}$	C_{max}
(S)-Zopiclone	5810		7070	
(R)-Zopiclone	8950		7620	
(S)-Desmethyl zopiclone[1]	1090		2310	
(R)- Desmethyl zopiclone	11800		13000	
Zopiclone N-oxide[1]	4830		4140	

1= For information only.

Table from sponsor present exposure and human ratio for these cpds:

Similar to the 7d dietary study, R-desmethyl zop followed by R-zop had the highest plasma concentration and exposure in both sexes, next highest is S-zop; the N-oxide had the lowest values.

The following study (Sepracor#190-510), is comparable in design and objective to the above study except rats in addition to mice were included in this study. This study was done to determine TK parameters after **dietary administration of RS-zop to male and female mice and rats at 100mg/kg/d RS-zop for 3 months.** This is because TK parameters of S-zop were not assessed in the 2 yr bioassay following oral dietary administration of RS-zop. The 100mg/kg/d dose used here because it was the top dose of RS-zop used in the 2- year lifetime bioassays conducted previously by RPR in these species. Therefore, results from this study can be used to better understand the relationship between RS-zop and S-zop and aid in assessing the relevance of using these data in caecinogenicity risk assessment. RS-zop was admixed in the diet and fed tc — B6C3F1 mice and — SD rats for 3months. There were 24 animals/sex; no control included because only drug PK parameters were measured. Clinical signs, B.wt and food intake were monitored. At end of 3month, blood was collected from 3/sex/species at 8 time points with animals having access to food till blood sampling. A validated — was used to determine R- and S-zop and their desmethyl metabolites as well as N-oxide. There were no drug related effects on mean B.wt, wt gain, or food intake; all animals survived till end of dosing. The results in both species showed diurnal differences with maximum concentrations observed at late night and early morning. Female mice had 37% of the S-zop exposure than males in contrast to the previous 3mo study (Sepracor#190-819A1), conducted in mice via oral gavage where no sex difference was observed in PK parameters (Summary tables from sponsor).

PK, Sepracor#190-510 (Cont.)

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The current sex difference could not be explained and the sponsor stated that further investigation was warranted.

In rats, sim[illegible] A1), the current die[illegible]. Mean plasma lev[illegible] ed between 5[illegible]

Table 5.D.3-14 Plasma Exposures in Mice after 3 Months of Dietary (RS)-Zopiclone

Analyte	Males		Females	
	Mean AUC_{0-24} (ng•h/mL)	C_{max} (ng/mL)	Mean AUC_{0-24} (ng•h/mL)	C_{max} (ng/mL)
(S)-Zopiclone	3,911	/	1,450	
(R)-Zopiclone	5,951		1,698	/
(S)-DMZ*	1,147		790	
(R)-DMZ	17,645	/	11,083	
Zopiclone N-Oxide	6,834		2,137	

* DMZ: desmethylzopiclone

Table 5.D.3-15 Zopiclone Exposures in Rats after 3 Months of Dietary (RS)-Zopiclone

Analyte	Males		Females	
	Mean AUC_{0-24h} (ng•h/mL)	C_{max} (ng/mL)	Mean AUC_{0-24h} (ng•h/mL)	C_{max} (ng/mL)
(S)-Zopiclone	12,884		29,465	
(R)-Zopiclone	3,960	/	6,338	/
(S)-DMZ*	13,099		8,093	
(R)-DMZ	14,878		6,939	
Zopiclone N-Oxide	8,124		8,285	

* DMZ: desmethylzopiclone

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PK, Sepracor#190-510 (Cont.)

Table below from sponsor present mean±s.d. of plasma concentrations in the rat.

Table 1. Mean±SD Plasma Concentrations (ng/mL) of Zopiclone and its Metabolites Following the Administration of 100 mg/kg/day (R,S)-Zopiclone via Dietary Admix for 90 Days to Male and Female Rats

Post-dose Time (h)*	(S)-(+)-Zopiclone			(R)-(-)-Zopiclone			(S)-(+)-Desmethyl Zopiclone			(R)-(-)-Desmethyl Zopiclone			Zopiclone N-Oxide		
	n	Mean	SD	n	Mean	SD	n	Mean	SD	n	Mean	SD	n	Mean	SD
Males															
0	3	405	236	3	94.5	93.5	3	823	598	3	865	595	3	295	255
2	3	425	171	3	28.6	49.5	3	856	379	3	881	373	3	258	71.7
4	3	305	39.5	3	91.7	26.9	3	706	114	3	871	144	3	218	98.0
8	3	210	212	3	44.3	76.8	3	199	113	3	261	106	3	551	64.1
12	3	443	203	3	171	107	3	258	60.9	3	300	69.2	3	356	120
16	3	963	121	3	311	66.4	3	818	95.4	3	865	120	3	146	49.3
20	3	899	117	3	334	53.3	3	536	373	3	651	498	3	490	179
24	3	327	189	3	47.0	44.8	3	601	387	3	666	203	3	243	71.1
Females															
0	3	1403	476	3	276	154	3	647	297	3	547	246	3	388	150
2	3	693	456	3	79.7	69.1	3	321	230	3	239	232	3	198	259
4	3	534	204	3	25.5	44.2	3	134	29.7	3	120	112	3	1266	1112
8	3	472	25.6	3	48.9	42.7	3	204	120	3	190	112	3	186	161
12	3	1387	523	3	438	159	3	200	134	3	131	136	3	166	144
16	3	1817	406	3	438	127	3	306	58.3	3	272	40.6	3	41.3	71.6
20	3	1933	599	3	392	124	3	594	179	3	508	161	3	311	70.5
24	3	1320	280	3	278	86.8	3	594	87.7	3	576	156	3	444	158

* Relative to 6:30 am on Day 90.

The following table from the sponsor compares the rat and mouse data to those from humans:

Plasma Exposures Following 3-Month Dietary Administration of (RS)-Zopiclone to Mice and Rats Relative to Maximum Anticipated Steady State Human Exposures*

Analyte	Males		Females	
	Mean AUC_{0-24h} (ng.h/mL)	Animal:Human Ratio	Mean AUC_{0-24h} (ng.h/mL)	Animal:Human Ratio
Mice				
(S)-Zopiclone	3,911	21	1,450	8
(S)-DMZ**	1,147	33	790	23
Combined	5,058	22	2,240	10
Rats				
(S)-Zopiclone	12,884	67	29,465	154
(S)-DMZ	13,099	374	8,083	231
Combined	25,983	115	37,548	166

* Plasma AUC_{0-24h} values were estimated to be 191 ng.h/mL for (S)-zopiclone and 35 ng.h/mL for (S)-DMZ after 3 mg/day for 7 days (Sepracor Study No. 190-002) ** DMZ: desmethylzopiclone

The highest exposure in male rats were those of R-DMZ, S-DMZ, and S-zop and in female rats, the highest exposure was that of S-zop; in both sexes in mice, the highest exposure was that of R-DMZ. When 24hr exposures in mice and rats were compared to the exposure in humans dosed 3mg/d for 7d, the safety factor of animal to human ratios for S-zop was 21&8 fold in male and female mice and 67&154 fold in male and female rats respectively. Therefore, there seem to be adequate margin of safety in the rat but not in mice specifically female mice. Similar correlation was seen for the active metabolite, S-DMZ, in both species. The sponsor indicated that further evaluations may be needed to clarify the sex differences noted in this study in mice that were not observed in previous 3month tox/TK study in mice dosed via oral gavage.

Another study in **mice** was done to evaluate exposure to S-zop and its S-desmethyl metabolite (SEP174559), following oral gavage administration of either cpd. (Sepracor#190-525). Male and female C57BL (different strain from the above 2 studies), were dosed **100, 200, or 300mg/kg/d S-zop** (groups 1,2,3) and **SEP 174559 was dosed at 100, 200, and 400mg/kg/d** (groups 4,5, and 6), for **90d.** Clinical signs, mortality, B.wt, and food intake were monitored. Blood was collected from 3/sex/timepoint between 0.5-24hr postdose after the last dose on d90. Animals were killed without further exam. Plasma levels of S-zop and SEP174559 were determined using a validated — method. There were 3 deaths in males (2 dosed 100mg/kg/d S-zop and 1 dosed 300mg/kg/d), and 6 deaths in females (1 dosed 300mg/kg/d S-zop, 3 dosed 100mg/kg/d SEP174559 and 2 dosed 200mg/kg/d SEP174559). The sponsor considered these deaths not drug related due to absence of dose response, however, in absence of gross and histopath exam a clear cause of death can not be determined and a drug effect can not be ruled out. Hypokinesia, loss of balance and half closed eyes were seen throughout the study. Both sexes were exposed to both cpds (table from sponsor) with large margin of safety to human exposure based on 3mg S-zop clinical dose for 7d.

Treatment	Dose (mg/kg/day)	Analyte	Male		Female	
			$AUC_{0.5-24h}$ (ng•h/mL)	Mouse:Human Ratio	$AUC_{0.5-24h}$ (ng•h/mL)	Mouse:Human Ratio
(S)-Zopiclone	100	(S)-Zopiclone	12400	65	17200	90
		SEP-174559	2290	59	5780	149
	200	(S)-Zopiclone	24000	126	24700	129
		SEP-174559	6090	157	7900	204
	300	(S)-Zopiclone	50000	262	42500	223
		SEP-174559	9170	237	11100	287
SEP-174559	100	SEP-174559	15900	411	23800	615
	200	SEP-174559	49700	1284	66600	1721
	400	SEP-174559	111000	2868	131000	3385

Plasma AUC_{0-24h} values were estimated to be 191 ng•h/mL for (S)-zopiclone and 38.7 ng•h/mL for SEP-174559 after 3 mg (S)-zopiclone/day for 7 days (Sepracor Study 190-002).

TK of **S-zopiclone** was also studied in **pregnant New Zealand white rabbits** (Sepracor# 190-416). Three time mated rabbits were administered via oral gavage **4 & 24mg/kg/d S-zop from gd6-18,** clinical sings, B.wt were recorded. Blood was collected on gd6&18 between 0.5-24hr postdose and on gd19 females were killed and necropsy done to confirm pregnancy. Plasma S-zop were determined by a validated ~ There were no deaths in any group. No clinical signs in low dose, fecal output was reduced in females dosed 24mg/kg/d (HD). Mean **B.wt** and **wt. gain** were reduced in HD after start of dosing on gd6 and continued throughout dosing with an overall loss of 160g. Mean wt loss was also seen in 4mg/kg/d dose at start of dosing gd6 but the wt fluctuated throughout dosing with an overall loss of 70g (table from sponsor). Mean maternal **food intake** in HD paralleled the decrease in B.wt and was decreased throughout dosing when compared to predose intake and intake of LD females (table from sponsor). Intake in LD was only slightly reduced relative to predose values [*initial body weights on gd0 were not comparable and the s.d. was relatively large in both groups]* (figure from sponsor).

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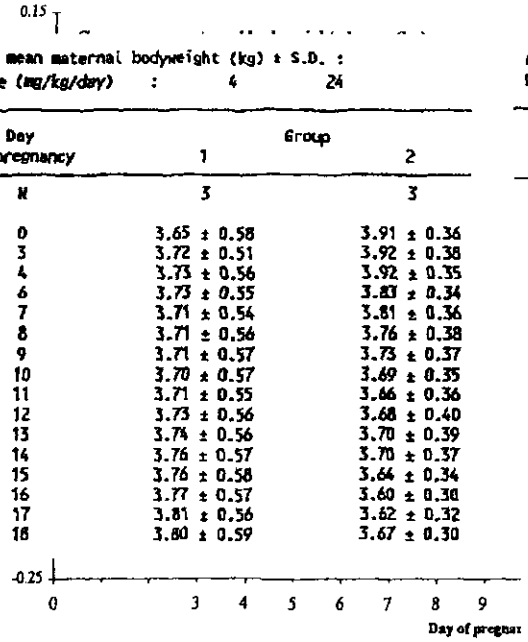
Oral rabbit TK study of S-zopiclone (Cont.)



Group mean maternal bodyweight (kg) ± S.D. :
Dosage (mg/kg/day) : 4 24

Day of pregnancy	Group 1	Group 2
N	3	3
0	3.65 ± 0.58	3.91 ± 0.36
3	3.72 ± 0.51	3.92 ± 0.38
4	3.73 ± 0.56	3.92 ± 0.35
6	3.73 ± 0.55	3.83 ± 0.34
7	3.71 ± 0.54	3.81 ± 0.36
8	3.71 ± 0.56	3.76 ± 0.38
9	3.71 ± 0.57	3.73 ± 0.37
10	3.70 ± 0.57	3.69 ± 0.35
11	3.71 ± 0.55	3.66 ± 0.36
12	3.73 ± 0.56	3.68 ± 0.40
13	3.74 ± 0.56	3.70 ± 0.39
14	3.76 ± 0.57	3.70 ± 0.37
15	3.76 ± 0.58	3.64 ± 0.34
16	3.77 ± 0.57	3.60 ± 0.38
17	3.81 ± 0.56	3.62 ± 0.32
18	3.80 ± 0.59	3.67 ± 0.30

Group mean maternal bodyweight change (kg) ± S.D.
Dosage (mg/kg/day) : 4 24

Day of pregnancy	Group 1	Group 2
N	3	3
0 to 6	0.08 ± 0.04	-0.08 ± 0.06
6 to 7	-0.02 ± 0.01	-0.02 ± 0.03
6 to 8	-0.02 ± 0.04	-0.07 ± 0.05
6 to 9	-0.02 ± 0.05	-0.10 ± 0.04
9 to 12	0.02 ± 0.03	-0.05 ± 0.06
12 to 16	0.04 ± 0.05	-0.08 ± 0.11
16 to 18	0.03 ± 0.05	0.06 ± 0.02
6 to 18	0.07 ± 0.04	-0.16 ± 0.10

Group mean maternal food consumption (g) ± S.D.
Dosage (mg/kg/day) : 4 24

Day of pregnancy	Group 1	Group 2
N	3	3
5 to 6	166 ± 20	113 ± 24
6 to 12	114 ± 5	35 ± 14
12 to 15	96 ± 27	40 ± 39
15 to 18	138 ± 16	67 ± 51
18 to 19	117 ± 37	78 ± 62
6 to 18	115 ± 12	44 ± 26

N = number of animals in mean

Oral rabbit TK study of S-zopiclone (Cont.)

All females were confirmed pregnant at necropsy. **TK** following single (gd6) and repeat dosing (gd18), to pregnant female rabbits showed measurable amounts of S-zop, S-zop desmethyl (DMZ), and zop-N-oxide (only in HD). Concentration and exposure increased more than proportional to dose and, moderate to relatively **large accumulation noted for S-zop and S-DMZ** (N-oxide was below quantitation limit of detection). The dose ratio was 6x between 4&24mg/kg/d but exposure increased **43 for S-zop in HD, 22&41x for S-DMZ in LD & HD** respectively. Similarly, C_{max} increased 39&46x for S-zop in LD&HD respectively, and 9&19x for S-DMZ respectively (table from sponsor). Dose proportionality and accumulation could not be assessed for the N-oxide because it was below detection limit on 1st day of dosing in both dose groups. Also stated that there were no measurable levels of R-zop or R-DMZ.

(S)-Zopiclone Oral Dose	(S)-Zopiclone Plasma TK		
	$AUC_{0.5\text{-}24h}$ (ng•h/mL)	C_{max} (ng/mL)	t_{max}* (h)
4 mg/kg/day			
Day 6 (Day 1 of dosing)	NC	79.8	0.5
Day 24 (Day 13 of dosing)	7233	3142	1
24 mg/kg/day			
Day 6 (Day 1 of dosing)	388	69.3	1
Day 24 (Day 13 of dosing)	16874	3203	4
	(S)-Desmethyl zopiclone Plasma TK		
4 mg/kg/day			
Day 6 (Day 1 of dosing)	748	312	1
Day 24 (Day 13 of dosing)	16462	2840	1
24 mg/kg/day			
Day 6 (Day 1 of dosing)	490	129	1
Day 24 (Day 13 of dosing)	20090	2487	4
	Zopiclone N-oxide Plasma TK		
4 mg/kg/day			
Day 6 (Day 1 of dosing)	NC	NC	NC
Day 24 (Day 13 of dosing)	906	425	NC
24 mg/kg/day			
Day 6 (Day 1 of dosing)	NC	NC	1
Day 24 (Day 13 of dosing)	716	427	2.5

NC = not calculated; *Median t_{max}

Based on these findings, it is concluded that oral gavage administration of S-zop to pregnant rabbits at 4&24mg/kg/d during organogenesis produced measurable amounts of S-zop, S-DMZ, and N-oxide (only at HD). Plasma concentrations and exposure increased more than proportional to dose and drug accumulated with repeated dosing.

3.3.4 METABOLISM:

RPR (1982), compared the metabolism of RS-zop in animals and humans using ^{14}C-label at the 4a, 5, 7, and 7a carbons or carbonyloxy chain of RS-zop (Sepracor#190-556). Drug was administered orally to SD rats and New Zealand white rabbits at **50-150mg/kg**, to dogs at **50mg/kg**, and humans at **5, 7.5, 10, or 15mg.** Urine and feces were collected from rat and rabbit (plasma from rats too), urine from dogs, and plasma and urine from humans. TLC and HPLC were used as the analytical methods and structural identification of cpds was done using [redacted] spectrometry.

Results:

Metabolites were recovered in urine and feces with 80% of radioactivity recovered in urine of animals. RS-zop in all animals and humans was extensively metabolized mainly by oxidation forming 14 metabolites. Metabolites were numbered I to XIV with unchanged parent being VIII. Major metabolites included N-oxide (XIII), N-desmethylzop (XII), and 2-amino-2-chloro pyridine (III). Similar metabolic profile was seen in rabbit, dog, and human.

RPR in 1975 measured RS-zop levels in serum and urine samples from dogs (2 males) and humans (n=6) following oral dose of **0.75&1mg/kg to dogs and 15&20mg to humans** (Sepracor#190-554). Blood collected between 1-48hr and urine between 0-6hr and up to 36-48hr postdose. Serum RS-zop in the dog were **150&240ng/ml** at 0.75 and 1mg/kg respectively, and remained at 100ng/ml at 2hr, concentrations declined rapidly thereafter. At 1mg/kg serum level was 100ng/ml at 6hr postdose and undetectable by 24hr postdose. Urinary elimination was 6% of dose at 0-72hr sample and desmethyl-zop plus 2-3 other metabolites were detected in urine; the amount of the desmethyl-zop was comparable to the RS-zop in urine samples up to 48hr. **Human serum** levels after 15mg dose were **63-130ng/ml** at 1hr and 23-92ng/ml at 6hr postdose respectively, and those after 20mg dose were **44-154ng/ml** and 47-90ng/ml after 1&6hr postdose respectively. No parent was detected in the 36hr serum sample. Human urinary elimination of unchanged drug was also low at 0.5-3% in the 72hr sample, there were several metabolites including the desmethyl which was found at moderate amounts up to the 48hr samples.

Another study conducted by RPR 3 years later than the above study (1978), compared metabolism between animals and humans but used unlabelled RS-zop (Sepracor#190-555). Single oral dose of RS-zop was administered to rats, rabbits, dogs, and humans and blood, urine, and feces collected at specific time points up to 72hr postdose for parent and metabolite analysis. In addition to isolation and identification of metabolites, serum protein binding of parent was determined in vitro (gel filtration) and in vivo (equilibrium dialysis). Concentrations of RS-zop, N-oxide, and N-desmethyl were measured by TLC (Sepracor#190-554).

Results:

Drug was well absorbed and peak serum levels reached within 3hrs in dogs and humans, 4-6hr in rabbits and 1hr in rat (table from sponsor). Both N-desmethyl and N-oxide as well as unchanged drug were detected in urine and feces.

Table 5.D.3-7 Serum Pharmacokinetics of (RS)-Zopiclone in Rats, Rabbits, Dogs, and Humans after Oral Dosing

	Rat	Rabbit	Dog	Human
Dose (zopiclone, mg/kg)	0.8	0.8/125	0.27/0.8	5 mg/10 mg
C_{max} (ng/mL)		—		
t_{max} (h)	1	4-6	2-3	3

Metabolism (Cont.)

Table 5.D.3-8 Urine and Fecal Excretion of (RS)-Zopiclone and Metabolites in Rats, Rabbits, Dogs and Humans over 0-72 Hours after Oral Dosing

	Rat	Rabbit	Dog	Human
Dose (zopiclone, mg/kg)	0 .8	0.8 / 125	0.27 / 0.8	5 mg / 10 mg
Urine excretion (% of dose)				
(RS)-Zopiclone	0.1-2.6	0.15 / 2.1-6.6	3.3 / 0.7-2.8	4-10
N-Desmethylzopiclone	1.4-3.9	1.5 / 11.1-17.4	NS / 1.7-5.5	2-8 / 4.8-12.4
Zopiclone N-oxide	0.4-0.8	2-2.56 / 4.2-8.5	NS / 0.9-2.9	NS
Fecal excretion (% of dose)				
(RS)-Zopiclone	0.5	0.07-0.6	0.2-0.5	NS

NS = No sample

From these tables, N-desmethyls seem to be the major metabolite in urine in all animal species and, unchanged drug was equally important in humans. RS-zop in vitro and in vivo protein binding was low. Main metabolic pathway seemed to be oxidation of the piperazine CH3, or piperazine-CH2-bond or formation of the N-oxide. In total, 14 metabolites were identified similar to the above study.

In vitro metabolism of RS-zop in rat was done to compare with the profile in vivo (RPR 1983; Sepracor#190-557). Rat livers were removed and perfused for 30min following catheterization of the common bile duct and the portal vein. Perfusions were carried out with 5mg RS-zop or 5mg RS-zop/^{14}C-RS-zop (RP 45494), 10-100uCi. Perfusate were collected between 3-120min from 3 livers to determine kinetics of RS-zop disappearance from this medium. Both the perfusate and bile were analyzed qualitatively and quantitatively by TLC and HPLC/UV detection and autoradiography was used for the ^{14}C-RS-zop, cpds. Total radioactivity in perfusion fluid, bile, and liver was determined by liquid scintillation counting. Results of this study confirmed those from other studies (Sepracor#190-555 and 190-556), where similar to the in vivo data, RS-zop is extensively metabolized at 95% of dose in vitro. The major metabolites as well as unchanged parent that were detected in perfusion fluid or in bile were also present in urine or feces. Parent cpd rapidly disappeared from perfusion fluid with $t_{1/2}$ of 0.23hr with <1% of perfused dose detected by 90min. About 70% of dose was excreted in bile as metabolites.

S-zop Metabolism and Protein Binding:

Blood-to-plasma partitioning and binding of ^{14}C-S-zop to protein in mouse, rat, dog and human plasma was measured at multiple concentrations based on MTD in mouse, rat, and dog and on therapeutic doses in

humans using ultrafiltration methods (Sepracor#190-528). Blood was collected from 3 male dogs and 1 human and pooled at [ ] (for the equilibrium data. Blood and plasma for mice and rats were obtained from [ ]). Protein binding of S-zop in all these species ranged between **28.8% in the rat to 58.9% in humans** over the concentration range tested. Blood-to-plasma ratios over the same concentration range as that for the protein, were **0.31 in humans to 0.58 in mice indicative of little or no uptake of S-zop by RBC**. The protein binding data were as follows: rat 28.8-46.7%, mouse 36.7-56.5%, dog 31.9-43.5%, and humans 52.2-58.9%. Blood-to-plasma partitioning ratios were as follows: rat 0.50-0.55, mouse 0.46-0.58, dog 0.44-0.52, and humans 0.31-0.34 at the concentration range tested in these species (humans 5-500ng/ml and 10-10,000ng/ml for the mouse, rat, and dog). Metabolism of S-zop (Cont.)

Another study investigated if the presence or absence of R-zop affect the in vitro metabolism of S-zop using animal and human hepatocytes (Sepracor#190-536). ^{14}C-S-zop 50 and 100uM for mouse, rat, and dog and 1&2uM for human as well as a mixture of ^{14}C-S-zop + R-zop (50/50uM for the mouse, rat, and dog and 1/1uM for human were incubated with hepatocytes from these species according to SOPs. Radio-HPLC chromatograms showed similar radioactivity profiles of ^{14}C-S-zop incubated with or without R-zop in all species. Comparable percent of radioactivity of S-zop and desmethylzop, N-oxide, and lactamol were measured in all species. Unknown metabolites accounted for $\leq$2% of total radioactivity. It was concluded that R-zop had no effect on in vitro metabolism of ^{14}C-S-zop incubated with hepatocytes from mouse, rat, dog, and humans.

The isoforms of hepatic P450 responsible for metabolism of S-zop in human microsomes were identified in Sepracor#190-516. The metabolism of S-zop in -/+ CYP isoform specific inhibitors was assessed by monitoring the disappearance of S-zop or appearance of its metabolits using HPLC/UV detection as the analytical method. Results showed that S-zop is mainly metabolized by **CYP2E1 and CYP3A4** in human liver microsomes.

In another study, the inhibitory potential of S-zopiclone on CYP450 1A2, 2A6, 2C9, 2C19, 2D6, 2E1, and 3A4 was determined in cryopreserved human hepatocytes (Sepracor# 190-518). Hepatocytes were isolated, prepared, and pooled from 3m and 2f donors. The IC_{50} values of S-zop was determined against CYP450 isozymes. The results showed that S-zop had no inhibitory effect on 2A6, 2C19, 2E1, or 3A4, and only minimal though dose-dependent inhibition of 1A2, 2C9, and 2D6 (IC_{50} value greater than the maximum tested concentration of 100uM).

The permeability of S-zop across the human intestinal Caco-2 monolayers was examined (Sepracor#190-542). S-zop showed high absorptive and secretory permeability at all concentrations tested and did not exhibit any active transport mechanism in either absorptive or secretory directions. S-zop also had no effect on the integrity of the cellular junctions at any of the concentrations tested.

The effect of R-zop on the intrinsic clearance of S-zop in cryopreserved human hepatocytes could not be determined. This was because the intrinsic Cl of S-zop could not be determined due to degradation and limited metabolism of S-zop by human hepatocytes (Sepracor#190-568).

3.4 TOXICOLOGY:

3.4.1 OVERALL TOXICOLOGY SUMMARY

Single dose acute toxicology studies were done in mice (oral) and rats (i.v.). The MLD in mice for the racemate was >3000mg/kg in males and 1500-3000mg/kg in females, for the S-zop it was 900-1200mg/kg both sexes and for the R-zop, the MLD was >1500mg/kg in both sexes. In the rat, the corresponding MLD values for the racemate were 25-50mg/kg in males and 150-200mg/kg in females, for the S-zop it was 1-10mg/kg males and 100-250mg/kg females and for the R-zop it was 100-150mg/kg males and 150-200mg/kg in females. Results form both species showed that the **most toxic cpd of all three based on mortality is the S-form**. Clinical signs were seen **in both species with all 3 isomers** and included muscle incoordination, prostration, convulsions, hypoactivity, cold to touch, and labored respiration. Repeat dose oral gavage toxicity studies of the **S-form** (plus an arm for the RS-zop in the 3mo studies)**,** were done in mouse, rat, and dog. Doses were up to 200mg/kg/d in mice, 300/200mg/kg/d in rats, and 25mg/kg/d in the dog; 2.5% hydroxy propyl methyl cellulose was the vehicle in all the species. **Death** occurred in 4 female mice after the 1st dose in a dose escalation study, in the 1month mouse tox study 1 female mouse dosed 300/200mg/kg racemate in the tox study was found dead on d27 of dosing with unknown cause in addition to 17 deaths in all dose groups (including the LD of 50mg/kg) occurred in the TK group. In 1month rat study, all rats dosed 300mg/kg/d S-zop were killed on d6 of dosing and all those in TK group were killed on d7 of dosing due to clinical signs. Death also occurred in 200mg/kg RS-zop and S-zop as well as in the 50&100mg/kg S-zop groups. Similar **clinical signs** were observed in all drug groups in all 3 species and were comparable between the S- and the RS-zop. Signs included: hunched posture, lethargy, unsteady gait, piloerection, distended abdomen, partially closed eyes, and irregular/shallow breathing. In addition to these signs, the following were observed in the dog: vocalization, abnormal color of pinnae, mouth, perinasal areas and paws, there were also lesions in these areas that took a week to heal while dosing continued; no clear indication whether these lesions were or were not drug related. In general, mean body wt and weight gain were reduced at all doses in mice and dogs (no effect in rats) in the 1mo studies without an effect on food intake. Howver, drug effects on B.wt or food intake were not recorded in the 3mo studies of all 3 species. There were no consistant and/or dose-dependent changes in hematology or clinical chemistry parameters and those parameters that reached statistical significance the effects were small and generally not dose related. It is noted that in the 3mo studies rats and dogs administered the mid and/or high doses urine volume and specific gravity were increased and decreased respectively, the significance of this is unclear. Mean ALT, AST and ALP levels were increased in all 3 species in all or the 2 highest doses of the S-zop as well as the RS-zop group. Only in the rat at both 1 and 3mo studies (though more so in the 3mo), mean liver, kidneys, ovaries and thyroid wts were increased in males and females dose dependently for the racemate and S-zop. In the 1mo study, mean thymus wt was decreased dose dependently in both rats and dogs, mean testes wt was decreased in

rats dosed the racemate only and prostate wt was decreased in drug treated dogs (no histopath in the dog). In the 3mo studies, male reproductive organs and sperm parameters were adversely affected in the rat with some of the findings persisting till end of 1mo recovery period. In the 1mo rat study the following was observed: *glandular and non-glandular stomach mucosal atrophy and minimal diffuse epithelial hyperplasia, pituitary hypertrophy and vacuolation, renal basophilic regenerating tubules, interstitial edema and epithelial vacuolation in the epididimydes (high incidence in all drug groups), follicular epithelial hypertrophy, and diffuse lobular hyperplasia of mammary glands.* In the 1 but not 3mo dog study, the only histopath finding was in the epididimydes where sperm granuloma, spermatocele, and granulomatous inflammation were seen in dogs dosed S-zop at ≥5mg/kg/d. The NOEL in the 1month studies was 2mg/kg/d in the dog, in the rat it could not be determined due to death in the lowest dose of 50mg/kg/d, and similarly in the mouse, due to death in the low dose of 50mg/kg, NOEL could not be determined. **It is noted that in the 3mo mouse study at end of wk12 at doses >50mg/kg/d S-zop and 200mg/kg/d RS-zop ocular effects were observed, they included bilateral retinal degeneration and moderate to severe retinal atrophy in both sexes. These effects were not seen in recovery animals or in any other toxicity study. The NOAEL in 3mo study in the mouse is 200mg/kg/d in both sexes with AUC_{0-24} of 29,430ng.hr/ml in males and 28,167ng.hr/ml in females on d91. The NOAEL in 3mo study in the rat was 100mg/kg/d in females and 25mg/kg/d in males due to adverse effects including histopathology observed in males, AUC_{0-24} at these doses were 9157ng.hr/ml in males and 67,203ng.hr/ml in females all measured on d89 of study. The NOAEL in the 3mo dog study is 2.5mg/kg/d in males with AUC_{0-24} of 5884ng.hr/ml and the NOAEL in females is 10mg/kg/d with AUC_{0-24} of 25,574ng.hr/ml.**

3.4.2 SINGLE DOSE TOXICITY STUDIES:

Study Title: Acute oral tox in mice with zopiclone.
Study No: Sepracor# 190-801 / 403-SE-001-95
Conducting laboratory and location:
Date of study initiation: not reported; Date protocol signed: Dec 1995/Final report date: Jul 1996
GLP compliance: Yes/OECD
QA- Report Yes () No (x)
Lot# for (+)-zopiclone: 784-62; (-)-zopiclone: 784-72A; racemate-zopiclone: CH-7360
Purity/Stability: responsibility of sponsor.
Methods:
Dosing: - species/strain: CD-1 mice
- #/sex/group: 5/sex/gr Age/B.wt: 4-6wks/18-28g
- dosage groups in administered units: **racemate: 900, 1500, 3000mg/kg;**
(-) isomer: 900, 1500mg/kg
(+) isomer: 900, 1200, 1500mg/kg
- route, form, volume, and infusion rate: oral/gavage/vol not reported.

Formulation/vehicle: **0.25% methylcellulose (MC).**
Observations and times:
- Clinical signs: 1&4hr post dose daily for 14days.
- Body weights: d0, d7, & d14.
- Gross necropsy: end of 14d observation period.

Results:
Mortality: as follows:

Drug	Dose (mg/kg)	# dead per total per dose
racemate	900	0/10
	1500	2/10
	3000	5/10
(-) isomer	900	5/10
	1500	4/20
(+) isomer	900	3/10
	1200	9/10
	1500	9/10

Based on mortality findings, the RS- was the least toxic followed by the R-isomer and the most toxic was the S-isomer.

Clinical signs: for all 3 cpds: decreased activity, decreased muscle tone, labored respiration, abnormal gait and stance, eyes shut, poor grooming, quivering, and prostration. Signs were seen at all doses.

It was concluded that the most tox cpd based on mortality is the (+) isomer followed by (-) and then the racemate. The median leathal doses (MLD) in the mouse for the 3 isomers are:

Zopiclone	MLD (mg/kg)	
	m	f
RS-	>3000	1500-3000
S-	900-1200	900-1200
R-	>1500	>1500

Study Title: Acute i.v. tox in Albino rats with zopiclone.

Study No: Sepracor# 190-802′ → 312024

Conducting laboratory and location: —

Date of study initiation/termination: Dec 1998/Feb 1999

GLP compliance: Yes/FDA

QA- Report Yes (x)

Methods:

Drug Lot#/Batch#: (+) zopiclone: H1249-21C; (-/+)zopiclone: 9809002;
(-) zopiclone TJ1209-77C.

Purity/Stability: >′ — for (+) and (-) zopiclone; (-/+) zopiclone: —

Dosing:

- species/strain: Albino rat/Sprague Dawley
- #/sex/group: 5/sex/gr Age/B.wt: 8-12wks/18-225o-350g m and 200-300g f at study initiation.
- dosage groups in administered units:

racemate: 25, 50, 75, 150, 200, & 250mg/kg,
(S) isomer: 1, 10, 25, 75, 100, 200mg/kg,
(R) isomer: 75, 100, 150, 250mg/kg

- route, form, volume, and infusion rate: oral/gavage/10ml/kg.

Formulation/vehicle: **0.1N HCl.**

Observations and times:

- Clinical signs: 1, 3&4gr post dose and daily for 14days.
- Body weights: d0, d7, & d14.
- Gross necropsy: end of 14d observation period.

The median lethal dose will be determined.

Results:
Mortality: table below shows MLD in all 3 isomers with death occuring within 1hr postdose at 25, 10, and 100mg/kg in the racemate, S- and R- isomers respectively. Clinical signs seen in all 3 isomers at all doses with no clear sex difference they included: muscle incoordination, prostration, convulsions, hypoactivity, cold to touch, pale extremities, and/or tremors. The median lethal doses were as follows:

Drug	MLD (mg/kg) Males	females
racemate	25-50	150-200
(-)-isomer	100-150	150-200
(+)-isomer	1-10	100-250

3.4.3 REPEATE DOSE TOXICOLOGY STUDIES:

Study Title: Oral MTD and 7d repeat dose tox study of S-zopiclone in mice.

Study No: Sepracor# 190-805A1' — .# OTI00512
Conducting laboratory and location: —

Date of study initiation: March 1st 1999/March 8th 1999
GLP compliance: Yes/FDA & EC
QA- Report Yes () No (x)
Lot#/Batch#/purity/stability: 120998A/NR
Dosing:

- species/strain: mice/CD-1
- #/sex/groupt: 4-5/sex/gr Age/weight: 5wks/20-25g
- satellite groups used for toxicokinetics or recovery: NA
- dosage groups in administered units: there were 2 phases for this study: phase 1 escalating range finding MTD study upto 20days (see table below), and phase 2 a 7d limit test.

Doses for phase 1 are as follows (note that no vehicle control group was used in this phase of the study):

days	Doses (mg/kg/d)
Group 1	
1-7	200
8-13	300
14-20	400

Phase 2 limit test doses (daily dosing for 7d):

Group 2	vehicle
Group 3	50mg/kg/d
Group 4	400/300*mg/kg/d

* Deaths occurred at 400mg/kg on d1 consequently the dose was reduced to 300mg/kg and dosing at the 300mg/kg started on d2 and continued for 6 days.

- route, form, volume, and infusion rate: oral/gavage/vol NR.
Formulation/vehicle: **2.5% hydroxy propyl methyl cellulose**
Observations and times:
- Clinical signs: daily and as needed; for the 1st day of dosing: immediately postdose, 15, 30, 45, 60, 75, 90min, 2.5&3hr postdose.
- Body weights: daily for both phases.
- Food intake: between day1&7 of phase 2
- Gross necropsy/phase 2: end of study.
- Organ wts: phase 2: adrenals, liver, testes/epididymides, heart, spleen, thymus, kidneys, ovaries.
- Histopath: 5micron sections, processed in 10% formalin. All above tissues/organs.

Oral MTD and 7d repeat dose tox study of S-zopiclone in mice (Cont.)

Results:
Mortality: phase 2: **4f dosed 400mg/kg died** after the 1st dose and a replacement female was killed in moribund on **d2 of dosing at 300mg/kg/d. Cause of death was unknown (table below from sponsor):**

Animal No.	Dose (mg/kg/day)	Day killed or found dead	Clinical observations	Necropsy findings	Cause of death
400F	400	1	Prostrate, unsteady gait, cold body surface, piloerection, labored breathing, subdued	N.A.D.*	Unknown
402F	400	1	Prostrate, unsteady gait, cold body surface, subdued	Bladder: distended (with urine)	Unknown
406F	400	1	Prostrate, unsteady gait, cold body surface, piloerection	N.A.D.	Unknown
408F	400	1	Prostrate, unsteady gait, cold body surface, subdued, piloerection	N.A.D.	Unknown
414F**	300	2	Prostrate, unsteady gait, cold body surface, subdued, labored breathing	N.A.D.	Unknown

* No abnormality detected
** Replacement animal

Clinical signs: seen in both dose grs (50&300mg/kg/d) throughout the 7d dosing. They included: hunched posture, prostrate, unsteady gait, piloerection, labored breathing, and cold to touch (whole body). Similar clinical signs were seen in mice in phase 1 (escalating dose).
B.wt/Food intake: mean wt, wt gain, and food intake were decreased in both drug groups during the 2wk observation period in phase 2 part of the study. Mean wt loss occurred during the 1-7d period as follows (values are means±s.d.; n=4-5/sex/dose): -0.42±0.63g and -1.78±1.25g in 50&300mg/kg males respectively, relative to a mean gain of 0.4±1.13g in control males; the corresponding values in females were -0.7±0.8 and -0.72±0.45g vs. a gain of 0.78±1.0g in control females. Similarly, food intake was reduced dose dependently in the 50&300mg/kg grs during the same period of dosing. However, males

and females in phase 1 gained 12&11% wt respectively, over the 1-19days relative to the control; no effect on food.
Organ wt: no clear drug effect on any tissue/organ wt.
Gross Necropsy: no drug related findings in any group.

Summary and Conclusion:
Mice could not tolerate 400mg/kg dose and the MTD appears to be 50mg/kg since 1 death occurred in the 300mg/kg group following 2 days of dosing. Based on clinical signs, a **NOEL could not be determined in this study**.

Study Title: S-zop 7d toxicity (dose confirmation) study by oral gavage in C57BL mice.

Study No: Sepracor# 190-881 – 22534 TCS
Conducting laboratory and location: –
Date of study initiation: Not provided/study report date: 2002
GLP compliance/QA: No, it's a preliminary study
Lot#/Batch#: Not provided
Purity/Stability: Not provided

This study was a dose ranger in C57BL/6 mice (the background strain for p53 transgenic mice).

Method, Results, Conclusion:
S-zop was administered via oral gavage at 200 and 300mg/kg/d to 6/sex/group mice for 7d, the vehicle 0.5% CMC/1% Tween 80 served as the control group; dose volume was 10ml/kg. Mortality, clinical signs, B.wt, food intake, and gross necropsy were done. No death in any group, clinical signs were reversible, seen in both drug groups and included hypoactivity, cold to touch, staggering gait, sedation, and half-closed eyes. Generally, B.wt and food were reduced in males with no other findings. It was concluded that S-zop is well tolerated up to 300mg/kg/d in C57BL/6 mice except for decrease in wt and food intake in males and reversible signs in both sexes of both doses. [limited data were presented since this was a preliminary study].

Study Title: 4wk oral gavage tox/TK study of S-zopiclone in mice.

Study No: Sepracor# 190-813/(–)TI00513
Conducting laboratory and location: (–
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Date of study initiation/termination:Mar 1999/Apr 1999
GLP compliance: Yes/FDA & EC
QA- Report Yes (x)

Lot#/Batch#: for (S) zopiclone: AT737 SA1001E,F/Raw material lot# 120998A; for (-/+) zopiclone suspension: AT737 SA1002E,F/Raw material lot # 9809002
Purity/Stability: responsibility of sponsor.
Methods:
Dosing:
- species/strain: mice/CD-1
- #/sex/group: main study: 12/sex/gr; TK: 18/sex/dose.
- age/b.wt: 5wks/20-25g
- satellite groups used for toxicokinetics or recovery: 18/sex/dose
- dosage groups in administered units: **S-zop 50, 100, 200mg/kg/d; racemic: 200mg/kg/d.** The control group received the vehicle.
- route, form, volume, and infusion rate: oral/gavage/vol NR.
Formulation/vehicle: **2.5% hydroxy propyl methyl cellulose**
Observations and times:
- Clinical signs & mortality: 2x daily and as needed.
- Body weights: at start of dosing and weekly thereafter.
- Food intake: weekly.
- Clinical Chemistry/hematology: end of study, blood will be collected from the orbital sinus.
- TK: blood was collected from all mice on days1&29. There were 3mice/time point/gr at 0.5, 1, 2, 4, 6, and 24hr postdose. Plasma levels were determined for: S- & R- zopiclone, S & R- zopiclone-N oxide, and S- & R- desmethylzopiclone. *Note that TK was done by* — *; not* — software used was WinNonlin.

- Gross necropsy: end of study.
- Organ wts: adrenals, kidneys, ovaries, salivary gland, thymus, brain, liver, pituitary, spleen, uterus, heart, lungs, prostate, testes/epididymides.
- Histopath of above tissues/organs: 5micron sections and stained with H&E and examined microscopically.

Results:
Mortality: total of 23 deaths including 1m control found dead on d4 as follows:

Dose (mg/kg)	Mortality	Cause of Death
Main Study:		
200 S	2m	killed in moribund on d2of dosing - **gavage error**
200 RS	2f	killed on d2&27 - **gavage error**
50 S	1f	killed on d2 - **gavage error**

Table below from sponsor presents the above deaths with clinical signs and cause of death:
Main group:

Animal No.	Dose (mg/kg/day)	Day killed or found dead	Clinical observations	Necropsy findings	Cause of death
509M	200 (S)-zopiclone	2	Prostrate, cold body surface, hunched, irregular breathing	Lungs: reddened; Thoracic cavity: contained brown fluid	Dosing error
513M	200 (S)-zopiclone	2	Prostrate, cold body surface, hunched, piloerection	Lung: reddened; Thoracic cavity: contained straw colored fluid.	Dosing error
300F	50 (S)-zopiclone	2	Hunched, subdued, unsteady gait, partially closed eye	Lung: reddened; Thoracic cavity: contained gray gelatinous material	Dosing error
210F	300/200 (RS)-zopiclone	27	Prostrate, cold body surface, irregular breathing, pale extremities	Pancreas: pale; Bladder: distended	Unknown
218F	300/200 (RS)-zopiclone	2	Prostrate, hunched posture, piloerection, shallow breathing	Lung: reddened; Thoracic cavity: contained straw colored fluid	Dosing error
109M	Control	4	Found dead	Lung: discolored, reddened; Thoracic cavity: contained clear fluid	Dosing error

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4wk oral tox/TK study of S-zopiclone in mice/Sepracor#190-813 (Cont.)

Satellite Group:

TK Satellite Groups:

200 RS	1m	found dead on d3
	2m	killed on days3&17
	1f	killed on d3
50 S	2m	found dead on d1
	1m/1f	killed on d2&3 respectively
100 S	3m	found dead on days2&3
	2f	killed on days 3&4
200 S	1m	found dead on d2
	3f	died during bleeding d2

Total dead or unscheduled deaths (m+f):

50mg/kg	5/60
100mg/kg	5/60
200mg/kg	6/60
200mg/kg RS	6/60

control 1/24

TK mice were not examined hitopathologically therefore, exact cause of death could not be determined. Total of 6 deaths in the main study (1 in control) were due to gavage error and drug un-related. The remaining **17 deaths were considered *by the sponsor* to be drug related as well as result of stress from anesthesia and/or blood sampling.**

Clinical signs: observed in all drug grs including those killed in moribund or found dead they were: hunched posture, prostrate, unsteady gait, piloerection, irregular breathing, distended abdomen, cold to touch, and agitation. Generally, signs were similar between the RS-zop and S-zop except that they were more frequent in animals dosed with the S-zop than those dosed with the RS-zop in both sexes.

B.wt: mean wt gain over the 29d dosing period was significantly but not dose dependently decreased in all 3 female S-zop groups (23-49% less control); no change in males. Note that mean wt gain in the corresponding female TK groups was unaffected though a decreasing trend was observed without reaching statistical significance.

Food Consumption: no effect in any group.

Hematology & Clinical Chemistry: no effect on any hematology parameter. No consistent or dose dependent finding in any group re. clinical chemistry. Random findings included a significant decrease in mean creatinine, urea nitrogen, and TG in males and/or females of all 3 S-zop groups.

Organ wts: no consistent or dose-dependent changes except for some changes in relative and/or absolute wts that reached statistical significance in males and/or females dosed either the S- or the RS-zop. The changes included: ↑ in liver and kidney wts, and ↓ in adrenal wts.

Histopath: there were some drug related findings in animals dosed both drugs that reached statistical significance. Hepatocyte centrilobular cytoplasmic pallor was found in 7/12f dosed 200mg/kg RS-zop and in 4, 6, 3 out of 12 mice in each S-zop groups. Reduced cytoplasmic rarefication was seen in 3/12f dosed RS-zop, 1/12f in S-zop 200mg/kg vs. 0/12 in control. Also, hepatocyte centrilobular hypertrophy was seen in 4/12f dosed 200mg/kg RS-zop with only 1/12 females dosed 100mg/kg S- and none in female/male control or 200mg/kg S-zop.

4wk oral tox/TK study of S-zopiclone in mice/Sepracor#190-813 (Cont.)

TK: animals were exposed to all cpds analyzed following administration of either drug except the R-zop and R-desmethyl zop were below detection limit following S-zop administration indicating that the S- did not convert to the R-zop after oral administration. The highest levels of exposure in both sexes after either drug, were to R-zop and R-desmethyl zop. (tables from sponsor). Generally females had higher levels than males particularly on d29. T_{max} for S-zop was reached between 0.5-2hr postdose in both sexes after RS-zop administration and 1-6hr after its own administration. There seem to be no accumulation with repeated dosing for the S-zop and S-desmethyl following administration of either S-zop or RS-zop. However, the N-oxide following either cpd administration and perhaps to lesser degree, the R-zop and R-desmethyl following RS-zop, showed accumulation following repeated dosing. Generally, there was less than proportional increase in exposure to S-zop and S-desmethyl following S-zop administration whereas, more than proportional increase noted for the N-oxide particularly between 50 and 100mg/kg/d doses in both sexes. Following S-zop but not RS-zop, there was sex difference in exposure to the cpds with females generally having higher levels than males. It is of note that exposure to S-zop after single or repeat dosing of S-zop at 100mg/kg/d or 200mg/kg/d (corrected), was much higher (>2 fold), than exposure measured after 200mg/kg/d RS-zop. Similarly, exposures to S-desmethyl following S-zop

administration were much higher (1-2x and up to 4x on d29 in f), than those measured after RS-zop (corrected for dose).

Summary and Conclusion:
Oral daily administration of RS-zop at 200mg/kg/d or S-zop at 50, 100, 200mg/kg/d for 1 month to male and female mice caused drug related deaths. Clinical signs were seen in all dose groups and were similar between the S- and RS-zop but more frequent in the former. Mean wt gain was significantly but not dose dependently reduced in all 3 female groups dosed the S-zop, no effect in males or in either sex of the TK satellite group; no wt effect in mice dosed the racemate. There were no drug related findings on hematology, clinical chemistry, or gross necropsy. Some random and not dose dependent changes noted in organ wts (liver, kidneys, and adrenals) that except for the liver, did not correspond to any histopathology. Liver centrilobular cytoplasmic pallor was noted in females dosed with both the S- and RS-zop (higher incidence in the latter), hepatocyte hypertrophy was seen in females dosed the 200mg/kg RS-zop 4/12, and in only 1/12f dosed 100mg/kg S-zop. It can be concluded that the **toxicity profile is similar for the S- and RS-zop. A NOEL for S-zop could not be determined in this study because a drug related cause of death could not be ruled out at the 50mg/kg/d S-zop. Therefore, the MTD was exceeded at the 50mg/kg/d S-zop in mice.**

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Table **Toxicokinetics of (S)-Zopiclone, (R)-Zopiclone, (S)-Desmethylzopiclone, (R)-Desmethylzopiclone and Zopiclone N-Oxide after 4 Weeks Oral Dosing of (S)-Zopiclone or (RS)-Zopiclone in Mice**

Zopiclone Dose (mg/kg/day)	(S)-Zopiclone				(S)-Desmethylzopiclone			
	Male		Female		Male		Female	
	AUC	Cmax	AUC	Cmax	AUC	Cmax	AUC	Cmax
Day 1								
(S), 50	17067		22345		7176	1390	11748	
(S), 100	31877		50538		12324	1960	27220	
(S), 200	46213		92633		19240	3700	55875	
(RS)-zop, 200	26978		19390		18458	2500	10548	
Day 29								
(S), 50	19210		27833		7540	2030	17913	
(S), 100	41805		41638		14930	2170	28180	
(S), 200	46145		59868		17708	2320	37058	
(RS), 200	17873		18220		7745	1820	6430	

Zopiclone Dose (mg/kg/day)	(R)-Zopiclone				(R)-Desmethylzopiclone			
	Male		Female		Male		Female	
	AUC	Cmax	AUC	Cmax	AUC	Cmax	AUC	Cmax
Day 1								
(RS), 200	39845		33995	6720	72133		54410	
Day 29								
(RS), 200	26650		47490	9790	57048		84660	

Zopiclone Dose (mg/kg/day)	Zopiclone N-Oxide			
	Male		Female	
	AUC	Cmax	AUC	Cmax
Day 1				
(S), 50	4073		2285	
(S), 100	17823		12843	
(S), 200	22930		23093	
(RS), 200	9443		14016	
Day 29				
(S), 50	7123		4054	
(S), 100	21848		13068	
(S), 200	36383		28630	
(RS), 200	8740		23458	

AUC_{0-24} = ng•h/mL; C_{max} = ng/mL

Study Title: 4wk oral tox study of S-zopiclone in rats.

Study No: Sepracor# 190-804' — # OTI00504
Conducting laboratory and location: —
Date of study initiation/termination:Feb 1999/Mar 1999
GLP compliance: Yes/FDA & EC
QA- Report Yes (x)
Lot#/Batch#: for (S) zopiclone:Raw material lot# 120998A; for racemic zopiclone suspension: Raw material lot # 9809002
Purity/Stability: responsibility of sponsor.
Methods:
Dosing:

- species/strain: SD rats
- #/sex/group: main study: 10/sex/gr; TK: 18/sex/dose.
- age/b.wt: 5wks/113-137g
- satellite groups used for toxicokinetics or recovery: 18/sex/dose

- dosage groups in administered units: **S- 50, 100, 200, 300mg/kg/d; RS- 300mg/kg/d***. the control group received the vehicle.

* dose reduced to 200mg/kg due to deaths in this gr starting on d2 of dosing.
- route, form, volume, and infusion rate: oral/gavage/vol 12ml/kg.
Formulation/vehicle: **2.5% hydroxy propyl methyl cellulose.**
Observations and times:
- Clinical signs & mortality: 2x daily and as needed.
- Body weights: predose, start of dose, 2x weekly, and end of dosing.
- Food intake: predose and weekly thereafter.
- Water intake: determined over a 7d period during the 3rd wk of study.
- Ophthalmology: predose and once during wk4 (indirect and/or direct method to include anterior portion, optic media, and ocular fundus).
- Clinical Chemistry/hematology: end of study, blood was collected from the orbital sinus.
Urinalysis: during wk4.
- TK: blood collected from non-fasted all TK rats on days1&28. There were 3rats/time point/gr at 0.5, 1, 2, 4, 6, and 24hr postdose. Plasma levels were determined for: S- & R-zopiclone, S & R-zop N-oxide, and S- & R-desmethylzopiclone. *Note that TK was done by — , not* — —
- Gross necropsy: end of study.
- Organ wts: adrenals, kidneys, ovaries, salivary gland, thymus, brain, liver, pituitary, spleen, uterus, heart, lungs, prostate, testes/epididymides.
- Histopath: 5 micron sections and stained with H&E and examined microscopically.

Results:
Clinical signs: similar to other studies, hunched posture, lethargic, unsteady gait, distended abdomen, piloerection, partially closed eyes, and shallow/irregular breathing. Signs were seen in all dose groups. The animals that were found dead or killed in moribund had marked and long lasting lethargic and unsteady gait with rats showing hunched posture, piloerection, shallow breathing, and cold body.
Mortality: all males and females dosed 300mg/kg S-zop were killed on d6 of dosing, 1m in the TK group dosed 300mg/kg was found dead on d1 of dosing.

4wk oral tox study of S-zopiclone in rats/Sepracor# 190-804 (Cont.)

Dose (mg/kg)	Mortality	Cause of Death
300/200 RS	7f	killed d2
	1f	found dead d3
	1f	killed d2
200 S	2f	killed on d2
300 S	1m	found dead d2
100 S	1m	killed on d29
50 S	1f	killed d29
Total deaths:	all rats in 300mg/kg S- killed on d6 in main study and those in TK study, killed d7	
	8/28 rats dosed 200mg/kg RS- killed d2	
	2/28 rats dosed 200mg/kg S- killed d2	
	1/28 rats dosed 100mg/kg S- killed d29	
	1/28 rats dosed 50mg/kg S- killed d29	

There were 9f rats in the 300mg/kg RS- group that were found dead or killed.
B. wt & Food Consumption: no drug related effects.
Hematology: there were changes in some parameters that reached statistical significance and some were dose related. These findings were as follows:

Dose (mg/kg)	Parameter	finding
Week 2		
100&200 (S)	neutrophils	↑m dose-dependently
all 3doses (S)	eosinophils	↓m
all 3 doses (S)	MCHC	↓f
all 3 doses (S)	lymphos ↓f	
100&200 (S)	RBC&Hb	↓f
all 3 doses (S)	basophils	↓m
100&200 (S)	retics	↑f
Week 4		
All 3 doses (S) & 200 RS	MCV&MCH	↑m&f dose dependently
100&200 (S)	HcT	↓f but ↑m
200 RS	APTT&fib	↑f
all 3 doses (S)		
100&200 (S)	RBC & Hb	↓f dose dependently
200 (S)	MCHC	↓m&f
all 3 doses (S)	WBC	↑m
100&200 (S)	neutros	↑m&f (not sig in f)
all 3 doses (S)	monos	↓f
all 3 doses (S) & 200 RS	baso	↓m&f
all 3 doses (S) & 200 RS	retics	↑m&f
100&200 (S)	platelet	↓m&f (not sig in 100mg/kg males)
200 RS	fibrinogen	↑m&f (not sig in m)

These changes ranged between 2-9.6% for the above parameters with 15-20% decrease in platelets in females, 8-23% increase in WBCs in males, and 40-60% increase in neutros in males and females; all relative to the corresponding control values.
4wk oral tox study of S-zopiclone in rats/Sepracor# 190-804 (Cont.)

Clinical Chemistry: the following changes reached statistical significance:

Dose (mg/kg)	Parameter	Finding
Week 2		
100&200 (S)	***ALP&ALT***	↑m&f dose dependently
all 3 doses (S)	***AST***	↑m&f (not sig in 50mg/kg males) dose dependently
all 3 doses (S) & 200 (-/+)	HBDH&LDH	↑f
100&200 (S)	creatinine	↑m&f (in males only in 200mg/kg)
100&200 (S)	Na	↑m&f
100&200 (S)	Ca	↓m&f
Week 4		
All 3 doses (S) & 200 RS	urea	↑m&f (not sig in all f groups nor in 50mg/kg m), dose dependent.
all 3 doses (S) & 200 (-/+)	creatinine	↑m dose dependently
100&200 (S) & 200 RS	***ALP***	↑m dose dependently (sig only in RS and 200mg/kg S-)
all 3 doses (S) & 200 RS	***ALT***	↑f dose dependent
all 3 doses (S) & 200 RS	T. protein & Glob	↑m&f (A/G ratio also increased in all grs)

Inconsistent findings for cholesterol, Ca, Na, and Cl between males and females.

The above changes ranged between 3-25% relative to the corresponding control values and for the liver enzymes, changes were 1-1.4 times the values in the control.

Organ wts: mean absolute and/or relative wts of the following organs were affected by the drug reaching statistical significance in all or some drug groups :
Liver & Kidneys: Absol and relative wt increased dose dependently in males and females of all drug groups with statistical significance in almost all dose groups relative to corresponding control. These changes ranged between 10-55% for the liver and 4.7-11% for the kidneys; compared with the corresponding controls.
Ovaries: absolute and relative wt increased dose dependently in females of all drug groups (10-18%).
Thyroid: only absolute wt in all females dosed 200mg/kg RS and S was increased (1.2-1.3x the control).
Thymus: absolute and relative wt decreased in all male and female drug groups (15-24%).
Adrenals: absolute and relative wt in all female drug groups decreased dose dependently but not significantly but, the standard deviation in the control group was very large (0.44±1.3%).
Heart: inconsistant findings for males and females but values reached significant levels relative to control.

Testes: absolute and relative wt decreased only in rats dosed the racemate (7%).

4wk oral tox study of S-zopiclone in rats/Sepracor# 190-804 (Cont.)

Gross Necropsy: in animals that were found dead or killed in moribund: females dosed RS-zop showed dilation of collecting tubules and ducts as follows: minimal 1/8, slight 3/8, moderate 2/8 (total 6/8), those dosed 300mg/kg (S)zop showed foci of basophilic/regenerating tubules minimal in 3/10f and papillary/medullary calculi in 3/10f and minimal mineralization in 1/10f.
Histopath:
Dead or killed rats; control incidence is 0 unless specified otherwise:
Spleen extramedullary erythropoiesis 6/8f in 200mg/kg RS-zop and 10/10f 300mg/kg S-zop.
Stomach- non glandular: 5/10f dosed 300mg/kg S-zop showed diffuse epithelial hyperplasia (minimal).

Terminal sacrifice animals; control incidence is 0 unless specified otherwise:
Epididimydes: interstitial edema, epithelial vacuolation in all drug groups, 9-10/10 RS-zop and 5-10/10 in each group dosed S-zop.

Kidneys: basophilic regenerating tubules increased incidence in all male and female drug groups relative to control as follows: males: 2/10, 9/10, 7/10, 5/10, 3/10 and females: 1/10, 8/10, 1/10, 4/10, and 5/10, in control, RS-zop and 50, 100, and 200mg/kg S-zop groups respectively.

Liver: hepatocyte centrilobular hypertrophy in Rs-zop and 200mg/kg S-zop male and female groups as follows: males: 10/10 & 10/10, females: 7/9 & 5/10, respectively.

Pituitary: diffuse hypertrophy and vacuolation 2/10, 10/10, 5/10, 10/10, and 10/10 males dosed control, RS- and S-zop groups respectively.

Stomach- non glandular: diffuse epithelial hyperplasia seen in 5/10 and 2/10 males dosed RS- and 200mg/kg S-zop respectively, and in 1/9 each in RS- and 200mg/kg S-zop.

Stomach – glandular: slight to minimal mucosal atrophy in 7/9f dosed RS- & 2/10f in 200mg/kg S-zop.

Mammary gland: diffuse lobular hyperplasia (minimal) 4/9f dosed RS-zop and 1/10 each in 100&200mg/kg S-zop dosed females.

Thyroid Gland: follicular epithelial hypertrophy (minimal to moderate) in females 8/9 dosed racemate, 4/10f dosed 50mg/kg S-zop, and 10/10 each in females dosed 100&200mg/kg S-zop. However, in males, this finding was seen in all 10 animals of each group including the control but the severity was more in drug groups (moderate vs. minimal in control).

TK:
Table below presents means±s.d. values:

Dose (mg/kg/d)		**Cmax (ng/ml)**			
		Day1		**Day28**	
		m	f	m	f
300/200 RS-	S-	6283±4041	11533±777	1009±1808	16633±2902
	S-desmethyl-	2867±1223	1374±689	10690±6992	4367±1085
	N-oxide	1955±944	1241±370	3867±1424	3273±452
	R-	4533±2816	8640±4818	6860±2302	11367±2669
	R-desmethyl	3257±1223	1580±387	10673±6996	4247±1392
50 S-	S-	2370±935	5083±4308	6373±1004	12853±4456
	S-desmethyl-	1583±968	947±562	3137±367	2953±227
	N-oxide	410±128	268±175	1053±157	939±249
100 S-	S-	5643±2695	7243±3946	8990±3595	17033±3371
	S-desmethyl-	2683±2168	892±346	9380±4608	3240±1858
	N-oxide	861±298	472±166	1295±383	1673±531
200 S-	S-	11400±608	22967±6292	14267±3347	25333±3512
	S-desmethyl-	2850±987	1840±1108	15333±3287	7725*
	N-oxide	1672±656	1099±309	4230±173	3697±798
300 S-	S-	14633±7539	22333±7158	-	-
	S-desmethyl-	5367±2870	3057±240	-	-
	N-oxide	1816±895	1053±177	-	-

- only 2 out of 4 rats were sampled therefore, no s.d. was calculated.

4wk oral tox study of S-zopiclone in rats/Sepracor# 190-804 (Cont.)

Dose (mg/kg/d)		AUC0-24hr (ng.hr/ml)			
		Day1		Day28	
		m	f	m	f
300/200 RS-	S-	65724	159493	75919	175346
	S-desmethyl-	41474	28878	140734	69534
	N-oxide	11765	11235	23237	21791
	R-	34245	62718	32822	42502
	R-desmethyl	38642	29644	146108	66762
50 S-	S-	18005	40344	36046	76831
	S-desmethyl-	13543	12611	41108	29791
	N-oxide	1614	2330	5132	8011
100 S-	S-	32546	49608	59952	119499
	S-desmethyl-	27165	13344	79404	51721
	N-oxide	2954	1780	11009	13021
200 S-	S-	70309	138607	102724	161642
	S-desmethyl	42142	26020	112144	69888
	N-oxide	7743	6646	25095	20261
300 S-	S-	101992	244180	-	-
	S-desmethyl	92578	57395	-	-
	N-oxide	10065	11593	-	-

Based on the above data and in general, Cmax and AUC increased non-linearly with dose with values going either direction i.e. > and < than proportional to dose. Also, values were higher on day 28 as opposed to the corresponding values on day 1 indicative of accumulation. Drug accumulation with repeated dosing may be due to lower clearance, non-linear kinetics, metabolites, and/or enzyme saturation. The latter may not be the case here since the parent as well as the metabolites were accumulating. The drug seemed to be readily absorbed, Tmax ranged between 2-6hr for the S-zop following administration of RS-zop and 0.5-1hr after dosing the S-zop. Mean Tmax values for the S-desmethyl were 1-4hr, for the N-oxide 0.5-1hr following administration of the S-zop and 2-6 and 0.5hr respectively, following dosing with the RS-zop.

The R-zopiclone mean values for Cmax and AUC0-24hr after dosing with the racemate:

Day 1 males: 4533±2816ng/ml females: 8640±4818ng/ml
34245ng.hr/ml 62718ng.hr/ml

Day 28 males:	6860±2302ng/ml	females:	11367±2669ng/ml
	32822ng.hr/ml		42502ng.hr/ml

The R-desmethyl values were:

Day 1 males:	3257±1223ng/ml	females:	1580±387
	38642ng.hr/ml		29644ng.hr/ml
Day 28 males:	10673±6996ng/ml	females:	4247±1392ng/ml
	146108ng.hr/ml		66762ng.hr/ml

4wk oral tox study of S-zopiclone in rats/Sepracor# 190-804 (Cont.)

Mean Tmax for the R-zop was 0.5-1hr and for its desmethyl metabolite was 2-6hr. PK values for the R- and its metabolites were below quantitation limit following administration of the S-zop.

Summary and Conclusion:

Oral gavage administration of 200mg/kg/d RS- and 50, 100, and 200mg/kg/d S- zop for 1month to rats caused death at all doses (1f at 50mg/kg/d), some of the animals died due to gavage accidents others, the cause of death was drug related. Clinical signs were seen in all drug groups and included: hunched posture, lethargic, unsteady gait, distended abdomen, piloerection, partially closed eyes, and shallow/irregular breathing. Signs were seen in all dose groups. There were findings in hematology and clinical chemistry that reached statistical significance but were small, random (not in both sexes), and non-dose dependent. Effects that were dose dependent included decrease in RBC & Hb in females on both wks2&4, increase in neutrophils in males wk2 and in both sexes on wk4 rats dosed 100&200mg/kg S-zop and, increase in MCV & MCH in all the S- groups and the 200mg/kg RS-zop on wk4. Dose dependent and significant clinical chemistry findings included increase in ALT, AST, & ALP in males and females dosed 100&200 or all 3 doses on wk2 and on wk4, levels of ALP were increased dose dependently in males dosed the RS and ≥100mg/kg S-; ALT was also increased dose dependently but only in S-zop treated females and in 200mg/kg RS-zop. Total proteins, globulin, and A/G ratio were increased in s-zop treated m+f and the RS-zop 200mg/kg group. Urea and creatinine increased in both sexes dosed all 3 doses of the S- and the 200mg/kg RS dose depndently. These changes were relatively small upto 23% of the control and the enzyme changes were <2x those of the control. Mean wts of the liver and kidneys in both sexes of all drug groups were increased relative to the control and accompanied by histopathology. The incidence of basophilic regenerating tubules increased in all male and female drug groups relative to control and the incidence of hepatocyte centrilobular hypertrophy was increased in the RS- and 200mg/kg S-zop m and f groups as follows: males: 10/10 & 10/10, females: 7/9 & 5/10, respectively. **Incidence of thyroid follicular epithelial hypertrophy was 8/9 females dosed the RS, 4/10f dosed 50mg/kg S-, and 10/10 each in females dosed 100&200mg/kg S-zop.** However, in males, this finding was seen in all 10 animals of each group including the control but the severity was more in drug groups (moderate vs. minimal in control). Also pituitary hypertrophy and stomach hyperplasia were seen. Mean Cmax and AUC did not increase linearly with dose, values seemed to accumulate with repeated dosing. The MTD was exceeded in females at 50mg/kg/d S-zop and 200mg/kg/d RS-zop. The 50mg/kg/d males may be considered the NOAEL though histopathology of epididymides was seen at this dose. A **NOAEL could not be determined in this study due to clinical signs, death, and histopathology in the lowest dose of 50mg/kg/d.**

APPEARS THIS WAY
ON ORIGINAL

Study Title: 4wk oral tox study of S-zopiclone in dogs

Study No: Sepracor# 190-810 – OTI00505

Conducting laboratory and location: –

Date of study initiation/termination: Mar 1999/Apr 1999

GLP compliance: Yes/FDA & EC

QA- Report Yes (x)

Lot#/Batch#: for (S) zopiclone: AT737 SA1001E,F/Raw material lot# 120998A; for (-/+) zopiclone suspension: AT737 SA1002E,F/Raw material lot # 9809002

Purity/Stability: responsibility of sponsor.

Methods:

Dosing:

- species/strain: beagle dogs
- #/sex/group: main study: 3/sex/gr.
- age/B.wt: 6-8months/10-15kg
- satellite groups used for toxicokinetics or recovery: NA
- dosage groups in administered units: **S- 2, 5, 10, 20mg/kg/d; RS- 20mg/kg/d *.** the control group received the vehicle.
- route, form, volume, and infusion rate: oral/gavage/vol 0.4-4ml/kg

Formulation/vehicle: **2.5% hydroxy propyl methyl cellulose**

Observations and times:

- Clinical signs & mortality: 2x daily and as needed.
- Body weights: 2x weekly and on day of necropsy.
- Food intake: daily during last wk of acclimatisation and dosing periods.
- Ophthalmoscopy: pre-dose and once during wk4.
- Clinical Chemistry/hematology: non-fasted blood samples collected from jugular vein before study, 1wk into study, and end of study during wk4.
- Urinalysis: 18hr urine samples collected before study and wk4 .
- EKG: prior to study, before dosing, and 1hr after dose during wk4. Leads I, II, and III, as well as AVR, AVL, AVF, and HR.
- TK: blood from non-fasted dogs was collected from all dogs on days1&28 at 0.5, 1, 2, 4, 6, and 24hr postdose. Plasma levels were determined for: S- & R- zopiclone, S- & R- zopiclone-N-oxide, and S- & R- desmethylzopiclone. *Note that TK was done by not*
- Gross necropsy: end of study.
- Organ wts: adrenals, kidneys, ovaries, salivary gland, thymus, brain, liver, pituitary, spleen, uterus, heart, lungs, prostate, testes/epididymides.

- Histopath: 5micron sections and stained with H&E and examined microscopically.

* an oral MTD study in dogs (study#OTI00515/Sepracor#190-803), was done to determine the doses for this study. One male and one female beagle dogs were dosed orally for 2wks with S- or RS-zop at 40mg/kg (5ml/kg). The vehicle was as in all other studies, 2.5% hydroxypropyl methyl cellulose at 25mg/ml. Marked clinical signs were seen in the 2 dogs, dosing was stopped after a single 40mg/kg dose and the dogs were drug free for 3days. Dose was reduced to 30mg/kg that also produced the same marked signs, and dosing was stopped; dogs were killed on d9. Dogs lost wt over the 9d dosing period, mean RBC, Hb, and HcT was reduced in the males. The LDH and HBDH were markedly reduced in both the RS- and S-zop. Lesions on the lips and red foci on bladder were considered drug related findings; one male had an enlarged liver. It was concluded that doses ≥30mg/kg are not well tolerated by the dog.

4wk oral tox study of S-zopiclone in dogs/Sepracor# 190-810 (Cont.)

A 2nd oral MTD study was done at lower doses (OTI00516) to determine doses for 1month dog study. In one part of the study, 1 beagle dog/sex was gavaged 5mg/kg/d for 1-7days, followed by 10mg/kg/d for 8-10d, 15mg/kg/d for 11-14d, and 20mg/kg/d for 15-20d. in the 2nd part of the study, 1dog/sex was dosed 20mg/kg/d for 8d. There were no deaths in any group, clinical signs were observed at all doses and were were similar to other studies: agitation, salivation, incoordination, unsteady gait, red pinnae and mouth, ulceration of nose and muzzle, and vocalization. Mean body wt was reduced in all groups without an effect on food intake. No other drug related findings except for decrease in RBC, Hb, and HcT in dogs in part 1 of study and at necropsy, reddening of duodenal mucosa. It was concluded that 20mg/kg/d is well tolerated in dogs.

Based on the above 2 studies, doses for the 1month study were selected to be up to 20mg/kg/d.

Results:
Mortality: none in any group.
Clinical signs: in all drug groups: unsteady gait, partially closed eyes, agitation, lethargy and vocalization. Abnormal color of pinnae, mouth, perinasal area and paws were seen in all drug groups. In addition, **lesions in these areas were found at doses ≥5mg/kg/d of S-zop and 20mg/kg RS-zop. The sponsor stated that these lesions took 1 week to heal while dosing continued. Its unclear whether these lesions are drug related.**
B.wt: animals in drug groups lost wt over the 1-28day period whereas, the control dogs gained 0.7% males and 0.8% females. The gains/losses were as follows over the 1-29 day period: males: -0.06, -0.52, -0.44, -0.58, and -0.34kg in 20mg/kg/d RS- zop, 2, 5, 10, and 20mg/kg/d S-zopiclone respectively, the corresponding values in females are: -0.72, -0.05, 0.05, -0.20, and -0.20kg respectively. These values represent loss of 2.7-4.4% in males and 0.4-1.7% in females dosed the S-form and 0.5&5.7% loss in m and f dosed the racemate; control males and females gained 0.7&0.8% respectively, and the females dosed 5mg/kg S-zopiclone gained 0.4%.
Food Consumption: no drug related effects.
Ophthalmoscopy: no drug related effect.
EKG: no drug related effect except for a relatively small decrease in mean HR relative to corresponding controls in males and females dosed 20mg/kg S-zop measured 1hr postdose on wk4 (104 vs. 121bpm in m and 115 vs. 126bpm in f).
Hematology: generally no effects however, a non significant and not dose dependent increase in mean platelet (1.2-2x over control), was seen in m&f of all doses during both measurement periods (wk2&4).

Also in females of all dose groups, mean lymphocyte number was decreased relative to the control (22-41% less than control), during both wks2&4.
Clinical Chemistry: the following were changes from control values but none reached statistical significance and generally were not dose dependent: females wks2&4: increases in ALP & HBDH (alpha-OHbutyrate dehydrogenase) and on wk2 levels of ALT were also increased in all drug groups. In males/wk2, elevation in ALP & AST were seen in 20mg/kg S-zop, and on wk4, ALP levels were increased in both 10&20mg/kg S-zop groups relative to the control.
Organ wts: dose dependent decrease in mean absolute and relative thymus wt in all female drug groups relative to the control and a decreasing trend observed in males for both absolute and relative wts except relative wts were increased in 5&10mg/kg/d S-zop groups relative to the control. Mean absolute and relative wt of the prostate was decreased not dose dependently in all drug groups relative to the control. A decreasing trend in mean absolute and relative wt of the spleen was also seen in all male drug groups relative to the control. No other findings and no effect on liver wts.
Histopath: no findings related to the drug except in epididymides (see summary).
4wk oral tox study of S-zopiclone in dogs/Sepracor# 190-810 (Cont.)

TK: dogs were exposed to all cpds and there was no sex difference in TK parameters. Exposure to S-zop after administration of S-zop was less than that measured after administration of the RS-zop i.e. no difference in exposure after administration of either cpd (table from sponsor):

Mean $AUC_{0\text{-}24h}$ (S)-zopiclone after (S)-zopiclone administration/ Mean $AUC_{0\text{-}24h}$ (S)-zopiclone after (RS)-zopiclone administration (dose-adjusted)

Day 1		Day 28	
Male	Female	Male	Female
72.8 %	73.8 %	86.4 %	106 %

Exposure to S-zop increased almost linearly with increase in dose in both sexes, there seem to be some accumulation with repeated dosing at 1.6-3.3x more on d28 than day1. For the S-desmethyl metabolite, apparent terminal $t_{1/2}$ after administration of S-zop at all doses and RS-zop at 20mg/kg/d ranged from 4-20hr (the sponsor stated that these estimates are not reliable). The kinetics of S-desmethyl were non-linear with respect to both time and dose; there seem to be no accumulation of S-desmethyl after repeated administration of S-zop and RS-zop. R-zop and R-desmethyl were below detection limit following administration of S-zop but the dogs were exposed to measurable levels after administration of RS-zop. The N-oxide was increased with increase in S-zop dose at more than proportional increase in exposure after repeated dosing (up to 4x more on d28 than values on d1). Tables from sponsor:

Table 35
(S)-Zopiclone Toxicokinetic Parameters Following Oral Administration of (RS)-Zopiclone at 20 mg/kg/day to Male Dogs

Day 1

Parameter	201	203	205	Mean	SD
C_{max} (ng/ml)				3957	1031
t_{max} (h)				2#	-
AUC_{last} (ng.h/ml)				41149	15337
AUC_{0-24h} (ng.h/ml)				41149	15337
$AUC_{0-\infty}$ (ng.h/ml)				43261	14847
λ_z (/h)				0.132	0.0502
$t_{1/2}$ (h)				5.72	1.80
R_T				1.07	0.0485

Day 28

Parameter	201	203	205	Mean	SD
C_{max} (ng/ml)				11620	2322
t_{max} (h)				0.5#	-
AUC_{last} (ng.h/ml)				44761	21156
AUC_{0-24h} (ng.h/ml)				61122	10059
λ_z (/h)				0.214	0.0281
$t_{1/2}$ (h)				3.27	0.401
R_o				1.57	0.354

4wk oral tox study of S-zopiclone in dogs/Sepracor# 190-810 (Cont.)

Table 36
(S)-Zopiclone Toxicokinetic Parameters Following Oral Administration of (RS)-Zopiclone at 20 mg/kg/day to Female Dogs

Day 1

Parameter	200	202	206	Mean	SD
C_{max} (ng/ml)				3280	178
t_{max} (h)				2#	-
AUC_{last} (ng.h/ml)				40812	7122
AUC_{0-24h} (ng.h/ml)				40812	7122
$AUC_{0-\infty}$ (ng.h/ml)				39002	-
λ_z (/h)				0.183	-
$t_{1/2}$ (h)				3.83	-
R_T				1.01	-

Day 28

Parameter	200	202	206	Mean	SD
C_{max} (ng/ml)				7383	1584
t_{max} (h)				1#	-
AUC_{last} (ng.h/ml)				61283	15509
AUC_{0-24h} (ng.h/ml)				61283	15509
λ_z (/h)				0.232	0.0360
$t_{1/2}$ (h)				3.03	0.466
R_o				1.53	0.408

\# = Median
NC = Not calculated
* = Unreliable estimate; only 3 data points used in the regression

Mean±s.d C_{max} and AUC_{0-24} of S-zop on d28 after administration of S-zop were as follows:

Dose (mg/kg/d)	C_{max} (ng/ml)	AUC_{0-24} (ng.hr/ml)
2	1420±671 m	6610±304m
	2647±1757 f	11,162±1656 f
10	3343±1033 m	42,831±13,424 m
	7397±2839 f	34,768±12,231 f
20	14,967±7342 m	105,673±33,180 m
	16,667±5354 f	129,388±26,148 f

Mean values of S-zop after administration of RS-zop were:

20 RS-zop	11,620±2322 m	61,122±10,059 m
	7383±1584 f	61,283±15,509 f

Summary and Conclusion:

Daily oral administration of S-zop and RS-zop for 4wks to dogs did not cause death upto 20mg/kg/d. Clinical signs were observed in all drug groups and included: unsteady gait, partially closed eyes, agitation, lethargy and vocalization. Abnormal color of pinnae, mouth, perinasal area and paws were seen in all drug groups. In addition, lesions in these areas were found at doses ≥5mg/kg/d of S-zop and 20mg/kg RS-zop. **The sponsor stated that these lesions took 1 week to heal while dosing continued. These lesions seems to be drug related since they have been seen in all 3 dog tox studies.** Mean body wt was decreased in all drug groups over the 1-28day period relative to a gain in control dogs. This wt loss ranged between -0.05 to -0.72kg, these values represent loss of 2.7-4.4% in males and 0.4-1.7% in females dosed S-zop and 0.5&5.7% loss in m and f dosed RS-zop. There were no drug related effects on EKG, a relatively small decrease in mean HR vs. the corresponding controls was recorded in males and females dosed 20mg/kg S-zop 1hr postdose on wk4 (104 vs. 121bpm in m and 115 vs. 126bpm in f). No consistent drug related effects were observed on hematology or clinical chemistry except for a 22-41% decrease in mean lymphocytes drug treated females during both wks2&4 and increases in ALP, AST, and HBDH in females and/or males. Dose dependent decrease in mean absolute and relative thymus wts were seen in all female drug groups relative to the control; a decreasing trend was seen in males for both absolute and relative wts except mean relative wts were increased in 5&10mg/kg/d S-zop groups relative to the control. Mean absolute and relative wt of the prostate was decreased not dose dependently in all drug groups relative to the control. A decreasing trend in mean absolute and relative wt of the spleen was also seen in all male drug groups relative to the control; no other findings None of the wt changes correlated with histopathology. Table below from the sponsor shows histopathology in the epididimydes. These effects were not dose dependent and because of the small number of dogs in the study, a clear and meaningful conclusion can not be made. The **NOEL in this study is 2mg/kg/d** note that although ***clear*** drug related effects were not seen upto 20mg/kg, there were trends of changes in organ wts, mean body wt, and clinical signs that were observed in all drug groups and histopath findings in the epididimydes in the 5mg/kg/d dose group, all relative to the control. Some of the of the findings in the dog are similar to those observed in rats and mice studies and they seem to be drug related.

Histopathology Findings in Male Dogs Following Oral Administration of (S)- and (RS)- Zopiclone

Organ/Finding (n=3)	Control	(S) Zopiclone 2 mg/kg/day	(S) Zopiclone 5 mg/kg/day	(S) Zopiclone 10 mg/kg/day	(S) Zopiclone 20 mg/kg/day	(RS) Zopiclone 20 mg/kg/day
EPIDIDYMIS						
Sperm granuloma (moderate)	0	0	0	2	1	0
Spermatocoele (slight)	0	0	1	0	0	0
Granulomatous inflammation (slight)	0	0	1	1	0	0
Total # of dogs affected	0	0	1	2	1	0

Study title: 3 month oral gavage toxicity study of S-zopiclone in mice with 1 month recovery/Sepracor# 190-819/ – .# 312046.
Conducting lab: ' —
Study Initiation/Termination Dates: Dec 1999/Apr 2000
GLP: Yes (x) Japanese, OECD, and US FDA
QA: Yes (x)
Drug Lot/Batch#/purity: for S-zop 0290002. — , for RS-zop Z9910002 purity —
Species/Strain/# per dose/sex: CD-1 mice; 26/sex/group; TK satellite group 18/sex/dose and the control vehicle group consisted of 8/sex.
B.wt./Age at study initiation: 25-39g for males and 20.8-29.7g for females/8wks old.
Doses: **50, 100, 200mg/kg/d S-zop* and 200mg/kg/d RS-zop**. Both cpds were prepared as oral suspensions in 0.5% hydroxymethylcellulose (HMC). Dosing volume was either 2.5, 5, or 10ml/kg calculated according to the most recent B.wt. TK satellite group was treated similarly to the main groups.
Duration and dosing regimen: once daily oral gavage administration for 3 months with 1 month drug free recovery period.

* doses were selected based on results from previous dose range finding study : — OTI00512) and a 1 month study — OTI00513) where doses ≥300mg/kg/d caused death.

Methods:
At end of 3 months of daily oral gavage dosing, 10/sex/group were killed for primary necropsy, 10/sex/dose were assigned for 28d recovery period, and 6/sex/dose were used for hormone analyses (TSH, T3, and T4) and for analysis of liver MFO activities (mixed function oxidase). The following parameters were determined in this study:
Clinical signs/mortality: 1-2x daily observations at 1-2hr postdose with weekly detailed exam.
B.wt. & food intake: weekly beginning 2wks pre-dose. Food intake was calculated as g/animal/day.
Hematology/blood chemistry: at scheduled necropsies on wks 13&17. Blood was collected from the vena cava of all tox groups at time of primary necropsy (wk13) and at recovery necropsy study wk17.
Serum Hormones: TSH, T3 & T4 determined on wk13. Sample analyses were done at —
—
Ophthalmology: predose and wks12&16. Slit lamp and indirect ophthalmoscope were done.
Necropsy: on wks13&17 on 10/sex/group cranial, thoracic, pelvic, and abdominal cavities were examined.
Organ wt: adrenals, brain, total and caudal epididymides, heart, kidneys, liver, ovaries, spleen, testes, thymus, thyroid (weighed after fixation), and uterus. These organs were collected and weighed from 10/sex/group.
Spermatogenic parameters: sperm count, morphology, and production rates analyzed in 10/dose on wk13.
Histopath: standard list of tissues/organs were isolated, fixed, and shipped to — where they were processed and stained with H&E. The prepared slides were then returned to ' — for microscopic exam. Histopath exam was done on all toxicology mice from vehicle, and HD S-zop and RS-zop also the following organs/tissues were examined for the 50&100mg/kg/d groups: liver, heart, kidneys, ovaries, testes, thyroids, and epididymides. In addition, livers from recovery groups dosed the vehicle, 100&200mg/kg/d S-zop, and 200mg/kg/d RS-zop, were also examined. Gross lesions from all mice were examined as appropriate. One of the protocol deviations was absence of mammary gland tissue from many slides sent by — therefore, wet tissues from 23 females of the primary necropsy groups were re-examined to ensure presence of mammary tissues and slides were prepared at — labs.

Liver Enzyme Analyses: liver samples from toxicology groups dosed 200mg/kg/d S-zop and 200mg/kg/d RS-zop were collected and stored frozen and sent to — for P450 and UDP-glucuronyl transferase induction analysis. Samples were also collected from the low and mid dose groups but kept at — for possible analysis depending on the results from — at the HD.

TK: blood samples were collected from the vena cava of the TK satellite groups at 0.5, 1, 2, 4, 8, and 24hr postdose on last day of dosing (3 mice/time point and each mouse sampled only once in a 24hr period). TK analyses were done by — Plasma levels were determined for total zop, total desmethyl zop*, and R-zop, S-zop, and S-desmethyl zop after treatment with RS-zop**. Brain samples were also collected from the TK groups for drug level measurements.

* the analytical method used was — because data from previous studies showed absence of interconversion between the isomers. ** — was used for these cpds.

Results:

Mortality: total of 3 deaths occurred: 1 gavage error in RS-zop died on wk6 of dosing, 1LD killed in moribund on d6 of dosing and 1HD S-zop died during 1st wk of recovery (study d92). The sponsor considered these 2 deaths not to be drug related because of lack of "dose- or time- related effects" and the "generally good health of remaining mice in these groups".

Clinical Signs: qualitatively similar for both drugs but frequency of occurrences was more in the S-zop than the RS-zop (table from sponsor for some of the findings). In general, clinical signs occurred 1hr postdose and were absent on next day prior to dosing. Also, generally, signs were observed mainly during the 1st 2months of dosing except for the reddening of the ears that was seen during the last month of dosing. Clinical signs included hypoactivity, partial closure of the eyes, excessive sleeping, red ears, and excessive rubbing of face on cage surface; there were no clinical signs during recovery period. The effects seemed to be dose related in frequency though no difference noted in number of animals affected.

SUMMARY OF CLINICAL FINDINGS: TOTAL OCCURRENCE/NO. OF ANIMALS

(1-HOUR POST-DOSING) TABLE RANGE:	12-22-99 TO 03-22-00				
GROUP:	1	2	3	4	5
		FEMALE			
BEHAVIOR/CNS					
-HYPOACTIVITY	1/ 1	56/23	108/23	132/25	115/23
-PARTIAL CLOSURE RIGHT EYE	0/ 0	52/22	130/22	156/25	123/24
-PARTIAL CLOSURE LEFT EYE	1/ 1	51/22	129/22	153/25	121/23
-PROSTRATE	0/ 0	2/ 2	0/ 0	0/ 0	0/ 0
-FULL CLOSURE RIGHT EYE	0/ 0	0/ 0	3/ 3	6/ 6	2/ 2
-FULL CLOSURE LEFT EYE	0/ 0	0/ 0	3/ 3	6/ 6	2/ 2
-IMPAIRED MUSCLE COORDINATION	0/ 0	1/ 1	4/ 4	7/ 6	0/ 0
-ANIMAL SLEEPING	0/ 0	271/25	474/26	675/26	518/25
-RUBBING FACIAL AREA ON CAGE SURFACES	0/ 0	8/ 6	146/24	291/26	149/24
-HYPERACTIVITY	0/ 0	0/ 0	1/ 1	6/ 4	0/ 0
-UNKEMPT APPEARANCE	1/ 1	0/ 0	0/ 0	0/ 0	0/ 0
-TREMORS	1/ 1	0/ 0	0/ 0	0/ 0	0/ 0
CARDIO-PULMONARY					
-RESPIRATION SHALLOW	0/ 0	1/ 1	0/ 0	0/ 0	0/ 0
-HEART RATE DECREASED	0/ 0	1/ 1	0/ 0	0/ 0	0/ 0
-RALES	0/ 0	0/ 0	2/ 1	0/ 0	0/ 0
EXCRETA					
-DRIED YELLOW MATERIAL VENTRAL TRUNK	0/ 0	0/ 0	0/ 0	1/ 1	0/ 0
-WET YELLOW MATERIAL UROGENITAL AREA	0/ 0	1/ 1	0/ 0	0/ 0	0/ 0
SPECIAL I					
-REDDENED RIGHT FORELIMB	0/ 0	2/ 2	2/ 2	1/ 1	0/ 0
-REDDENED RIGHT EAR	0/ 0	4/ 4	35/10	40/11	28/ 7
-REDDENED LEFT EAR	0/ 0	4/ 4	35/10	39/10	28/ 7
-REDDENED FACIAL AREA	0/ 0	0/ 0	1/ 1	3/ 2	0/ 0
-REDDENED LEFT FORELIMB	0/ 0	2/ 2	0/ 0	1/ 1	0/ 0

1- 0 MG/KG/DAY 2-50 MG/KG/DAY(S) 3-100 MG/KG/DAY(S) 4-200 MG/KG/DAY(S) 5-200 MG/KG/DAY(R)

SUMMARY OF CLINICAL FINDINGS: TOTAL OCCURRENCE/NO. OF ANIMALS

(1-HOUR POST-DOSING) TABLE RANGE:	12-22-99 TO 03-22-00				
GROUP:	1	2	3	4	5
		MALE			
BEHAVIOR/CNS					
-HYPOACTIVITY	0/ 0	123/25	159/26	170/26	148/26
-PARTIAL CLOSURE RIGHT EYE	0/ 0	133/25	159/25	186/26	161/24
-PARTIAL CLOSURE LEFT EYE	0/ 0	130/25	159/25	184/26	156/24
-FULL CLOSURE RIGHT EYE	0/ 0	0/ 0	13/13	14/14	4/ 4
-FULL CLOSURE LEFT EYE	0/ 0	0/ 0	13/13	14/14	4/ 4
-IMPAIRED MUSCLE COORDINATION	0/ 0	4/ 4	2/ 2	1/ 1	1/ 1
-ANIMAL SLEEPING	1/ 1	283/26	749/26	987/26	630/26
-RUBBING FACIAL AREA ON CAGE SURFACES	0/ 0	16/ 6	176/24	417/25	186/25
-HYPERACTIVITY	0/ 0	1/ 1	0/ 0	0/ 0	0/ 0
-UNKEMPT APPEARANCE	0/ 0	3/ 1	0/ 0	0/ 1	0/ 0
BODY/INTEGUMENT					
-DERMAL ATONIA	0/ 0	3/ 1	0/ 0	0/ 0	0/ 0
-DEHYDRATED	0/ 0	1/ 1	0/ 0	0/ 0	0/ 0
-EMACIATED	0/ 0	4/ 1	0/ 0	0/ 0	0/ 0
CARDIO-PULMONARY					
-BODY COOL TO TOUCH	0/ 0	3/ 1	0/ 0	0/ 0	0/ 0
EXCRETA					
-DRIED YELLOW MATERIAL UROGENITAL AREA	1/ 1	1/ 1	2/ 1	26/ 4	15/ 5
SPECIAL I					
-SWOLLEN FACIAL AREA	0/ 0	0/ 0	1/ 1	0/ 0	0/ 0
-REDDENED RIGHT FORELIMB	0/ 0	0/ 0	1/ 1	1/ 1	0/ 0
-SWOLLEN ABDOMINAL AREA	0/ 0	6/ 1	0/ 0	0/ 0	0/ 0
-REDDENED RIGHT EAR	0/ 0	5/ 2	14/ 6	30/ 9	16/ 9
-REDDENED LEFT EAR	0/ 0	4/ 1	14/ 6	27/ 8	17/ 9
-REDDENED LEFT FORELIMB	0/ 0	0/ 0	1/ 1	1/ 1	0/ 0

1- 0 MG/KG/DAY 2-50 MG/KG/DAY(S) 3-100 MG/KG/DAY(S) 4-200 MG/KG/DAY(S) 5-200 MG/KG/DAY(R)

B.wt., Food Intake, hematology, serum chemistry, and serum hormones*: no drug related findings on any of these parameters by either the S- or RS-zop (table from sponsor).

WEEK 13		----- MALE -----			
ANALYSIS GROUP:	0 MG/KG/DAY	50 MG/KG/DAY(S)	100 MG/KG/DAY(S)	200 MG/KG/DAY(S)	200 MG/KG/DAY(R)
TSH (uG/mL)					
MEAN	0.08	0.10	0.15	0.12	0.12
S.D.	0.023	0.061	0.114	0.027	0.069
N	6	6	6	6	6
TOTAL T3 (ng/dL)					
MEAN	41.45	40.90	59.95	62.38	65.27
S.D.	12.220	12.398	19.931	16.080	16.784
N	6	4	6	5	6
TOTAL T4 (uG/dL)					
MEAN	7.18	8.70	8.18	6.62	7.16
S.D.	1.571	1.734	0.890	0.818	2.157
N	5	4	5	6	5

		-----FEMALE -----			
ANALYSIS GROUP:	0 MG/KG/DAY	50 MG/KG/DAY(S)	100 MG/KG/DAY(S)	200 MG/KG/DAY(S)	200 MG/KG/DAY(R)
TSH (uG/mL)					
MEAN	0.03	0.05	0.07	0.04	0.04
S.D.	0.012	0.011	0.056	0.018	0.012
N	6	5	6	6	5
TOTAL T3 (ng/dL)					
MEAN	54.86	60.88	50.68	57.32	56.43
S.D.	9.485	10.467	8.682	7.469	9.560
N	5	4	5	5	4
TOTAL T4 (uG/dL)					
MEAN	7.10	7.42	6.02	6.50	5.80
S.D.	0.453	0.669	1.374	1.056	0.781
N	5	5	5	5	3

uG/dL = MICROGRAMS/DECILITER, uG/mL = MICROGRAMS/MILLILITER, ng/dL = NANOGRAMS/DECILITER

3month oral gavage toxicity study of S-zopiclone in mice/Sepracor#190-819 (Cont.)

Ophthalmology: ocular effects were observed on wk12 in all drug groups of both sexes as follows:

Finding			cont.	50	100	200	200 RS-zop
Bilateral retinal degeneration		males	0/26	1/25	**5/21**	**1/25**	**4/22**
		females	0/25	0/25	**4/22**	**2/24**	**4/21**
Opacity		males	0/10	0/10	0/10	0/10	**2/10**
		Females	0/10	0/10	0/10	**1/10**	0/10
Retinal atrophy		males	6/10	NA	NA	6/10	3/10
	Moderate		6/6	NA	NA	6/6	0/3
	Severe		none	NA	NA	none	**3/3**
		females	3/10	NA	NA	5/10	3/10
	Moderate		3/3	NA	NA	1/5	none
	Severe		none	NA	NA	**4/5**	**3/3**

The retinal atrophy was characterized as being bilateral, diffuse loss of outer regions of the retina beyond the inner nuclear layer. Lack of correlation was suggested by the sponsor because retinal atrophy was not observed in the mice with retinal degeneration and that these mice had normal retinal histology. The sponsor therefore, concluded that the retinal degeneration is of no toxicological significance because it was not accompanied by trauma or inflammation, no histopath or "clear" dose response, not observed at recovery period, and no such retinal effects observed in the 3month rat or dog studies — 12047 & — 12045 respectively). Because of these arguments, the sponsor suggested that the retinal degeneration be more appropriately termed "vascular pallor or retinal hypoperfusion" the sponsor goes on to state that although retinal degeneration may have represented a "non-specific 2nd effect", the finding is of no toxicological significance. **The reviewer can not exclude a drug effect** although some points made by the sponsor are valid, such as absence of any ocular effects by end of recovery.

Organ wts & Gross Exam:. There were no drug related changes on any organ wt. The following small changes were recorded: relative liver wt to B.wt in 200mg/kg/d S-zop and RS-zop in males and females was 5-10% increased over the corresponding control values (end of dosing wk13). Mean liver wt relative to brain wt was increased 4&9% (not significantly) in males dosed 200mg/kg/d S-zop and RS-zop respectively, whereas, a 15% increase ($p<0.05$) noted only in females dosed RS-zop 200mg/kg/d with a not significant decrease in all 3 S-zop female groups. During wk17 recovery, mean relative liver wt to B.wt was increased significantly (13%) in females dosed 200mg/kg/d S-zop and RS-zop relative to the control. Similarly at recovery period, mean liver wt relative to brain wt in females was increased though not significantly, 10.5&16% in S-zop 100&200mg/kg/d and 3% in RS-zop, vs. corresponding controls.

Histopathology & Liver Enzyme Analysis: There were *minimal to mild liver centrilobular hypertrophy in HD RS-zop and S-zop that the sponsor considered to be physiological adaptation to the liver enzyme induction that was described as weak phenobarbital-type of induction.* S-zop and RS-zop at 200mg/kg/d caused in both sexes a significant increase in P450 content and several other enzyme activities (tables below from sponsor) compared to the control values. Both Z-zop and RS-zop 200mg/kg/d caused a 4.7&5.2 fold increase in CYP2B10 in male mice relative to the control. Also, the RS-zop but not the S-zop, significantly increased UDPGT activity as reflected by the increase in thyroxine glucuronidation.

Table 1: Effect of (*S*)-Zopiclone on mouse liver microsomal protein yield, cytochrome b_5 levels, cytochrome P450 levels and NADPH-cytochrome *c* reductase activity

Treatment	Protein Yield (mg protein/g liver)	Cytochrome b_5 (nmol/mg protein)	Cytochrome P450 (nmol/mg protein)	NADPH-cytochrome *c* reductase (nmol/mg protein/min) [d]
Samples from Sponsor [a]				
Male				
Vehicle Control: 0 mg/kg/day	13.5 ± 1.5	0.566 ± 0.067	0.884 ± 0.112	68.7 ± 13.1
(*S*)-Zopiclone: 300 mg/kg/day	15.8 ± 3.9	0.675 ± 0.041	1.27 ± 0.17 *	93.8 ± 11.4 *
	(1.2)	(1.2)	(1.4)	(1.4)
racemic-Zopiclone: 280 mg/kg/day	18.2 ± 3.6	0.788 ± 0.096 *	1.46 ± 0.20 *	101 ± 15 *
	(1.3)	(1.4)	(1.7)	(1.5)
Female				
Vehicle Control: 0 mg/kg/day	12.6 ± 1.7	0.571 ± 0.051	0.711 ± 0.036	104 ± 15
(*S*)-Zopiclone: 200 mg/kg/day	16.2 ± 3.2*	0.699 ± 0.011 *	1.19 ± 0.08 *	123 ± 16
	(1.3)	(1.2)	(1.7)	(1.2)
racemic-Zopiclone: 280 mg/kg/day	17.8 ± 2.4*	0.766 ± 0.050 *	1.13 ± 0.11 *	136 ± 27 *
	(1.4)	(1.3)	(1.6)	(1.3)
Samples from testing facility [b]				
Male				
Untreated	ND	0.505	1.36	114
Corn Oil	ND	0.557	1.15	155
β-Naphthoflavone	ND	0.963	1.48	286
3-Methylcholanthrene	ND	1.197	2.46	197
Phenobarbital	ND	0.73	2.53	270
Dexamethasone	ND	0.71	3.57	262
Pregnenolone-16α-carbonitrile	ND	0.649	2.42	225
Clofibric Acid	ND	0.750	1.57	201

[a] Values are the mean ± standard deviation of individual data from six mice, with the exception of female mice treated with racemic-Zopiclone (n=5). In cases where individual values were determined to be statistical outliers, these values were not included in the statistical analysis (See Appendix 3).
[b] Historical data for b_5 and P450. Values are the average of duplicate determinations of pooled samples for NADPH-Cytochrome *c* reductase.
* Significantly different according to Dunnett's test ($p < 0.05$).
ND: Not Determined
Values in parenthesis indicate the fold increases over control or the fraction of control (0 mg/kg/day vehicle control).

Table 2: Effect of (*S*)-Zopiclone on mouse liver microsomal cytochrome P450 and UGT enzyme activities

Treatment	EROD [c,d]	PROD [c,e]	Testosterone oxidation [e,f]			4-Nitrophenol hydroxylation [g]	Lauric acid 12-hydroxylation [d]	4-MU glucuronidation [d,h]	Thyroxine glucuronidation [d]
			6β	7α	16β				
Samples from Sponsor [a]									
Male									
Vehicle Control: 0 mg/kg/day	222 ± 33	39.9 ± 16.0	2570 ± 440	223 ± 93	198 ± 29	2.37 ± 0.89	1590 ± 470	137 ± 16	26.7 ± 2.4
(*S*)-Zopiclone: 200 mg/kg/day	305 ± 50 *	187 ± 22 *	5280 ± 1430*	226 ± 86	279 ± 80	2.38 ± 0.41	2220 ± 210 *	153 ± 25	27.8 ± 1.3
	(1.4)	(4.7)	(2.1)	(1.0)	(1.4)	(1.0)	(1.4)	(1.1)	(1.0)
racemic-Zopiclone: 200 mg/kg/day	259 ± 41	208 ± 40 *	5760 ± 1780*	260 ± 132	238 ± 60	2.67 ± 0.44	2250 ± 260*	195 ± 34 *	35.3 ± 3.0 *
	(1.2)	(5.2)	(2.2)	(1.2)	(1.2)	(1.1)	(1.4)	(1.4)	(1.3)
Female									
Vehicle Control: 0 mg/kg/day	207 ± 36	60.4 ± 5.3	2770 ± 460	217 ± 112	205 ± 53	1.96 ± 0.27	1070 ± 60	120 ± 9	37.8 ± 2.0
(*S*)-Zopiclone: 200 mg/kg/day	443 ± 56 *	177 ± 26 *	6250 ± 1070*	321 ± 160	651 ± 46*	2.67 ± 0.33 *	1530 ± 230 *	138 ± 9 *	41.5 ± 1.3
	(2.1)	(2.9)	(2.3)	(1.5)	(3.2)	(1.4)	(1.4)	(1.2)	(1.1)
racemic-Zopiclone: 200 mg/kg/day	322 ± 55 *	184 ± 23 *	4870 ± 660*	346 ± 100	431 ± 103*	2.24 ± 0.35	1420 ± 110 *	161 ± 17 *	43.1 ± 4.1 *
	(1.6)	(3.0)	(1.8)	(1.6)	(2.1)	(1.1)	(1.3)	(1.3)	(1.1)
Samples from testing facility [b]									
Male									
Untreated	195	19.3	6210	851	683	3.15	3360	232	40.3
Corn Oil	241	19.0	4250	415	375	3.39	3490	183	38.4
β-Naphthoflavone	1610	94.6	4180	306	728	4.49	2700	240	41.7
3-Methylcholanthrene	ND	101	3560	440	473	4.96	2290	251	45.5
Phenobarbital	599	284	12000	439	1070	5.09	5220	245	54.6
Dexamethasone	363	435	26800	562	490	4.91	5550	219	36.6
Pregnenolone-16α-carbonitrile	528	34.0	15900	265	648	3.76	4080	203	46.9
Clofibric Acid	230	17.8	3270	378	350	4.48	10700	210	39.9

[a] Values are the mean ± standard deviation of individual data from six mice, with the exception of female mice treated with racemic-Zopiclone (n=5). In cases where individual values were determined to be statistical outliers, these values were not included in the statistical analysis (See Appendix 3).
[b] Values are the average of duplicate determinations of pooled samples.
[c] EROD: 7-Ethoxyresorufin *O*-dealkylation
[d] Rates are expressed as pmol/mg protein/minute
[e] PROD: 7-Pentoxyresorufin *O*-dealkylation
[f] The abbreviations denote the hydroxylated metabolite of testosterone formed (e.g., 16β denotes 16β-hydroxytestosterone).
[g] Rates are expressed as nmol/mg protein/minute.
[h] 4-MU: 4-Methylumbelliferone
* Significantly different from control according to Dunnett's test ($p < 0.05$).
ND: Not Determined
Values in parenthesis indicate the fold increase over the control or the fraction of control (0 mg/kg/day vehicle control).

3month oral gavage toxicity study of S-zopiclone in mice/Sepracor#190-819 (Cont.)

Sperm Parameters: no drug effect.

TK: generally there was no sex difference in TK parameters with slightly lower values in females than males. Concentration and exposure increased non-linearly with dose (> proportional). Tables from sponsor:

Table 7. Zopiclone Toxicokinetic Parameters Following Oral Administration of 50, 100, or 200 mg/kg/day (S)-Zopiclone or 200 mg/kg/day (RS)-Zopiclone via Oral Administration to Male and Female Mice

	Male Mice*			Female Mice*		
Gavage Dose	$AUC_{0.5\text{-}24h}$ (ng•h/mL)	C_{max} (ng/mL)	t_{max} (h)	$AUC_{0.5\text{-}24h}$ (ng•h/mL)	C_{max} (ng/mL)	t_{max} (h)
Day 91						
50 mg/kg/day (S)	1917		1	1536		0.5
100 mg/kg/day (S)	5902		1	5335		0.5
200 mg/kg/day (S)	29430		1	28167		0.5
200 mg/kg/day (RS)	16339		1	12967		0.5
200 mg/kg/day (RS) (R)-isomer	33208		1	25611		0.5

*Results are for the (S) isomer unless otherwise noted.

Table 8. Desmethyl Zopiclone Toxicokinetic Parameters Following Oral Administration of 50, 100, or 200 mg/kg/day (S)-Zopiclone or 200 mg/kg/day (RS)-Zopiclone via Oral Administration to Male and Female Mice

	Male Mice*			Female Mice*		
Capsule Dose	$AUC_{0.5\text{-}24h}$ (ng•h/mL)	C_{max} (ng/mL)	t_{max} (h)	$AUC_{0.5\text{-}24h}$ (ng•h/mL)	C_{max} (ng/mL)	t_{max} (h)
Day 91						
50 mg/kg/day (S)	1015		1	1536		0.5
100 mg/kg/day (S)	2697		1	4257		4
200 mg/kg/day (S)	5721		1	8149		2
200 mg/kg/day (RS)	2986		1	4538		0.5
200 mg/kg/day (RS) (R)-isomer	35201		1	28499		1

*Results are for the (S) isomer unless otherwise noted.

Table 9. Zopiclone N-Oxide Toxicokinetic Parameters Following Oral Administration of 50, 100, or 200 mg/kg/day (S)-Zopiclone or 200 mg/kg/day (RS)-Zopiclone via Oral Administration to Male and Female Mice

	Male Mice			Female Mice		
Capsule Dose	$AUC_{0.5\text{-}24h}$ (ng•h/mL)	C_{max} (ng/mL)	t_{max} (h)	$AUC_{0.5\text{-}24h}$ (ng•h/mL)	C_{max} (ng/mL)	t_{max} (h)
Day 91						
50 mg/kg/day (S)	4503		0.5	3711		0.5
100 mg/kg/day (S)	13890		0.5	7539		0.5
200 mg/kg/day (S)	44813		0.5	36620		0.5
200 mg/kg/day (RS)	50247		1	45842		0.5

3month oral gavage toxicity study of S-zopiclone in mice/Sepracor#190-819 (Cont.)

Summary and Conclusion:
Oral gavage administration of S-zop up to 200mg/kg/d and RS-zop at 200mg/kg/d to mice was well tolerated. The cause of death in the 2 females dosed 50&200mg/kg/d S-zop could not be determined and was considered by the sponsor not to be drug related. Clinical signs were reversible and similar between the S- and the racemate they included: hypoactivity, partial closure of eyes, sleep, excessive rubbing of face on cage area and red ears. There were no drug related findings in body wt, food intake, clinical pathology, or serum hormones. Bilateral retinal degeneration and atrophy were observed in high dose S- and RS- zop groups though there seem to be lack of correlation because not the same mouse had the 2 lesions. The sponsor dismissed these eye findings as drug related due to absence of such findings in rat and dog studies, no dose response, no findings at end of recovery period. There were minimal to no changes in organ wts, mild to minimal liver centrilobular hypertrophy that might have been due to the liver P450 enzyme induction caused by HD S-zop and RS-zop. Concentration and exposure increased more than proportional to dose and generally no sex difference noted (slight increase in f than m). Based on these results, the NOAEL in this study for S-zop in both sexes is 200mg/kg/d.

3 month oral gavage toxicity study of S-zopiclone in rats with 1 month recovery
Sepracor# 190-818/ — Study# — ,12047
Conducting lab —
Study Initiation/Termination Dates: Dec 1999/Apr 2000
GLP: Yes (x) Japanese, OECD, and US FDA
QA: Yes (x)
Drug Lot/Batch#/purity: for S-zop 0290002 — ,; for RS-zop Z9910002 purity —
Species/Strain/# per dose/sex: Sprague-Dawley rats; 15/sex/group; TK satellite group 18/sex/dose and the control vehicle group consisted of 6/sex.
B.wt./Age at study initiation: 263-277g for males and 192-205g for females/8wks old.
Doses: **25, 50, 100mg/kg/d S-zop* and 100mg/kg/d RS-zop**. Both cpds were prepared as oral suspensions in 0.5% CMC. Dosing volume was 2.5, 5, or 10ml/kg. The TK satellite group was treated in the same way as the main groups.
Duration and dosing regimen: once daily oral by gavage administration for 3mo with 1mo drug free recovery period.

* doses were selected based on results from previous 1month study (— OTI00504) where doses ≥200mg/kg/d caused death.

Methods:
At the end of 3mo of daily oral gavage dosing, 10/sex/group were killed for primary necropsy, the remaining 5/sex/dose were assigned for **49d* recovery** period.
* the study title refers to 30d recovery period whereas study protocol and conduct refers to 45d (wk20); QA inspection?

The following parameters were determined in this study:
Clinical signs/mortality: 1-2x daily observations at 1-2hr postdose with weekly detailed exam.
B.wt. & food intake: weekly beginning 1wk pre-dose. Food intake was calculated as g/animal/day.
These were done for all groups.

Hematology/blood chemistry: for all toxicology groups at scheduled necropsies on wks 13&20. Blood was collected from the vena cava of overnight fasted rats at time of necropsy.
3 month oral gavage toxicity study of S-zopiclone in rats/Sepracor#190-818 (Cont.)

Urinalysis: for all toxicology groups, overnight urine was collected using metabolic cages prior to the day of blood sampling.
Serum Hormones: for all toxicology groups, TSH, T3, T4, estradiol, LH, testosterone, and prolactin were determined on wk13&20.
Ophthalmology: for all toxicology groups, predose and wk12/Slit lamp and indirect ophthalmoscope.
Necropsy: complete necropsy to include external surfaces, all orifices, cranial, thoracic, pelvic, and abdominal cavities were examined for all toxicology animals. At both necropsy times, liver samples from 5/sex/group were collected for potential EM processing.
Organ wt: adrenals, brain, total and caudal epididymides, heart, kidneys, liver, ovaries, pituitary, spleen, testes, thymus, thyroid (weighed after fixation), and uterus. These organs were collected and weighed from all rats at scheduled necropsies.
Spermatogenic parameters: sperm count, morphology, and production rates for all males from primary and recovery necropsies.
Histopath: standard list of tissues/organs were removed, fixed and shipped to — — where they were processed and stained with H&E. The prepared slides were then returned to — for microscopic exam. Histopath exam was done on all toxicology rats from vehicle, and HD S-zop and RS-zop also the following organs/tissues were examined for the 25&50mg/kg/d groups: liver, heart, kidneys, ovaries, right testes, thyroids, and right epididymides. Gross lesions from all rats were examined as appropriate.
Liver Enzyme Analyses: liver samples from all TK groups (those in the 8&24hr collection times) were collected and stored frozen. Those from control, high dose S-zop and RS-zop were sent to — — for cytochrome P450 and UDP-glucuronyl transferase induction analysis. Samples from the low and mid dose groups were kept at — for possible analysis depending on the results from or the high dose.
TK: blood samples were collected from the retro orbital sinus of the TK groups at 0.5, 1, 2, 4, 8, and 24hr postdose on days 0, 27, and 89 of dosing (3 rats/time point and no single rat sampled more than once in 24hr period). TK analyses were done by — The following table from sponsor layout the use of the TK groups:

Group	Group Size	Sample	30 Minute	1 Hour	2 Hour	4 Hour	8 Hour	24 Hour
1A	6/sex (3/sex/ time-point)	Blood	-	Yes	-	-	-	Yes
		Brain	-	At 24 hr point	-	-	-	Yes
		Liver	-	At 24 hr point	-	-	-	Yes
2A	18/sex (3/sex/ time-point)	Blood	Yes	Yes	Yes	Yes	Yes	Yes
		Brain	Yes	Yes	Yes	Yes	At 24 hr point	Yes
		Liver	X	X	X	X	At 24 hr point	Yes
3A	18/sex (3/sex/ time-point)	Blood	Yes	Yes	Yes	Yes	Yes	Yes
		Brain	Yes	Yes	Yes	Yes	At 24 hr point	Yes
		Liver	X	X	X	X	At 24 hr point	Yes
4A	18/sex (3/sex/ time-point)	Blood	Yes	Yes	Yes	Yes	Yes	Yes
		Brain	Yes	Yes	Yes	Yes	At 24 hr point	Yes
		Liver	X	X	X	X	At 24 hr point	Yes
5A	18/sex (3/sex/ time-point)	Blood	Yes	Yes	Yes	Yes	Yes	Yes
		Brain	Yes	Yes	Yes	Yes	At 24 hr point	Yes
		Liver	X	X	X	X	At 24 hr point	Yes

- Indicates that no animal scheduled for that time point. X Indicates that sample will not be collected.
Yes Indicates that sample will be collected as scheduled.

Plasma levels of total zop, total desmethyl zop, and R-zop, S-zop, and S-desmethyl zop after treatment with RS-zop**. Brain samples were also collected from the TK groups for drug level measurements on d89.*
3 month oral gavage toxicity study of S-zopiclone in rats/Sepracor#190-818 (Cont.)

** the analytical method used was — because data from previous studies showed absence of interconversion between the isomers. ** — was used for these cpds.]*

Results:
Mortality: two gavage accidental deaths occurred, 1m in RS-zop and 1f in S-zop 100mg/kg/d dose were found dead during wks 17&7 respectively. No more deaths in any group.
Clinical signs: seen in all drug groups and included hypoactivity, partial closure of eyes, impaired muscle coordination, impaired equilibrium, sensitive to touch and hyperactivity. These signs occurred 1hr postdose and were absent by next day dose and were not dose dependent. No clinical signs observed during recovery period.
B.wt./Food Intake: the only effect was transient reduction in mean B.wt and wt. gain during the period of 0 to 1 or 2 weeks of dosing that reached statistical significance ($p<0.01$), in males dosed 50&100mg/kg/d S-zop and 100mg/kg/d RS-zop and all female groups. These reductions in mean wts were small, 3-5% relative to the corresponding controls and mean wt gain reductions were 18-30% in males and 35-46% in females (not dose dependent)(wk0-1 females: 28, 15, 17, 18, 17g in control, 25, 50, 100mg/kg/d S-zop and RS-zop respectively). In general, transient body wt changes early on in a study usually reflects animal daptation to the drug and are of no toxicological significance. Body weight changes during wk0-1 in males dosed RS-zop corresponded to decrease in mean food intake otherwise, food intake was increased in 50mg/kg/d S-zop females and both sexes of S-zop and RS-zop 100mg/kg/d throughout the dosing period reaching statistical significance.
Hematology, Clinical Chemistry, & Ophthalmology: no drug related findings, any changes that reached statistical significance were either small or not dose dependent and therefore, not considered of toxicological significance.
Urinalysis: mean total urine volume was significantly increased in both sexes at end of dosing wk13: 13, 20, 34.5*, 24, 29ml in male cont., 25, 50, 100mg/kg/d S-zop and RS-zop respectively, * $p<0.05$, ** $p<0.01$; and the corresponding values in females were 8, 11, 21, 35**, 37**ml respectively. Consequently, mean specific gravity was decreased in females dosed 100mg/kg/d S-zop and RS-zop ($p<0.01$); no other meaningful findings were recorded.
Serum Hormones: there was a marked inter-animal variation in the data as reflected by the large s.d. values in all hormone measurements of all groups therefore, a meaningful conclusion could not be made.
The sponsor stated that this study was unable to replicate the increase in TSH levels observed in the preious study conducted by Rhone-Poulene Roerer.
Macroscopic Exam: drug related findings that correlated with organ wt changes and/or histopathology included the epididymides and testes, at the following incidences:
Small R &/or L epididymides: 0, 0, 1, 3, 0 in cont, 25, 50, 100mg/kg/d S-zop and RS-zop respectively, each out of 10 animals per group.
Small and Soft R &/or L testes: 0, 0, 0, 3, 0 in cont, 25, 50, 100mg/kg/d S-zop and 100mg/kg/d RS-zop respectively, each out of 10 animals per group.
No other drug related macroscopic findings were noted.

Organ wt: mean absolute and relative **liver** wt was dose-dependently and significantly increased relative to corresponding control in males and females treated groups. The increase over the control values in liver wts relative to B.wt in males were 15.5, 17, 28% in 25, 50, 100mg/kg/d S-zop 25% in RS -zop relative to the control value respectively, the corresponding values in female rats were 14, 34, 50, 61% respectively. In recovery period, mean liver wt were still increased in females dosed 100mg/kg/d S-zop and both sexes dosed RS-zop, relative to the control. These liver wt increases did not correlate with

histopathology but there was an increase in P450 enzyme induction. Mean absolute and relative **kidney** wts were increased in males dosed 100mg/kg/d S-zop and both sexes dosed RS-zop, comparable wts were measured at recovery period; kidney wt changes did not correlate with histopathology. At wk13 necropsy, mean absolute and relative **epididymides** and cauda wts were significantly reduced in all male drug groups relative to the control. Decreased wt of epididymides correlated with small size and histopath finding of subacute inflammation, edema, and epithelial dysplasia. During recovery period, the decrease in epididymides wt in 100mg/kg/d S-zop and RS-zop was less pronounced than that measured at wk13 indicative of reversal in effect. Mean absolute and relative wt of the **testes** was decreased in 100mg/kg/d S-zop relative to the control and correlated with small and soft testes and seminiferous tubule degeneration; no drug effect at recovery period (table from sponsor). Mean absolute and relative wt of **thymus** in females dosed 100mg/kg/d S-zop and RS-zop was decreased relative to the control but with no effect in males and no histopath correlate. At recovery period, slight thymus wt reduction was still observed in the RS-zop female group.

ORGAN WEIGHTS RELATIVE TO FINAL BODY WEIGHTS (GRAMS/100 GRAMS) WEEK 13

GROUP:	0 MG/KG/DAY	25 MG/KG/DAY-SZ	50 MG/KG/DAY-SZ	100 MG/KG/DAY-SZ	100 MG/KG/DAY-RZ
	----- M A L E -----				
HEART					
MEAN	0.343	0.350	0.344	0.340	0.345
S.D.	0.0292	0.0277	0.0286	0.0198	0.0173
N	10	10	10	10	10
RT TESTIS					
MEAN	0.446	0.453	0.448	0.342**	0.423
S.D.	0.0271	0.0176	0.0390	0.1459	0.0433
N	10	10	10	10	10
LT TESTIS					
MEAN	0.441	0.451	0.454	0.390	0.431
S.D.	0.0251	0.0284	0.0371	0.1393	0.0467
N	10	10	10	10	10
RT EPIDIDYMIS					
MEAN	0.176	0.141*	0.110**	0.107**	0.119**
S.D.	0.0441	0.0126	0.0072	0.0149	0.0344
N	10	10	10	10	10
LT EPIDIDYMIS					
MEAN	0.156	0.145	0.116**	0.106**	0.113**
S.D.	0.0083	0.0211	0.0107	0.0235	0.0088
N	10	10	10	10	10
RT CAUDA EPID					
MEAN	0.080	0.057	0.043**	0.035**	0.055
S.D.	0.0368	0.0115	0.0047	0.0065	0.0324
N	10	10	10	10	10
LT CAUDA EPID					
MEAN	0.063	0.062	0.046**	0.046**	0.048**
S.D.	0.0025	0.0111	0.0042	0.0121	0.0074
N	10	10	10	10	10

** - Significantly different from the control group at 0.01 using Dunnett's test

Histopathology: no drug effect in any female group. Findings in males were limited to the testes and epididymides. Seminiferous tubule degeneration of the testes was observed in 100mg/kg/d S-zop. This finding correlated as indicated above, with smaller testes and decrease in serum testosterone levels (though data were highly variable). Epididymal subacute inflammation, interstitial edema, and dyplasia of epithelial lining were observed in all drug groups compared to minimal to no findings in control. The severity of these findings were mainly mild (1/10 moderate inflammation noted in

control and 50mg/kg/d S-zop) and 3/10 severe hypospermia in 100mg/kg/d S-zop relative to 0/10 in control. The dysplasia was descibed as follows: "tubules decreased in diameter and lined by disorganized epithelium, with areas of epithelial hyperplasia (piling up of epithelium), epithelial necrosis, variation in nuclear size and shape, some mitotic figures, and transmigration of inflammatory cells". No other drug related histopath findings.

Sperm Parameters: sperm concentration (# of sperm per gm tissue), and production rates in the testes were significantly ($p<0.05$) reduced in 100mg/kg/d S-zop, sperm concentration in epididymides was also significantly reduced in 50&100mg/kg/d S-zop and RS-zop ($p<0.01$). Sperm motility was reduced significantly ($p<0.01$ or 0.05), and dose dependently in S-zop and RS-zop (85.5, 71.6, 8.4, 6.0, 7.0% in control, 25, 50, 100mg/kg/d S-zop and RS-zop respectively). Sperm morphology was affected in 50&100mg/kg/d S-zop and RS-zop as follows: normally shaped head separated from flagellum or head absent with normal flagellum, were seen in all drug groups. These effects correlated with both macro- and micro- scopic findings in testes and epididymides and organ/tissue wt reductions. Generally, all changes in sperm parameters were absent at recovery period.

Enzyme Induction: several enzymes were induced by zop and the induction profile is suggested by the sponsor to be similar to that of phenobarbital. Tables from sponsor:

Table 1: Effect of (*S*)-Zopiclone on rat liver microsomal protein yield, cytochrome b_5 content, cytochrome P450 content and NADPH-cytochrome *c* reductase activity

Treatment	Protein yield (mg protein/g liver)	Cytochrome b_5 (nmol/mg protein)	Cytochrome P450 (nmol/mg protein)	NADPH-cytochrome *c* reductase (nmol/mg protein/min)
Samples from Sponsor [a]				
Male				
0 mg/kg/day (vehicle control)	16.2 ± 1.3	0.476 ± 0.040	0.670 ± 0.078	68.4 ± 8.0
100 mg/kg/day: (*S*)-Zopiclone	18.0 ± 0.0	0.540 ± 0.090	0.745 ± 0.070	73.9 ± 18.4
	(1.1)	(1.1)	(1.1)	(1.1)
100 mg/kg/day: racemic-Zopiclone	20.1 ± 0.0 *	0.514 ± 0.052	0.640 ± 0.087	74.4 ± 12.2
	(1.2)	(1.1)	(.96)	(1.1)
Female				
0 mg/kg/day (vehicle control)	14.0 ± 0.0	0.490 ± 0.026	0.547 ± 0.062	59.4 ± 8.1
100 mg/kg/day: (*S*)-Zopiclone	14.3 ± 0.0	0.571 ± 0.053 *	0.564 ± 0.059	101 ± 21 *
	(1.0)	(1.2)	(1.0)	(1.7)
100 mg/kg/day: racemic-Zopiclone	20.8 ± 2.2 *	0.499 ± 0.074	0.516 ± 0.069	83.1 ± 9.5 *
	(1.5)	(1.0)	(.94)	(1.4)
Samples from testing facility [b]				
Rat Male Saline	ND	0.387	0.796	140
Rat Female Saline	ND	0.590	0.900	160
Rat Male β-Naphthoflavone	ND	1.10	2.38	162
Rat Male Phenobarbital	ND	0.689	2.31	249
Rat Male Isoniazid	ND	0.570	1.13	141
Rat Male Dexamethasone	ND	0.668	2.38	305
Rat Male Clofibric acid	ND	0.700	1.91	256

a Values are mean ± standard deviation of five or six rats. A standard deviation of 0 indicates that the standard deviation is <1% of the mean.

b Historical data for b_5 and P450. Values are the average of duplicate determination of pooled samples for NADPH-cytochrome *c* reductase.

* Significantly different according to Dunnett's test ($p < 0.05$).

ND: Not Determined

Values in parentheses indicate the fold increases over control or the fraction of control (0 mg/kg/day vehicle control).

Table 2: Effect of (S)-Zopiclone on rat liver microsomal cytochrome P450 enzyme activities

Treatment	EROD [c,d]	PROD [c,e]	Testosterone oxidation [f]				4-Nitrophenol hydroxylation [g,h]	Lauric acid 12-hydroxylation [i]	4-MU glucuronidation [h,j]	Thyroxine [k] glucuronidation
			16β	2α	7α	6β				
Samples from Sponsor [a]										
Male										
0 mg/kg/day (vehicle control)	163 ± 23	9.38 ± 1.07	61.2 ± 5.5	2050 ± 250	297 ± 52	1700 ± 140	0.605 ± 0.174	800 ± 115	108 ± 19	27.3 ± 1.9
100 mg/kg/day: (S)-Zopiclone	366 ± 36 * (2.2)	161 ± 55 * (17)	239 ± 62 * (3.9)	1560 ± 360 * (0.76)	345 ± 80 (1.2)	3510 ± 280 * (2.1)	0.835 ± 0.297 (1.4)	1190 ± 120 * (1.5)	206 ± 28 * (1.9)	36.9 ± 5.0 * (1.4)
100 mg/kg/day: racemic-Zopiclone	293 ± 70 * (1.8)	124 ± 52 * (13)	176 ± 54 * (2.9)	1290 ± 200 * (0.63)	272 ± 14 (0.92)	3050 ± 540 * (1.8)	1.27 ± 0.280 * (2.1)	932 ± 71 (1.2)	305 ± 43 * (2.8)	32.7 ± 5.1 (1.2)
Female										
0 mg/kg/day (vehicle control)	178 ± 61	0.459 ± 0.399	56.0 ± 10.2	26.8 ± 8.2	1000 ± 230	101 ± 7	1.56 ± 0.570	796 ± 109	136 ± 17	26.6 ± 3.9
100 mg/kg/day: (S)-Zopiclone	280 ± 35 * (1.6)	21.3 ± 11.3 (46)	154 ± 15 * (2.8)	37.2 ± 2.4 (1.4)	863 ± 80 (0.86)	750 ± 130 * (7.4)	1.09 ± 0.290 (0.70)	979 ± 109 * (1.2)	200 ± 24 * (1.5)	25.2 ± 2.2 (0.95)
100 mg/kg/day: racemic-Zopiclone	216 ± 50 (1.2)	50.3 ± 33.5 ‡ (110)	142 ± 58 * (2.5)	32.0 ± 7.8 (1.2)	817 ± 116 (0.82)	510 ± 165 * (5.0)	0.997 ± 0.077 (0.64)	884 ± 76 (1.1)	241 ± 62 * (1.8)	28.6 ± 4.0 (1.1)
Samples from testing facility [b]										
Rat Male Saline	169	9.88	42.9	1840	273	1860	1.44	989	192	32.3
Rat Female Saline	197	2.66	11.9	ND	776	67.4	1.26	1550	197	26.9
Rat Male β-Naphthoflavone	3740	40.3	28.2	320	1040	1770	4.05	1110	868	166
Rat Male Phenobarbital	1000	999	2300	1620	808	8030	2.68	1350	453	52.0
Rat Male Isoniazid	166	11.2	54.7	581	381	1170	7.46	1240	245	32.9
Rat Male Dexamethasone	90.9	15.3	311	282	549	22400	3.85	2420	150	143
Rat Male Clofibric acid	306	46.0	192	2500	516	5540	1.49	11800	208	45.4

[a] Values are means ± standard deviation of individual data from six rats. In cases where individual values were determined to be statistical outliers, these values were not included in the statistical analysis (See Appendix 3).
[b] Values are the average of duplicate determinations of pooled samples.
[c] EROD: 7-Ethoxyresorufin O-deethylation
[d] Rates are expressed as pmol/mg protein/min.
[e] PROD: 7-Pentoxyresorufin O-deethylation, NOTE: Male rat rates and positive controls were taken from the original data, however, female rat rates were taken from the repeated data
[f] The abbreviations denote the hydroxylated metabolite of testosterone formed (e.g., 16β denotes 16β- hydroxytestosterone).
[g] 4-Nitrophenol hydroxylase: Data was taken from repeated assay.
[h] Rates are expressed as nmol/mg protein/min.
[i] 4-MU: 4-methylumbelliferone
[j] Significantly different according to Dunnett's test ($p < 0.05$).
[k] Significantly different according to Dunn's test ($p < 0.05$).
Values in parentheses indicate the fold increases over control or the fraction of control (0 mg/kg/day vehicle control).
ND: Not Detected

Based on these data, 100mg/kg/d S-zop and RS-zop caused a moderate induction of liver enzymes (note the 46x increase in CYP2B1/2 in females dosed 100mg/kg S-zop). The sponsor considered zopiclone induction profile to be similar to that of phenobarbital with an effect ½ of that observed with phenobarbital.

TK: table from sponsor:

Gender (S) or (RS)-Zopiclone (mg/kg/day)	Zopiclone Results* $AUC_{0.5\text{-}24h}$ (ng•h/mL)			C_{max} (ng/mL)			t_{max} (h)		
	Day 0	Day 27	Day 89	Day 0	Day 27	Day 89	Day 0	Day 27	Day 89
Males									
25 (S)	5511	7152	9157				1	0.5	0.5
50 (S)	15362	12699	18598				0.5	0.5	1
100 (S)	33334	38844	44578				0.5	2	0.5
100 (RS)	19965	26784	25172				0.5	0.5	1
100 (RS) (R)-isomer	8975	13610	12500				0.5	0.5	1
Females									
25 (S)	24477	18990	20098				1	0.5	0.5
50 (S)	62820	29016	39549				2	0.5	1
100 (S)	93167	49648	67203				4	1	0.5
100 (RS)	57025	44805	54911				4	1	0.5
100 (RS) (R)-isomer	27689	16045	17830				4	1	0.5

*Results are for the (S) isomer unless otherwise noted.

3 month oral gavage toxicity study of S-zopiclone in rats/Sepracor#190-818 (Cont.)

Females had much higher concentrations and exposure than males at all doses. Concentration in both sexes increased linearly on d0 (after single dose), but less than proportional on days 27&89. Increase in exposure on the other hand, was variable. Females took longer to achieve C_{max} relative to males (1-4hrs vs. 0.5-1hr respectively). Exposure to the R-isomer accounted nearly to half the amount of the racemate.

Summary & Conclusion:
Oral gavage administration of S-zop at 25, 50, or 100mg/kg/d and RS-zop at 100mg/kg/d to male and female rats caused no drug related deaths and was well tolerated up to 90days of daily dosing. The **NOAEL in males is <25mg/kg/d** based on findings in reproductive tissues, and **in females is 100mg/kg/d**. There were no significant drug related effects in either sex on clinical pathology parameters, ophthalmology, urinalysis, or gross morphology and histopathology (in females) up to 100mg/kg/d S-zop and RS-zop. Target organs of toxicity in males were the reproductive tissues, testes and epididymides wts were reduced, a small decreasing trend noted in serum testosterone, and histopathology findings were observed. Mean liver and kidney wts were significantly increased but none of these wt changes correlated with histopathology. Data on serum hormone levels were inconclusive and did not support the findings reported previously by RPR for RS-zop. S-zopiclone 100mg/kg/d and RS-zop 100mg/kg/d caused mild to moderate induction of liver enzymes and the sponsor stated that the effect was qualitatively similar to but only ½ that of phenobarbital. Exposure and concentration were higher in females than males and the latter increased linearly following single but not repeated dosing. Changes in exposure on the other hand, were variable. **Exposure after 25mg/kg/d on d89 in males was 9157ng.hr/ml and in females 20,098ng.hr/ml.**

3 month oral capsule toxicity study of S-zopiclone in dogs with 1 month recovery
Sepracor# 190-817/ — ,tudy# ~ 312045
Conducting lab —
Study Initiation/Termination Dates: Nov 1999/Mar 2000
GLP: Yes (x) Japanese, OECD, and US FDA
QA: Yes (x)
Drug Lot/Batch#/purity: for S-zop 0290002' — for RS-zop Z9910002 purity —
Species/Strain/# per dose/sex: Beagle dogs; 6/sex/group; 4/sex/group were killed at end of 3month dosing and 2/sex/dose were kept drug free for 1month.
B.wt./Age at study initiation: 6.5-8months old at start of dosing.
Doses: **0, 2.5, 10, and 25mg/kg/d S-zop* and 10mg/kg/d RS-zop**. Both cpds were administered in gel caps, control dogs received empty gel caps.
Duration and dosing regimen: once daily oral capsule administered for 3 months with 1 month drug free recovery period.

* doses were selected based on results from previous 1month study ' — ' OTI00505) and 2 dose range finding studies ' — OTI00515 & OTI00516). These studies showed 30mg/kg/d was not tolerated but 20mg/kg/d was well tolerated (no more detail was provided for these studies).
The following parameters were determined in this study:

Clinical signs/mortality: 2x daily observations at 1&4hr postdose with weekly detailed exam. Only once daily observations at recovery period. Animals at postdose were taken out of their individual cages and allowed to move freely in the animal room and were observed for any abnormalities.
3 month oral capsule toxicity study of S-zopiclone in dogs/Sepracor#190-817 (Cont.)

B.wt. & food intake: weekly beginning 1wk pre-dose. Food intake was measured daily and weekly means reported along with B.wt intervals; food intake calculated as g/animal/day. Final fasted B.wt was recorded prior to each necropsy.
Hematology/blood chemistry/Urinalysis: blood and urine were collected from all dogs predose on -wk1, end of dosing wk12, and once at end of recovery period study wk16. Blood was collected from the jugular vein of overnight fasted dogs placed in metabolic cages for urine collection. The following *serum hormones were also determined: TSH, T3 & T4.*
Ophthalmology: predose -wk1 and wk12. Slit lamp and indirect ophthalmoscope were done.
ECG: once predose (-wk1), and end of dosing during wk12 from all dogs between 2-3hrs postdose; Leads I, II, III, aVR, aVL, and aVF were used. ECG records were evaluated by —

TK: blood samples were collected from 4/sex/group on d0 (1st dose), 1month, and at 3months (d88 of dosing) at 0.5, 1, 2, 4, 8, and 24hr postdose. Frozen samples were shipped overnight to — a 3rd party contractor; the data were not audited by — Plasma levels of total zop and total desmethyl zop were determined by a non-chiral method and levels of R- & S-zop and R- & S- desmethyl zop following dosing with RS-zop were determined by a — method and TK parameters calculated.
Necropsy: complete necropsy to include external surfaces, all orifices, cranial, thoracic, pelvic, and abdominal cavities was done on all dogs at wks 13&17 and those found dead.
Liver Samples: at primary necropsy (wk13), from each dog in control, 25mg/kg/d S-zop, and 10mg/kg/d RS-zop, 10g section of the liver was collected from the right median lobe immediately after isolation of the liver, sectioned, rinsed in saline, frozen in liquid nitrogen, and shipped to — for cytochrome p450 and UDP-glucuronosyltransferase induction analyses. Results from — were not audited by — but are included in this report. Also, liver samples and processing were done on livers from dogs in remaining groups and those at end of recovery period but kept at — for potential enzyme analysis if warranted by results from primary necropsy. In addition to the above, small section of the left lobe of the liver was collected, placed in a drop of McDowell-Trump fixative and cut with a razor blade into 1mm cubes. These cubes were then placed in vials containing McDowell-Trump fixative and stored at — for potential EM.
Organ wt: the following organs were isolated and weighed: adrenals, brain, total and caudal epididymides, heart, kidneys, liver, ovaries, pituitary, spleen, testes, thymus, and thyroid.
Histopath: standard list of tissues/organs from all dogs were isolated, fixed, stained with H&E, and examined by —

Results:
Mortality: there were 3 total deaths that were considered drug related: 2MDm (#6777&6792) found dead, the 1st was found dead within 1hr postdose on d89 and the 2nd male was found dead on 2nd day of recovery period; 1HDf (#6834) was found dead on 2nd day of recovery period. Clinical signs in these dogs were similar to those in other dogs except for rales in male# 6777.
Clinical Signs: signs were observed in all S- and RS- zopiclone drug groups and included: red ears, "injected" sclera, salivation, diarrhea (females only), and the following CNS effects: hypoactivity, excessive sleepiness, tremors, muscle incoordination, circling, prostrate and/or hyperactivity, excessive

chewing, and sensitivity to touch. Most signs occurred within 1-4hr postdose lasting till next day. Signs observed throughout dosing and into 1st wk of recovery period before they resolved.

B.wt & Food Intake: there was no drug related effect on food intake. A small, transient and not statistically significant change was seen in mean wt of mid and high dose females. Mean wt in MD&HDf was slightly lower than controls starting on wk6 and up. A transient decrease in wt gain

was seen in all drug groups including RS-zop during wks 1-2 reaching statistical significance in HDf ($p<0.01$; -0.7±0.16kg vs. -0.2±0.08kg in cont. f). In males dosed 25mg/kg/d S-zop mean wt gain was significantly increased through the entire 13wk of dosing (0-13wk 3.4±0.64kg vs. 2.0±1.13kg in cont.m); no change in females. Some transient decreases and increases that reached statistical significance were observed in both sexes. All changes in B.wt and/or wt gain were small, not dose dependent, and were reversible by end of recovery period.

Hematology: no drug related findings except for platelet count. Mean platelet count was significantly increased in HDm on wk13 but comparable to control value by end of recovery wk16 (40% more than the control; 452 vs. 323thous/ul). Mean platelet count was significantly increased in MD&HDf S-zop and RS-zop groups relative to values in control on wk13 and comparable to control by end of recovery (410, 436thous/ul for MD&HDf and 410thous/ul for RS-zop vs. 311thous/ul in cont.f).

Clinical Chemistry: dose related increase in mean ALP in both sexes dosed 10&25mg/kg/d S-zop at end of dosing ($p<0.05$; males: 2x & 2.4x the control respectively, females: 1.8x & 2.9x the control respectively), no other drug related findings. The ALP changes were comparable to control at end of recovery period. Similarly, there was no drug effect on any of the serum hormones measured.

Urinalysis: total urine volume on wk12 was increased in HDm (3x) and MD&HDf (3x&2.7x respectively) compared to the corresponding controls ($p<0.05$ or 0.01) also specific gravity was decreased in MDf. Non of these findings were considered drug related.

ECG, Ophthalmology, Macroscopic Exam: no findings.

Organ wts: the only organ wt effect was an increase ($p<0.05$ or 0.01) in mean absolute and relative (to body and brain wts) liver wts in both sexes reaching statistical significance in HD and sometimes in MD. The absolute liver wt changes in males were 1x, 1.2x, 1.6x, and 1.2x in low, mid, and high S-zop and RS-zop relative to control respectively, and the values in females were 1.2&1.3 in MD&HD respectively, relative to control. Relative liver wt to B.wt increases in males were 1.1x, 1.2x, 1.4x, and 1.3x in low, mid, high S-zop and RS-zop respectively, the corresponding values in females were 1x, 1.3x, 1.4x, and 1x. These liver wt changes were reversible and wts were comparable to controls during recovery period.

Liver Enzymes: cytochrom b5, P450 content, and activities of NADPH-cytochrom c reductase, 7-benzoyloxyresorufen O-delakylase (CYP2B11; ≅6x increase in males), and testosterone 16α- and 16β-hydroxylase were increased ($p<0.05$) in HD dogs (m+f) dosed S-zop comapred to the corresponding control values. Female dogs dosed 25mg/kg/d also had an increase in testosterone 6β-hydroxylation and thyroxine glucuronidation, HDm also had an increase in 4-nitrophenol hydroxylation and 4-methylumbelliferone glucuronidation (but none of these enzymes were affected in female dogs); all changes relative to control values. RS-zop caused in both sexes an increase ($p<0.05$) in activities of 7-benzyloxyresorufin O-delakylase (5x over control), and testosterone 16β- and 16α-hydroxylase and in males, RS-zop caused an increase in cytochrome b5, cytochrome P450 content, NADPH cytochrome c reductase, and 4-nitrophenol hydroxylase. The sponsor indicated that this induction profile of zopiclone is similar to that of phenobarbital and that zopiclone's induction is third to half that of phenobarbital.

Histopathology: the only organ affected was the liver in all drug groups including the RS-zop. Hepatocellular hypertrophy was seen at wk13 necropsy and correlated to increase in liver wt and liver enzyme induction. The incidence was as follows: 0/4, 3/4, 4/4, & 4/4 in males and in females 0/4, 1/4, 3/4,

4/4, & 3/4 in cont., low, mid, high S-zop and RS-zop respectively. The hypertrophy was described by the sponsor as follows: "increased cellular size, rounded cellular borders, acidophilic/"ground glass" cytoplasm with basophilic clumping of cytoplasmic organelles. Moreover, there was variable degree of loss of hepatocellular plate linearity and structure. At recovery, liver hypertrophy was observed only in 25mg/kg/d S-zop male (2/2), and not present in any other group. The severity of all liver hypertrophy in both sexes was "minimal".

TK: all cpds were detectable following S- and RS-zop administration. Generally, no sex difference in TK was observed, any differences were small and limited to one dose. There seem to be no cpd accumulation with time except perhaps for S-zop in both sexes and R-zop in males (table from sponsor).
Increase in exposure and concentration was not dose proportional in S-zop in either sex.

Table 1: Toxicokinetics of (S)-zopiclone, (R)-zopiclone (S)-desmethyl zopiclone and zopiclone N-oxide after 13 weeks oral dosing of (S)-zopiclone or (RS)-zopiclone

Dose (mg/kg/day)	(S)-zopiclone				(S)-desmethyl zopiclone			
	Male		Female		Male		Female	
	AUC	Cmax	AUC	Cmax	AUC	Cmax	AUC	Cmax
Day 0								
(S)-zop, 2.5	5191		1436	116	2380		1842	133
(S)-zop, 10	13775		18339	1233	5190		4027	275
(S)-zop, 25	45689		49446	3215	10075		9591	625
(RS)-zop, 10	10805		11377	962	2621		2782	194
Day 28								
(S)-zop, 2.5	5899		1738	161	1426		1255	91
(S)-zop, 10	16652		21699	1806	3235		2111	179
(S)-zop, 25	71335		63066	5665	6103		5598	395
(RS)-zop, 10	11728		12286	1685	1365		1656	1131
Day 88								
(S)-zop, 2.5	5884		2395	314	1166		1415	119
(S)-zop, 10	19399		25574	2671	3431		2729	201
(S)-zop, 25	7954		57413	5245	6042		5328	343
(RS)-zop, 10	12540		14318	2293	1504		1910	157

Dose (mg/kg/day)	(R)-zopiclone				(R)-desmethyl zopiclone			
	Male		Female		Male		Female	
	AUC	Cmax	AUC	Cmax	AUC	Cmax	AUC	Cmax
Day 0								
(RS)-zop, 10	13701		12744	1321	4091		3523	
Day 28								
(RS)-zop, 10	8446		8435	1448	2980		2678	
Day 88								
(RS)-zop, 10	8568		9502	1951	3614		3574	

Dose	lone N-oxide			
(mg/kg/day)	Male		Female	
	AUC	Cmax	AUC	Cmax
Day 0				
(S)-zop, 2.5	1415		1105	103
(S)-zop, 10	5356		9848	839
(S)-zop, 25	20106		17172	1155
(RS)-zop, 10	2264		2922	264
Day 28				
(S)-zop, 2.5	1382		936	78
(S)-zop, 10	4517		8913	804
(S)-zop, 25	15261		14722	1242
(RS)-zop, 10	2406		2884	419
Day 88				
(S)-zop, 2.5	1086		1138	155
(S)-zop, 10	5051		7062	661
(S)-zop, 25	17659		11768	1041
(RS)-zop, 10	2436		3704	556

$AUC_{0\text{-}24}$ = ng•hr/mL; Cmax = ng/mL

Summary & Conclusion:
Oral administration of S-zop at 2.5, 10, & 25mg/kg/d and RS-zop at 10mg/kg/d to male and female dogs for 3 months caused death in 2MDm (but no deaths in HD) and 1HDf with no deaths in RS-zop though the sponsor referenced a published article by Tamura et al., 1983 where mortality was observed in RS-zop at 5months in a 6month study in dogs dosed 10&25mg/kg/d but not at 5mg/kg/d; no deaths observed in the current study at 10mg/kg/d RS-zop. Clinical signs were seen in all drug groups, no drug effect on the parameters studied including ECG and serum hormone levels. Zopiclone increased absolute and relative liver wts in both sexes dosed 25mg/kg/d and in females dosed 10mg/kg/d. Liver wt changes correlated with enzyme induction and hepatocelluar hypertrophy. The MTD for S-zop in males is 2.5mg/kg/d due to death in MD and, in females is 10mg/kg/d due to death at 25mg/kg/d. These doses can also be considered NOAEL since findings were limited to liver wt changes and minimal liver histopathology. Plasma $AUC_{0.5\text{-}24hr}$ at 2.5mg/kg/d in males is 5884 and that at 10mg/kg/d in females is 25,574ng.hr/ml.

Study Title: 1 year oral toxicity study in the dog/Sepracor# 190-832
Conducting Lab: — , study# 347-028), —
Date of Study Initiation: August 29 1980
GLP: Yes
QA: Yes
Drug Lot#/purity: lot#8 (CA78 160 00 and CA80 311 00)

Methods:
Pure bred beagle dogs 8-9months old purchased from — weighed 9-16kg and 9-14kg males and females respectively, there were 5/sex/group. Dogs were housed individually and identified by ear tags. RS-zop was orally administered as capsules at **0, 1, 5, 25mg/kg/d for 1yr***, the control group received ß-d lactose capsules at 25mg/kg/d. It is noted that the drug at the high dose was administered 2x per day instead of once daily starting on d1 of wk37 in the attempt to reduce mortality that was observed in this group.

* basis for dose selection was not provided.
Parameters assessed:
Clinical Signs and mortality: daily with weekly detailed exam.
B.wt/Food intake: weekly beginning 1wk predose.
Water inake: start on d6/wk3 water bottles were offered instead of water pans because differences in intake between control and drug groups were observed. Intake was recorded 2x per week starting wk7.
Ophthalmoscopy: all groups predose, 3, 6, 9, and 12months of dosing. Indirect ophthalmoscope with pupillary dilation.
EKG: all dogs predose and 3,6,9,12months of dosing. Records were sent to Dr. — for radiology consult.

Clinical Pathology, Hematology, and Urinalysis: on all dogs predose, 1,2,3,6,9, and 12months of study. Blood was collected from the jugular vein of overnight fasted dogs. Standard hematology and clinical chemistry parameters were measured also serum T3 & T4 were determined. Urinalysis was done on fasted dogs placed in metabolic cages, parameters included color, volume, pH, specific gravity, Alb, Bilirubin, occult blood, glucose, protein and microscopic exam of sediment.

1 yr dog toxicity study/Sepracor#190-832 (Cont.)

Organ wts: at end of study the following organs were weighed: adrenals, heart, kidneys, testes, liver, thyroids, pituitary, ovaries.
Gross exam: standard exam of orifices, abdominal cavity, and skin.
Histopath: standard set of tissues/organs from all dogs were processed for H&E staining. Similar exam done for dogs that died in extremis or found dead.

Results:
Mortality: The following deaths occurred as a result of convulsion, all were found dead except for #2119 was killed in moribund after a severe convulsive episode:

	1st appearance	# of times convulsing	Wk of death
MDm # 2119	31	2	34
HDm# 2117	27	2	46
HDm# 2128 *	25	6	53
HDf# 2136	27	1	27
HDf# 2145	29	2	42

To minimize death, drug was administered in 2 divided doses for dog#s 2117&2145 from wk37 onwards though clearly this approach did not seem to help since these 2 dogs died later due to convulsions.
* terminal kill though the dog convulsed 6x during the study.
Additional death #2114 HDm (found dead), did not convulse cause of dearth unknown.
Clinical Signs: convulsions were observed as indicated above and also in dog# 2128 but did not lead to death. Emesis occurred at higher frequency in drug groups than in control throughout the study. Dogs in HD showed effects that were extension of the drug pharmacology such as sleep, lethargy, and hypoactivity mainly observed shortly postdose. Pre-dose, trembling and convulsions were 1st observed between wks27-39 in 5&25mg/kg/d dogs. These 2 effects disappeared in 5mg/kg/d but remained in the 25mg/kg/d till end of study again noted that they occurred predose except 1HDm convulsed postdose. Hypersalivation (ptyalism) was observed in all drug groups but none in controls. Irregular heart beat was recorded in 2/5 dogs dosed 25mg/kg/d during 6&9month measurements and none in low dose or control dogs (1 in 5mg/kg). Incidence of conjunctivitis was higher in HDf relative to controls.
B.wt and Food Intake: no clear drug related effects. Mean B.wt in MDf was significantly increased during wks 26,39, and 52 of study relative to control. Similarly, food intake was in general, higher in drug groups than control throughout the study.
Water intake: consumption was clearly increased in drug groups relative to control from wk7-51 (time when water bottles replaced water pans). The significance of this is unclear.
Hematology: some values reached statistical significance at one or more time points but in general, there seem to be no clear drug related effects. It is noted however, that platelets were increased significantly and dose dependently in both sexes in 5&25mg/kg/d groups relative to control at more than one time point.

Clinical Chemistry: ALP activity was increased significantly and dose dependently in 5&25mg/kg/d males (all 4 time points in 5mg/kg however, the sponsor stated that the values in 5mg/kg though reached statistical significance, were within the historical range for the lab for this strain). ALP was also increased in mid and high dose females though did not reach significant level in mid dose. Mean T4 in HDm was increased relative to control but not significantly (3.7±1.6 pg/dl vs. 1.5±0.36 control); no effect on T3 in either sex.

Organ wt: absolute and relative liver wts were significantly increased in both sexes dosed 25mg/kg/d relative to control wt. (wt relative to B.wt in males was 56% more and 30% more in f than cont wts).

1 yr dog toxicity study/Sepracor#190-832 (Cont.)

Gross morphology: no drug effect.

Histopath: drug related effects were observed only in the liver. Moderate hepatocyte vacuolation observed in 1/3 HD male and none in control or other drug groups, slight in 1/5 male control and none in other groups, and very slight in 1/4 MDm and none in others. In females liver vacuolation: very slight in 1/5MD and 1/3HD (none in control), slight in 1/5 LD, 2/5 MD, and 2/3 HD (none in control). Hyaline bodies were seen in 1 each HDm&f (none in others). In this reviewer's opinion, these liver findings are very mild and may reflect enzyme induction reflected as increase in liver enzyme activities and liver wt.

Summary & Conclusion:

RS-zop was administered daily by oral capsule to male and female beagle dogs at 0, 1, 5, and 25mg/kg for 1yr. Convulsions and death occurred in one male dog dosed 5mg/kg and five dogs dosed 25mg/kg therefore, 5mg/kg/d exceeded the MTD in males and 25mg/kg/d exceeded MTD in females. Other clinical signs were extension of the pharmacology and included hypoactivity, excitability, and sleepiness. No drug effect on ECG or ophthalmology. Some parameters in hematology and clinical chemistry reached statistical significance but were small, random, and not dose related. Mean wt and food intake were increased in HD and some MD dogs. Mean absolute and relative liver wt was increased in HD dogs and histopathology showed some vacuolation and hyaline droplets, no other histopath findings were seen in any organ. Mean serum T4 in HDm was increased relative to control though not reaching statistical significance and no changes in T3 or any histopath correlate. The NOAEL is therefore, 5mg/kg/d in females and 1mg/kg/d in males which is also a NOEL.

Study Title/#: 18month oral toxicity study of RS-zop in the rat/6month interim report/Sepracor# 190-831F' ~~~ 347-017. *See Carcinogenicity section for more discussion on the thyroid findings.*

Conducting Lab and Location: ~~~

Date of Study Initiation: July 18th 1977

GLP: Yes/No*

QA: Yes/No *

* for both, the final report was QA and reviewed under GLP however, the conduct of the study was not GLP. This is because the study was initiated prior to establishment of GLP regulation in 1979.

Drug Lot#/purity: Lot# 4 CA 76 260 00 and lot 5 CA 77 194 00/purity not reported.

Formulation/vehicle: NA, dietary administration.

Methods:

CD rats from — . 50/sex/group weighed 58-134g males and 66-123g females and were 5-6wks old at initiation of study. They were housed individually in wire mesh hanging cages with food and water available ad libitum. RS-zop was administered in the **diet at 0, 2, 20, or 200mg/kg/d** for 18months with 15/sex/group interim kill at 6month. Parameters assessed:

Clinical Signs and mortality: daily with weekly detailed exam.

B.wt/Food intake: weekly for the 1st 26wks and once monthly thereafter.

Ophthalmoscopy: all groups predose, 3, 6, 12, and 18months of dosing. Indirect ophthalmoscope with pupillary dilation.

APPEARS THIS WAY ON ORIGINAL

Clinical Pathology, Hematology, and Urinalysis: done at 3 and 6month of dosing on 10/sex/group with few exceptions. Rats were fasted overnight and blood collected from the orbital sinus. Hematology parameters: RBC, WBC (total and differential), Hb, and HcT. Clinical Pathology parameters: glucose, BUN, ALP, ALT, total protein, Alb, Na, K, and A/G ratio. Urinalysis included color, volume, pH, specific gravity, Alb, Bilirubin, occult blood, and microscopic exam of sediment.

Organ wts at 6&18months: heart, kidneys, spleen, testes, liver, thyroids, pituitary, ovaries, and adrenals (last 4 were weighed after fixation).

Gross exam: at 6months of dosing, 15/sex/group were killed by CO2 and standard necropsy was done. At 18months, surviving rats were killed and subjected to standard necropsy (external body surfaces and body orifices and body cavities).

Histopath: H&E stained tissue sections from control and HD (200mg/kg/d), were examined as well those from rats that died. In addition, in both sexes dosed 20mg/kg/d, the liver, spleen, testes, and kidneys were examined as well as the kidneys in females dosed 2mg/kg/d and thyroids in 20mg/kg/d and thyroids in males dosed 2mg/kg/d.

Results:

Mortality and Clinical signs: No drug related mortalities or clinical signs up to 200mg/kg/d at both interim and 18month of dosing. There was 1m in each drug group and 1f in LD that were found dead; cause of death unknown.

LDm# 61847 wk15 had severe lung congestion and edema and minimal histopath.

LDf# 61887 wk13 had minimal gross and histopath such as "very slight" lung congestion, accumulation of yellow pigment in the spleen, etc.

MDm# 61939 wk13 had congested liver, scattered very small red foci in lungs and thymus, and hemorrhage in the base of the midbrain.

HDm# 62040 wk13 had marked congestion in lungs and dark red material in right eye plus minimal histopath.

Since no remarkable or unusual findings were observed in any of these rats, the sponsor considered these deaths not drug related.

Survival on wk78 was as follows:

Dose (mg/kg/d)	**# survivors/total**	
	Males	Females
0	34/35	33/35
2	31/35	30/35

20	29/35	31/35
200	33/35	30/35

B.wt/Food Intake: at 6months, no drug related effects on mean wt or intake up to 20mg/kg/d. Mean wt HDm&f was reduced 12&17% respectively, relative to corresponding controls. Mean food intake was reduced 8% in HDm, no change in females. At end of study, mean wt at 200mg/kg/d was significantly reduced at all test intervals, $p<0.01$, table below shows mean wt and percent in () difference from control during wk78:

Dose (mg/kg/d)	**# survivors/total**	
	Males	Females
0	786	426
2	743 (5.5%)	424 (0.5%)
20	732 (7%)	406 (5%)
200	560 (29%)	306 (28%)

Rat 18month study/6mo interim, Sepracor#190-831F (Cont.)

Hematology, Clinical Chemistry, Ophthalmoscopy, and Urinalysis: no drug related findings.
Organ wts: at 6months, liver, thyroid, and pituitary wts in both sexes were significantly increased in all drug groups relative to control (except HDf pituitary wt was significantly decreased), however, no histopathology correlate in any of these organs. At terminal kill, the absolute liver wt in HDf was significantly increased, the significant increase in absolute and relative thyroid wt at all doses at 6month was not dose related and did not correlate with histopath therefore, not considered by the sponsor to be drug related. Increase in absolute and relative thyroid wt was also observed at 18month in HDm but only in those males with adenomas.
Gross morphology: at 6months, no drug related findings except for the enlarged organs noted above. At terminal kill, the only drug related finding was enlarged thyroids in 5 of 33 rats dosed 200mg/kg/d.
Histopath: at 6months, 12/30 rats in HD showed "very slight" portal bile duct proliferation/hyperplasia vs. zero incidence in controls; the significance of this finding was unclear as stated by the sponsor however, at 18month, this finding was not observed.
At terminal kill, the following was observed:
Thyroid: incidence of thyroid follicular cell adenomas was significantly increased in HDm compared to concurrent controls. The incidence was 9/33 in HDm vs. 1/34 in control males. The adenomas were described as small, occasionally multiple, and considered by the pathologist typical of those seen spontaneously in aged rats. However, the pathologist did recognize that this finding is likely to be drug related as it correlated with enlarged thyroids and increase in thyroid wts in this drug group; there were 1/31 and 3/29 in low and mid dose males respectively, and in 2/5 dead males, but none in females.
Liver: clear increase in hepatocellular hypertrophy in HD males and females relative to the control. Also, 3/33 "neoplastic nodules" and 1/33 hepatocellular carcinoma were found in HDm but not statistically significantly different from the control.
Testes: increased incidence of testicular tubular atrophy in 20&200mg/kg/d groups relative to control with the occasional finding of spermatocele in HD. However, the sponsor did not consider these effects to be drug related because they were small (see summary section).
Spleen: increased incidence of hemosiderin pigment of both sexes in drug groups compared to control. However, this was not considered drug related because of absence of evidence of erythrocyte breakdown (normal clinical path and bone marrow).

Summary and Conclusion:

Daily oral dietary administration of RS-zop to rats at 2, 20, or 200mg/kg for 6months was well tolerated without drug related effects on survival, clinical signs, clinical pathology parameters, urinalysis, ophthalmology, or gross morphology. Mean wt at end of 6month was significantly reduced in HDm&f (12&17% respectively) relative to control wts and about half of the rats had portal bile duct proliferation vs. none in controls, however, this finding was absent at 18month.. At end of 18months, similarly, RS-zop was well tolerated without death with very good survival at 1-2 deaths in control, 4-5 deaths in 2mg/kg/d, 4-6 deaths in 20mg/kg/d and 2-5 deaths in 200mg/kg/d, each out of 35/sex/group. No clinical signs up to 200mg/kg/d. Mean B.wt in both sexes dosed 200mg/kg/d continued to decline as observed at end of the 6month with decreases reaching statistical significance relative to the corresponding controls at all time points analyzed i.e. every 3month (28-29% less than control means at wk78). Mean food intake was decreased only in HDm with 8-10% less intake relative to control. Some parameters in hematology and clinical chemistry reached statistical significance relative to the control however, they were small and not dose related. Thyroids seemed enlarged upon gross exam and their wts was increased in HDm which may be related to the histopathological findings in this group. Incidence **of thyroid follicular cell adenomas** was significantly increased in HDm (9/33, $p<0.05$) compared to control 1/34

Rat 18month study/6mo interim, Sepracor#190-831F (Cont.)

(1/31 in LDm and 3/29 in MDm also in 2/5 rats that were dead; none in females)(also see Dr —— report Sepracor# 190-858 under carcinogenicity section). **Liver hyperplastic nodules** were seen in 3/33 HDm and **liver carcinoma** in 1/33 HDm compared to 0 incidence in control for both findings and in LD&MD. **Liver hypertrophy** was found in both sexes dosed 200mg/kg/d at 18month which is in support of the lesion findings:

Liver hypertrophy:

Cont	8/34m	2/32f
20mg/kg/d	5/29m	1/31f
200mg/kg/d	21/33	15/30f

The sponsor nevertheless, dismissed the liver neoplasia and considered them spontaneous and not drug related (also see —— report Sepracor# 190-858). It is of note that except for the liver hypertrophy, other hepatic parameters *in this study*, did not support drug related lesions: there were no changes in liver wts (though thyroid wt increases did reach statistical significance), at end of 18months in HD rats and the changes in serum liver enzymes were inconsistent (HDm SGOT ↑ 30% at 3month, ↓ 32% at 6month, ↑16% in 12month and no effect in females except a 34% ↑ 12month all relative to control values). In addition, mean ALP values was *decreased* (not increased), in HDf at all time points relative to control. As results showed in later studies, RS-zop is a liver enzyme inducer in rodents and these liver findings are likely the result of this enzyme induction and not a direct drug effect as a true liver carcinogen. The increased incidence of bile duct hyperplasia/proliferation was not observed at end of 18month. The incidence of testicular tubular atrophy was increased in 20&200mg/kg/d groups relative to control with the occasional finding of spermatocele in HD. Though the sponsor in this report did not consider this to be drug related due to its small value, as shown in later reproductive and other toxicity studies, RS-zop as well as S-zop have marked effects on the male reproductive parameters.

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3.4.4 GENETIC TOXICOLOGY:

Study Title: Bacterial reverse mutation test of zopiclone

Study No: Sepracor# 190-811. # OTI00506
Study Type: pre-incubation and plate incorporation
Conducting Laboratory:
GLP compliance: Yes/OECD
QA- Reports Yes (x)
Date of Study Initiation/completion: Mar 1999/Apr 1999
Drug Lot Number: responsibility of sponsor.
Study Endpoint: increase in number of revertant colonies over negative control.

Methodology:
- Strains/Species/Cell line: TA98, TA100, TA1535, and TA1537, E.coli WP2uvrA
- Dose Selection Criteria: cytotoxicity (inihibtion of background lawn) and/or precipitation of tes article.
- Basis of dose selection: dose range finder cytotoxicity assay in -/+ S9 using TA98 and WP2uvrA.
- Range finding studies: only TA98 and WP2uvrA strains were tested in duplicate cultures in presence and absence of S9. The concentrations tested were 0, 1.6, 8, 40, 200, 1000, and 5000ug/plate; background lawn was assessed 66hrs after incubation.
- Test Agent Stability: stable, dark tubes used.
- Metabolic Activation System: S9 from livers of male Fischer 344 rats induced by Aroclor 1254.
- Controls:

Positive Controls:

Strain	-S9	+S9
TA1535	Na azide	2-aminoanthracene
TA1537	9-aminoacridine	2-aminoanthracene
TA98	2-nitrofluorene	2-aminoanthracene
TA100	Na azide	2-aminoanthracene

WP2uvra 4-NQ-N-oxide 2-aminoanthracene

- Vehicle: DMSO
- Negative Controls: DMSO

- Comments: the **R- , S-, and RS-zop** were tested in presence and absence of S9 using both methods.
- Exposure Conditions:
    - Incubation and sampling times: according to guidelines, 20min for the preincubation.
    - Doses used in definitive study: 0, 8, 40, 200, 1000, and 5000ug/plate.
- Analysis:
    - No. slides/plates/replicates: 2 plates per concentration for the cytotox assay per strain and 3 plates per concentration per strain for the main assay.
    - Counting method: not reported.
    - Cytotoxic endpoints: reduction in background lawn.
    - Genetic toxicity endpoints/results: increase in number of revertants over the control.
    - Statistical methods: Dunnett's test.
- Other: the vehicle and positive control values were compared with historical data from the lab.

Bacterial Gene Mutation, Sepracor#190-811 (Cont.)

- Criteria for Positive Results: statistically significant and dose dependent increase in mean number of revertants per plate of at least one tester strain over the mean revertants per plate of the appropriate vehicle control.

Results:

- Study Validity: valid
- Study Outcome: negative.

Summary and Conclusion:

The R- , S-, and racemate-zopiclone were not mutagenic in the bacterial reverse gene mutation assay up to 5000ug/plate concentration in either the – or + of S9 using both pre-incubation and plate incorporation method.

Study Title: Bacterial reverse mutation test of zopiclone

Study No: Sepracor# 190-800 — 301-SE-001-94

Study Type: plate incorporation

Conducting Laboratory: —

GLP compliance: Yes/OECD; EPA, FDA

QA- Report Yes (x)

Date of Study Initiation/completion: April 1994/April 1994; study report 1997

Drug Lot Number: responsibility of sponsor.

Results:

- Study Validity: No, due to lack of assessment of TA100 as a result of microbial contamination and E.coli strain was not included in the assay.
- Study Outcome: negative.

Methods, Results, and Conclusion:
This study was conducted prior to the above (Sepracor#190-811), and by a different lab (— —). Same standard method as in the above study was used here including the bacterial strains tested with and without Aroclor-induced rat liver S9 however, only the plate incorporation method was tested. RS-zop in a prescreen assay was not toxic to either TA1538 or TA100. Therefore, concentrations tested in the main assay in -/+S9 were 50, 167, 500, 1670, 5000, and 10000ug/plate in TA98, TA100, TA1535, TA1537, and TA1538 (no E.coli was tested). The cpd was slightly insoluble at 10,000ug/ml and TA100 could not be scored in both the original (1st) assay and the repeat assay due to microbial contamination. In the 1st assay, a small 2.1x increase in revertants observed in TA1538/–S9 and a 2x increase in TA98/+S9. The assay was repeated for these 2 strains under identical conditions with all the doses and revertant frequency was comparable between drug and concurrent control values; all positive controls in both assays produced the anticipated positive responses. Therefore, **RS-zop up to 10,000ug/plate was negative** in the Ames bacterial reverse gene mutation in -/+ S9; note that TA100 could not be evaluated due to microbial contamination and, E.coli was not tested in this assay.

Study Title:In vitro mammalian cell cytogenetic test of RS-, R-, and S-zop in CHO cells

Study No: **Sepracor# 190-808/ — ; OTI00507**
Study Type: cytogenetic
Conducting Laboratory: —

GLP compliance: Yes/OECD
QA- Report Yes (x)
Date of Study Initiation/completion: Feb 1999/Apr 1999
Drug Lot Number: responsibility of sponsor.
Study Endpoint: increase in chromosomal aberrations above the corresponding control values.

Methodology:
- Strains/Species/Cell line: Chinese hamster ovary
- Dose Selection Criteria:
  - Basis of dose selection: cytotox assay.
  - Range finding studies: the data were not presented however, according to the protocol, 5 concentrations were tested in -/+S9, both vehicle and culture medium (untreated) controls were tested. Incubations in +S9 were for 3hrs and in –S9, they were 3+1.5 cell cycle, with a harvest time of 1.5 cell cycle.
- Test Agent Stability: stable
- Metabolic Activation System: S9 from Aroclor 1254-induced livers of male Fischer 344 rats.
- Controls:
  - Vehicle: DMSO
  - Negative Controls: vehicle control and culture medium (untreated).

- Positive Controls: MMC in absence of S9 and CP in presence of S9.
- Comments: two main assays were done.

- Exposure Conditions:
  - Incubation and sampling times: in +S9 incubations for 3hrs then cells were washed with fresh culture and incubation continued till cells are harvested on the next day. For -S9, treatment for 3hrs +1.5 cell cycle time, cells were harvested after 1.5cell cycle after end of culture treatment, centrifuged, fixed, and slides prepared for chromosome aberration scoring.
  - Doses used in definitive study: only 3 concentrations were tested: 240, 1200, and 2400ug/ml both in -/+S9 in experiments 1&2.
  - Study design:
  Doses were selected so that the maximum dose would produce 50-75% cytotox relative to the solvent control. If this level is not achieved, then the highest concentration of 5mg/ml or 10mM or the dose that exceeds the solubility limit, will be tested.
- Analysis:
  - No. slides/plates/replicates: 2/concentration for the drug and 4 for the solvent control.
  - Counting method: total of 200 cells will be scored per dose except for the solvent control 400 cells will be scored. The sponsor indicated that scoring may be terminated after a minimum of 25 cells have been scored where level of aberrations excluding gaps exceeds 30%. The sponsor stated that the scoring was done by an independent lab:

CHO cytogenetic assay, Sepracor#190-808 (Cont.)

  - Cytotoxic endpoints: measurement of total protein/Mitotic index inhibition.
  - Genetic toxicity endpoints/results: increase in frequency of chrom abs above the concurrent control.
  - Statistical methods: Fischer's exact test.
- Criteria for Positive Results: dose response with at least one point having statistical significance relative to the vehicle control ($p<0.01$) in both experiments.

Results:
- Study Validity: valid.
- Study Outcome: **positive.**

Comments:
For the 3 cpds, a ppt was observed in the medium at 2400ug/ml.
- Osmolality and pH were checked for medium control, solvent control and test article medium of both -/+S9. At the 2400ug/ml R-zop slight reduction in osmolality compared to solvent control was observed.
The sponsor stated that there were no extreme environmental conditions in any culuture.
Also note, that the results for the dose range finder were not reported. The sponsor indicated that the top concentration tested was 5000ug/plate in absence and presence of S9.

Summary and Conclusion:
S-zop:

Two experiments were done, based on the protocol, if negative or equivocal findings are seen in the 1st experiment, then a 2nd study with 2 harvest times will be done (immediately after treatment and 24hr later) with exposure for 1.5 cell cycle length in –S9. In the 1st experiment, the highest MI inhibition of 38% was seen at the highest concentration tested, 2400ug/ml. This inhibition is less than the 50% recommended by the OECD as well as that in the study protocol (inhibition of 50-70% of control). In experiment 1 (tables below from sponsor), in **–S9, there was a non dose dependent increase in numerical aberration (hyperploidy) and a 5.5 fold increase in chromosomal deletions in 2400ug/ml relative to the vehicle control** (this was mainly due to the increase seen in one of the 2 cultures (10 in culture A and only 1 in culture B). Also, percent of cells with aberrations at 2400ug/ml was 3.5 vs. 1.5 in the vehicle control. The findings were negative in +S9.

In experiment 2, a **significant but not dose dependent increase in percent of cells with aberrations was seen in –S9 in the cells collected immediately after treatment (4x control), and in +S9 at 2400ug/ml after 2nd harvest of 24hr post treatment** (3.5% vs. 0.5% in control or 7x; table below from sponsor). The chromatid deletions in the 2 highest concentrations 1st harvest in –S9, were increased dose dependently, over the vehicle control in both cultures (8&21 vs. 3 in culture A and, 16&23 vs. 8 in culture B for the vehicle control, 1200 & 2400ug/ml respectively; tables from sponsor). Also, polyploidy in experiment 2 at 2400ug/ml in –S9/2nd harvest was 15 vs. 9 in control. This was due to 10 vs. 4 in culture A and 5 vs. 5 polys in culture B. The sponsor indicated that the increase in percent of cells with aberrations in +S9/2nd harvest (3.5 vs. 0.5%), was caused by the exceptionally low vehicle control and that the value of 3.5% in the 2400ug/ml concentration was within the vehicle control data in the study of 1.5-7.5%. However, from the historical control table provided by the sponsor, the vehicle control value seems to be within the mean±s.d. of 1.88±1.54 which corresponds to 0.34-3.42 (lower and upper values), therefore, the control is acceptable.

CHO cytogenetic assay, Sepracor#190-808 (Cont.)

S-zop Data

Experiment 1

Dose	Total	Aberrant	Mitotic	Gaps	Chromatid		Chromosome		Multiple	Numerical aberrations			% cells with aberrations	
µg/ml	cells	cells	Index		deletion	exchange	deletion	exchange	aberrations	Poly	Endo	Hyper	with gaps	without gaps
Without Metabolic Activation														
240	200	9	4.05	2	2	0	5	3	0	7	0	3	4.5	3.5
1200	200	5	6.10	4	0	0	1	0	0	3	0	4	2.5	0.5
2400	200	10	3.45	3	3	0	11	2	0	6	0	2	5.0	3.5
Control	400	15	5.55	10	2	0	2	2	0	6	0	0	3.8	1.5
MMC	50	32	3.45	2	27	23	3	0	2	0	0	0	64.0	62.0
With Metabolic Activation														
240	200	23	2.50	3	3	1	0	19	1	5	1	8	11.5	10.5
1200	200	19	4.60	0	1	0	1	18	0	8	0	8	9.5	9.5
2400	200	9	4.35	1	0	1	0	7	0	3	0	0	4.5	4.0
Control	400	33	3.88	5	2	0	1	29	0	13	0	2	8.3	7.5
CPA	50	23	1.10	1	7	24	2	7	2	0	0	0	46.0	44.0

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CHO cytogenetic assay, Sepracor#190-808 (Cont.)

The following are aberration data by culture for S-zop:

Experiment 1 With S9

Dose	Total	Aberrant	Mitotic		Chromatid		Chromosome		Multiple	Numerical aberrations			% cells with aberrations	
µg/ml	cells	cells	Index	Gaps	deletion	exchange	deletion	exchange	aberrations	Poly	Endo	Hyper	with gaps	without gaps
With Metabolic Activation, Culture A														
240	100	4	3.00	0	0	0	0	5	0	2	0	0	4.0	4.0
1200	100	13	4.50	0	0	0	1	13	0	7	0	6	13.0	13.0
2400	100	5	3.80	1	0	0	0	4	0	1	0	0	5.0	4.0
Control	200	12	3.35	0	1	0	0	12	0	4	0	0	6.0	6.0
CPA	25	11	0.60	0	3	15	2	5	0	0	0	0	44.0	44.0
With Metabolic Activation, Culture B														
240	100	19	2.00	3	3	1	0	14	1	3	1	8	19.0	17.0
1200	100	6	4.70	0	1	0	0	5	0	1	0	2	6.0	6.0
2400	100	4	4.90	0	0	1	0	3	0	2	0	0	4.0	4.0
Control	200	21	4.40	5	1	0	1	17	0	9	0	2	10.5	9.0
CPA	25	12	1.60	1	4	9	0	2	2	0	0	0	48.0	44.0
[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	24.8
With Metabolic Activation, 1st Harvest														
240	200	15	3.30	5	2	0	1	7	0	1	0	1	7.5	5.0
1200	200	11	5.20	3	1	0	1	6	0	2	0	3	5.5	4.0
2400	200	11	6.05	3	2	0	0	6	0	0	0	1	5.5	4.0
Control	400	23	4.53	10	2	0	1	12	0	4	0	5	5.8	3.8
CPA	127	46	3.05	7	15	27	11	9	1	0	0	3	36.2	32.3
Without Metabolic Activation, 2nd Harvest														
2400	200	0	4.15	0	0	0	0	0	0	15	0	3	0.0	0.0
Control	400	2	3.63	0	0	0	0	2	0	9	0	5	0.5	0.5
With Metabolic Activation, 2nd Harvest														
2400	200	9	9.10	2	1	1	2	4	0	1	0	0	4.5	3.5
Control	400	4	6.98	2	4	0	0	1	0	5	0	3	1.0	0.5

Experiment 1 Without S9

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Dose	Total	Aberrant	Mitotic		Chromatid		Chromosome		Multiple	Numerical aberrations			% cells with aberrations	
µg/ml	cells	cells	Index	Gaps	deletion	exchange	deletion	exchange	aberrations	Poly	Endo	Hyper	with gaps	without gaps
Without Metabolic Activation, Culture A														
240	100	5	4.90	2	1	0	1	2	0	2	0	1	5.0	3.0
1200	100	3	5.80	2	0	0	1	0	0	1	0	2	3.0	1.0
2400	100	7	3.80	1	3	0	10	2	0	2	0	2	7.0	6.0
Control	200	6	6.70	5	1	0	1	0	0	2	0	0	3.0	1.0
MMC	25	15	2.80	1	9	8	3	0	1	0	0	0	60.0	56.0
Without Metabolic Activation, Culture B														
240	100	4	3.20	0	1	0	4	1	0	5	0	2	4.0	4.0
1200	100	2	6.40	2	0	0	0	0	0	2	0	2	2.0	0.0
2400	100	3	3.10	2	0	0	1	0	0	4	0	0	3.0	1.0
Control	200	9	4.40	5	1	0	1	2	0	4	0	0	4.5	2.0
MMC	25	17	4.10	1	18	15	0	0	1	0	0	0	68.0	68.0

CHO cytogenetic assay, Sepracor#190-808 (Cont.)

S-zop:
Experiement 2 With S9

Dose	Total	Aberrant	Mitotic		Chromatid		Chromosome		Multiple	Numerical aberrations			% cells with aberrations	
µg/ml	cells	cells	Index	Gaps	deletion	exchange	deletion	exchange	aberrations	Poly	Endo	Hyper	with gaps	without gaps
With Metabolic Activation, Culture A														
240	100	5	3.50	0	0	0	1	4	0	1	0	1	5.0	5.0
1200	100	6	5.50	1	1	0	0	4	0	1	0	1	6.0	5.0
2400	100	5	6.10	0	1	0	0	4	0	0	0	0	5.0	5.0
Control	200	9	4.10	6	2	0	0	3	0	1	0	3	4.5	2.5
BP	100	36	2.40	6	12	15	11	4	0	0	0	3	36.0	31.0
With Metabolic Activation, Culture B														
240	100	10	3.10	5	2	0	0	3	0	0	0	0	10.0	5.0
1200	100	5	4.90	2	0	0	1	2	0	1	0	2	5.0	3.0
2400	100	6	6.00	3	1	0	0	2	0	0	0	1	6.0	3.0
Control	200	14	4.95	4	0	0	1	9	0	3	0	2	7.0	5.0
BP	27	10	3.70	1	3	12	0	5	1	0	0	0	37.0	37.0
With Metabolic Activation, 2nd harvest culture A														
2400	100	7	9.20	1	1	1	2	3	0	0	0	0	7.0	6.0
Control	200	1	8.10	0	0	0	0	1	0	2	0	1	0.5	0.5
With Metabolic Activation, 2nd harvest culture B														
2400	100	2	9.00	1	0	0	0	1	0	1	0	0	2.0	1.0
Control	200	3	5.85	2	4	0	0	0	0	3	0	2	1.5	0.5

Experiment 2 Without S9

Dose	Total	Aberrant	Mitotic		Chromatid		Chromosome		Multiple	Numerical aberrations			% cells with aberrations	
µg/ml	cells	cells	Index	Gaps	deletion	exchange	deletion	exchange	aberrations	Poly	Endo	Hyper	with gaps	without gaps
Without Metaholic Activation, 1st Harvest Culture A														
240	100	11	3.60	1	3	6	0	3	0	2	0	1	11.0	10.0
1200	25	11	0.80	1	8	7	1	1	0	0	0	0	44.0	40.0
2400	100	22	1.90	4	21	2	0	2	1	3	0	0	22.0	20.0
Control	200	10	3.25	0	3	2	0	7	0	3	0	5	5.0	5.0
MMC	100	27	1.90	10	11	7	1	2	2	0	0	1	27.0	21.0
Without Metabolic Activation, 1st Harvest Culture B														
240	100	6	3.70	1	3	0	0	3	0	3	0	2	6.0	5.0
1200	25	10	0.20	1	16	0	0	1	0	1	0	0	40.0	40.0
2400	100	23	1.00	1	23	0	0	0	1	3	0	3	23.0	22.0
Control	200	13	3.00	3	8	1	0	3	1	4	0	7	6.5	6.0
MMC	25	11	2.70	2	9	5	0	1	1	0	0	0	44.0	40.0
Without Metabolic Activation, 2nd harvest culture A														
2400	100	0	5.20	0	0	0	0	0	0	10	0	1	0.0	0.0
Control	200	0	4.10	0	0	0	0	0	0	4	0	3	0.0	0.0
Without Metabolic Activation, 2nd harvest culture B														
2400	100	0	3.10	0	0	0	0	0	0	5	0	2	0.0	0.0
Control	200	2	3.15	0	0	0	0	2	0	5	0	2	1.0	1.0

CHO cytogenetic assay, Sepracor#190-808 (Cont.)

R-zop

R-zop was negative in experiment 1 in both -/+S9. However, in experiment 2, a 6.7x increase was seen in % cells with abs as well as a 6x increase in chromatid deletions at 2nd harvest in –S9 at 2400ug/ml. However, only 89 cells were evaluated, it is unclear why this is the case since there was no inhibition of MI (table from sponsor):

Dose	Total	Aberrant	Mitotic		Chromatid		Chromosome		Multiple	Numerical aberrations			% cells with aberrations		Relative growth
µg/ml	cells	cells	Index	Gaps	deletion	exchange	deletion	exchange	aberrations	Poly	Endo	Hyper	with gaps	without gaps	Sulforhodamine B
Without Metabolic Activation, 1st harvest															
240	200	17	1.75	6	5	3	0	7	0	4	0	0	8.5	6.0	93%
1200	3	2	0.00	1	2	0	0	0	0	0	0	0	66.7	33.3	91%
2400	27	7	0.05	3	5	0	0	0	2	0	0	0	25.9	14.8	108%
Control	400	25	2.93	4	6	2	0	15	0	0	0	1	6.3	5.3	100%
MMC	44	21	0.80	2	18	5	0	2	1	2	0	2	47.7	45.5	
With Metabolic Activation, 1st harvest															
240	200	20	3.20	2	2	1	2	14	0	1	0	8	10.0	9.0	110%
1200	200	14	2.05	0	4	2	0	10	0	0	0	3	7.0	7.0	57%
2400	200	13	2.30	0	3	0	1	10	0	5	0	5	6.5	6.5	50%
Control	400	26	1.95	3	4	1	0	20	0	5	0	6	6.5	6.0	100%
BP	50	20	0.60	0	8	12	1	6	0	0	0	0	40.0	40.0	
Without Metabolic Activation, 2nd harvest															
2400	89	20	2.40	7	19	1	0	1	0	1	0	4	22.5	18.0	
Control	255	12	2.38	9	3	1	0	3	0	6	0	5	4.7	2.7	
With Metabolic Activation, 2nd harvest															
2400	200	17	4.65	8	3	0	3	5	0	1	0	1	8.5	5.0	
Control	400	51	4.25	29	11	5	3	8	0	3	0	5	12.8	6.3	

RS-zop

There were increases in aberrant cells in both experiments that reached statistical significance (tables from sponsor).

Experiment 1 RS-zop

Dose	Total	Aberrant	Mitotic		Chromatid		Chromosome		Multiple	Numerical aberrations			% cells with aberrations		Relative growth
µg/ml	cells	cells	Index	Gaps	deletion	exchange	deletion	exchange	aberrations	Poly	Endo	Hyper	with gaps	without gaps	Sulforhodamine B
Without Metabolic Activation															
240	200	11	5.05	2	2	0	5	2	0	5	0	3	5.5	4.5	96%
1200	200	11	4.75	4	1	0	5	2	0	4	0	2	5.5	3.5	88%
2400	200	15	3.55	6	2	2	7	3	0	5	0	2	7.5	5.5	79%
Control	400	15	5.55	10	2	0	2	2	0	6	0	0	3.8	1.5	100%
MMC	50	32	3.45	2	27	23	3	0	2	0	0	0	64.0	62.0	
With Metabolic Activation															
240	200	17	3.85	0	0	2	0	16	0	9	0	6	8.5	8.5	101%
1200	200	16	4.10	0	1	0	0	17	0	6	0	6	8.0	8.0	106%
2400	200	24	4.55	1	3	5	5	15	1	7	0	4	12.0	11.5	124%
Control	400	33	3.88	5	2	0	1	29	0	13	0	2	8.3	7.5	100%
CPA	50	23	1.10	1	7	24	2	7	2	0	0	0	46.0	44.0	

CHO cytogenetic assay, Sepracor#190-808 (Cont.)

Experiment 2 RS-zop

Dose	Total	Aberrant	Mitotic	Gaps	Chromatid		Chromosome		Multiple	Numerical aberrations			% cells with aberrations	
µg/ml	cells	cells	Index		deletion	exchange	deletion	exchange	aberrations	Poly	Endo	Hyper	with gaps	without gaps
Without Metabolic Activation, 1st harvest														
240	200	34	1.10	7	23	7	0	11	0	7	1	3	17.0	16.0
1200	18	15	0.25	2	38	1	1	1	0	3	0	3	83.3	83.3
2400	200	31	0.75	2	18	5	2	7	2	5	0	3	15.5	14.5
Control	400	72	2.35	10	38	11	4	24	1	26	0	9	18.0	16.3
MMC	50	39	0.50	4	40	26	1	5	7	0	0	0	78.0	78.0
With Metabolic Activation, 1st harvest														
240	200	8	2.60	7	6	2	2	3	0	7	1	3	4.0	4.0
1200	125	22	3.60	4	4	1	1	15	0	2	0	1	17.6	15.2
2400	125	20	1.75	2	6	5	2	9	0	0	0	0	16.0	15.2
Control	400	36	2.23	13	6	1	1	19	0	3	0	0	9.0	6.3
BP	50	32	1.50	3	7	25	7	13	1	0	0	0	64.0	60.0
Without Metabolic Activation, 2nd harvest														
2400	50	19	0.85	5	17	4	4	3	2	1	0	0	38.0	36.0
Control	325	38	2.95	10	11	2	7	17	1	5	0	0	11.7	9.2
With Metabolic Activation, 2nd harvest														
2400	50	28	1.70	10	40	1	0	0	1	6	0	1	56.0	50.0
Control	400	36	3.33	17	16	2	0	3	1	29	0	7	9.0	5.5

Conclusions:

RS-, R-, and S-zop were tested for their potential to induce chromosomal aberrations in the CHO cells. The S-zop was clastogenic at the 2 highest concentrations in both experiments in –S9 and positive response in +S9 only in experiment 2. R-zop in –S9 was negative in experiment 1 but positive in experiment 2 at highest concentration, negative in +S9. RS-zop, showed increases in aberrant cells in both experiments in -/+S9 with clear response in -S9 Exp.1 and in +S9 Exp.2. However, no conclusion could be made in the 2nd harvest in both -/+S9 due to small number of cells. The sponsor indicated that additional study is recommended to further assess these findings for all 3 test cpds specially since only 3 concentrations were used and data in mid point could not be analyzed because of small number of cells.

Study Title:Chromosome aberrations in CHO cells with RS-, R-, and S-zop

Study No: **Sepracor# 190-815A1** — . **study#20960-0-437**
Study Type: Cytogenetic
Conducting lab: . .
GLP: Yes (x) OECD
QA: Yes (x) No ()
Study Initiation/Termination dates: Dec 1999/Mar 2000
Drug batch/lot# S-zopiclone 120998A; RS-zopiclone 9809002; R-zopiclone 121198A
Vehicle: DMSO for all 3 cpds
Negative/Positive controls: the vehicle control cultures were treated with 10ul/ml DMSO. The positive control in absence of S9 was MMC tested at 2 doses 0.75&1.5ug/ml for the 3hr treatment and 0.2&0.4ug/ml for the 17.5hr treatment. Cyclophosphamide (CP) was the positive control in presence of S9 and tested at 5&10ug/ml to induce chrom abs in CHO cells. Both CP & MMC were *dissolved in water*.

This assay is a repeat study in view of the positive findings in the above assay (— . study# OTI00507; Sepracor#190-808).

Methods:
Each test cpd was dissolved in DMSO to the limit of solubility which was found to be 1000ug/ml for all 3 cpds. There were 2 complete trials/assays in presence and absence of S9 with 4 analyzable concentrations each. In the 1st trial, treatment period was 3hr in both -/+S9 and cultures harvested 20hrs after treatment initiation. In the 2nd trial, treatment period was 17.5hr in -S9 and 3hrs in +S9 with all cultures harvested 20hrs after treatment initiation. The CHO cell line was derived from a single female Chinese hamster and the CHO cells in this assay were obtained from a permanent cell line and originally came from the lab of — . The CHO cells were grown in **McCoy's 5a** culture medium that was supplemented with standard nutrients. The S9 was obtained from rat livers induced with Aroclor™ (500mg/kg single dose). Replicate cultures (except for trial IIA with RS/+S9, where triplicate flasks were tested), were used for each concentration of test cpd, vehicle controls, and for each of 2 concentrations of positive control. The chrom ab assay was carried out according to OECD guidelines and the lab SOPs. Cytotoxicity was assessed by percent of cell confluency within the culture flasks and by percent inhibition of mitotic index relative to the concurrent controls. The latter was evaluated from vehicle control and test cpd concentrations by analyzing the # of mitotic cells in 1000 cells and the ratio expressed as % of mitotic cells. Chromosomal aberrations were analyzed using 100 cells per replicate or triplicate when possible, from all 3 test cpds, vehicle controls, and one dose of positive control. Polyploidy and endoreduplication were also analyzed by assessing at least 100 metaphases. All analyses were done blindly.

Assay Acceptance Criteria:
All of the following should be satisfied for an accepted assay:
(1)Negative/untreated controls and vehicle controls must contain <5% cells with abs and the positive controls should show statistically significant response ($p \leq 0.01$), relative to vehicle controls. (2) at least 3 analyzable concentrations should be available.

Positive Response:

Significant ($p \leq 0.01$) increase in # cells with chrom abs at 1 or more concentrations with dose response relationship. Statistical evaluation of % of cells with >1 abs provided indication of severity of the positive finding.

CHO repeat study, Sepracor#190-815A1 (Cont.)

Results:

All 3 test cpds and in both -/+ S9 in 2 trials, cytotoxicity was either not achieved or minimal and solubility (in DMSO), limited the use of higher concentrations. Therefore, the maximum concentration used in all cpds was up to 1000ug/ml in -/+S9. Except in one condition (see next), none of the 3 cpds, R-, S-, or RS-zopiclone, caused an increase in chrom abs in presence or absence of S9 up to 17.5hr treatment in -S9. Percent of cells with abs of RS-zop in trial II, +S9/3hr at 698ug/ml was **12.6x higher** than mean % cells with abs of the vehicle control (24% in culture B), also % cells with >1 abs was **1.7 vs. 0** in control (4% in culture B), and, % endoreduplication was **3 vs. 0** in vehicle control (5% in culture B)(table below). However, this increase was not dose dependent since the cpd was negative at the next higher concentration of 930ug/ml. Nevertheless, these positive findings occurred at minimal cytotoxicity with MI inhibition of 10&35% at 698&930ug/ml respectively. This assay was repeated using similar concentrations (total of 5 including 700&1000ug/ml), with no increase noted in chrom abs at any concentration relative to the vehicle control, again in absence of cytotoxicity up to 1000ug/ml (0 MI inhibition).

CHROMOSOME ABERRATIONS IN CHINESE HAMSTER OVARY CELLS

Cells Fixed 20.0 Hours After Initiation of Treatment, 3.0 Hour Treatment

Assay No.: 20960 Trial #: II Date: 01/10/00 Lab #: Metabolic Activation: +S9

Compound: (RS)-zopiclone

				CELLS SCORED	NUMBER AND TYPE OF ABERRATION: NOT COMPUTED TG	NOT COMPUTED SG	NOT COMPUTED UC	SIMPLE TB	SIMPLE SB	COMPLEX ID	COMPLEX TR	COMPLEX QR	COMPLEX CR	COMPLEX D	COMPLEX R	COMPLEX CI	COMPLEX DF	OTHER GT	# OF ABERRATIONS PER CELL	% CELLS WITH ABERRATIONS	% CELLS WITH >1 ABERRATIONS	% PP	% E
CONTROLS NEGATIVE:	McCoy's 5a		A100	5															0.00	0.0	0.0	1.0	1.0
			B100	2	1														0.00	0.0	0.0	1.0	1.0
			A+B200	7	1														0.00	0.0	0.0	1.0	1.0
VEHICLE:	DMSO	10.0µL/mL	A100	1	2									1		1			0.02	2.0	0.0	2.0	0.0
			B100	3															0.00	0.0	0.0	2.0	0.0
			A+B200	4	2									1		1			0.01	1.0	0.0	2.0	0.0
POSITIVE:	CP	5.00µg/mL	A25	1	2		4			1	2	3						1	0.60	40.0	8.0	1.0	1.0
			B25	2				2		3	5	3	1	1					0.60	44.0	16.0	1.0	1.0
			A+B50	3	2		4	2		4	7	6	1	1				1	0.60	42.0*	12.0*	1.0	1.0
TEST ARTICLE		233µg/mL	A100	3							2								0.02	2.0	0.0	3.0	3.0
			B100	4								1							0.01	1.0	0.0	1.0	2.0
			A+B200	7							2	1							0.02	1.5	0.0	2.0	2.5
		465µg/mL	A100																0.00	0.0	0.0	1.0	0.0
			B100	3	1						1								0.01	1.0	0.0	1.0	2.0
			A+B200	3	1						1								0.01	0.5	0.0	1.0	1.0
	→	698µg/mL	A100	2	1	1						1	1	2					0.04	4.0	0.0	1.0	1.0
			B75	8	2			13	1		1	6		2					0.31	24.0	4.0	1.0	3.0
			A+B175	10	3	1		13	1		1	7	1	4					0.15	12.6*	1.7	1.0	3.0
		930µg/mL	A100	9	2			5		2	4					1			0.12	7.0	3.0	2.0	1.0
			B100	8	2											1			0.01	1.0	0.0	1.0	2.0
			A+B200	17	4			5		2	4					2			0.07	4.0	1.5	1.5	1.5

* Significantly greater than the vehicle controls, $p \leq 0.01$.

Positive, vehicle, and negative controls produced the expected responses in trial II but exceeded historical range in 1 or more cultures in trial I as follows:

In **-S9 at 3hr** treatment and only in **trial I**, negative control culture A (%MI 15.2 vs. 3.2-14.9% historical range) and vehicle control culture B (%MI 20.3 vs. 2.4-16.2 historical control range)(table 1) were outside the historical range. These values were used for all 3 cpds in -S9 at 3hr treatment: RS-zop (table 1), R-zop (table 11), and S-zop (table 19). The positive control in **-S9 at 3hr** treatment, values for % cells with abs exceeded historical range (72 vs. 25.5-70 range)(table 20). This value of positive control was used for all 3 cpds in -S9 at 3hr treatment: RS-zop (table 2), R-zop (table 12), and S-zop (table 20).

CHO repeat study, Sepracor#190-815A1 (Cont.)

In **+S9 at 3hr** treatment **trial 1**, vehicle control culture A (%MI 16.4 vs. 1.9-15.3 historical range)(table 5) exceeded historical range. This value was used for all 3 cpds in +S9 at 3hr treatment trial 1: RS-zop (table 5), R-zop (table 15), and S-zop (table 23). The positive control values for the polyploidy in +S9 at 3hr treatment trial 1 exceeded historical range (10 vs. 0-6.5 range)(table 24). This positive control was also used for the S-zop (table 24) and RS-zop (table 6). All control values in trial II were within historical range.

Summary & Conclusion:
This is a repeat assay in view of the positive findings in the previous CHO assay (— # OTI00507; Sepracor#190-808). All 3 forms of zopiclone in this assay did not increase chrom abs, except in trial II/+S9 RS-zop at 1 concentration. It is noted that all 3 cpds lacked cytotoxicity in this assay. According to guidelines, in absence of cytotoxicity, higher concentrations of the cpd should be tested however, in this case cpd solubility prevented increasing the concentration above 1000ug/mL **Limited solubility prevented testing higher concentrations for all 3 cpds in this assay. It is unclear to this reviewer why cpd solubility was decreased when the same vehicle, DMSO, did not impose such limit on solubility in the previous CHO assay with concentrations up to 2400ug/ml. Positive clastogenic response was observed at 2400ug/ml and 1200ug/ml in that assay. It is of note that in this study 1 or more of the 3 controls under one or more experimental conditions, had values outside the historical range. Therefore, accurate conclusion on the results in this CHO assay can not be assessed and the study can be considered invalid. Justification for the limit of solubility of all 3 cpds in the current assay is needed.**

Study Title: L5178Y TK$^{+/-}$ mouse lymphoma forward mutation assay with a confirmatory assay with RS-, R-, and S-zopiclone

Study No: Sepracor# 190-816 — study# 20960-0-431 ICH
Conducting lab —
GLP: Yes (x); OECD, ICH S2B
QA: Yes (x)
Study Initiation/Termination dates: Nov 1999/Jan 2000
Drug batch/lot# S-zopiclone 120998A; RS-zopiclone 9809002; R-zopiclone 121198A
Vehicle: DMSO for all 3 cpds
Negative/Positive controls: the vehicle control was exposed to the same concentration of 1% as the test cultures. There were single vehicle control cultures in the range finder assay and 3 vehicle control cultures in the main assays. MMS is a direct acting mutagen, it was used in duplicate cultures at 13ug/ml in -S9 at 4hr incubation and at 6.5ug/ml in -S9 at the 24hr treatment. Methylcholanthrene (MC), needs activation by microsomal enzymes and was used at 2 & 4ug/ml as a positive control in +S9. The S9 was

obtained commercially → and derived from SD rats dosed with single 500mg/kg Aroclor 1254 as the inducer and the S9 was prepared 5days later.

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MLA Assay, Sepracor#190-816 (Cont.)

Methods:
A preliminary dose range finding study was done in -S9 (4&24hr treatment) and in +S9 (4hr treatment) using range of concentrations based on solubility and toxicity profile of the drugs. All 3 cpds showed a ppt at the top concentration and the latter was limited to 1000ug/ml (2000ug/ml for the RS-zopiclone 24hr treatment), which represents 2x the solubility limit in culture medium. Cytotoxicity was assessed based on 10-20% cell survival. A total of 2 assays in absence and presence of S9 were done, the conditions for the 1st initiation assay were as follows: vehicle controls in triplicate, 2 positive controls and 10 different dose levels at 1 culture per dose. Standard expression period of 2 days was allowed for mutant recovery, growth and expression of the $TK^{-/-}$ phenotype and cell densities were determined on Day 1 and adjusted to $3x10^5$ cells/ml in 20ml of growth medium. A total of $3x10^6$ cells from each tube were suspended in selection medium in ***soft agar*** to recover the TFT-resistant mutants. The mutant frequency (MF) was counted as the ratio (total number of mutant colonies / total viable colonies) x $2x10^{-4}$ and MF was given in units of 10^{-6}. If 1 dish in either set was lost due to contamination or other causes, the colony count of the missing dish was determined by a proportion equation based on the wts of the 3 dishes of the set and the colony counts in the 2 acceptable dishes. Cytotoxicity in the main assays was determined by the relative suspension growth of the cells over the 2 day expression period for the 4hr treatment or the relative suspension growth over the 3 day treatment and expression period for the 24hr treatment in the -S9 confirmatory assay, multiplied by the relative cloning efficiency at time of selection. As the sponsor indicates, the relative total growth (RTG) is not a clear measure of cell survival, it is used as a determinant of effectiveness of treatment and as basis for selecting doses for other future assays. The confirmatory assay was done with exposure for 24hr and treatment of 4hr and all other conditions identical to the initiation assay. Both small and large colonies were quantified for all cultures and a bimodal curve was generated and colonies quantified by AUCs (an Automated Colony Counter was used).

Assay Acceptance Criteria:
For vehicle controls: Cloning Efficiency (CE), should be between 60-130% a value >100% is acceptable due to errors in cell counts usually $\pm$10%. The normal range for background MF (mean of vehicle control values), is $30x10^{-6}$ to $120x10^{-6}$ anything outside this range is invalid.
Positive Controls: at least 1 of the positive control cultures in each trial should produce MF of at least $200x10^{-6}$.
Test cpd: any assay should include concentrations that reduce RTG to 10-20% of the mean vehicle control or achieve max concentration recommended. *This RTG requirement is waived if the concentration of the highest analyzable dose was at least 75% of a higher, excessively cytotoxic dose or if the highest concentration was at least 2x the solubility limit of the cpd in the culture medium.*

Criteria for a positive response:

Dose dependent increase of 2x or more in MF over the mean concurrent control cultures. The sponsor also indicates that it is "desirable" to obtain the 2x increase in at least 3 doses, but this depends on the dose steps selected and the cytotoxicity level where mutagenicity was observed. The dose dependency is waived if a 4x increase in MF is seen in a single dose at or near the top analyzable concentration. Such increase in MF has to be reproducible in a 2nd assay.

Results:

All 3 cpds produced a white opaque suspension around 100 and/or 200mg/ml in **DMSO**. In the treatment medium, RS-zop was soluble between 750-1000ug/ml, R-zop and S-zop were soluble up to 250ug/ml but formed a ppt between 375 and 1000ug/ml.

MLA Assay, Sepracor#190-816 (Cont.)

Dose Range Finding:

Cytotoxicity for all 3 cpds ranged between none to moderate in both presence and absence of S9. Since no cytotox was observed at the top concentration of 981ug/ml of RS-zop/4hr treatment in either -/+S9, the top concentration in the main assay was 2000ug/ml in -/+S9, which was 2x the solubility limit in the medium. Similarly, for the R-zop/4hr treatment in -/+S9, though no cytotoxicity was seen at the top concentration of 999ug/ml, this concentration was the max used in the main assays in both -/+S9, due to ppt seen at 500&999ug/ml. For the S-zop, top concentration in the range finding and the main assays for both -/+S9, was 1000ug/ml because of ppt seen at 500&1000ug/ml. The sponsor also conducted a dose range finding study in -S9 with 24hr treatment for all 3 cpds. The RS-zop concentration that did not show cytotxicity at the 4hr treatment in either -/+S9, showed high toxicity at 1000ug/ml and excessive toxicity at 2000ug/ml. The R-zop/24hr in -S9 showed high toxicity at 999ug/ml. The S-zop/24hr in -S9 showed no toxicity up to 999ug/ml.

Main Assays:

RS-zop/-S9 at 4hr (tables 7&8)/initial assay: No increase in MF or colony size relative to the negative control up to 2000ug/ml. However, in +S9/4hr, RS-zop **showed dose dependent increase in MF in 5 concentrations starting at 500ug/ml this increase specifically was reflected in small colony (tables 19&20). In the confirmatory assay/24hr (tables 13&14), the significant increase in MF was repeatable only in the top concentration of 500ug/ml in -S9 and dose dependent trend was also observed starting at 250ug/ml (again small colony reflected this increase in MF)(table 14).** In the confirmatory assay in +S9/4hr, the significant increase in MF was seen at top concentration of 2000ug/ml and 1000ug/ml but not significant at 1500ug/ml (tables 21&22). Nevertheless, a positive trend is observed from the 500ug/ml onward except at 1500ug/ml (table 21). Note all these findings occurred at acceptable level of cytotoxicity.

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MLA Assay, Sepracor#190-816 (Cont.)

E. TREATMENT DATE: 12/14/1999
F. CELLS ANALYZED: $3x10^6$
G. TREATMENT PERIOD: ~4 hours

TABLE 19: INITIAL MUTATION ASSAY WITH ACTIVATION WITH (RS)-ZOPICLONE

Test Condition	Daily Cell Counts (Cell/ML, 10E5 Units)		Cumulative RSG[a]		Total Mutant Colonies	Total Viable Colonies	Cloning Efficiency[b]		Relative Growth(%)[c]	Mutant Frequency (10E-6 Units)[d]
	Day 1	Day 2								
S9-Activation Controls[e] S9 Batch Number: 955			AVG VC				AVG VC			
Vehicle Control	11.2	14.1	17.5		179	508	84.7		106.7	70.4
Vehicle Control	10.3	16.3	18.7		185	479	79.8		106.9	77.4
Vehicle Control	10.0	12.3	13.7	16.6	171	521	86.9	83.8	85.2	65.7
MCA 2 µg/mL	8.6	12.7	12.1		753	407	67.8		59.1	370.0[f]
MCA 4 µg/mL	6.4	14.2	10.1		838[h]		LOST TO CONTAMINATION			
Test Compound µg/mL			Relative to Vehicle Control (%)				Relative to Vehicle Control (%)			
62.5	7.2	18.4	88.6		183	436[h]	86.7		76.8	84.0
125	6.2	16.8	69.6		228	388[h]	77.2		53.7	117.5
250	7.4	14.5	71.7		335	482[h]	95.9		68.8	138.9
500	7.6	11.9	60.5		388	513	102.0		61.6	151.5[f]
750	5.1	15.7	53.5		273[h]	372	74.0		39.6	146.6[f]
1000	6.5	9.1	39.5		355	424	84.4		33.4	167.1[f]
1500	4.0	7.5	20.1		308	359	71.4		14.3	171.4[f]
2000	4.5	6.3	19.0		332	306[h]	60.8		11.5	217.0[f]

[a]RSG = (Day 1 Count/3) * (Day 2 Count)/3 (or Day 1 Count if not subcultured)

[b]Cloning Efficiency = Total Viable Colony Count/Number of Cells Seeded * 100

[c]Relative Growth = (Relative Suspension Growth * Relative Cloning Efficiency) / 100

[d]Mutant Frequency = (Total Mutant Colonies/Total Viable Colonies) * 2x10E-4

Decimal is moved to express the frequency in units of 10E-6

[e]Vehicle Control = 1% DMSO

Positive Control: MCA = Methylcholanthrene

[f]Mutagenic. Exceeds Minimum Criterion of 142.4 X 10E-6

[h]One plate lost to contamination. Total counts calculated by using a weight proportion

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MLA Assay, Sepracor#190-816 (Cont.)

APPEARS THIS WAY
ON ORIGINAL

APPEARS THIS WAY
ON ORIGINAL

TABLE 20: SIZING DATA FOR INITIAL MUTATION ASSAY WITH ACTIVATION WITH (RS)-ZOPICLONE

E. TREATMENT DATE: 12/14/1999

Test Condition	Conc.	Cum. RSG (%)[a] Day 1	Cum. RSG (%)[a] Day 2	Cloning Efficiency[b] Abs %	Cloning Efficiency[b] Rel %	Relative Growth[c]	Mutant Frequency ($x10^{-6}$)[d] Total	Small	Large
Vehicle Control[e]									
	1%	106.7	105.6	84.7	101.1	106.7	70.4	38.2	32.2
	1%	98.1	112.2	79.8	95.2	106.9	77.4	46.0	31.4
	1%	95.2	82.2	86.9	103.7	85.2	65.7	33.9	31.8
MCA[f] (µg/mL)									
	2	81.9	73.0	67.8	80.9	59.1	370.0	249.9	120.1
	4	61.0	60.7	LOST TO CONTAMINATION					
Test Article(µg/mL)									
	62.5	68.6	88.6	72.7	86.7	76.8	84.0	49.0	35.0
	125	59.0	69.6	64.7	77.2	53.7	117.5	74.8	42.7
	250	70.5	71.7	80.4	95.9	68.8	138.9	113.5	25.3
	500	72.4	60.5	85.5	102.0	61.6	151.5	116.2	35.3
	750	48.6	53.5	62.0	74.0	39.6	146.6	116.4	30.2
	1000	61.9	39.5	70.7	84.4	33.4	167.1	130.6	36.5
	1500	38.1	20.1	59.8	71.4	14.3	171.4	136.2	35.3
	2000	42.9	19.0	50.9	60.8	11.5	217.0	197.1	20.0

[a]Cum. RSG = Cumulative Suspension Growth Relative to the Average Vehicle Control Suspension Growth

[b]Cloning Efficiency = Total Viable Colony Count/Number of Cells Seeded * 100

[c]Relative Growth = (Relative Suspension Growth * Relative Cloning Efficiency) / 100

[d]Mutant Frequency = (Total Mutant Colonies/Total Viable Colonies) * 2x10E-4

Decimal is moved to express the frequency in units of 10E-6

Expressed as Total Mutant Frequency, Small Colony Mutant Frequency and Large Colony Mutant Frequency

[e]Vehicle Control = DMSO

[f]Positive Control: MCA = Methylcholanthrene

Colony Counts increased by 9.099% to compensate for area of dish not scanned

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TABLE 13: CONFIRMATORY MUTATION ASSAY WITHOUT ACTIVATION – 24 HOUR TREATMENT WITH (RS)-ZOPICLONE

E. TREATMENT DATE: 12/14/1999
F. CELLS ANALYZED: $3x10^6$
G. TREATMENT PERIOD: ~24 hours
H. EXPRESSION PERIOD: 2 days

Test Condition	Daily Cell Counts (Cell/ML, 10E5 Units)			Cumulative RSG[a]		Total Mutant Colonies	Total Viable Colonies	Cloning Efficiency[b]		Relative Growth (%)[c]	Mutant Frequency (10E-6 Units)[d]
	Day 1	Day 2	Day 3								
Nonactivation Controls[e]					AVG VC				AVG VC		
Vehicle Control	13.2	9.4	9.6	44.1		140[h]	609	101.5		108.1	46.1
Vehicle Control	15.0	7.6	10.4	43.9		166	573	95.5		101.2	57.9
Vehicle Control	13.0	7.1	14.0	47.9	45.3	121	464	77.3	91.4	89.3	52.2
MMS 6.5 µg/mL	7.7	8.4	7.4	17.7		1043	LOST TO CONTAMINATION				
MMS 6.5 µg/mL	8.6	9.0	7.9	22.6		890	287	47.8		26.2	620.5[f]
Test Compound µg/mL				Relative to Vehicle Control (%)				Relative to Vehicle Control (%)			
15.7	11.6	7.7	12.4	90.6		165	484	88.3		80.0	68.0
31.3	9.1	10.0	14.1	104.9		140	429	78.2		82.0	65.1
62.5	11.1	9.3	14.3	120.7		122	398	72.6		87.6	61.4
125	9.0	8.5	14.2	88.8		123	412	75.2		66.8	59.8
250	8.2	5.5	12.9	47.6		153	413	75.4		35.9	73.9
375	4.2	7.4	12.9	32.8		176	367	66.8		21.9	95.8
500	6.1	3.8[g]	13.6	20.4		259	471	85.9		17.5	109.7[f]

[a]RSG = [Treatment termination (Day 1) cell density/$3x10^5$] x [Day 2 cell density/$3x10^5$ or Day 1 density if not split back] x [Day 3 cell density/$3x10^5$ or Day 2 density if not split back]

[b]Cloning Efficiency = Total Viable Colony Count/Number of Cells Seeded * 100

[c]Relative Growth = (Relative Suspension Growth * Relative Cloning Efficiency) / 100

[d]Mutant Frequency = (Total Mutant Colonies/Total Viable Colonies) * 2x10E-4
Decimal is moved to express the frequency in units of 10E-6

[e]Vehicle Control = 1% DMSO
Positive Control: MMS = Methyl methanesulfonate

[f]Mutagenic. Exceeds Minimum Criterion of 104.1 X 10E-6

[g]Not Subcultured

[h]One plate contaminated. Total counts calculated by using a weight proportion

TABLE 14: SIZING DATA FOR CONFIRMATORY MUTATION ASSAY WITHOUT ACTIVATION – 24 HOUR TREATMENT WITH (RS)-ZOPICLONE

E. TREATMENT DATE: 12/14/1999

Test Condition	Conc.	Cum. RSG (%)[a] Day 1	Day 2	Day 3	Cloning Efficiency[b] Abs %	Rel %	Relative Growth[c]	Mutant Frequency ($x10^{-6}$)[d] Total	Small	Large
Vehicle Control[e]										
	1%	96.1	112.7	97.4	101.5	111.0	108.1	46.1	23.5	22.5
	1%	109.2	103.5	96.9	95.5	104.4	101.2	57.9	31.2	26.7
	1%	94.7	83.8	105.7	77.3	84.5	89.3	52.2	30.1	22.1
MMS[f] (µg/mL)										
	6.5	56.1	58.7	39.1	LOST TO CONTAMINATION					
	6.5	62.6	70.3	50.0	47.8	52.3	26.2	620.5	449.4	171.1
Test Article(µg/mL)										
	15.7	84.5	81.1	90.6	80.7	88.3	80.0	68.0	32.0	36.0
	31.3	66.3	82.6	104.9	71.5	78.2	82.0	65.1	39.7	25.4
	62.5	80.8	93.7	120.7	66.4	72.6	87.6	61.4	35.6	25.8
	125	65.5	69.5	88.8	68.7	75.2	66.8	59.8	35.4	24.3
	250	59.7	41.0	47.6	68.9	75.4	35.9	73.9	42.7	31.1
	375	30.6	28.2	32.8	61.1	66.8	21.9	95.8	62.5	33.3
	500	44.4	21.0	20.4	78.6	85.9	17.5	109.7	69.9	39.8

[a]Cum. RSG = Cumulative Suspension Growth Relative to the Average Vehicle Control Suspension Growth

[b]Cloning Efficiency = Total Viable Colony Count/Number of Cells Seeded * 100

[c]Relative Growth = (Relative Suspension Growth * Relative Cloning Efficiency) / 100

[d]Mutant Frequency = (Total Mutant Colonies/Total Viable Colonies) * 2x10E-4

Decimal is moved to express the frequency in units of 10E-6

Expressed as Total Mutant Frequency, Small Colony Mutant Frequency and Large Colony Mutant Frequency

[e]Vehicle Control = DMSO

[f]Positive Control: MMS = Methyl methanesulfonate

Colony Counts increased by 9.099% to compensate for area of dish not scanned

APPEARS THIS WAY
ON ORIGINAL

TABLE 21: CONFIRMATORY MUTATION ASSAY WITH ACTIVATION WITH (RS) ZOPICLONE

E. TREATMENT DATE: 1/11/2000
F. CELLS ANALYZED: $3x10^6$
G. TREATMENT PERIOD: ~4 hours
H. EXPRESSION PERIOD: 2 days

Test Condition	Daily Cell Counts (Cell/ML, 10E5 Units)		Cumulative RSG[a]		Total Mutant Colonies	Total Viable Colonies	Cloning Efficiency[b]		Relative Growth (%)[c]	Mutant Frequency (10E-6 Units)[d]
	Day 1	Day 2								
S9-Activation Controls[e] S9 Batch Number: 955			AVG VC				AVG VC			
Vehicle Control	16.1	13.0	23.3		166	532	88.7		108.8	62.3
Vehicle Control	15.6	15.1	26.2		166	457	76.2		105.1	72.6
Vehicle Control	16.2	11.4	20.5	23.3	133	475	79.1	81.3	85.6	56.1
MCA 2 µg/mL	12.2	12.3	16.7		506	494	82.4		72.4	204.9[f]
MCA 4 µg/mL	11.0	14.0	17.1		580	476	79.3		71.5	244.0[f]
Test Compound µg/mL			Relative to Vehicle Control (%)				Relative to Vehicle Control (%)			
62.5	14.2	13.4	90.7		228	490	100.4		91.0	93.1
125	13.2	13.6	85.5		201	528	108.2		92.6	76.0
250	10.5	14.1	70.6		209	479	98.1		69.2	87.5
500	8.2	15.6	61.0		221	434	89.0		54.2	102.0
750	9.2	13.1	57.4		212	347	71.1		40.8	122.0
1000	7.9	13.2	49.7		226	338	69.3		34.4	133.5[f]
1500	6.1	11.6	33.7		202	335	68.6		23.1	120.5
2000	4.7	8.1	18.1		236	312	63.9		11.6	151.0[f]

[a]RSG = (Day 1 Count/3) * (Day 2 Count)/3 (or Day 1 Count if not subcultured)
[b]Cloning Efficiency = Total Viable Colony Count/Number of Cells Seeded * 100
[c]Relative Growth = (Relative Suspension Growth * Relative Cloning Efficiency) / 100
[d]Mutant Frequency = (Total Mutant Colonies/Total Viable Colonies) * 2x10E-4
Decimal is moved to express the frequency in units of 10E-6
[e]Vehicle Control = 1% DMSO
Positive Control: MCA = Methylcholanthrene
[f]Mutagenic. Exceeds Minimum Criterion of 127.3 X 10E-6

TABLE 22: SIZING DATA FOR CONFIRMATORY MUTATION ASSAY WITH ACTIVATION WITH (RS) ZOPICLONE

E. TREATMENT DATE: 1/11/2000

Test Condition	Conc.	Cum. RSG (%)[a]		Cloning Efficiency[b]		Relative Growth[c]	Mutant Frequency ($x10^{-6}$)[d]		
		Day 1	Day 2	Abs %	Rel %		Total	Small	Large
Vehicle Control[e]									
	1%	100.8	99.7	88.7	109.1	108.8	62.3	28.7	33.6
	1%	97.7	112.3	76.2	93.7	105.1	72.6	32.0	40.6
	1%	101.5	88.0	79.1	97.2	85.6	56.1	29.9	26.2
MCA[f] (µg/mL)									
	2	76.4	71.5	82.4	101.3	72.4	204.9	100.7	104.2
	4	68.9	73.4	79.3	97.5	71.5	244.0	121.6	122.5
Test Article(µg/mL)									
	62.5	88.9	90.7	81.6	100.4	91.0	93.1	45.9	47.2
	125	82.7	85.5	88.0	108.2	92.6	76.0	39.3	36.8
	250	65.8	70.6	79.8	98.1	69.2	87.5	46.5	41.0
	500	51.4	61.0	72.4	89.0	54.2	102.0	68.3	33.7
	750	57.6	57.4	57.8	71.1	40.8	122.0	74.2	47.8
	1000	49.5	49.7	56.4	69.3	34.4	133.5	86.5	47.1
	1500	38.2	33.7	55.8	68.6	23.1	120.5	81.4	39.1
	2000	29.4	18.1	52.0	63.9	11.6	151.0	109.8	41.3

[a]Cum. RSG = Cumulative Suspension Growth Relative to the Average Vehicle Control Suspension Growth

[b]Cloning Efficiency = Total Viable Colony Count/Number of Cells Seeded * 100

[c]Relative Growth = (Relative Suspension Growth * Relative Cloning Efficiency) / 100

[d]Mutant Frequency = (Total Mutant Colonies/Total Viable Colonies) * 2x10E-4

Decimal is moved to express the frequency in units of 10E-6

Expressed as Total Mutant Frequency, Small Colony Mutant Frequency and Large Colony Mutant Frequency

[e]Vehicle Control = DMSO

[f]Positive Control: MCA = Methylcholanthrene

Colony Counts increased by 9.099% to compensate for area of dish not scanned

APPEARS THIS WAY
ON ORIGINAL

The R-zop/-S9 at 4hr in initial assay, showed no increase in MF up to 999ug/ml. In the confirmatory assay in -S9/24hr (tables 15&16), though there was no significant increase in MF, note the 3rd vehicle control MF value was too high compared to the other 2 cultures (85.9 vs. 47.6&58.8; table 15). If this high value was not considered in the calculation, then MF would've shown a 2.6, 2.1, and 1.8 fold increase in MF at the top 3 concentrations of the R-zop with emphasis on small colony size (table 16).

TABLE 15: CONFIRMATORY MUTATION ASSAY WITHOUT ACTIVATION – 24- HOUR TREATMENT WITH (R)-ZOPICLONE

A. TEST ARTICLE: (R)-ZOPICLONE
B. GENETICS ASSAY NO.: 20960-0-431 ICH
C. VEHICLE: DMSO
D. SELECTIVE AGENT: TFT 3.0 µg/mL
E. TREATMENT DATE: 12/14/1999
F. CELLS ANALYZED: $3x10^6$
G. TREATMENT PERIOD: ~24 hours
H. EXPRESSION PERIOD: 2 days

Test Condition	Daily Cell Counts (Cell/ML, 10E5 Units)			Cumulative RSG[a]		Total Mutant Colonies	Total Viable Colonies	Cloning Efficiency[b]		Relative Growth (%)[c]	Mutant Frequency (10E-6 Units)[d]
	Day 1	Day 2	Day 3								
Nonactivation Controls[e]					AVG VC				AVG VC		
Vehicle Control	11.8	10.1	13.3	58.7		141[h]	591	98.6		96.3	47.6
Vehicle Control	11.5	9.5	18.1	73.2		172	586	97.6		119.0	58.8
Vehicle Control	11.3	8.6	20.1	72.3	68.1	177	411	68.6	88.2	82.5	85.9
MMS 6.5 µg/mL	8.8	9.0	12.3	36.1		983	285	47.5		28.5	690.4[f]
MMS 6.5 µg/mL	8.6	7.3	15.3	35.6		824	251	41.8		24.8	656.5[f]
Test Compound µg/mL				Relative to Vehicle Control (%)				Relative to Vehicle Control (%)			
31.3	9.2	9.6	21.0	100.9		175	504	95.2		96.0	69.3
62.5	11.2	9.1	14.5	80.4		157	463[i]	87.5		70.3	67.8
125	7.0	7.9	21.1	63.5		172	502	94.8		60.2	68.7
250	6.2	11.1	12.0	44.9		201	432	81.6		36.6	92.9
375	5.2	8.8	13.9	34.6		192	399	75.4		26.1	96.2
500	6.6	7.1	16.1	41.0		229	382	72.1		29.6	120.0
749	6.8	4.9	19.2	34.8		219	383	72.3		25.2	114.5
999	3.6[g]	9.9	11.9	19.2		224	467	88.2		17.0	95.8

[a]RSG = [Treatment termination (Day 1) cell density/$3x10^5$] x [Day 2 cell density/$3x10^5$ or Day 1 density if not split back] x [Day 3 cell density/$3x10^5$ or Day 2 density if not split back]

[b]Cloning Efficiency = Total Viable Colony Count/Number of Cells Seeded * 100

[c]Relative Growth = (Relative Suspension Growth * Relative Cloning Efficiency) / 100

[d]Mutant Frequency = (Total Mutant Colonies/Total Viable Colonies) * 2x10E-4

Decimal is moved to express the frequency in units of 10E-6

[e]Vehicle Control = 1% DMSO

Positive Control: MMS = Methyl methanesulfonate

[f]Mutagenic. Exceeds Minimum Criterion of 128.3 X 10E-6

[g]Not subcultured

[h]One plate contaminated. Total counts calculated by using a weight proportion

[i]One plate broken. Total counts calculated by using a weight proportion

TABLE 16: SIZING DATA FOR CONFIRMATORY MUTATION ASSAY WITHOUT ACTIVATION – 24-HOUR TREATMENT WITH (R)-ZOPICLONE

E. TREATMENT DATE: 12/14/1999

Test Condition	Conc.	Cum. RSG (%)[a]			Cloning Efficiency[b]		Relative Growth[c]	Mutant Frequency ($x10^{-6}$)[d]		
		Day 1	Day 2	Day 3	Abs %	Rel %		Total	Small	Large
Vehicle Control[e]										
	1%	102.3	109.8	86.2	98.6	111.7	96.3	47.6	23.8	23.8
	1%	99.7	100.7	107.5	97.6	110.6	119.0	58.8	33.5	25.3
	1%	98.0	89.5	106.2	68.6	77.7	82.5	85.9	54.1	31.8
MMS[f] (µg/mL)										
	6.5	76.3	73.0	53.0	47.5	53.8	28.5	690.4	495.0	195.4
	6.5	74.6	57.8	52.2	41.8	47.4	24.8	656.5	463.5	193.0
Test Article(µg/mL)										
	31.3	79.8	81.4	100.9	84.0	95.2	96.0	69.3	46.3	22.9
	62.5	97.1	93.9	80.4	77.2	87.5	70.3	67.8	34.9	33.0
	125	60.7	51.0	63.5	83.6	94.8	60.2	68.7	42.6	26.1
	250	53.8	63.4	44.9	72.0	81.6	36.6	92.9	52.5	40.4
	375	45.1	42.2	34.6	66.6	75.4	26.1	96.2	63.4	32.8
	500	57.2	43.2	41.0	63.6	72.1	29.6	120.0	73.7	46.3
	749	59.0	30.7	34.8	63.8	72.3	25.2	114.5	82.1	32.5
	999	31.2	27.4	19.2	77.8	88.2	17.0	95.8	58.9	36.9

[a]Cum. RSG = Cumulative Suspension Growth Relative to the Average Vehicle Control Suspension Growth

[b]Cloning Efficiency = Total Viable Colony Count/Number of Cells Seeded * 100

[c]Relative Growth = (Relative Suspension Growth * Relative Cloning Efficiency) / 100

[d]Mutant Frequency = (Total Mutant Colonies/Total Viable Colonies) * 2x10E-4

Decimal is moved to express the frequency in units of 10E-6

Expressed as Total Mutant Frequency, Small Colony Mutant Frequency and Large Colony Mutant Frequency

[e]Vehicle Control = DMSO

[f]Positive Control: MMS = Methyl methanesulfonate

Colony Counts increased by 9.099% to compensate for area of dish not scanned

In the initial assay of R-zop/4hr +S9, a dose dependent increase though not significant in MF was seen at 250ug/ml dose (tables 23, 24). In the confirmatory assay +S9/4hr (tables 25&26), there was no cytotoxicity up to 1000ug/ml and the increase in MF was thus not observed; higher concentrations than 1000ug/ml could not be tested due to a ppt.

TABLE 23: INITIAL MUTATION ASSAY WITH ACTIVATION WITH (R)-ZOPICLONE

A. TEST ARTICLE: (R)-ZOPICLONE
B. GENETICS ASSAY NO.: 20960-0-431 ICH
C. VEHICLE: DMSO
D. SELECTIVE AGENT: TFT 3.0 µg/mL
E. TREATMENT DATE: 12/14/1999
F. CELLS ANALYZED: $3x10^6$
G. TREATMENT PERIOD: ~4 hours
H. EXPRESSION PERIOD: 2 days

Test Condition	Daily Cell Counts (Cell/ML, 10E5 Units)		Cumulative RSG[a]		Total Mutant Colonies	Total Viable Colonies	Cloning Efficiency[b]		Relative Growth(%)[c]	Mutant Frequency (10E-6 Units)[d]
	Day 1	Day 2								
S9-Activation Controls[e] S9 Batch Number: 955			AVG VC				AVG VC			
Vehicle Control	10.4	12.8	14.8		168	502[g]	83.7		111.9	66.9
Vehicle Control	7.8	12.6	10.9		191	578	96.4		95.1	66.0
Vehicle Control	9.2	10.6	10.8	12.2	161[g]	555[g]	92.5	90.9	90.5	58.2
MCA 2 µg/mL	5.1	12.5	7.1		C	514[g]	85.6		54.8	C
MCA 4 µg/mL	5.4	10.7	6.4		907	554	92.4		53.6	327.2[f]
Test Compound µg/mL			Relative to Vehicle Control (%)				Relative to Vehicle Control (%)			
7.85	10.4	11.1	105.3		230	587	107.7		113.4	78.4
15.7	6.7	20.4	124.7		163	464	85.1		106.0	70.1
31.3	9.8	11.6	103.7		238	540	99.1		102.7	88.1
62.5	7.8	13.4	95.3		213	578	106.1		101.1	73.7
125	9.6	9.8	85.8		298	609[g]	111.7		95.8	97.8
250	7.7	12.6	88.5		219	529	97.1		85.9	82.9
500	5.6	13.4	68.4		227	425	78.1		53.4	106.7
999	4.6	5.6	23.5		227	393	72.0		16.9	115.6

[a]RSG = (Day 1 Count/3) * (Day 2 Count)/3 (or Day 1 Count if not subcultured)
[b]Cloning Efficiency = Total Viable Colony Count/Number of Cells Seeded * 100
[c]Relative Growth = (Relative Suspension Growth * Relative Cloning Efficiency) / 100
[d]Mutant Frequency = (Total Mutant Colonies/Total Viable Colonies) * 2x10E-4
Decimal is moved to express the frequency in units of 10E-6
[e]Vehicle Control = 1% DMSO
Positive Control: MCA = Methylcholanthrene
[f]Mutagenic. Exceeds Minimum Criterion of 127.4 X 10E-6
C = contaminated
[g]One plate lost to contamination. Total counts calculated by using a weight proportion

TABLE 24: SIZING DATA FOR INITIAL MUTATION ASSAY WITH ACTIVATION WITH (R)-ZOPICLONE

A. TEST ARTICLE: (R)-ZOPICLONE
B. GENETICS ASSAY NO.: 20960-0-431 ICH
C. VEHICLE: DMSO
D. SELECTIVE AGENT: TFT 3.0 µg/mL
E. TREATMENT DATE: 12/14/1999

Test Condition	Conc.	Cum. RSG (%)[a] Day 1	Cum. RSG (%)[a] Day 2	Cloning Efficiency[b] Abs %	Cloning Efficiency[b] Rel %	Relative Growth[c]	Mutant Frequency ($x10^{-6}$)[d] Total	Small	Large
Vehicle Control[e]									
	1%	113.9	121.4	83.7	92.2	111.9	66.9	29.5	37.3
	1%	85.4	89.6	96.4	106.1	95.1	66.0	43.0	23.0
	1%	100.7	88.9	92.5	101.8	90.5	58.2	32.0	26.2
MCA[f] (µg/mL)									
	2	55.8	58.1	85.6	94.3	54.8	CONTAMINATED		
	4	59.1	52.7	92.4	101.7	53.6	327.2	198.4	128.7
Test Article(µg/mL)									
	7.85	113.9	105.3	97.8	107.7	113.4	78.4	50.6	27.9
	15.7	73.4	124.7	77.3	85.1	106.0	70.1	51.3	18.8
	31.3	107.3	103.7	90.0	99.1	102.7	88.1	56.2	31.9
	62.5	85.4	95.3	96.4	106.1	101.1	73.7	48.6	25.1
	125	105.1	85.8	101.5	111.7	95.8	97.8	53.8	44.1
	250	84.3	88.5	88.2	97.1	85.9	82.9	62.3	20.6
	500	61.3	68.4	70.9	78.1	53.4	106.7	67.7	39.0
	999	50.4	23.5	65.5	72.0	16.9	115.6	75.0	40.6

[a]Cum. RSG = Cumulative Suspension Growth Relative to the Average Vehicle Control Suspension Growth
[b]Cloning Efficiency = Total Viable Colony Count/Number of Cells Seeded * 100
[c]Relative Growth = (Relative Suspension Growth * Relative Cloning Efficiency) / 100
[d]Mutant Frequency = (Total Mutant Colonies/Total Viable Colonies) * 2x10E-4
Decimal is moved to express the frequency in units of 10E-6
Expressed as Total Mutant Frequency, Small Colony Mutant Frequency and Large Colony Mutant Frequency
[e]Vehicle Control = DMSO
[f]Positive Control: MCA = Methylcholanthrene
Colony Counts increased by 9.099% to compensate for area of dish not scanned

TABLE 25: CONFIRMATORY MUTATION ASSAY WITH ACTIVATION WITH (R)-ZOPICLONE

A. TEST ARTICLE: (R)-ZOPICLONE
B. GENETICS ASSAY NO.: 20960-0-431 ICH
C. VEHICLE: DMSO
D. SELECTIVE AGENT: TFT 3.0 µg/mL
E. TREATMENT DATE: 1/11/2000
F. CELLS ANALYZED: 3x10[6]
G. TREATMENT PERIOD: ~4 hours
H. EXPRESSION PERIOD: 2 days

Test Condition	Daily Cell Counts (Cell/ML, 10E5 Units)		Cumulative RSG[a]		Total Mutant Colonies	Total Viable Colonies	Cloning Efficiency[b]		Relative Growth (%)[c]	Mutant Frequency (10E-6 Units)[d]
	Day 1	Day 2								
S9-Activation Controls[e] S9 Batch Number: 955			AVG VC				AVG VC			
Vehicle Control	12.4	13.5	18.6		160	612	102.0		84.4	52.4
Vehicle Control	13.9	19.0	29.3		152	507	84.6		110.3	59.8
Vehicle Control	14.0	15.7	24.4	24.1	166	559	93.1	93.2	101.1	59.4
MCA 2 µg/mL	11.0	21.5	26.3		455	492	82.0		95.8	184.9[f]
MCA 4 µg/mL	11.3	15.1	19.0		613	592	98.7		83.2	207.0[f]
Test Compound µg/mL			Relative to Vehicle Control (%)				Relative to Vehicle Control (%)			
7.85	19.0	14.7	128.7		132	458	81.9		105.4	57.6
15.7	11.8	19.0	103.3		172	736	131.7		136.0	46.8
31.3	13.2	17.9	108.8		146	600	107.3		116.8	48.7
62.5	12.7	19.1	111.7		153	599	107.1		119.7	51.0
125	14.8	15.1	102.9		193	531	95.0		97.8	72.7
250	10.7	18.3	90.2		175	526	94.0		84.8	66.4
500	12.3	17.2	97.4		168	339	60.7		59.1	99.0
1000	11.2	11.1	57.3		194	603	107.9		61.8	64.4

No cytotox

[a]RSG = (Day 1 Count/3) * (Day 2 Count)/3 (or Day 1 Count if not subcultured)
[b]Cloning Efficiency = Total Viable Colony Count/Number of Cells Seeded * 100
[c]Relative Growth = (Relative Suspension Growth * Relative Cloning Efficiency) / 100
[d]Mutant Frequency = (Total Mutant Colonies/Total Viable Colonies) * 2x10E-4
Decimal is moved to express the frequency in units of 10E-6
[e]Vehicle Control = 1% DMSO
Positive Control: MCA = Methylcholanthrene
[f]Mutagenic. Exceeds Minimum Criterion of 114.4 X 10E-6

TABLE 26: SIZING DATA FOR MUTATION ASSAY WITH ACTIVATION WITH (R)-ZOPICLONE

E. TREATMENT DATE: 1/11/2000

Test Condition	Conc.	Cum. RSG (%)[a] Day 1	Cum. RSG (%)[a] Day 2	Cloning Efficiency[b] Abs %	Cloning Efficiency[b] Rel %	Relative Growth[c]	Mutant Frequency ($x10^{-6}$)[d] Total	Mutant Frequency ($x10^{-6}$)[d] Small	Mutant Frequency ($x10^{-6}$)[d] Large
Vehicle Control[e]									
	1%	92.3	77.1	102.0	109.4	84.4	52.4	27.1	25.3
	1%	103.5	121.6	84.6	90.7	110.3	59.8	27.1	32.7
	1%	104.2	101.2	93.1	99.9	101.1	59.4	37.5	21.9
MCA[f] (µg/mL)									
	2	81.9	108.9	82.0	88.0	95.8	184.9	81.2	103.8
	4	84.1	78.6	98.7	105.9	83.2	207.0	84.0	123.0
Test Article(µg/mL)									
	7.85	141.4	128.7	76.4	81.9	105.4	57.6	35.2	22.4
	15.7	87.8	103.3	122.7	131.7	136.0	46.8	22.2	24.6
	31.3	98.3	108.8	100.0	107.3	116.8	48.7	28.7	20.0
	62.5	94.5	111.7	99.8	107.1	119.7	51.0	24.8	26.2
	125	110.2	102.9	88.6	95.0	97.8	72.7	45.2	27.5
	250	79.7	90.2	87.6	94.0	84.8	66.4	33.2	33.2
	500	91.6	97.4	56.5	60.7	59.1	99.0	61.7	37.3
	1000	83.4	57.3	100.6	107.9	61.8	64.4	37.3	27.1

[a]Cum. RSG = Cumulative Suspension Growth Relative to the Average Vehicle Control Suspension Growth

[b]Cloning Efficiency = Total Viable Colony Count/Number of Cells Seeded * 100

[c]Relative Growth = (Relative Suspension Growth * Relative Cloning Efficiency) / 100

[d]Mutant Frequency = (Total Mutant Colonies/Total Viable Colonies) * 2x10E-4

Decimal is moved to express the frequency in units of 10E-6

Expressed as Total Mutant Frequency, Small Colony Mutant Frequency and Large Colony Mutant Frequency

[e]Vehicle Control = DMSO

[f]Positive Control: MCA = Methylcholanthrene

Colony Counts increased by 9.099% to compensate for area of dish not scanned

There was no increase in MF of S-zop in -S9 in both the initial and confirmatory assays but noted, was the little cytotoxicity in the -S9/4hr treatment. However, in +S9 initial assay (table 27), there was dose dependent increase in MF starting at 125ug/ml with increases in small colony (table 28). Though a dose dependent increase in MF was observed in the confirmatory assay in +S9 (tables 29 & 30) at the same concentrations tested, this increase was small with only 1.7x increase over the mean MF of the concurrent vehicle control at the top concentration of 1000ug/ml.

MLA Assay, Sepracor#190-816 (Cont.)

TABLE 27: INITIAL MUTATION ASSAY WITH ACTIVATION WITH (S)-ZOPICLONE

TEST ARTICLE: (S)-ZOPICLONE
GENETICS ASSAY NO.: 20960-0-431 ICH
VEHICLE: DMSO
SELECTIVE AGENT: TFT 3.0 µg/mL

E. TREATMENT DATE: 12/14/1999
F. CELLS ANALYZED: $3x10^6$
G. TREATMENT PERIOD: ~4 hours
H. EXPRESSION PERIOD: 2 days

Test Condition	Daily Cell Counts (Cell/ML, 10E5 Units)		Cumulative RSG[a]		Total Mutant Colonies	Total Viable Colonies	Cloning Efficiency[b]		Relative Growth(%)[c]	Mutant Frequency (10E-6 Units)[d]
	Day 1	Day 2								
-Activation Controls[e] 39 Batch Number: 955				AVG VC				AVG VC		
Vehicle Control	9.4	21.3	22.2		166	549	91.5		111.4	60.4
Vehicle Control	9.7	19.1	20.6		158	469	78.2		88.1	67.4
Vehicle Control	10.4	17.8	20.6	21.1	170	538	89.6	86.4	100.9	63.3
MCA 2 µg/mL	5.8	16.3	10.5		576	467	77.8		44.8	246.7[f]
MCA 4 µg/mL	7.9	16.7	14.7		666	664	110.7		88.9	200.3[f]
Test Compound µg/mL			Relative to Vehicle Control (%)				Relative to Vehicle Control (%)			
3.93	7.9	20.4	84.7		157	495	95.5		80.9	63.4
7.85	9.9	16.8	87.4		199	620	119.5		104.5	64.1
15.7	9.6	19.2	96.9		185	501	96.6		93.6	74.1
62.5	9.2	16.0	77.4		235	535	103.1		79.8	87.8
125	10.3	16.5	89.4		195[g]	472	91.1		81.4	82.4
250	6.6	19.7	68.4		265	464	89.4		61.1	114.4
500	6.8	14.5	51.8		326	443[g]	85.4		44.3	147.3[f]
999	5.7	9.3	27.9		355	425	82.0		22.9	166.7[f]

:SG = (Day 1 Count/3) * (Day 2 Count)/3 (or Day 1 Count if not subcultured)
:loning Efficiency = Total Viable Colony Count/Number of Cells Seeded * 100
:elative Growth = (Relative Suspension Growth * Relative Cloning Efficiency) / 100
/lutant Frequency = (Total Mutant Colonies/Total Viable Colonies) * 2x10E-
Decimal is moved to express the frequency in units of 10E-6
'ehicle Control = 1% DMSO
Positive Control: MCA = Methylcholanthrene
lutagenic. Exceeds Minimum Criterion of 127.4 X 10E-6
)ne plate lost to contamination. Total counts calculated by using a weight proportion

TABLE 28: SIZING DATA FOR INITIAL MUTATION ASSAY WITH ACTIVATION WITH (S)-ZOPICLONE

A. TEST ARTICLE: (S)-ZOPICLONE
B. GENETICS ASSAY NO.: 20960-0-431 ICH
C. VEHICLE: DMSO
D. SELECTIVE AGENT: TFT 3.0 µg/mL
E. TREATMENT DATE: 12/14/1999

Test Condition	Conc.	Cum. RSG (%)[a]		Cloning Efficiency[b]		Relative Growth[c]	Mutant Frequency ($x10^{-6}$)[d]		
		Day 1	Day 2	Abs %	Rel %		Total	Small	Large
Vehicle Control[e]									
	1%	95.6	105.3	91.5	105.8	111.4	60.4	39.0	21.5
	1%	98.6	97.4	78.2	90.5	88.1	67.4	40.0	27.4
	1%	105.8	97.3	89.6	103.7	100.9	63.3	36.5	26.8
MCA[f] (µg/mL)									
	2	59.0	49.7	77.8	90.0	44.8	246.7	124.3	122.4
	4	80.3	69.4	110.7	128.1	88.9	200.3	113.6	86.7
Test Article(µg/mL)									
	3.93	80.3	84.7	82.6	95.5	80.9	63.4	40.1	23.3
	7.85	100.7	87.4	103.3	119.5	104.5	64.1	35.2	28.9
	15.7	97.6	96.9	83.5	96.6	93.6	74.1	47.5	26.6
	62.5	93.6	77.4	89.1	103.1	79.8	87.8	58.8	29.0
	125	104.7	89.4	78.7	91.1	81.4	82.4	51.8	30.6
	250	67.1	68.4	77.3	89.4	61.1	114.4	80.9	33.4
	500	69.2	51.8	73.8	85.4	44.3	147.3	101.0	46.3
	999	58.0	27.9	70.9	82.0	22.9	166.7	134.4	32.3

[a]Cum. RSG = Cumulative Suspension Growth Relative to the Average Vehicle Control Suspension Growth
[b]Cloning Efficiency = Total Viable Colony Count/Number of Cells Seeded * 100
[c]Relative Growth = (Relative Suspension Growth * Relative Cloning Efficiency) / 100
[d]Mutant Frequency = (Total Mutant Colonies/Total Viable Colonies) * 2x10E-4
Decimal is moved to express the frequency in units of 10E-6
Expressed as Total Mutant Frequency, Small Colony Mutant Frequency and Large Colony Mutant Frequency
[e]Vehicle Control = DMSO
[f]Positive Control: MCA = Methylcholanthrene
Colony Counts increased by 9.099% to compensate for area of dish not scanned

TABLE 29: CONFIRMATORY MUTATION ASSAY WITH ACTIVATION WITH (S)-ZOPICLONE

A. TEST ARTICLE: (S)-ZOPICLONE
B. GENETICS ASSAY NO.: 20960-0-431 ICH
C. VEHICLE: DMSO
D. SELECTIVE AGENT: TFT 3.0 µg/mL

E. TREATMENT DATE: 1/11/2000
F. CELLS ANALYZED: $3x10^6$
G. TREATMENT PERIOD: ~4 hours
H. EXPRESSION PERIOD: 2 days

Test Condition	Daily Cell Counts (Cell/ML, 10E5 Units)		Cumulative RSG[a]		Total Mutant Colonies	Total Viable Colonies	Cloning Efficiency[b]		Relative Growth (%)[c]	Mutant Frequency (10E-6 Units)[d]
	Day 1	Day 2								
S9-Activation Controls[e] S9 Batch Number: 955				AVG VC				AVG VC		
Vehicle Control	19.5	14.0	30.3		180	565	94.2		103.0	63.7
Vehicle Control	17.7	16.0	31.5		173	550	91.6		104.0	63.1
Vehicle Control	18.7	13.4	27.8	29.9	204	555	92.6	92.8	92.9	73.5
MCA 2 µg/mL	13.6	17.0	25.7		532	497	82.9		76.8	214.0[f]
MCA 4 µg/mL	8.9	16.2	16.0		656	495	82.6		47.7	264.8[f]
Test Compound µg/mL			Relative to Vehicle Control (%)				Relative to Vehicle Control (%)			
7.85	22.1	12.9	106.0		197	527	94.6		100.3	74.9
15.7	21.2	15.7	123.8		165	443	79.6		98.5	74.4
31.3	19.9	15.2	112.5		178	445	79.9		89.9	79.9
62.5	15.6	13.8	80.1		188	527	94.6		75.8	71.2
125	20.7	11.3	87.0		228	515	92.5		80.4	88.6
250	13.5	16.0	80.3		260	550	98.8		79.3	94.4
500	16.2	12.4	74.7		236	417	74.9		55.9	113.1
1000	10.0	7.5	27.9		269	466	83.7		23.3	115.7

[a]RSG = (Day 1 Count/3) * (Day 2 Count)/3 (or Day 1 Count if not subcultured)
[b]Cloning Efficiency = Total Viable Colony Count/Number of Cells Seeded * 100
[c]Relative Growth = (Relative Suspension Growth * Relative Cloning Efficiency) / 100
[d]Mutant Frequency = (Total Mutant Colonies/Total Viable Colonies) * 2x10E-4
Decimal is moved to express the frequency in units of 10E-6
[e]Vehicle Control = 1% DMSO
Positive Control: MCA = Methylcholanthrene
[f]Mutagenic. Exceeds Minimum Criterion of 133.5 X 10E-6

MLA Assay, Sepracor#190-816 (Cont.)

TABLE 30: SIZING DATA FOR CONFIRMATORY MUTATION ASSAY WITH ACTIVATION WITH (S)-ZOPICLONE

A. TEST ARTICLE: (S)-ZOPICLONE
B. GENETICS ASSAY NO.: 20960-0-431 ICH
C. VEHICLE: DMSO
D. SELECTIVE AGENT: TFT 3.0 µg/mL
E. TREATMENT DATE: 1/11/2000

Test Condition	Conc.	Cum. RSG (%)[a] Day 1	Cum. RSG (%)[a] Day 2	Cloning Efficiency[b] Abs %	Cloning Efficiency[b] Rel %	Relative Growth[c]	Mutant Frequency ($x10^{-6}$)[d] Total	Mutant Frequency ($x10^{-6}$)[d] Small	Mutant Frequency ($x10^{-6}$)[d] Large
Vehicle Control[e]									
	1%	104.7	101.5	94.2	101.5	103.0	63.7	40.9	22.8
	1%	95.0	105.3	91.6	98.8	104.0	63.1	43.3	19.8
	1%	100.4	93.2	92.6	99.7	92.9	73.5	38.9	34.6
MCA[f] (µg/mL)									
	2	73.0	86.0	82.9	89.4	76.8	214.0	91.7	122.4
	4	47.8	53.6	82.6	89.0	47.7	264.8	140.1	124.7
Test Article(µg/mL)									
	7.85	118.6	106.0	87.8	94.6	100.3	74.9	36.9	38.1
	15.7	113.8	123.8	73.8	79.6	98.5	74.4	38.9	35.5
	31.3	106.8	112.5	74.2	79.9	89.9	79.9	39.2	40.7
	62.5	83.7	80.1	87.8	94.6	75.8	71.2	36.0	35.2
	125	111.1	87.0	85.8	92.5	80.4	88.6	48.7	39.8
	250	72.5	80.3	91.6	98.8	79.3	94.4	67.9	26.6
	500	86.9	74.7	69.5	74.9	55.9	113.1	86.4	26.7
	1000	53.7	27.9	77.6	83.7	23.3	115.7	83.8	31.9

[a]Cum. RSG = Cumulative Suspension Growth Relative to the Average Vehicle Control Suspension Growth
[b]Cloning Efficiency = Total Viable Colony Count/Number of Cells Seeded * 100
[c]Relative Growth = (Relative Suspension Growth * Relative Cloning Efficiency) / 100
[d]Mutant Frequency = (Total Mutant Colonies/Total Viable Colonies) * 2x10E-4
Decimal is moved to express the frequency in units of 10E-6
Expressed as Total Mutant Frequency, Small Colony Mutant Frequency and Large Colony Mutant Frequency
[e]Vehicle Control = DMSO
[f]Positive Control: MCA = Methylcholanthrene
Colony Counts increased by 9.099% to compensate for area of dish not scanned

HISTORICAL CONTROL DATA

Nonactivation Studies

Pooled negative and vehicle control mutant frequencies

Mean (± SD)	$53.0 \pm 22.0 \times 10^{-6}$
Range	20.5 to 114.8×10^{-6}
Number of experiments	52
Number of controls	156

Positive control mutant frequencies (5.0 nl/mL methyl methanesulfonate)

Mean (± SD)	$272.7 \pm 135.7 \times 10^{-6}$
Range	115.9 to 632.1×10^{-6}
Number of experiments	51
Number of controls	51

Positive control mutant frequencies (10.0 nl/mL methyl methanesulfonate)

Mean (± SD)	$483.9 \pm 315.2 \times 10^{-6}$
Range	176.1 to 1996.4×10^{-6}
Number of experiments	52
Number of controls	52

Activation Studies

Pooled negative and vehicle control mutant frequencies

Mean (± SD)	$65.3 \pm 27.1 \times 10^{-6}$
Range	27.6 to 150.3×10^{-6}
Number of experiments	54
Number of controls	162

Positive control mutant frequencies (2.0 µg/mL 3-methylcholanthrene)

Mean (± SD)	$454.5 \pm 166.2 \times 10^{-6}$
Range	204.8 to 787.8×10^{-6}
Number of experiments	54
Number of controls	54

Positive control mutant frequencies (4.0 µg/mL 3-methylcholanthrene)

Mean (± SD)	$567.0 \pm 248.3 \times 10^{-6}$
Range	218.9 to 1414.9×10^{-6}
Number of experiments	54
Number of controls	54

Because some experiments contained multiple controls, the number of independent control cultures exceeded the number of experiments.

Summary and Conclusion:

The RS-zopiclone produced a concentration dependent and reproducible positive response (increase in MF), in the **presence of metabolic activation** in both the initial and main assays. RS-zop was not mutagenic nor was it clastogenic in -S9 at 4hr in the initial assay but a positive response was observed in the confirmatory assay in -S9/24hr (dose dependent but significant only at the top concentration).

The R-zopiclone was negative in -S9 at the 4 and 24hr treatment periods but noted was the 3rd outlier value of the negative control that raised the mean of the vehicle control causing a negative response with the test cpd. Otherwise, a positive trend was seen at the higher doses with 1.8-2.6 fold increase in MF over the vehicle control. The R-zopiclone although did not meet the sponsor's criteria for a positive response in +S9, showed a positive trend at the 4hr/+S9 and the confirmatory assay was invalid due to absence of any cytotoxicity at the top concentration. Therefore, it may be concluded that there was a positive trend particularly in +S9, although according to sponsor's criteria for a positive response the R-zop is negative.

The S-zopiclone was negative in -S9 at 4hr treatment but in confirmatory assay -S9/24hr treatment, a positive trend (but negative response under sponsor's criteria), was observed at the top concentrations. In presence of S9/4hr treatment a clear dose dependent positive and statistically significant response (increase in MF), was seen in the top 3 concentrations relative to vehicle control. However, in the repeat assay, only a positive trend was observed with increase in small colony, but with no statistical significance. In conclusion, the S-zopiclone was considered negative by the sponsor since it did not meet criteria for a positive response except in 1 of 2 assays in +S9. However, it is the reviewer's opinion that based on the clear positive response in 1 assay and presence of positive trend in drug concentration in both absence and presence of S9, that S-zop was positive in the MLA.

It is therefore, concluded that the RS-zop was clearly positive in +S9 with a weak positive or equivocal response in -S9. The R-zop was negative though a very weak positive trend was observed. The S-zopiclone showed a stronger positive signal than did the R-zop and in +S9/4hr a clear dose response and statistically significant positive response, though according to the sponsor's criteria. **It almost seems that there is some potentiation of clastogenic/mutagenic effect in the RS-zop than each enantiomer alone. But the results of each enantiomer particularly those of the S-zopiclone, do not exclude a positive response.**

Study Title: In vivo mouse micronucleus (MN) assay with RS-, R-, and S-zopiclone.

Study No: Sepracor# 190-820/ — study# 20960-0-455 OECD

Conducting lab: —

GLP: Yes (x); OECD

QA: Yes (x)

Study Initiation/Termination dates: Nov 1999/Mar 2000

Drug batch/lot# S-zopiclone 120998A; RS-zopiclone 9809002; R-zopiclone 121198A

Negative vehicle/Positive controls: the vehicle control was 2.5% hydroxypropylmethylcellulose (HPMC), for all 3 cpds, the positive control was CP dissolved in deionized water and dosed at 80mg/kg. Dosing volume in all cases was 10ml/kg. Noted was that CP for the 1st trial was purchased from — . and for trial 2 from —

In vivo mouse Micronucleus assay, Sepracor#190-820 (Cont.)

Methods:
Young adult male and female CD-1 mice (8-10wks old), were purchased from — for the dose range finder study and from — for the MN assay. Animals were housed up to 6 per cage. Each animal's body wt was determined prior to dosing and dose adjusted accordingly. All 3 cpds were administered by oral gavage at 50, 100, 200, 300, and 400mg/kg for the dose range finding study to 3/sex/group CD-1 mice and animals were observed for 2days postdose. Clinical signs were observed in all 3 cpds and were dose dependent. There was no sex differences in toxicity profile in the RS-zop and S-zop therefore, only male mice were tested in the main assay. However, female mice were more sensitive to the R-zop with 2 of 3 females found dead on day 2 postdose of 400mg/kg in the dose range finding (although 1 male dosed R-zop in the main assay was also found dead on day1 of dosing). Therefore, male and female mice dosed R-zop were tested in the main assay. For all 3 cpds, the following doses were tested in **males** in the main assay: **100, 200, and 400mg/kg** and doses for **female** mice dosed R-zop, were **62.5, 125, and 250mg/kg.** it should be noted that female mice were dosed S-zop by mistake therefore, a 2nd trial was conducted using females only and dosed with R-zop.

At approximately 24hr postdose, bone marrow from each mouse in each group was removed and processed according to SOPs, additional samples were removed 48hr postdose from mice in vehicle control and high dose test cpds. Cytotoxicity of bone marrow was assessed by scoring the number of PCEs and NCEs from at least 200 cells per each slide. The MNPCE frequency was evaluated by scoring at least 2000 cells per animal according to SOPs.

Assay Acceptance Criteria:
Statistical analyses were done separately for each sex and harvest time. Criteria for positive response was detection of statistically significant increase in MNPCE for at least 1 dose AND a statistically significant dose response. A test cpd not inducing both of these responses was negative. The sponsor also indicated that in addition to statistical significance, biological relevance was also considered in the assessment. The historical background frequency of MN for CD-1 mice ir — is 0.0-0.4%, which is within the range reported in published literature.

Results & Conclusion:
Dose Range Finding Study: clinical signs were similar for all 3 cpds and in general increased in severity with increase in dose. Signs in the RS- and S-zop included: ataxia, hunched posture, and hypoactivity without death. For the R-zop additional clinical signs included tremors and coldness to touch with death in 2 of 3 females on d2 postdose, male mice recovered with no deaths. Although S-zop was more toxic to mice than the RS-zop based on clinical signs in the dose range finding, doses selected for the main assay using male mice for RS-, R-, and S-zop were 100, 200, and 400mg/kg. Female mice were dosed R-zop and S-zop at 62.5, 125, and 250mg/kg.

Main Assay: Trial 1: 1male mouse dosed 400mg/kg R-zop was found dead on d1 postdose, no other deaths in any group. All mice in vehicle and positive control groups were normal and without clinical signs. Clinical signs in drug groups included: hunched posture and slight hypoactivity in ≥100mg/kg RS-zop 1hr postdose, ataxia and labored breathing 13min post 200&400mg/kg in addition, in these 2 groups hypoactivity and coldness to touch were also observed within 1hr postdose; all signs were absent by 24hr postdose. Clinical signs in male mice dosed R-zop were only seen at 1hr postdose at 200&400mg/kg and included slight hypoactivity, cold to touch, and only at 400mg/kg ataxia (2/6 at 24hr harvest and 5/6 at 48hr

harvest), and hunched posture. In males dosed S-zop group, ataxia observed in all doses, hunched posture and cold to touch noted in 200&400mg/kg groups, labored breathing was seen in 1/6 males dosed
In vivo mouse Micronucleus assay, Sepracor#190-820 (Cont.)

400mg/kg at 24hr harvest; all signs were absent by 24hr postdose. Females dosed S-zop, showed similar signs to those seen in other groups. Only ataxia and hunched posture were observed at 62.5&125mg/kg, hypoactivity and cold to touch noted at 250mg/kg. **Trial 2** for females dosed R-zop, clinical signs were limited to hunched posture and slight hypoactivity at 1hr postdose at 250mg/kg (the former was the only sign noted at 125mg/kg); no signs observed at 62.5mg/kg.

Bone marrow **cytotoxicity** as demonstrated by decrease in PCE/NCE ratio, was **absent** from all 3 test cpds including the 48hr harvest time at high dose. There were no statistically significant increase in MNPCE at any dose in any group of test drug. In contrast, positive control group in both trials induced a significant increase in MNPCE; the vehicle control group values were within the historical range.

Based on these results, it is concluded that **R-zop, RS-zop, and S-zop were negative** in the mouse bone marrow micronucleus assay following single oral gavage dose of up to 400mg/kg.

Reviewer's Comments:

1. The following were noted though they may not have had an impact on study outcome: qualitatively, the clinical signs were similar in all 3 test cpds and between the dose range finding study and main assay. However, there were differences in incidence, time to onset, and duration as well as the number of signs per animal between animals in the dose range finding study and those in main assay. It is unclear why such differences were seen at the same dose and under the same experimental conditions. One factor may be the animals in the 2 assays since they were purchased from 2 different locations of [redacted].

2. It is also the reviewer's opinion that accurate conclusion can not be made based on these results. Although presence of adverse signs usually indicates adequacy of doses selected, bone marrow cytotoxicity is the more evident end point that ensures target site exposure to the cpd. Therefore, absence of such cytotoxicity in all 3 assays, makes the results less reliable. However, higher doses in the R-zop & S-zop may not have been possible due to the death.

Study Title: UDS / DNA repair in primary culture of rat hepatocytes.

Study No: Sepracor# 190-843/RPR# 22-379-E

Conducting lab: [redacted]

GLP: Yes (x)

QA: Yes (x)

Study Initiation/Termination dates: Dec 1985

Drug batch/lot# GUN 1255

Negative vehicle/Positive controls: RS-zop was dissolved in 5% DMSO and the latter served as the vehicle control as well as pyrene at $5x10^{-5}$M. Mitomycin C at 10^{-5}M was the positive control.

Methods:

6wk old male SD rats were received from — , and were 7wks old at start of study. Hepatocytes were isolated and cultured according to standard methods. RS-zop was tested at 5 concentrations and 3 slides per concentration were evaluated. Cellular viability of adherent cells was checked for all cpds 18hr after incubation. When cell adhesion was judged to be satisfactory, medium was aspirated and replaced with fresh Williams medium minus serum and containing 10uCi/ml
UDS in primary rat hepatocyte (Cont.)

^{3}H-thymidine plus either RS-zop or positive or negative controls. Slides were prepared for autoradiography and 8days after depositing the photographic emulsion, autographs were developed, slides stained, and grains counted using Artek counter connected to a microscope. 150 cells were counted per concentration. The sponsor stated that the counter was capable of producing counts in either grain mode or area mode, the latter was used because it allowed aggregates to be viewed as discrete grains. The net nuclear grain counts were calculated by subtracting the mean cytoplasmic count from the total nuclear count.

Criteria for a positive result (genotoxic): if NNG (net nuclear grain) is >5
Criteria for a possible genotoxic: if NNG is between 0-5 and,
Criteria for a negative response: if NNG is <0

Results & Conclusion:
RS-zop was tested in the UDS DNA repair assay in primary rat hepatocytes in culture at concentrations tested were: $5x10^{-6}$, 10^{-5}, $5x10^{-5}$, 10^{-4}, and $5x10^{-4}$M. Cytotoxicity was observed at the highest concentration. RS-zop did not increase the NNG up to 10^{-4}M compared to the negative control; MMC produced the expected increase in NNG.

As indicated in the PK/TK section, S-zop is metabolized to the active metabolite S-desmethyl zop. The latter is currently — and has been investigated with several general toxicology studies, reproductive/developmental studies, as well as genetic toxicology studies. The following genetic toxicology assays were conducted with S-desmethyl zop and the results are briefly summarized (for detail, refer to original review for —

Bacterial reverse gene mutation (Sepracor#192-807) Results:negative
In vitro CHO cells chromosomal aberration assay (Sepracor#192-805) Results:positive
In vitro mammalian human lymphocyte assay (Sepracor#192-810) Results:positive
In vivo chromosomal aberration and MN assay in mouse bone marrow (Sepracor#192-806)
Results:negative up to 350mg/kg
^{32}P postlabelling analysis of DNA adducts in calf thymus DNA incubated in vitro with S-desmethyl zop
Results: equivocal.

Overall Genetic Toxicology Summary:

All 3 forms of zopiclone: RS-, S-, and R-, tested **negative** in the bacterial Ames gene mutation assay, in the in vivo mouse bone marrow MN assay up to 400mg/kg oral dose, and RS-zop was negative in UDS DNA repair using primary rat hepatocytes in culture. However, **positive** responses were observed in the in vitro mammalian CHO chromosomal aberration assay and MLA. **RS-zop and S-zop were positive** in the 1st CHO assay where S-zop was positive in both trials in –S9 and in +S9 in trial 1, RS-zop was positive

in both -/+S9 in both trials; the R-zop showed a weak positive response in trial2 in –S9. All 3 cpds tested **negative in the repeat CHO** assay except for RS-zop in +S9 tested positive for structural and numerical abs. The valididty of this 2nd assay is questionable. The positive findings in the previous CHO assay were observed at concentrations higher than those in the repeat assay, it is unclear why solubility was limited since the same vehicle, DMSO, was used in both studies. Therefore, unless an explanation is provided to justify the solubility issue, the clear positive response observed in the 1st assay remains as the valid one for this assay. In the MLA assay, RS-zop was positive in +S9 at both 4&24hr incubation times with clear increase in small colony, moreover, a positive trend was seen in –S9at the 24hr. The S-zop was positive in +S9 at 4hr with increase in small colony and questionable at the 24hr/+S9. However, S-zop +S9/4hr was negative in the repeat assay but, with a positive trend; S-zop was negative in –S9 at 4hr and 24hr (but positive trend for the latter time point). The R-zop in the MLA was positive in –S9 at 24hr and +S9 at 4hr otherwise negative at –S9/4hr. Of importance to these findings are the results of the S-desmethyl zop, the active metabolite of S-zop. This cpd is being studied under IND# 63,056 and was tested **positive in the CHO and mammalian human lymphocyte assays, further supporting the positive findings of S-zop in the above assays.** It is noted that S-zop and RS-zop were tested in the alternative **P53 bioassay in mice with negative findings.**

The results of the CHO and MLA for the 3 cpds were consulted to the Genetic Toxicology Cmtt of CDER and their conclusions were in agreement to those of the reviewer and the CROs. **This reviewer concludes that results for the in vitro chromosomal aberrations lean towards a positive clastogenic response for S-zop and RS-zop.** The sponsor however, continues to state that RS-zop and S-zop are not clastogenic in these assays, and that a threshold phenomenon is playing a role (Dr — Sepracor#190).

3.4.5. CARCINOGENICITY

The following are reviews of the RS-zop mouse and rat 2yr carcinogenicity studies and 18month rat toxicity study plus the S-zop P53 alternative in vivo mouse study. In addition, all consultant and Pathology Working Group reports are discussed, each of the 4 tumors are discussed within each study as well as separately, assessment and relevance to human cancer risk are addressed in the Special Toxicology section of this review.

The mouse and rat studies were submitted by Sepracor on June 22nd 2001 serial# 40. They were reviewed and presented to the CDER Executive CAC on October 2nd 2001. the following is that review:

MOUSE CARCINOGENICITY STUDY:

Study design was that of the NTP: 2 control groups and 3 dose groups; the study was GLP

Species/Strain/# per sex per dose: B6C3F1 mice; 52/sex/group. Mice housed 4/cage

Doses/Route/Duration: **0, 0, 1, 10, 100mg/kg/d** RS-zop for at least 102wks via the DIET.

Results:

Mortality/Clinical Signs: No drug effect; survival in all groups at end of study was >20%

Food Intake: No drug effect

B.wt/B.wt Gain: mean B.wt was unaffected at any period of study in either sex of any dose. Mean B.wt **gain** was **increased** in HDf during wks0-14 of dosing ($p<0.001$), no drug effect in males during this period (tables from sponsor). Mean B.wt gain was statistically significantly **decreased in both sexes in HD** during wks 14-102 (66% m and 41% f less than the corresponding controls), and during wks0-102 (17% m and 18% f less than the corresponding controls). Note that wt gain in the 10mg/kg/d dose in males was significantly increased over the control values during these periods 14-102 and 0-102wks (tables from sponsor).

Table 4: Body Weight Gains (g) by Interval Weeks in Male Mice Receiving (RS)-Zopiclone for at Least 104 Weeks

Week Interval	Control		(RS)-Zopiclone (mg/kg/day)		
	0	0	1	10	100
0 – 14	16.8	16.5	16.3	16.6	16.7
14 – 102[1]	6.6	6.4	5.4	8.2**	2.2***
0 – 102	23.1	22.4	21.7	24.8**	18.9***

Table 5: Body Weight Gains (g) by Interval Weeks in Female Mice Receiving (RS)-Zopiclone for 104 Weeks

Week Interval	Control		(RS)-Zopiclone (mg/kg/day)		
	0	0	1	10	100
0 – 14	9.8	9.1	8.4**	10.1	12.7***
14 – 102[1]	21.5	21.3	20.4	20.3	12.7***
0 – 102	31.4	30.5	28.7	30.4	25.4***

** p<0.01; *** p<0.001.

[1] Body weights were assessed only through Week 102; animals were necropsied during Weeks 106-107.

RS-zop Mouse Car study (Cont.)

Organ wt: the only drug effect was a small but statistically significant increase in mean relative wt of the liver in HDm+f (table from sponsor). This increase was 33&38% more than the corresponding control values in males and females respectively, or 1.3x the liver wt in the control.

Table 6: Mean Absolute (g) and Relative (%) Liver Weights in Male and Female Mice Receiving (RS)-Zopiclone for at Least 104 Weeks

Group		Control		(RS)-Zopiclone (mg/kg/day)		
		0	0	1	10	100
Males	Abs	2.4	2.4	2.2	2.3	2.8
	Rel	5.9	6.1	5.5	5.5	8.0*
Females	Abs	2.2	1.9	1.8	2.2	2.5
	Rel	4.9	4.1	4.2	5.1	6.2**

* p<0.05; ** p<0.01.

Gross Exam: No findings except for subcutaneous masses in HDm (see below).
Non-Neoplastic Findings: None
Neoplastic Findings: HDm skin fibrosarcoma
HDf pulmonary adenoma/adenocarcinoma

The incidences of both tumor types were statistically significant relative to the concurrent control incidence as well as exceeded historical data for these tumor types.

Sepracor's Conclusion:

Skin tumors are related to the bite wounds leading to incrustations that consequently caused the tumors. Male B6C3F mice are known for their aggressive behavior when housed together, **RS-zop increased/exaggerated the fighting behavior in these mice at high dose that led to the tumors. Therefore, this tumor type is irrelevant to humans and is not a direct effect of RS-zop.**

Pulmonary tumors are common in rodents and when the data were reevaluated for statistical significance using current criteria and guidance, these tumors were found to be spurious and irrelevant to humans.

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RS-zop Mouse Car study (Cont.)

The following is a detailed discussion of the findings, expert opinions, and conclusions:

Male Mouse Skin Tumors:
Table from sponsor:

Table 7: Incidence of Fibromas or Sarcomas in Male Mice Receiving (RS)-Zopiclone for at Least 104 Weeks

Tumor Type	Control		(RS)-Zopiclone (mg/kg/day)		
	0	0	1	10	100
(Number examined)	*(52)*	*(52)*	*(52)*	*(52)*	*(52)*
Fibroma	1[a]	1	0	0	2
Sarcoma	1	1	2	0	13**
Fibroma or Sarcoma	2	1[b]	2	0	14**[c]

** p<0.001, versus combined controls. [a]Number of animals exhibiting the finding.

[b]One animal (No. 55) had both a fibroma and a sarcoma, therefore the incidence was counted only once for the combined total.

[c]One animal (No. 244) had both a fibroma and a sarcoma, therefore the incidence was counted only once for the combined total.

These tumors were also referred to as malignant mesenchymal tumors. They were not found in female mice or in either sex of the rat. [incidence of fibroma or sarcoma in female mice was 3, 1, 1, 3, and 2 each out of 52 mice per group in 0, 0, 1, 10, and 100mg/kg/d respectively].

Historical control incidence from 12 carcinogenicity studies conducted at — between 1979-1984 for gang caged (4/cage), B6C3F1 mice was 2.75% (0-11%) and 4.9% (0-10%) for s.c. fibroma and sarcoma respectively. Therefore, the **sarcoma incidence in HDm mice of 25%** clearly exceeded the historical range from 12 studies. The historical incidence from these same 12 studies for dorsal incrustations in gang caged mice (4/cage), was 14% (2-33%) but 0% in one study where mice were individually housed. The **incidence of encrustations in HDm in this study was 52%**, far exceeded the historical range.

The sponsor stated the following:

- There was no difference between drug groups and controls in tumor onset (1st sarcoma appeared at wk 97, 54, 61, and 75 for control 1, control 2, 1mg/kg/d, and 100mg/kg/d groups respectively, with corresponding mean times of 99, 54, 78, and 88weeks respectively,

- There was no difference between drug groups and controls in the skin location of these tumors (all except 1 tumor was seen on the dorsal surface of the skin which is a similar incidence/skin location reported for historical control data),

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RS-zop Mouse Car study (Cont.)

- The cause of death in mice with these s.c. tumors was not determined by the pathologist however, there was no difference in mortality between drug and control groups leading the sponsor to conclude that these tumors were not lethal,

- The incidence of dorsal incrustations-indicative of fighting/biting, was significantly higher in HDm than that for the controls or any other drug group (table from sponsor); no drug effects on incrustations or skin tumors were observed in female groups including the controls,

Table 10: Incidence (%) of Dorsal Encrustations in Male Mice Receiving (RS)-Zopiclone for at Least 104 Weeks

Tumor Type	Control		(RS)-Zopiclone (mg/kg/day)		
	0	0	1	10	100
(Number examined)	(52)	(52)	(52)	(52)	(52)
Number affected (%)	3 (6)	5 (10)	3 (6)	3 (6)	27 (52)
Mean Duration (weeks)	0.5	0.7	0.2	0.1	6.3

The historical control **duration** of incrustations from the 12 carcinogenicity studies was 1.4wks (0.1-3.8wks). The incidence for the controls, low, and mid dose RS-zop groups fell within this historical range **but the HD far exceeded it (6.3wks).**

- Although the encrustations in HDm in this study exceeded both the historical control range for the incidence and duration of encrustations, the sponsor indicated that further analyses of the historical data showed a correlation between these incrustations and sarcoma development such that only 3% sarcoma found in males without dorsal incrustations but 29% found in males with incrustations, incidences similar to those in RS-zop of 5&19% respectively.

The sponsor concluded that RS-zop at high dose exaggerated the aggressiveness of gang caged male mice and secondarily leading to development of sarcoma as a result of biting wounds/incrustations.

The correlation between aggressive behavior in male B6C3F1 mice housed in group and sarcoma incidence has been reported by Boorman G. (1985) in a correspondence with F. Roe (see detail later). Based on this observation, the NTP began recommending individual housing of these mice. RPR , conducted a 2nd carcinogenicity study at . (1990) to test this proposal. The protocol consisted of 2 concurrent studies (Sepracor#190-844). In the 1st study, male B6C3F1 mice were housed individually and 4/cage and administered either 100mg/kg/d RS-zop or control drug-free diet for 106wks and killed on wk107 (n=100 per group). Unlike the original study where there was no difference in mortality was recorded, incidence of mortality in this study was increased in gang caged mice compared to the single caged mice (table from sponsor). Most of these deaths occurred in the 1st yr of dosing.

Table 11: Incidence of Mortality by Interval Weeks in Male Mice Receiving 0 or 100 mg/kg/day of (RS)-Zopiclone for 106 Weeks

Week Interval	Single Housed		Group Housed[1]	
	0	100 mg/kg	0	100 mg/kg
0 – 52	1	0	5	17
53 – 78	6	9	4	9
79 – 92	18	10	17	9
93 – 107[2]	18	10	10	22
0 – 107[2]	43	29	36	57

[1] n = 4/cage [2] Includes animals sacrificed through Week 107.

RS-zop Mouse Car study (Cont.)

There was a clear correlation between skin encrustations and mice housed as gang and absence of encrustations in mice housed individually (table from sponsor). This correlation also extended to higher incidence of sarcoma in gang-housed mice compared to zero incidence in mice housed individually.

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Table 12: Incidence (%) of Subcutaneous Sarcomas in Male Mice Receiving (RS)-*Zopiclone for 106 Weeks*

Dose (mg/kg/day)	Caging			
	Individual		Group[1]	
	0	100	0	100
(Number per group)	(100)	(100)	(100)	(100)
Dorsal Encrustations [2]	0	0	28	71
Fibromas	0	0	0	6
Sarcomas	0	0	3	13
Mean Duration (weeks) [3]	-	-	2.1	13.0

[1] n = 4/cage.
[2] 0-52 weeks; encrustations were not considered after 52 weeks as it was thought that tumor development might possibly cause encrustations.
[3] Encrustation.

The sponsor did not believe the increased deaths in 100mg/kg/d zop group was due to sarcoma. Although there were 9 of the 13 mice with sarcoma that died on drug or were killed in moribund compared with 3 of 3 mice in control. The sponsor indicated that there was no evidence that this higher incidence of sarcoma led to increased mortality because mortality in the 2nd study (see below), was lower in drug group 100mg/kg/d than in the control (16 vs. 24 on wk107, respectively).

Also noted in this study a "shift" in severity of encrustations/bite wounds from grade 1 in control gang housed to grade 2-3 in RS-zop mice. **Mean duration of encrustation (through wk52) was 2.1 wks in control and 13.0 wks in RS-zop (historical mean 1.4wks).** As indicated above, individually housed mice, control or drug, had no encrustations and no sarcomas/fibromas.

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RS-zop Mouse Car study (Cont.)

The 2nd part of the study included 52 male B6C3F1 mice housed 4/cage and dosed 1 or 100mg/kg/d RS-zop and 1 control group received the drug free diet. The 1mg/kg/d group was terminated on wk26 of

dosing*. As indicated above, there was no difference in mortality between the 100mg/kg/d RS-zop and the control. The incidence of sarcoma was increased in mice showing high incidence of encrstations (table from sponsor).

Table 14: Incidence (%) of Subcutaneous Sarcomas in Male Mice Receiving 0 or 100 mg/kg/day of (RS)-Zopiclone for 106 Weeks

Dose (mg/kg/day)	Caging Group[1]	
	0	100
(Number per group)	(52)	(52)
Dorsal Encrustations[2]	5	30
Fibromas	0	2
Sarcomas	0	2
Mean Duration (weeks)[3]	*1.0*	*7.7*

[1] n = 4/cage.
[2] 0 - 52 weeks; encrustations were not counted after this as it was thought that tumor development might possibly cause encrustations.
[3] Duration of encrustations.

Moreover, the mean duration of encrustations (through wk52) was much higher in drug group, 7.7wks, compared with 1wk in the control.

It should be noted that the incidence of encrustation in this part of the study was much lower (5&30 each per 52 mice, table 14), than that in the 1st part of this study (table 12; 14 & 37 each in 52 mice in control and 100mg/kg/d respectively). The sponsor contributed this difference to "enviromental factors" such as marked differences in location and cage rack density.

The sponsor concluded that this additional study confirmed the proposal that gang-caged B6C3F1 male mice causes animals to fight → encrustations → sarcomas and that zopiclone exaggerates the aggressive behavior of these mice : —, 1988 "paradoxical aggressive reaction induced in male B6C3F1 mouse in a non-benzodiazepine hypnotic, zopiclone; RP report —

* see statistical review dated Feb 1991 for detail as well as more comprehensive discussion of this study later in this review..

Consultant Opinions on the Skin tumors
Drs. —) were consulted by Sepracor for the tumor findings in mice and rats. All consultants noted these skin tumors to have a number of patterns ranging from undifferentiated sarcomas to well-differentiated fibro-proliferative lesions that "may not represent a neoplastic process". They referenced an article by Brand et al., 1975 that showed s.c. sarcomas in rodents can

RS-zop Mouse Car study (Cont.)

develop due to chronic injury (foreign body carcinogenesis) as seen from repeated s.c. injections of cpds including food additives; a model that was dismissed later as a screening method. These consultants also referred to the 2nd study as an additional support to the conclusion that the increased incidence of skin sarcoma in high dose male mice was caused by the increased aggressiveness induced by RS-zop in gang caged male mice that led to encrustations and ultimately sarcomas.

The sponsor concluded that RS-zop acted indirectly through a non-genetoxic mechanism to exacerbate the aggressive behavior of male B6C3F1 mice when gang housed, similar to other cpds that act through the bzd receptors and is unique to rodents. Therefore, group housing is the "sole" cause of these tumors since encrustations and sarcomas were absent when male mice were housed individually. Therefore, these tumors are irrelevant to human risk assessment.

See more details of reviews and reports of these and other consultant reports regarding the male skin tumors.

Female Mouse Pulmonary Tumors:
Table from sponsor:

Table 15: Incidence of Pulmonary Tumors in Female Mice Receiving (RS)-Zopiclone for up to 106 Weeks

Tumor Type	Control		(RS)-Zopiclone (mg/kg/day)		
	0	0	1	10	100
(Number examined)	(52)	(52)	(52)	(52)	(52)
Adenoma	6[1]	5	5	2	10
Adenocarcinoma	0	0	1	1	4*
Adenoma + Carcinoma	6	5	6	3	14**[a]

* p<0.05; ** p<0.01 (Fischer's Exact Test).
[a] 1 animal (# 470; terminal sacrifice) was diagnosed with both an adenoma and an adenocarcinoma; this animal was counted only in the adenocarcinoma column.

Only adenocarcinoma reached statistical significance in HDf compared to the control. There were no statistical differences in these tumors in male mice or in either sex of the rat. The sponsor reported the incidence of adenocarcinoma for male mice. Table below from the sponsor:

Incidence of male mice with pulmonary tumours

	Dosage (mg/kg/day)				
	Controls		1	10	100
No. examined	52	52	52	52	52
Adenoma	13	12	14	9	7
Adenocarcinoma	1	2	4	1	3
Adenoma or adenocarcinoma	14	14	18	10	10

All values not significantly different from controls, P > 0.05

RS-zop Mouse Car study (Cont.)

Historical control incidence for pulmonary tumors from 5 "contemporary" (time period not reported), carcinogenicity studies conducted at — in female B6C3F1 mice was 4.2% (1.7-6.8%) for adenomas and 2.1% (0-5.8%) for adenocarcinomas. Therefore, the **adenocarcinoma incidence in this study in HDf mice of 8% exceeded the historical range of 5.8% from these 5 studies.[the combined incidence for adenoma and carcinoma was 27% in this study].**

The sponsor stated the following:

- Lung tumors are common in mice and there are several types,
- Pulmonary tumors are usually seen in both sexes, a finding not observed in this study,
- Generally, cpds that induces pulmonary tumors increase not only the number of tumor-bearing animals but also tumor multiplicity and incidence of pre-neoplastic lesions. Neither of these parameters was observed in this study:

Table 18: Incidence of Non-neoplastic Pulmonary Lesions in Female Mice Receiving (RS)-Zopiclone for up to 106 Weeks

Lesion	Control		(RS)-Zopiclone (mg/kg/day)		
	0	0	1	10	100
Moribund Sacrificed/Died on Test	(13)	(10)	(12)	(14)	(15)
Chronic Pulmonitis	1	0	2	0	1
Peribronchial lymphoid hyperplasia	2	0	1	3	1
Perivascular lymphoid hyperplasia	7	1	3	5	4
Terminal Sacrificed	(39)	(42)	(40)	(38)	(37)
Chronic Pulmonitis	5	3	2	6	9*
Peribronchial lymphoid hyperplasia	0	2	1	0	2
Perivascular lymphoid hyperplasia	22	20	16	18	27*

* p<0.05. Number in parenthesis indicates the number of animals examined.

There were no alveolar hyperpla sia but higher incidence of perivascular lymphoid hyperplasia was observed in terminal kill HDf, the latter as indicated by the sponsor, represents an inflammatory response and not a preneoplastic lesion.

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RS-zop Mouse Car study (Cont.)

- Absence of dose dependent response for any tumor type and no indication of early onset of any tumor (tables from sponsor).

Table 16: Incidence of Pulmonary Tumors in Female Mice Receiving (RS)-Zopiclone That Died on Test or Were Moribund Sacrificed

Tumor Type	Control		(RS)-Zopiclone (mg/kg/day)		
	0	0	1	10	100
(Number Examined)	(13)	(10)	(12)	(14)	(15)
Adenoma	2[a]	0	1[b]	0	2[d]
Adenocarcinoma	0	0	0	1[c]	2[e]

[a] animal (No. 264) died at Wk 87 and (No. 271) at Wk 93.
[b] animal (No. 412) died at Wk 98.
[c] animal (No. 434) died at Wk 91.
[d] animal (No. 478) died at Wk 102 and (No. 516) at Wk 90.
[e] animal (No. 498) died at Wk 85 and (No. 507) at Wk 104.

Table 17: Incidence of Pulmonary Tumors in Female Mice Receiving (RS)-Zopiclone for up to 106 Weeks - Terminally Sacrificed

Tumor Type	Control		(RS)-Zopiclone (mg/kg/day)		
	0	0	1	10	100
(Number Examined)	(39)	(42)	(40)	(38)	(37)
Adenoma	4	5	4	2	8[a]
Adenocarcinoma	0	0	1	0	2

[a] 1 animal (No. 0470) had both an adenoma and a carcinoma -- only the carcinoma was counted in this table.

- As indicated earlier, no statistically significant increase in pulmonary tumors was seen in male mice or either sex in the rat carcinogenicity study.
- Morphologically, these tumors were similar to spontaneous tumors.
- The absence of any pulmonary adenocarcinoma in the 2 female control groups contributed to the statistical significance observed in HDf.

Based on all of the above, the sponsor concluded that there is a weak weight of evidence in support of a drug effect.

Consultant Opinions on the Pulmonary Tumors

Drs. [redacted] were previously consulted and each provided their opinions on the female mice pulmonary tumors. Recently, Sepracor convened a Pathology Working Group (PWG), to review the findings and reevaluate the histopathological slides of female mice with alveolar/bronchiolar lesions in this study. The chair of this group was Dr. [redacted], plus 4 expert toxicologic pathologists: Dr. [redacted]. These experts used the diagnostic criteria defined by Dixon et al., (1999) and the microscopic evaluation was done blindly (group designation was unknown to the evaluator). All slide evaluations were reviewed by the PWG and a consensus was

reached at the same sitting. At the end of the discussion and diagnosis of each slide, the blind was broken; *note that there were 10 sections of each lung per animal all of which were evaluated.*

RS-zop Mouse Car study (Cont.)

[This was a mistake/oversight by the original lab, normally only 2-3 sections are prepared per lung not per lobe which was the case here]. The following table from the sponsor presents the conclusions of the PWG regarding the pulmonary tumors in female mice:

Table 19: Incidence of Alveolar/Bronchiolar (A/B) Tumors in Female Mice Receiving (RS)-Zopiclone for up to 106 Weeks – PWG Evaluation

Tumor Type	Control		(RS)-Zopiclone (mg/kg/day)		
	0	0	1	10	100
SP[a]: Total A/B Tumors	6	5	6	3	14
PWG Evaluation					
A/B Adenoma	5	4[b]	5	3	10[d]
A/B Carcinoma	0	0	1	0[c]	2[c]
Total A/B Tumors	5	4	6	3	12

[a] SP = original study pathologist diagnosis (refer to Table 15).
[b] Animal No. 352 was diagnosed as an A/B hyperplasia rather than an adenoma as was previously noted by the SP.
[c] Animal No. 343 was diagnosed as an A/B adenoma rather than a carcinoma as was previously noted by the SP.
[d] Animal No. 482 and 497 were diagnosed as A/B hyperplasia rather than an adenoma as was previously noted by the SP.
[e] Animal No. 470 and 493 were diagnosed as an A/B adenoma rather than a carcinoma as was previously noted by the SP.

The PWG group diagnosed some of the neoplasia as hyperplasia rather than adenoma and some as adenomas instead of carcinomas as previously thought. The group did not consider these tumors to be drug related for the following reasons:

- Absence of dose response
- Single sex, single dose, and single species (not seen in either sex of the rat)
- Morphologically, these lesions were similar to spontaneous lesions in mice
- The alveolar/bronchiolar hyperplasia was seen at low incidence and was associated with chronic inflammation. Pulmonary tumors generally, induce high incidence of A/B hyperplasia that later progress to neoplasia; such progression was not observed in this study.
- Chemically-induced A/B tumors and not spontaneous ones, generally metastasize. This was not seen in RS-zop tumors, 2 A/B carcinomas had limited metastasis: 1 in LD metastasized to the bronchial lymph node, and the 2nd in HD infiltered to the pleura and mediastinum
- The high incidence of pulmonary tumors in B6C3F1 high dose females is within the historical control range of the NTP database (Haseman, JK et al., 1998, A/B adenoma mean incidence was 5.9% (range 0-24%), A/B alveolar carcinoma mean incidence was 2.4% (range 0-8%), for female B6C3F1 mice NTP feeding study of 1341 controls).

Sponsor's conclusions on the female mice pulmonary tumors:
The female mice A/B tumors are not RS-zop related. The reasons were those of the PWG stated above in addition to the following points:

- B6C3F1 mice are not unique for these tumors, CD-1 mice has similar incidences for pulmonary adenomas (mean 14.4%) and adenocarcinomas (mean 12.1%) in female mice (Maita et al., 1988).

RS-zop Mouse Car study (Cont.)

- The NTP data reported that occasionally the incidence of pulmonary carcinomas in the control group is 0. Therefore, statistical significance of small increases in incidence of common tumors should be considered within the "total context of the biological evidence".
- Based on recently proposed FDA statistical guidance document (Guidance for Industry: Statistical Aspects of the Design, Analysis, and Interpretation of chronic Rodent Carcinogenicity Studies of Pharmaceuticals; draft guidance, May 2001) for common tumors, those with incidence generally >1%, a p value of <0.005 or according to Haseman (1990), a p value <0.01 should be used (for trend and pair-wise analyses). The incidence of pulmonary adenocarcinoma with the original incidence or the re-analyzed slides by the PWG, would not meet these tests and the difference would not achieve statistical significance even with the original cutoff p value of <0.05. However, based on the reanalysis by the CRO, statistical significance was shown with the pair-wise but not with trend test.

The following is detailed review of the 2nd carcinogenicity study in mice (males only), discussed earlier.

Investigation of factors affecting the development of tumors of the subcutis in male B6C3F1 mice receiving zopiclone for 2yr in the diet/Sepracor# 190-844
Conducting lab. — , study # 90/RHS017/016
Study Date: August 19 1987 for both studies (see below)
Batch# for RS-zop: CA 8410103 lot 7
QA/GLP: Yes, OECD, FDA, and Japanese regulations for GLP.

Objective: in previous 2yr mouse carcinogenicity study (Sepracor# 190-834), the incidence of s.c. tumors was increased in male mice dosed 100mg/kg/d RS-zop. The sponsor stated that this was due to drug related increase in aggressive behavior of gang-housed mice (4/cage) leading to increase fighting and skin encrustations. Two studies combined in this 1 report were done to determine if preventing fighting by housing male mice individually, will reduce or prevent these tumors i.e. these tumors are not directly drug related.

Methods:
Male B6C3F1 mice were purchased from — . and 108 mice assigned initially per group. 2wks after start of dosing, number was reduced to 100 per group. This was done in the 100mg/kg/d RS-zop group (gang housed), by selecting the 2 cages MOST AFFECTED by fighting and killing off these 8mice; these mice were not examined postmortem. This was then followed by random selection of 8mice from all other groups. The study design was as follows (control mice received drug free diets):

Study Design for Two-Year Mouse Studies

Group Number	Treatment	Dosage (mg/kg/day)	No. of mice per group	No. of mice per cage	Sacrifice (weeks)
1	Control	0	100	1	106
2	Zopiclone	100	100	1	106
3	Control	0	100	4	106
4	Zopiclone	100	100	4	106
5	Control	0	52	4	106
6	Zopiclone	1	52	4	26
7	Zopiclone	100	52	4	106

Investigation of s.c. tumors in male mice Sep#190-844 (Cont.)

Parameters assessed: mortality, clinical signs, B.wt, food intake, gross exam (limited to skin and subcutis), histopathology of the skin and subcutis (one "standard" section of the mid dorsal region and any abnormalities or masses), including all masses, lesions, and encrustation observed in vivo. Sections were cut at 5micron thickness and stained with H&E.

Results & Conclusion:

Generally, no drug effect on clinical signs, B.wt or food intake. Mortality was significantly increased in gang caged mice dosed RS-zop relative to gang-caged controls, a finding not observed in the previous study. There was an increase in incidence, severity, and duration of dorsal encrustations and s.c. tumors in mice housed 4/cage and administered 100mg/kg/d RS-zop compared to single-housed controls. No incidence of skin tumors observed in single-housed mice of control or drug group (tables from sponsor). It is concluded that RS-zop increased the aggressive behavior of gang-housed male B6C3F1 mice causing increase in fighting behavior (manifested as dorsal skin encrustations), and consequent skin tumors. Therefore, absence of skin tumors in male mice housed individually and administered 100mg/kg/d indicate that these tumors are not a directl effect.

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Male Mouse Car Study Sepracor#190-844 (Cont.)

Text-table 2

Incidence, severity and duration of dorsal encrustations and incidence of tumours of the dorsal subcutis (fibroma and sarcoma)

Study	RHS/017/ZOP				RHS/016/ZOP	
Group	1	2	3	4	5	7
Treatment	Control	Zopiclone	Control	Zopiclone	Control	Zopiclone
Dosage (mg/kg/day)	0	100	0	100	0	100
No. of mice per cage	1	1	4	4	4	4
No. of mice per group	100	100	100	100	52	52
Dorsal encrustations (Weeks 0-52*)						
Total number of animals bearing dorsal encrustations						
- Any grade	0	0	28	71	5	30
- Grade 1+	0	0	24	42	5	25
- Grade 2+	0	0	4	20	0	5
- Grade 3+	0	0	0	9	0	0
Mean duration (weeks) of encrustation#	-	-	2.1	13.0	1.0	7.7
Tumours of dorsal subcutis						
Number of animals bearing tumours of the dorsal subcutis						
Fibroma	0	0	0	6	0	2
Sarcoma	0	0	3	13	0	2
Total	0	0	3	19	0	4

\+ Maximum severity observed. See Annex 1 for explanation of severity rating

* Encrustations seen after Week 52 were not considered as it was thought that tumour development might possibly cause encrustations

\# Calculated from all mice in group; mice without encrustations were considered to be affected for zero weeks.

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Male Mouse Car Study Sepracor#190-844 (Cont.)

Text-table 3

Association between dorsal encrustations and dorsal tumours of the subcutis (fibroma and sarcoma)

Groups	RHS/016/ZOP 5+7	RHS/017/ZOP 3+4	Total 3+4 + 5+7
Incidence (%) of mice with tumours among mice with no encrustations (Weeks 0-52*)	0/69 (0%)	5/101 (5%)	5/170 (3%)
Incidence (%) of mice with tumours among mice bearing dorsal encrustations (Weeks 0-52*)	4[a]/35 (11%)	17[b]/99 (17%)	21[c]/134 (16%)

* Encrustations seen after Week 52 were not considered as it was thought that tumour development might possibly cause encrustations
a Significantly different, $P < 0.05$
b Significantly different, $P < 0.01$
c Significantly different, $P < 0.001$

Text-table 4

Association between the number of weeks dorsal encrustations were observed and dorsal tumours (fibroma and sarcoma)

	Weeks dorsal encrustations observed 0	1-5	6-10	11-20	21-52*
Incidence (%) of mice with tumours among mice bearing encrustation for the specified period.					
RHS/016/ZOP Groups 5+7	0/69 (0%)	1/10 (10%)	0/5 (0%)	1/15 (7%)	2/5[c] (40%)
RHS/017/ZOP Groups 3+4	5/101 (5%)	1/32 (3%)	3/15 (20%)	5/25 (20%)	8/27[c] (30%)
Total Groups 3+4 + 5+7	5/170 (3%)	2/42 (5%)	3/20 (15%)	6/40 (15%)	10/32[c] (31%)

* Encrustations observed after Week 52 were not considered as it was thought that tumour development might possibly cause encrustations
c Significant trend, $P < 0.001$ (Cochrane Armitage)

In another follow up study, RPR, (—), carried out a study in brown B6C3F1 mice — ., to also address the proposal that RS-zop induces increased aggressive behavior in male

mice that lead to the skin tumors observed in the 2yr carcinogenicity study. The sponsor refers to the literature on benzodiazepines and states that although they are known to reduce aggressive behavior, Bzd have the tendency under "certain conditions" to increase spontaneous aggressiveness. The fighting behavior in mice induced by pain resulting from electric shock to the foot was increased as evidenced by higher number of bites when these mice dosed chlordiazepoxide for 10days (Renzi, 1982). Fox et al. (1970, 1972, 1974), showed chronic low dose administration of a number of benzodiazepines increased the aggressive behavior (bites and sometimes death) in these mice. Guaitani et al. (1971), administered N-desmethyldiazepam for 6mo to groups of male and female mice and found large cutaneous necrosis in males only. They concluded that this cpd, increased spontaneous aggressiveness in group housed male mice since these findings were much reduced when mice housed individually. RS-zop in earlier study caused anti-aggressive behavior in mice exposed to foot shock (Julou et al., 1983). In the current study (Sepracor#190-842), RS-zop was administered in the diet at 10 or 100mg/kg/d and chlordiazepoxide at 50mg/kg/d for 4wks to 24/group mice housed 4/cage. The foot shock test was used to provoke aggression and compare responses to spontaneous aggression. In all groups, provoked aggression was increased as evidenced by the number of aggressive pairs and number of bites. However, RS-zop induced a more rapid increase and the number of fighting episodes occurred earlier after 100mg/kg/d (at 1wk as opposed to wk2), than in control. During wk1 the number of aggressive pairs was 11/12 in 100mg/kg RS-zop vs. 5/12 in control. Increase in number of aggressive pairs was also seen in chlordiazepoxide but the data was noisy to make a clear conclusion. The spontaneous aggression was also increased in 100mg/kg/d RS-zop at 30% after 10d and 22% for 25d compared to control; at 10mg/kg/d 12% of mice showed increase in spontaneous aggression. For chlordiazepoxide, up to 22% increase in spontaneous aggression but also severity of the bites was high (e.g. absence of external ears). This study concluded that RS-zop at 100mg/kg/d under certain conditions may induce paradoxical aggression in male mice.

In another report entitled "Origin of fibrosarcoma in male B6C3F1 mice, prepared in May 30th 1988 (Sepracor#190-854), Dr — of RPR referenced Drs. Boorman of NTP and Ward from the NCI where they stated that "fibrosarcomas in B6C3F1 mice may be the result of skin lesions in males regularly exhibiting fighting behavior". In order to examine if RS-zop-induced skin lesions are due to increased fighting, — (the contract lab that did the mouse carcinogenicity study), was asked by RPR to re-examine the clinical sign data for the RS-zop in the car study and compare the results to historical control from other car studies done using the same strain. Re-analysis showed that the extent of fighting was increased in male mice dosed 100mg/kg/d RS-zop compared to concurrent control and that a strong correlation was seen between dorsal skin encrustations and tumors at these sites. The following table was generated by Dr. — showing the incidence of s.c. tumors in the 2yr mouse study indicating that there was no difference in mortality rate/survival among any of the treated groups and control and mean wt was reduced (-18%), in both sexes dosed 100mg/kg/d relative to combined controls.

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Incidence of subcutaneous tumours

Group	1	2	3	4	5
Dose (mg/kg)	0	0	1	10	100
		MALES			
Fibroma	1	1	0	0	2
Sarcoma	1	1	2	0	13***
Fibroma or sarcoma	2	1(a)	2	0	14*** (a)
		FEMALES			
Fibroma	0	0	0	0	0
Sarcoma	3	1	1	3	2
Fibroma or sarcoma	3	1	1	3	2

*** p < 0.001
(a) In groups 2 and 5 there were single animals presenting both a fibroma and a sarcoma.

The incidence (25 %) of sarcomas in males treated at 100 mg/kg was higher than the historical data for this mouse strain available at —) - 10 %).

The incidence (27 %) of tumors of the subcutis (fibroma and sarcoma) was also outside the historical control range at — (0 - 18 %).

Dr. — Report, Sepracor# 190-854 (Cont.)

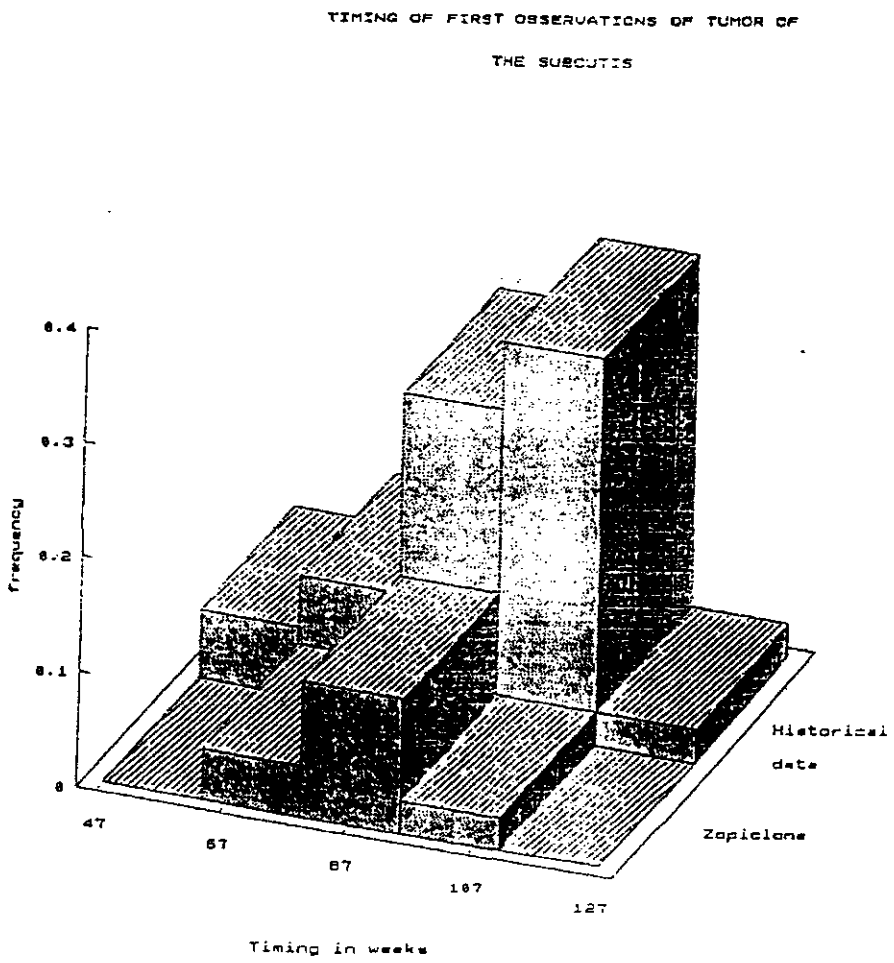
It is clear from this table that **the incidence of fibrosarcoma far exceeded the — historical range** however, Dr — nade arguments re. the skin tumors as follows:

1. He referenced the NTP **historical data** base for skin fibrosarcomas (information from Dr. Gary Boorman at NTP/NIEHS). Most if not all of the mouse studies at the NTP are done using B6C3F1 strain and that fibrosarcoma are common tumor type in these mice and were recorded in controls from 25 of 44 studies. Increased incidence according to Dr. — was "always" observed in males and never in female mice with a wide range between the studies as well as between groups within the same study. Of the 44 NTP studies the incidence was between 10-20% in 5 studies, >20% in only 2 studies (29&26%). Therefore, Dr. — concluded that the incidence of 25% with RS-zop is within the historical range [though only 2 of 44 studies showed such a range].

2. Another parameter is the **tumor location**, RS-zop did not change tumor sites compared to historical data i.e. skin tumors are prevelant on the dorsal as opposed to ventral or lateral surface.

3. **Multiple tumors were not increased** by RS-zop. Most of the mice in drug and historical data argued Dr — bore one fibroma or sarcoma.

4. Time to tumor onset was not modified by RS-zop compared to historical data as presented in the figure below from Dr. — eport:



5. Support for increased fighting in RS-zop leading to skin wounds and hence tumors. Dr. [redacted] presented the table below showing percent of mice with dorsal encrustations in the 2yr study:

Percentage of animals presenting dorsal encrustations

Dose (mg/kg zopiclone)	0	0	1	10	100
Males	6	10	6	6	46
Females	0	0	0	0	0

The 46% exceeded the mean historical range from 13 studies at [redacted] at 14% (range 2-33%) for dorsal encrustations. Noted one carcinogenicity study was done in individually housed mice with 0 incidence of cutaneous encrustations therefore, suggestive that encrustations are likely caused by bites. This finding led to the conclusion that RS-zop at high dose of 100mg/kg/d increased fighting in gang-housed male B6C3F1 mice.

6. Next comes "**duration of dorsal encrustations**". Mean duration of dorsal encrustations considered both mean duration of encrustation per animal + incidence of animals presented with encrustations. The mean value in historical and in-study control was 1.3wk (range 0.02-3.8) and in RS-zop 6.7wk concluding that the latter modified the fighting behavior in these mice.

7. **Association between fighting and s.c. tumors** was assessed by comparing tumor incidence among mice with encrustations for 1 or more wks, with tumor incidence with those mice that did not have encrustations, table below from the report showed such an association:

Association between dorsal encrustations and tumors of the subcutis
Historical and within-study control data

	Males without encrustations	Males with dorsal encrustations
Number observed	682	112
Number with fibroma or fibrosarcoma	35	20
Percent	5.1	17.9

The difference between these two incidences was highly significant using Fisher's Exact Probability Test, two-tailed test ($p < 0.0005$).

Clearly some mice may have been bitten without developing dorsal encrustations. Therefore, distribution of tumors as function of the number of mice bitten per cage, should also be considered and the report did that. Results showed bites played an important role in development of the s.c. tumors in these mice.

Conclusions:

Dr. concluded that the incidence of s.c. tumors at 100mg/kg/d RS-zop in male mice was unusually high and exceeded several historical data bases, that there is a good correlation between the incidence of dorsal tumors and incidence and duration of dorsal bites leading to the conclusion that fighting causes tumors in the subcutis of male B6C3F1 mice. This fighting behavior in RS-zop is paradoxical phenomenon of long term exposure to high dose of RS-zop. RS-zop modified the animal fighting behavior increasing the aggressiveness of these mice leading to biting and mutilation with consequent lesion formation. Therefore, the fibrosarcoma in male mice are not a direct effect of RS-zop but indirect mediated by increased aggressiveness and biting. Based on this explanation of the origin of these tumors, the author concluded that this finding has no relevance to humans treated at therapeutic dose of RS-zop.

In a report by Dr. — in 1986 (Sepracor#190-848), he reached similar conclusion to the above report i.e. that RS-zop likely increased aggressiveness in male mice which was responsible for increased biting and skin encrustations with consequent tumor formation. He also reference and has personal communications with Dr. Boorman of NTP on the skin tumors. He stated that Dr. Boorman made 3 points: (1) variability in the incidence of s.c. tumors in male mice is not seen in female mice, (2) in none of the 42 NTP carcinogenicity studies did they make a conclusion that a chemical is a carcinogen on the basis that it increased risk of s.c. tumors in male mice and, (3) NTP has no idea why the s.c. tumors are so variable in this strain of mice. The likelihood that it is due to fighting was at the time being investigated by housing male mice individually.

See Reviewer conclusion on lung and skin tumors in section 3.4.8 Special Toxicology Studies

S-zop potential tumorigenic effects in P53 transgenic mice/Sepracor# 190-838

Conducting lab: —
Study Date: July 26 2001
Batch# for S-zop: 029-0015
QA/GLP: Yes, OECD, FDA, and Japanese regulations for GLP.

Methods:

Heterozygous $P53^{+/-}$ from C57BL/6 strain were purchased from —
Mice were 10-12wk of age at start of dosing with B.wt of 27 and 22.6g for males and females respectively, there were 25/sex/gr, the design of study as follows:

Vehicle	Group 1	CMC 0.5% w/w + Tween 80 1%w/w
Untreated control	Group 2	
S-zop 100mg/kg/d	Group 3	
200mg/kg/d	Group 4	
300mg/kg/d	Group 5	
p-cresidine 400mg/kg/d in vehicle	Group 6	
p-cresidine 400mg/kg/d in corn oil	Group 7	

S-zop doses were selected based on 28d (Sepracor# 190-813 by —) and 3mo (Sepracor# 190-819 by —), general toxicity studies in mice where the NOAEL in both studies was 200mg/kg/d; the 300mg/kg/d was estimated by the sponsor to be the MTD. The 100mg/kg/d gave a reasonable multiple of human exposure; dose volume was 10ml/kg/d for all groups. Parameters assessed: mortality, clinical signs, B.wt, food intake, hematology, standard macroscopic gross exam and palpations, organ wts, histopathology of standard organs/tissues. Blood samples for hematology were taken from the orbital sinus of overnight fasted mice in all groups prior to daily dosing. Blood for TK analysis was taken from 5/sex/group at end of study from orbital sinus of non-fasted mice. Blood was sent to —
for TK analysis for S-zop and its metabolite S-desmethyl-zop using — method. H&E stained slides from vehicle control and HD group were examined for histopath plus any animals that died or killed in moribund and all lesions from all mice in low and mid dose at end of study. Target tissues for tumors (liver, urinary bladder, and kidney) were also examined as well as any gross lesions in both positive control groups. All slides were peer reviewed by —

Results:

Statistics was **NOT** done on survival and tumor data because there was no difference in mortality among any group and no difference in tumor formation of vehicle control and S-zop groups (tumors were observed in the positive controls as expected). S-zop was well tolerated up to 300mg/kg/d, considered as the NOAEL.

Clinical Signs and Mortality: Hypoactivity and half closed eyes were seen from d1 till end of study in 300mg/kg/d S-zop, the latter sign was seen in 100&200mg/kg/d from d20 till end of study and the former from after d43 onward. Orange-colored urine was seen in the cresidine groups in addition to hypokinesia/hypoactivity, piloerection was also seen but at small incidence. A total of 25 deaths occurred in all groups distributed as follows (table from sponsor):

Group	1		2		3		4		5		6		7	
Dose-level (mg kg day)	0 (vehicle)		None (-ve control)		100 (test item)		200 (test item)		300 (test item)		400 (+ve control)		400 oil (+ve control)	
Total	4 M + 0 F		0 M + 3 F		1 M + 1 F		1 M + 1 F		1 M 0 F		4 M + 3 F		3 M + 3 F	
% mortality	16	0	0	12	4	4	4	4	4	0	16	12	12	12

S-zop P53 study (Cont.)

There were 9 deaths due to tumors, 11 cause undetermined, 4 due to non-neoplastic reasons, and 1 gavage error. Out of the 12 deaths in control and drug groups, 10 were killed in moribund, 2 found dead and, out of the 13 deaths in p-cresidine, 7 were killed in moribund and 6 found dead. It is of note that **only 3 of the 6 deaths** in the cresidine group in corn oil, were due to tumors of urinary bladder or kidney, one due to osteosarcoma of the femoral bone, and the remaining 2 cause unknown. None of the 7 deaths in cresidine/vehicle were due to bladder or kidney tumors, only 1 died due to malignant fiber histiocytoma of the skin, the others cause was unknown and 1 was gavage error.

B.wt: mean B.wt gain was reduced in all drug groups and positive controls relative to vehicle and untreated control values (tables from sponsor). Mean wt gain reduction reached statistical significance in males from wk2 and females from wks 8/9 compared to controls. The decline in wt gain in females correlated with decrease in food intake.

Mean body weight change (g) for males

Group	1	2	3	4	5	6	7
Dose-level	0	None	100	200	300	400	400
(mg/kg/day)	(vehicle)	(-ve control)	(test item)	(test item)	(test item)	(+ve control)	(-ve control)
Period							
week 1-4	1.9	2.9	-0.3	-0.5	-0.4	0.2	-0.9
week 4-8	1.7	3.5	1.4	1.3	1.4	-0.3	-0.8
week 8-13	2.8	2.4	1.6	0.8	0.7	-0.5	-0.2
week 1-13	6.4	8.8	2.7	1.6	1.7	-0.6	-1.9
week 13-27	5.3	7.7	4.5	3.6	3.3	0.3	0.3
week 1-27	**11.7**	**16.5**	**7.2**	**5.2**	**5.0**	**-0.3**	**-1.6**
FBW (g)	39.0	43.1	34.7	32.7	32.2	26.1	26.0
% diff.		-10.5	-11.0	-16.2	-17.4	-33.1	-33.3

FBW: final body weight

% diff.: % difference compared to controls.

Mean body weight change (g) for females

Group	1	2	3	4	5	6	7
Dose-level	0	None	100	200	300	400	400
(mg/kg/day)	(vehicle)	(-ve control)	(test item)	(test item)	(test item)	(+ve control)	(-ve control)
Period							
week 1-4	0.5	1.2	-0.7	0.0	0.2	-0.4	-0.2
week 4-8	1.5	2.1	0.8	0.2	0.1	0.2	-0.3
week 8-13	1.4	1.1	0.3	0.3	0.9	0.3	0.2
week 1-13	3.4	4.4	0.4	0.5	1.2	0.1	-0.3
week 13-27	4.4	5.5	1.1	0.9	0.7	0.7	0.7
week 1-27	**7.8**	**9.9**	**1.5**	**1.4**	**1.9**	**0.8**	**0.4**
FBW (g)	29.9	31.9	24.6	24.3	24.6	23.6	22.8
% diff.		+6.7	-17.7	-18.7	-17.7	-21.1	-23.7

FBW: final body weight

% diff.: % difference compared to controls.

S-zop P53 study (Cont.)

Food Intake: there was no drug related effect on food intake in males whereas a non-dose dependent reduction noted in all female groups relative to the controls. Mean cumulative food consumption per animal over the entire period in males was -3 to 0% and -7 to -5% in females. Food intake was consistently lower in both the p-cresidine groups relative to the controls and the cresidine in oil group ate much less than that in vehicle. The latter was contributed to the compensatory calorific value of the oil; there was no difference in B.wts between these 2 groups. This reduction in food intake correlated with the wt reduction in the positive controls, mean cumulative food consumption per animal over the entire period for oil/aqueous groups was -15 to -30% in males and -16 to -31 in females.
Hematology: both RBC and WBC counts were reduced significantly in all drug groups in both sexes relative to controls; similar findings for the positive controls (tables from sponsor). The reduction in WBCs in drug groups and positive controls was due to reduced neutrophils and lymphocytes.

Group	1	2	3	4	5	6	7
Dose-level (mg/kg/day)	0 (vehicle)	None (-ve control)	100 (test item)	200 (test item)	300 (test item)	400 (+ve control)	400 (+ve control)
- Males							
Parameter (unit)							
. WBC (G/L)	9.11	10.22	4.75	5.04	4.97	4.96	5.57
		12.2	*-47.9*	*-44.7*	*-45.4*	*-45.6*	*-38.9*
. RBC (T/L)	10.53	10.45	10.48	9.94	9.97	8.86	8.38
		-0.8	*-0.5*	*-5.6*	*-5.3*	*-15.9*	*-20.4*
. Hemoglobin (g/dL)	15.7	16.0	15.9	15.3	15.5	12.1	10.7
		1.9	*1.3*	*-2.5*	*-1.3*	*-22.9*	*-31.8*
. Hematocrit (L/L)	0.47	0.47	0.47	0.46	0.46	0.36	0.33
		0.0	*0.0*	*-2.1*	*-2.1*	*-23.4*	*-29.8*
. MCV (fl)	44.8	44.7	45.2	45.7	46.5	40.6	40.0
		-0.2	*0.9*	*2.0*	*3.8*	*-9.4*	*-10.7*
. MCH (pg)	14.9	14.9	15.2	15.4	15.5	13.7	12.8
		0.0	*2.0*	*3.4*	*4.0*	*-8.1*	*-14.1*

Group	1	2	3	4	5	6	7
Dose-level (mg/kg/day)	0 (vehicle)	None (-ve control)	100 (test item)	200 (test item)	300 (test item)	400 (+ve control)	400 (+ve control)
- Females							
Parameter (unit)							
. WBC (G/L)	4.19	4.77	3.16	3.61	2.32	3.26	3.20
		13.8	*-24.6*	*-13.8*	*-44.6*	*-22.2*	*-23.6*
. RBC (T/L)	10.64	14.25	10.02	10.06	10.18	9.09	8.19
		33.9	*-5.8*	*-5.5*	*-4.3*	*-14.6*	*-23.0*
. Hemoglobin (g/dL)	15.1	14.3	15.6	15.6	16.0	12.9	11.4
		-5.3	*3.3*	*3.3*	*6.0*	*-14.6*	*-24.5*
. Hematocrit (L/L)	0.48	0.66	0.47	0.47	0.48	0.40	0.36
		37.5	*-2.1*	*-2.1*	*0.0*	*-16.7*	*-25.0*
. MCV (fL)	44.6	47.5	46.8	47.0	47.7	44.2	44.3
		6.5	*4.9*	*5.4*	*7.0*	*-0.9*	*-0.7*
. MCH (pg)	14.3	10.9	15.5	15.4	15.7	14.1	13.9
		-23.8	*8.4*	*7.7*	*9.8*	*-1.4*	*-2.8*

S-zop P53 study (Cont.)

Organ wts: absolute and relative wt of the epididymides were significantly increased dose dependently in males relative to controls and correlated with histopathology; smaller change noted in positive controls. Other changes included increase in absolute and relative adrenal wts in drug groups and positive control males relative to control however, no histopath correlation and small change in females.
Gross exam: the only drug related effect was enlarged epididymides in 200 and 300mg/kg/d S-zop and kidney changes in p-cresidine that correlated with histopath (irregular and granular surface).
Histopathology: Non-Neoplastic findings: none in females and little in 100 and 200mg/kg/d dose groups. At 300mg/kg/d the following was observed in epididymides: high incidence 92%, sperm retention with chronic interstitial inflammation and some sperm granuloma. The static spermatozoa lead to degeneration of semniferous tubules in the testes with occasional sperm granuloma, similar changes in testes and epididymides but at lower incidence, were seen in 100 and 200mg/kg/d groups. Minimal hepatocyte vacuolation in 300mg/kg/d S-zop male and female mice considered of biological but not toxicological significance. In p-cresidine groups but none in S-zop, slight to marked hepatocellular degeneration/necrosis was seen. Only in p-cresidine groups, renal tubular basophilia, nephropathy and papillary necrosis were were reocrded at high incidence (table from sponsor):

Incidence of non-neoplastic histopathological findings in the kidney

Group	1		2		3		4		5		6		7	
Dose-level (mg/kg/day)	0 (vehicle)		None (-ve control)		100 (test item)		200 (test item)		300 (test item)		400 (+ve control)		400 (+ve control)	
Sex	M	F	M	F	M	F	M	F	M	F	M	F	M	F
Tubular basophilia	2/25	-	-	-	-	-	-	-	-	-	21/24	15/24	7/25	11/25
Chronic tubulo-interstitial nephropathy	1/25	-	-	-	1/2	-	1/2	-	-	-	3/24	-	21/25	6/25
Papillary necrosis	1/25	-	-	-	-	-	-	-	-	-	22/24	8/24	25/25	22/25

-: no findings or not analysed

Similarly, urinary bladder hyperplasia, metaplasia, and dysplasia were seen in p-cresidien groups and none in drug groups (table from sponsor):

Incidence of non-neoplastic proliferative findings in the urinary bladder

	Males				Females			
Dose-level (mg/kg/day)	0 (vehicle)	300 (test item)	400 (+ve control) aqueous vehicle	400 (+ve control) oil vehicle (a)	0 (vehicle)	300 (test item)	400 (+ve control) aqueous vehicle	400 (+ve control) oil vehicle
Findings								
. Urothelium hyperplasia	0	0	23	23	0	0	25	25
. Squamous metaplasia	0	0	12	16	0	0	13	19
. Urothelium dysplasia	0	0	7	2	0	0	5	5

S-zop P53 study (Cont.)

Neoplastic Findings: no drug related tumors were observed up to 300mg/kg/d S-zop in either sex relative to controls. As expected, increased incidence of urinary bladder transitional cell carcinoma and submucosal mesenchymal tumors was seen in the positive control groups relative to the controls (table from sponsor).

Incidence of neoplastic lesions

Group	1		2		3		4		5		6		7	
Dose-level (mg/kg/day)	0 (vehicle)		None (-ve control)		100 (test item)		200 (test item)		300 (test item)		400 (+ve control)		400 (+ve control)	
Sex	M	F	M	F	M	F	M	F	M	F	M	F	M	F
Bone (skull)														
- osteosarcoma	1/25	-	-	-	-	-	-	-	-	-	-	-	-	-
Brain														
- glioblastoma	-	-	-	-	-	1/1	-	-	-	-	-	-	-	-
Femur														
- osteosarcoma	-	-	-	-		-	-	-	-	-	-	-	1/3	-
Hemolymphoreticular system														
- lymphoblastic malignant lymphoma	-	-	-	-		-	-	1/2	-	-	-	-	-	-
Lung														
- bronchio-alveolar carcinoma	-	-	-	-	-	-	-	1/2	-	-	-	-	-	-
Prostate														
- adenocarcinoma	1/25	-	-	-	-	-	-	-	-	-	-	-	-	-
Skin														
- malignant fibrous histiocytoma	1/25	-	-	1/3	-	-	3/3	-	1/25	-	-	1/1	-	-
Thymus														
- benign thymoma	-	-	-	-	1/2	-	-	-	-	-	-	-	-	-
Urinary bladder														
- (transitional cell) carcinoma	-	-	-	-	-	-	-	-	-	-	3/25	3/25	3/24	6/25
- submucosal mesenchymal tumour	-	-	-	-	-	-	-	-	-	-	3/25	2/25	2/24	0/25
Uterus														
- histio-sarcoma	-	-	-	-	-	1/6	-	-	-	-	-	-	-	-

M: male, F: female
-: no findings or not analysed

It is noted that the incidence of palpable masses was small and comparable between vehicle and drug groups as well as positive control: 2/100 control, 0/50 100mg/kg/d, 1/50 200mg/kg/d, 0/50 300mg/kg/d S-zop and, 2/100 for cresidine groups. Except for the 1 palpable mass in cresidine/vehicle group that appeared on wk10, all other masses were detectable during wks20-23.

S-zop P53 study (Cont.)

TK: both S-zop and S-desmethyl zop were detectable in all drug groups and their concentrations increased with dose with generally higher levels in females than males (table from sponsor):

Mean (± standard deviations) plasma concentrations (ng/mL) of (S)-ZOPICLONE and (S)-DESMETHYL ZOPICLONE in the test treated groups are shown in the table below:

Group	Dose (mg/kg/day)	(S)-ZOPICLONE		(S)-DESMETHYL ZOPICLONE	
		Male	Female	Male	Female
3	100	2416 ± 480	7568 ± 6324	428 ± 70	1590 ± 422
4	200	5622 ± 1336	7960 ± 2378	939 ± 268	1870 ± 591
5	300	9374 ± 1513	10656 ± 2989	1512 ± 188	2322 ± 530

Summary and Conclusion:

S-zop up to 300mg/kg/d administered orally for 26wks to heterozygous P53 mice was well tolerated with no difference in mortality and mild clinical signs. Mean wt gain was reduced in both sexes in a dose related manner being 22 and 33% of the control in males and females dosed 300mg/kg/d over the entire 26wks. The reduction in female B.wt correlated with decrease in food intake. Mean WBC and RBC counts were reduced in all drug groups relative to control. In males dosed 200&300mg/kg/d enlarged epididymides with sperm retention and consequent testicular degeneration and necrosis was observed. There was no induction of tumors by S-zop up to 300mg/kg/d whereas, p-cresidine prepared in vehicle or corn oil induced the expected urinary bladder tumors [**in this reviewer's opinion the positive tumor response in the positive control seems weak**]. The sponsor considered 100mg/kg/d as the MTD based on B.wt gain changes, hematological findings, and histopath of the epididymides and testes in males. Based on these findings **S-zop up to 300mg/kg/d was not carcinogenic in this bioassay.**

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RAT CARCINOGENICITY STUDY:

An 18month chronic toxicity study and a 2 year carcinogenicity study were done in the rat in addition to other specialized studies conducted specifically to address tumor findings in the long term studies.

18 Month Chronic Toxicity Study/Sepracor#190-831F (see Chronic Toxicity section for the interim data and other detail:

This study was done at —— (final report on Feb 1981), to address the long term toxicity of RS-zop in the rat.

Species/Strain/# per sex per dose: CD rat, 50/sex/dose. There was a 6month interim sacrifice.

Doses/Route/Duration: **0, 2, 20, 200mg/kg/d** via the **diet** for 78 weeks.

Results:

Mortality/Clinical Signs: No drug effect

Food Intake: No drug effect

B.wt/B.wt Gain: mean B.wt in HD rats was significantly reduced (28% in both sexes), compared to the control values or other drug groups.

Hematology: No drug effect

Clinical Chemistry: some increases in HD rats in serum proteins, albumin, and globulin.

Organ wt: mean liver and thyroid wts in HD rats were statistically significantly increased relative to the corresponding control wts (tables from sponsor). Increases were relatively **small at 1-2x** the control wt. There were no other drug related effects on any organ.

Table 21: Mean Absolute (ABS) and Relative (REL) Weights of the Thyroid in Male and Female CD Rats Receiving (RS)-Zopiclone for 78 Weeks

Group	Control	(RS)-Zopiclone (mg/kg/day)		
	0	2	20	200
Males				
ABS (mg)	42	38	41	58*
REL (%)[1]	5.5	5.3	5.8	10.5**
Females				
ABS (mg)	34	33	33	31
REL (%)[1]	8.4	8.2	8.6	10.4**

* p<0.05; ** p<0.01 from control.
[1] Values given as x1000.

Table 22: Mean Absolute (Abs.) and Relative (Rel.) Weights of the Liver in Male and Female CD Rats Receiving (RS)-Zopiclone

Group	Control	(RS)-Zopiclone (mg/kg/day)		
	0	2	20	200
Males				
ABS (g)	25.5	25.5	25.2	27.7
REL (%)	3.3	3.6*	3.5*	4.9**
Females				
ABS (g)	14.7	15.2	15.6	17.1**
REL (%)	3.6	3.7	4.0**	5.6**

* p<0.05; ** p<0.01 from control.

Gross Exam: No findings except for 5/33 enlarged thyroids in HDm.

18mo Rat Tox study, Sepracor#190-831F (Cont.)

Non-Neoplastic Findings: hepatocellular hypertrophy* in HDm+f relative to the control. Proliferation of portal bile duct was increased in HD rats only at interim sacrifice but not at 18mo.

* Dr. A. Wilk (previous Pharm/Tox reviewer), in a supplement dated May 1983, mentioned an increased incidence of liver **hyperplastic nodules** in HDm. She also reported the opinions of 2 consultants: Drs. — Dr. — evaluated the liver slides and considered 3 liver adenomas to be foci of cellular alteration. Dr. — on the other hand, did not evaluate the slides but was provided a table from the sponsor showing 7 vs.1 liver nodules in 200mg/kg/d males and control respectively. Consultant's conclusion was that RS-zop was not a liver carcinogen because of the low incidence, these are spontaneous tumors in aging rats in addition to absence of foci of cellular alteration. The latter though apparently not observed at the end of 18month, was seen in the 24month rat carcinogenicity study as the incidence of basophilic and eosinophilic foci in the liver increased in males dosed 100mg/kg/d — 1988), at 22/50 and 8/50 respecitvely, vs. 12/50 and 2/50 in control groups respectively.

Thyroid follicular cell hyperplasia in HDm (table from sponsor).

Table 24: Incidence of Thyroid Follicular Cell Hyperplasia in Male and Female CD Rats Receiving (RS)-Zopiclone for up to 18 Months

Group	Control	(RS)-Zopiclone (mg/kg/day)		
	0	2	20	200
Males	(35) [a]	(34)	(34)	(34)
- Hyperplasia	0	0	0	2
Females	(35) [a]	(34)	(35)	(35)
- Hyperplasia	1	0	0	0

[a] Number examined includes terminal sacrifices, deaths and unscheduled sacrifices through 78 weeks but does not include animals killed at the 6-month interim sacrifice.

Neoplastic Findings: MDm & HDm thyroid follicular cell adenomas, dose related in mid and high dose males (table from sponsor).

Table 23: Incidence of Thyroid Follicular Cell Adenomas in Male and Female CD Rats Receiving (RS)-Zopiclone for up to 78 Weeks Original and Supplemental[1] Microscopic Readings

Group	Control	(RS)-Zopiclone (mg/kg/day)		
	0	2	20	200
Males	(35) [2]	(34)	(34)	(34)
Original : Adenoma	1	1	5	9*
Supplemental : Adenoma	-	0	-	0
Females	(35) [2]	(34)	(35)	(35)
Original : Adenoma	0	0	0	0
Supplemental : Adenoma	-	0	1	-

* $p<0.05$, from control.

[1] Addendum to original report (dated July 21, 1982), i.e. second slide reading. Dashes indicate that no slides from those groups were evaluated in the supplemental read.

[2] Number examined includes terminal sacrifices, deaths and unscheduled sacrifices through 78 weeks but does not include the 6-month interim sacrificed animals.

Liver carcinoma was seen in 1/33 HDm vs. 0 incidence in male control and other drug groups.

18mo Rat Tox study, Sepracor#190-831F (Cont.)

Slides from low dose rat and mid dose female rat groups were not examined in the original study. In an amendment dated Jul 1982, original thyroid slides for these groups were examined and only 1 additional adenoma was found in MDf and no additional hyperplasia was found. There were no drug related changes in mammary glands of either sex however, there was a significant decrease in incidence of pituitary tumors in males: 47% control, 16% HDm; $p<0.05$ and in females: 26% control and 21% in HDf; p not significant.

Because of the thyroid and liver findings, TSH, T3, and T4 hormone levels were measured in a 1month oral **gavage** study in SD rats (RP21439 Aug 1982/Sepracor# 190-853; see special Tox section for mechanistic studies). Male and female rats (15/sex/group; 5/cage) were administered RS-zop at **0, 0.2, 2, 20, or 200mg/kg/d**. Groups of 5/sex/dose were killed by ether anesthesia, on wks 1, 2, and 4 for serum hormone determination, thyroid and pituitary wts plus macro- and micro-scopic examinations. Drug related findings were transitory and included clinical signs (exaggerated pharmacology), only in the 2 high doses during 1st wk of dosing, decrease in B.wt in HDm only during 1st 2wks of dosing, no drug effect on organ wts or macroscopic findings. Microscopic findings showed a *small* dose related increase in follicular cell hyperplasia indicative of increased thyroid activity in males dosed 20&200mg/kg/d; no changes in any female rat group. Mean TSH level was significantly increased in HDm relative to the control in all 3 measurement periods (n=1 on wk2). Mean T3 levels were affected only mildly by RS-zop in HDm and the sponsor contributed the statistical significance on wk4 to the low value of the control (table 26 from sponsor). Mean T3 was also significantly increased in mid and high dose males on wk2 relative to the control value. Note that the control value of 62 during wk2 is not too different from 58 on wk4 deemed low by the sponsor. Mean T3 levels were also significantly and dose dependently increased in 20&200mg/kg/d *female* rats (note that there were no changes in thyroid histology in females in the 18month study).

Table 26: Mean Serum T_3 Levels (ng/100 ml) in CD Rats Receiving up to 200 mg/kg/day of (RS)-Zopiclone for 1 Month

Week	Males					Females				
	0	0.2	2	20	200	0	0.2	2	20	200
1	78	74	84	88	83	74	91	90	115*	123*
2	62	67	68	83*	84[a]	71	78	80	97*	92
4	58	58	59	70	87*	61	80	63	83*	85*

* $p<0.05$ relative to the control group. [a] n = 1.

Table 25: Mean Serum TSH Levels (µg/ml) in CD Rats Receiving up to 200 mg/kg/day of (RS)-Zopiclone for 1 Month

Week	Males					Females				
	0	0.2	2	20	200	0	0.2	2	20	200
1	0.49	0.70	0.71	0.59	0.87*	0.39	0.44	0.39	0.50	0.54
2	0.62	0.62	0.64	0.62	1.81[a]	0.43	0.30	0.41	0.46	0.53
4	0.58	0.82	1.08	1.14	1.34*	0.40	0.40	0.38	0.40	0.50

Consultant Opinions on the Thyroid Findings:

Drs. [redacted] considered the follicular cell hyperplasia in HDm in the 18month oral dietary study to be a drug effect on the thyroid/pituitary pathway. This was supported by the 1month hormone study that showed an increase in TSH serum levels in HDm. The consultants concluded that increase in T4 elimination caused by liver enzyme induction was the likely cause of TSH elevation in HDm. Therefore, RS-zop affects thyroid hormones secondarily to its effect on the liver through enzyme

induction. Such effect is indicative of a THRESHOLD phenomenon below which these findings may not be observed (see 24 month rat study for more detail).

24 Month Rat Carcinogenicity Study/Sepracor#190-833:

Study design was that of the NTP: 2 control groups and 3 dose groups; GLP

Species/Strain/# per sex per dose: CD rats; 50/sex/group including control.

Doses/Route/Duration: **0, 0, 1, 10, 100mg/kg/d*** RS-zop for at least 105wks via the DIET.

*** note that these doses were lower than those used in the 18month study where thyroid tumors were observed (2, 20, 200mg/kg/d).**

Results:

Mortality/Clinical Signs: No drug effect; survival in all groups at end of study was 34-44%

B.wt/B.wt Gain:mean B.wt gain in HD rats was significantly reduced (25% m &35% f) relative to the gains in corresponding controls. In females, this decrease in wt gain paralleled decrease in food intake during the latter period of the study. Mean B.wt and food intake in MDf were increased during the 1st 13wks but were reduced ($p<0.001$) during the end of the study.

Organ wt:although mean absolute wts of the liver in MD&HDm were significantly increased relative to the controls, this increase was small at 1.2x the control. Similarly, the relative liver wt increased only 1.6x the control value without reaching statistical significance (table from sponsor). Moreover, mean absolute liver wt in HDf was significantly decreased compared to the control value and no effect on relative wt in this group. There was no other drug related effect on any organ including the thyroids.

Table 28: Mean Absolute and Relative Liver Weights in Male and Female CD Rats Receiving (RS)-Zopiclone for up to 108 Weeks

Group	Control		(RS)-Zopiclone (mg/kg/day)		
	0	0	1	10	100
Males					
Absolute (g)	27.8	26.0	27.9	31.1**	32.4***
Relative (%)	3.3	3.1	3.1	3.7	5.0
Females					
Absolute (g)	22.5	22.1	21.9	21.1	20.4**
Relative (%)	3.9	3.5	3.5	4.0	5.1

** p<0.01; *** p<0.001 from control.

Non-Neoplastic Lesions: HDm: hepatocellular foci of baso- or eosino- philia, epididymal and testicular changes, and pituitary hyperplasia. HDf: enlarged thyroid follicles (see tables 34&35).

Table 34: Incidence of Non-Neoplastic Thyroid Lesions in Male CD Rats Receiving (RS)-Zopiclone for up to 108 Weeks

Tissue Finding	Control		(RS)-Zopiclone (mg/kg/day)		
	0	0	1	10	100
(Number Examined)	(49)	(50)	(50)	(50)	(49)
- Enlarged Follicles with Basophilic Epithelium	1	1	0	0	4
- Enlarged Follicles	2	2	1	3	4
- Follicular Cell Hypertrophy	0	0	0	0	0

Table 35: Incidence of Non-Neoplastic Thyroid Lesions in Female CD Rats Receiving (RS)-Zopiclone for up to 108 Weeks

Tissue Finding	Control		(RS)-Zopiclone (mg/kg/day)		
	0	0	1	10	100
(N° Examined)	(50)	(50)	(50)	(50)	(49)
- Enlarged Follicles with Basophilic Epithelium	0	0	0	1	0
- Enlarged Follicles	0	0	0	1	4*
- Follicular Cell Hypertrophy	0	0	0	2	0

* p<0.05, compared to the combined controls.

Neoplastic Findings: HDm: **increased incidence of thyroid follicular cell carcinomas.**
HDf: increased incidence of mammary gland adenomas and carcinomas.
HDm&f: decrease in incidence of pituitary tumors relative to the corresponding controls (42% control male groups vs. 20% HDm ($p<0.05$); 64% control female groups vs. 39% HDf ($p<0.01$)).

Thyroid Follicular Cell Tumors in Male Rat:
Carcinoma but not adenoma incidence, was significantly increased in HDm relative to the control and any other drug group; no difference in adenoma incidence (table from sposor).

Table 30: Incidence of Thyroid Follicular Cell Tumors in Male CD Rats Receiving (RS)-Zopiclone for up to 108 Weeks

Follicular Cell Tumor	Control		(RS)-Zopiclone (mg/kg/day)		
	0	0	1	10	100
(Number Examined)	(49)	(50)	(50)	(50)	(49)
Adenoma	2	2	5	0	3
Carcinoma	0	0	1	0	6**
Adenoma or Carcinoma	2	2	6	0	9**

** $p<0.01$, compared to the combined controls.

Table 31: Incidence of Thyroid Follicular Cell Tumors in Male CD Rats Receiving (RS)-Zopiclone for up to 108 Weeks – Unscheduled Deaths

Follicular Cell Tumor	Control		(RS)-Zopiclone (mg/kg/day)		
	0	0	1	10	100
(Numbered Examined)	(33)	(34)	(29)	(34)	(32)
Adenoma	2[a]	1[b]	3[c]	0	1[e]
Carcinoma	0	0	1[d]	0	6**[f]
Adenoma or Carcinoma	2	1	4	0	7*

* $p<0.05$; ** $p<0.001$, compared to the combined controls.
[a] Animals No. 31 (Died- Wk 98) and No. 50 (Sacrificed- Wk 99).
[b] Animal No. 62 (Sacrificed- Wk 106).
[c] Animals No. 129 (Died- Wk 73), No. 133 (Sacrificed- Wk 79) and No. 137 (Died- Wk 98).
[d] Animal No. 111 (Sacrificed- Wk 70).
[e] Animal No. 214 (Sacrificed- Wk 102).
[f] Animals No. 212 (Died- Wk 101), No. 225 (Died- Wk 80), No. 227 (Sacrificed- Wk 105), No. 238 (Sacrificed- Wk 93), No. 240 (Died- Wk 105) and No. 246 (Sacrificed- Wk 106).

Table 32: Incidence of Thyroid Follicular Cell Tumors in Male CD Rats Receiving (RS)-Zopiclone for up to 108 Weeks – Terminal Sacrifice

Follicular Cell Tumor	Control		(RS)-Zopiclone (mg/kg/day)		
	0	0	1	10	100
(Number Examined)	(16)	(16)	(21)	(16)	(17)
Adenoma	0	1[a]	2[b]	0	2[c]
Carcinoma	0	0	0	0	0
Adenoma or Carcinoma	0	1	2	0	2

[a] Animal No. 80.
[b] Animals No. 107 and No. 117.
[c] Animals No. 205 and No. 207.

All tumors occurred in the unscheduled death rats with 3 of the 6 carcinomas found near end of study after wk104; study pathologist did not determine cause of death in these or any rats. Tumor distribution appeared random on a per cage basis. There were 2HDm (#240&246) that had thyroid carcinoma in *both lobes otherwise they were unilateral*, another HDm (#205), had adenoma in both lobes otherwise all adenomas were unilateral. Historical control mean incidence for thyroid follicular cell **adenomas** in — in CD rats was **4.2%** for 524 rats or 1.5-8.6% range and for **carcinoma 0.4%** for 524 rats or a range of 0-1.5%. Thyroid **carcinoma in HDm was 12%** far exceeding this range with the incidence of thyroid tumors in control, low, and mid dose groups within this historical range. Non-Neoplastic thyroid lesions shown above in tables 34&35 from sponsor where incidence of enlarged follicles was slightly and not significantly increased in HDm (4/49; table 34), but the incidence was significantly increased in HDf (4/49 vs. 0/100). There was no follicular cell hypertrophy in any male group or female group except in MDf, relative to the controls (table above from sponsor). The statistical significant increase in enlarged follicles in HDf was attributed to the 0 incidence in the control and therefore, was considered of no toxicological significance specially since the incidence of thyroid tumors in females was not affected.

Special Studies:

To investigate these tumors many studies were done (see Special Toxicity section for detail). Serum thyroid hormones were measured in a 9wk study in SD rats (RP# 22387, May 1985; Sepracor#190-850). Single-housed rats (40/sex/group) were administered via the **diet,** RS-zop at **0, 1, 10, or 100mg/kg/d,** for 2, 4, and 9wks, 10/sex/group were killed under ether anesthesia. Serum thyroid hormones, RS-zop levels, and microscopic exams of thyroid, liver, adrenals, pituitary, ovaries, and mammary glands were done during these periods as well as liver P450 content, aniline hydroxylase, and aminopyrine N-demethylase activities were determined. There were no drug effects on mortality, clinical signs, or body wt. Mean liver wt increased 33% at end of 9wks in HDm and 43% in HDf over the corresponding control values. This increase in liver wt did not cause an increase in liver enzyme activities nor any histopathology. Mean serum levels of TSH showed marked variability in the data (table from sponsor; s.d. ≥50% including the control group), accurate conclusion could not be made. Also noted, is absence of any histopathology of the thyroids.

Table 36: Mean Serum TSH Levels (ng/ml) in CD Rats Receiving up to 100 mg/kg/day of (RS)-Zopiclone for up to 9 Weeks

Week	Males				Females			
	0	1	10	100	0	1	10	100
0	756	528	547	438	300	245	251	261
2	707	1148	758	1193	260	307	292	427
4	1760	1372	1372	1807	336	251	415	418
9	870	1241	792	739	194	266	260	359***

*** p<0.001compared to controls.

Because of the non-supportive results in this dietary study and the one conducted previously both via the diet, a 3rd study was done to measure thyroid hormones following dietary and gavage administration of RS-zop (RP#93, Dec 18th 1987/Sepracor#190-849). Male and female SD rats (40/sex/group; 4/cage), were given by **gavage 0, 1, 10, 100, or 200mg/kg/d or via the diet 100 or 200mg/kg/d for 4 or 10wks of dosing.** Rats were killed at 2 and 11hr postdose on wks 4 and 10 of dosing (10/sex/group). Serum TSH, T3, rT3*, and T4, histopath of thyroid, liver, adrenals, pituitary, ovaries, and mammary glands, and RS-zop blood levels were determined. * rT3 is 3,3',5'-tri-iodothyronine (breakdown of T4 via 5-monodeiodinase in the liver and is inactive form of T3).

Clinical signs of exaggerated pharmacology were seen in 100&200mg/kg/d groups via gavage only. Slight decrease in mean wt gain in 100mg/kg/d males (gavage and diet) and females (diet) was seen. Similar to previous study, there was large variability in the data as seen from the >50% s.d. However, changes in mean TSH were recorded in male rats dosed 200mg/kg/d during both wks4&10 (table from sponsor). Note that change at 100mg/kg/d was less marked than at 200mg/kg/d and recorded only at 4wks (this is

Table 37: Mean Serum TSH Levels (ng/ml) in Male CD Rats Receiving up to 200 mg/kg/day of (RS)-Zopiclone for 4 or 10 Weeks

Sacrifice Time	Gavage					Diet		
	0	1	10	100	200	0	100	200
Wk 4 - 2 h	339	381	537	521	754**	499	509	1164**
Wk 4 - 11 h	310	211	202	464	840***	320	310	713***
Wk 10 - 2 h	390	356	444	294	535	309	480**	770***
Wk 10 - 11 h	276	267	245	323	900***	239	373*	593***

* p<0.05; ** p<0.01; *** p<0.001, compared to the respective control group.

the dose with the tumors, no tumors at 200mg/kg/d).
The sponsor indicated that changes in T3 were not obvious but levels of rT3 (wk4 at 12hr only), and T4 were significantly reduced in HD rats. Females in HD showed similar increase in TSH but the changes in T3 were not as marked and levels of T3&T4 were similarly reduced. These hormone changes may be reflective of the small increases in mean thyroid wts at 200mg/kg/d of both sexes via both routes of administration. Thyroids were hyperactive in 200mg/kg/d wk4 and at wk10 for 100&200mg/kg/d groups.

Mean plasma RS-zop levels increased non-linearly with dose (less than proportional in HD), via either route of administration (table from sponsor):

Table 38: Mean Plasma Levels[a] (µg/ml) of (RS)-Zopiclone in Male CD Rats Receiving (RS)-Zopiclone for 4 and 10 Weeks

Sacrifice Week	Gavage				Diet	
	1	10	100	200	100	200
4	0.094	0.435	7.815	8.472	1.875	1.591
10	0.077	1.130	10.940	8.806	2.311	2.392

[a] n =5; samples taken approximately 2 h after dosing.

More studies were done to further examine the thyroid findings: *in vitro* and 2 *in vivo* studies in rats. The *in vitro* study utilized liver homogenates from SD rats (6/sex/dose) dosed via the diet, RS-zop at **0 or 200mg/kg/d for 36 or 56days**. Liver homogenates were incubated with T4 and free T3 and 5'monodeiodinase activity measured (table from sponsor). Mean T3 levels in males increased 1.5x over the control on d56 but no effect in females and no significant effect on enzyme activity in either sex except for a non-significant 1.4x increase in activity in males over the control value.

Table 39: Free T_3 and 5'- Monodeiodinase Activity in Male Rats Receiving 200 mg/kg/day of (RS)-Zopiclone for 4 and 10 Weeks

Treatment	Males				Females			
	Free T_3[1]		5'- MDI[2]		Free T_3		5'- MDI	
	36 d	56 d	36 d	56 d	36 d	56 d	36 d	56 d
Control	14.2	18.8	0.12	0.18	15.5	17.5	0.12	0.16
Zopiclone	18.1	27.5**	0.15	0.25	19.5	17.8	0.15	0.18

** p<0.01, compared to the control value.

[1] Activity at 120 minutes, in units of pmole/g protein.

[2] 5'- monodeiodinase activity at 120 minutes, in units of pmole/min/g protein.

RS-zop was administered via the **diet at 0 or 200mg/kg/d** to male and female CD rats for **10wks**. Control and drug groups were injected 3uCi ^{125}I 24hr pre-sacrifice, at necropsy, thyroids were weighed and *total* radioactivity was measured. Thyroglobulin binding was assessed in thyroid homogenate supernatant using chromatography. No increase in ^{125}I uptake in males but increased incorporation of radioactivity uptake was seen in females (no thyroid tumors in females). No drug effect on thyroglobulin binding in either sex. These results indicated at least in males, thyroid hormone synthesis was unaffected by drug treatment but also note 200mg/kg/d is not the dose where thyroid tumors were observed.

APPEARS THIS WAY ON ORIGINAL

Another *in vivo* study in CD rats (30/sex/group) examined liver enzyme activity and thyroid hormone changes after dietary administration of RS-zop at 0 or **200mg/kg/d for 10wks** (RPR report# SM566, Dec 23, 1991). ^{125}I-thyroxine was administered to 6/sex/group rats to determine the clearance over 72hr, serum T3, T4, and TSH, and 5'monodeiodinase activity in the liver microsomes. Mean serum free T4 & TSH were increased significantly in both sexes over the control values as well as clearance of T4 (tables from sponsor).

Table 40: Mean Serum FT_3 (pg/ml), FT_4 (pg/ml) and TSH Levels (ng/ml) in CD Rats Receiving 200 mg/kg/day of (RS)-Zopiclone for 10 Weeks

Treatment	Males			Females		
	FT_3	FT_4	TSH	FT_3	FT_4	TSH
Control	5.09	16.67	9.62	5.88	13.94	5.23
200 mg/kg	4.99	13.50**	16.43**	6.46	10.47***	15.27***

Table 41: ^{125}I-Thyroxine Kinetics in CD Rats Receiving 200 mg/kg/day of (RS)-Zopiclone for 10 Weeks

Treatment	Males			Females		
	C_{max} [a]	$AUC_{(0\text{-}inf)}$ [b]	Cl [c]	C_{max} [a]	$AUC_{(0\text{-}inf)}$ [b]	Cl [c]
Control	4.23	59.0	3.84	3.06	41.3	5.53
200 mg/kg	2.87*	44.7***	5.06***	1.68***	25.2***	9.13***

** p<0.01; *** p<0.001, compared to the respective control group. [a] cpm x 10^3/ml. [b] h· cpm x 10^3/ml. [c] ml/k/kg.

Table 42: 5'- Monodeiodinase and T_4 UDP-GT Activity in Male Rats Receiving 200 mg/kg/day (RS)-Zopiclone for 10 Weeks

Treatment	Males		Females	
	5'-MDI[1]	T_4 – UDP-GT[2]	5'-MDI	T_4 – UDP-GT
Control	8.94	121.79	11.85	102.68
200 mg/kg	11.56	249.02***	15.00*	201.45***

** p<0.001, compared to the control value. [1] 5'-monodeiodinase activity in units of 10^{-3} pmol/min/g liver. [2] UDP-GT in units of pmol/min/g liver.

From the above table, liver 5'deiodinase activity was significantly increased in female rats but only minimal increase in males. UDP-GT activity responsible for clearance of T4 was also increased in both sexes relative to the control. These results indicate increased elimination of T4 due to induction of hepatic UDP-GT with lesser effect with 5'deiodinase. Therefore, continued lower levels of T4 will signal TSH to maintain T4 production leading to thyroid hyperactivity → hyperplasia → neoplastic response.

In a recent 90 day rat toxicity/TK study of S-zop and RS-zop, thyroid function was also determined. SD rats (15/sex/dose) were administered via oral gavage S-zop at **0, 25, 50, or 100mg/kg/d and RS-zop at 100mg/kg/d** (Sepracor#190-818A). Blood TSH, T3 and T4 were determined at end of dosing and after 1 month recovery. Necropsy and microscopic exams were unremarkable for the thyroid and livers. Similar to other studies, data were highly variabile and any drug related effect could not be detected in any group. Mean liver wts were only slightly increased in S- and RS- zop groups, both cpds seemed to induce a number of liver microsomal enzymes including UGT1A1 responsible for T4 clearance (in HDm, no increase in females). Elevation in activity of UGT1A1 is indicative of increased clearance of T4 consequently leading to continued stimulation of TSH and hyperactive thyroid eventually leading to thyroid tumors.

Sponsor's Conclusion on the Rat Thyroid Tumors:
Thyroid tumors observed in 18 month and 2 year rat carcinogenicity studies are secondary RS-zop-induction of liver enzyme activities that increases clearance of T4 through 5' deiodinase (minor pathway) and UDP-GT (major pathway). Increased elimination of T4 → ↑ TSH → hyperactive thyroid → hyperplasia → adenoma/carcinoma. **Based on absence of increase in thyroid hormones in humans, these findings are irrelevant to human cancer risk assessment**. The sponsor cited and provided copies of published literature in support of their conclusion. The sposnor indicated that RS-zop enzyme induction profile is similar to that of phenobarb where increased breakdown of T4 due to liver enzyme induction, is responsible for thyroid cell proliferation. Other drugs with thyroid lesions related to disruption in TSH activity include midazolam, oxazepam, amiodarone, and vidarabine. No evidence to date supports similar induction of these drugs of thyroid effects in humans. Moreover, the sponsor stated that no non-radioactive chemical exposure is known to cause thyroid tumors in humans. The sponsor also stated the differences between rodent/rat and human thyroids: **half life of T4 is shorter in rodents compared to humans leading to higher basal thyroid activity in the former, transport proteins of the thyroid hormones are different between the 2 species, and in the rat, only relatively small increases in serum TSH are needed to stimulate thyroid follicular cell proliferation.** IARC 1999, published criteria for identification of agents that cause thyroid follicular cell tumors in rodents via hormonal changes:
1) The cpd and its metabolites must be non-mutagenic or non-clastogenic *in vitro* and *in vivo* assays (RS-zop and S-zop were not mutagenic in a number of assays sponsor's conclusion however, this reviewer and members of the CDER genetic toxicology cmtt found both cpds to to be clastogenic).
2) Hormonal imbalance must be chronically demonstrated under conditions of carcinogenicity assay (RS-zop increased TSH and decreased T4 in a number of studies; see above).
3) The mechanism of the cpd that leads to the hormone imbalance is defined. In this case RS-zop induced liver enzymes 5'deiodinase and UDP-GT increasing T4 breakdown and TSH stimulation.

A clinical study showed no changes in TSH, T3, T4, or thyroglobulin following 30mg/d dose of RS-zop for 1 month in human volunteers.

Therefore, thyroid tumors were observred in one sex, one species, and at high doses, in sponsor's opinion RS-zop is not genetoxic drug. Also, there are differences between human and rodent thyroid profiles that makes such tumors irrelevant to human cancer risk based on this mechanism.

Consultant Opinions on the Thyroid Findings:
The following experts evaluated the data and each submitted a written report:

— Feb 25 1985:did not re-read the slides. Reviewed the results of both the 18 month and 24 month rat studies.

— Jun 5, 1985: re-read all slides of thyroid and pituitary from the rat 24 month study. Reviewed reports of thyroid function study RP# 21439 dated Aug 16 1982 and clinical report by — dated 1982, genetox assays, and carcinogenic ity studies.

— , Jan 20, 1986: did not re-read the slides. Reviewed the results of both the 18 month and 24 month rat studies and the rat mechanistic thyroid studies conducted at that time, 1982.

— Jan 22, 1990: did not re-read the slides. Reviewed the results of the thyroid tumors in the 24 month rat carcinogenicity study and results from the thyroid function tests.

All consultants concurred on the conclusion that the thyroid tumors in male rats are hormonally mediated and their incidence demonstrate a **THRESHOLD** effect since tumors were not observed at doses that did not increase blood TSH levels.

A recent panel of experts, Pathology Working Group, PWG, reviewed the data and re-evaluated the slides of the thyroid lesions in the rat 2 yr study. Dr. ' — neaded the group that included: Drs. — All experts used the common diagnostic criteria of Hardisty et al., 1990 and Botts et al., 1991, slides were read blindly, and review and discussion was done at the same sitting to reach a consensus on tumor type. Results and Conclusions were as follows:

1. The PWG believes that the likely mechanism of thyroid tumors is secondary to liver enzyme induction leading to increased metabolism of T4 and increase in TSH levels. Increase in TSH leads to chronic stimulation of the thyroid and consequent proliferative lesions of follicular cells.

2. Liver enzyme induction leading to distruption of the hypothalamic-pituitary-thyroid axis is a known mechanism of thyroid stimulation. RS-zop in the 2 yr study caused an increase in liver wts in HDm and there was centrilobular hypertrophy, findings collectively reflective of enzyme induction

3. Thyroid wts were increased in HDm rats indicative of hyperactivity.

4. Several neoplasia diagnosed originally as adenomas were reclassified by the PWG as follicular cell hyperplasia. In addition, tumors originally diagnosed as carcinomas became adenomas (table from sponsor).

Table 43: Incidence of Thyroid Follicular Cell Proliferations in Male Mice Receiving (RS)-Zopiclone for up to 108 Weeks
- PWG Evaluation -

Proliferative Lesion	Control		(RS)-Zopiclone (mg/kg/day)		
	0	0	1	10	100
SP Evaluation [1]					
Adenoma	2	2	5	0	3
Carcinoma	0	0	1	0	6
Total Tumors	2	2	6	0	9
PWG Evaluation					
Adenoma	0[a]	0[b]	3[c]	0	4[e]
Carcinoma	0	0	0[d]	0	3[f]
Total Tumors	0	0	3	0	7

[1] SP = original study pathologist's diagnosis (same as reported in Table 32).
[a] Animals Nos. 031 and 050 were diagnosed by the PWG with follicular cell hyperplasia rather than adenomas as was previously noted by the SP.
[b] Animals Nos. 062 and 080 were diagnosed by the PWG with follicular cell hyperplasia rather than adenomas as was previously noted by the SP.
[c] Animals Nos. 129, 133 and 137 were diagnosed by the PWG with follicular cell hyperplasia rather than adenoma as was previously noted by the SP. The slide for Animal No. 107 (adenoma) was missing but for comparative purposes was considered with an adenoma by the PWG (as per the SP's diagnosis).
[d] Animal No. 111 was diagnosed by the PWG with a follicular cell adenoma rather than a carcinoma as was previously noted by the SP.
[e] Animals Nos. 214 and 214 were diagnosed by the PWG with follicular cell hyperplasia rather than with an adenoma as was previously noted by the SP.
[f] Animals Nos. 225, 227 and 246 were diagnosed by the PWG with follicular cell adenoma rather than a carcinoma as was previously noted by the SP.

Reviewer's Comments:

This reviewer agrees with these conclusions based on the liver findings that included one or more of the following: liver wt increase, enzyme elevation, and/or histopathology in absence of clear direct effect on the thyroids. This opinion is formulated based on these results observed in one or more of the general toxicity studies, special studies, and the 2yr carcinogenicity studies. Thereofe, RS-zop seems to have caused the thyroid tumors indirectly and secondarily through liver enzyme induction. **However, it should be noted that this conclusion was reached by all previous consultants as well as the PWG with the assumption that RS-zop is not a mutagen. This is not the conclusion of this reviewer nor the CDER Genetic Toxicology committee where RS-zop was found to be clastogen in the MLA and equivocal in the Chinese hamster ovary cell conducted conducted recently*. Therefore, although the RS-zop induced thyroid tumors may be secondary and indirect to liver induction, the possibility remains open for an mutagenic effect.**

* positive mutagenic responses were also observed for the S-zop and its active metabolite, S-desmethyl.

Mammary Gland Tumors in Female Rats/24 month study:

Table from sponsor presents the incidence in female rats:

Table 45: Incidence of Mammary Tumors in Female CD Rats Receiving (RS)-Zopiclone for up to 108 Weeks
- Died on Test or Were Moribund Sacrificed -

Mammary Gland	Control	(RS)-zopiclone (mg/kg/day)

Table 48: Incidence of Mammary Tumors in Female CD Rats Receiving (RS)-Zopiclone for up to 108 Weeks: Terminally Sacrificed

Mammary Gland	Control		(RS)-Zopiclone (mg/kg/day)		
	0	0	1	10	100
Number Examined	25	24	18	16	22
Adenoma	18	15	16	10	11
Carcinoma	2	3	2	2	4
Adenoma + Carcinoma	20	18	18	12	15

It is of note that there seem to be a discrepancy or a *different way of looking at the tumor numbers* reported here by the sponsor and those reported in Dr. Ann Wilk's review dated Mar 1988 (previous reviewer of the studies). In her review, the following table from sponsor was presented: this table lists # of adenoma as 26/50 in HDf compared to 19/50 in table 44 from the sponsor but the number of carcinomas and total number of tumors was similar in both tables.

Incidence of all female rats bearing one or more mammary gland neoplasms

Group and sex	1F	2F	3F	4F	5F
Number examined	50	50	47	50	50
Benign fibroepithelial tumour	37	33	37	32	26[a]
Carcinoma	4	9	6	8	18[b]
Benign and/or malignant tumour	37	36	38	37	37

a Significantly different from controls, P < 0.05.
b Significantly different from controls, P < 0.01.

Pair-wise and trend test showed the significant increase in carcinoma and significant decrease in adenomas in HDf (table 44). The overall incidence was not increased in HDf but rather, there was a **shift in the tumor progression from adenoma to carcinoma and from well differentiated to poorly differentiated pathology in HDf**. The combined incidence of adenomas and carcinomas in control rats was high at **72 and 74%.** The historical control incidence ir – for **adenomas is 64.7%** range 50-75% (341/527), and for **carcinomas** is **9.2%** range 5.0-18.6% (49/527). Clearly, the incidence of carcinoma in HDf far exceeded the historical range (**36%**; 18/50), however adenoma incidence in this and other groups including the controls were within the historical range. Note that **the carcinoma incidence was increased in rats that died or killed in moribund** (table 45) yet these tumors do not seem to be the cause of death in these rats since there was no difference in mortality between this group and control. The sponsor indicated (tables from sponsor), that there were no differences in tumor multiplicity since most rats with carcinoma also had 1 or more adenomas. Table 48 from sponsor presents tumor incidence in terminally kill rats. Based on the above data, there was no difference in the incidence of mammary gland tumors in any group of treated rats killed at end of study and those in the control groups.

The following tables from the sponsor present the number of adenomas and carcinomas in individual animals the wk of unscheduled death and the multiplicity of tumors.

Table 47: Incidence of Mammary Carcinomas in Female CD Rats Receiving (RS)-Zopiclone for up to up to 108 Weeks: Unscheduled Deaths and Moribund Sacrifice

Control 1			Control 2			1 mg/kg			10 mg/kg			100 mg/kg		
N°	Wk	M	N°	Wk	M	N°	Wk	M	N°	Wk	M	N°	Wk	M
267	102	+	311	73		359	94	+	415	70	+	451	100	+
275	98	+	329	68		360	104	+	422	94		453	96	+
			332	100	+	387	104		426	91		456	100	
			333	89	+	395	104	+	434	106	+	463	70	+
			334	78					441	75		470	35	
			336	53	+				447	73	+	475	95	
												480	93	+
												484	88	+
												485	96	
												488	88	
												492	107	
												496	83	+
												497	83	+
												498	82	+

N° = Animal Number. Animals Nos. 434 and 492 died just prior to the scheduled sacrifice (Weeks 105-108).
Wk = Week of death or sacrifice.
M = multiplicity, *i.e.* more than 1 adenoma and/or carcinoma on slide section (+).

Table 46: Incidence of Mammary Adenomas in Female CD Rats Receiving (RS)-Zopiclone for up to 108 Weeks: Unscheduled Deaths and Moribund Sacrifice

Control 1			Control 2			1 mg/kg			10 mg/kg			100 mg/kg		
N°	Wk	M	N°	Wk	M	N°	Wk	M	N°	Wk	M	N°	Wk	M
251	72	+	301	89		351	91		401	95	+	457	92	+
259	93		303	99	+	356	91	+	406	95		458	103	+
263	94	+	312	78		357	89		410	99		459	72	
264	98	+	317	82	+	365	91		413	88	+	472	97	+
265	72		325	97		368	88	+	414	86	+	477	102	
271	83		330	90	+	369	103	+	416	81	+	478	98	
272	81		338	84	+	372	92	+	417	93		482	71	
276	74		339	97	+	373	105		418	106		499	83	
277	91	+	342	93		377	105		424	86				
279	94	+	343	102	+	378	98	+	430	99	+			
282	102	+	344	65		379	98		433	97	+			
285	89	+	348	82	+	384	74	+	435	84				
288	98	+				388	90		437	101				
299	82					396	101	+	438	102				
300	93	+				398	86		439	89				
						399	102	+	440	77	+			
									444	101				
									448	102	+			
									450	103	+			

N° = Animal Number. Animal No. 418 died just prior to the scheduled sacrifice (Weeks 105-108).
Wk = Week of death or moribund sacrifice.
M = multiplicity, *i.e.* more than 1 adenoma on the slide section (+).

From these tables, many rats with carcinoma also exhibited adenomas. The sponsor stated that occasionally, hyperplasia was seen but there was no difference in incidence between treated and control groups. The sponsor next **attributed these mammary tumors to increases in 17beta estradiol and provided some literature references.** As stated in this review, serum levels of 17beta estradiol were highly inconsistent and variable among the several studies conducted to address this issue therefore, accurate conclusion could not be made.

Consultant's Opinions on Mammary Tumors:

Expert pathologists reviewed these tumor data (some re-read the slides blindly) and provided written reports. These experts were: [redacted] The following is summary of their assessment:

- All agreed that mammary gland tumors were increased in high dose female rats administered 200mg/kg/d zop compared to the concurrent and historical control values for these tumors.
- This high incidence was seen in rats that died on drug or killed in moribund but no difference in incidence of these tumors in rats that survived till end of study/terminal necropsy.
- These mammary adenocarcinomas were not the cause of death since there was no difference in mortality rate in any group.
- Mammary gland tumors and endocrine tumors in general, are common tumors in aged, overfed, and untreated rats.
- There was no evidence of invasion and/or metastasis.
- The overall incidence of these tumors between the groups was not increased but there was a **statistically significant shift in progression of the pathology from adenomas (decrease in #) to carcinomas (increase) in HDf.**
- Drs. [redacted] (from the original pathologists), re-read the slides using more defined diagnostic criteria (invasion, papillary pattern, mitotic activity, and extent of pleomorphism), and concurred that

there is **NO doubt that high dose of RS-zop had some effect on mammary gland as seen from higher number of female rats with carcinomas with multi-layering and/or cribiform structure,** that the NOEL is 10mg/kg/d, no significant increase in number of rats with tumors showing muscle invasion or metastasis (10 of the 18 rats had invasion of only the connective tissue), and therefore, therapeutic doses of RS-zop is unlikely to impose an increased risk of breast cancer in humans i.e. Threshold effect.

- Dr — re-read the slides blindly and used a grading scale to differentiate the extent of any tumor progression using both cytological and histological endpoints (using atypia or lack of differentiation as a feature). He came up with the table below:

Mammary Tumors in Female Rats Receiving (RS)-Zopiclone in the Diet for 105 Weeks
- Dr — Diagnosis* -

Mammary Tumors	Dose (mg/kg) of (RS)-Zopiclone				
	0	0	1	10	100
N° of Tumors/Group	37/50	36/50	38/50	37/50	37/50
Tumor Classification					
Grade 2-3	10	18	18	20	12
Grade 4	3	6	1	4	11
Mean N° Tumors / Rat	2.6	2.3	2.5	2.4	2.1
Mean Histological Grade	1.6	2.2	1.7	2.2	2.4

* Grading scale: 1 – adenoma/fibroadenoma with atypia;
2 – well-differentiated carcinoma with slight atypia;
3 – moderately differentiated carcinoma with moderate atypia; and
4 – poorly differentiated carcinoma with severe atypia.

From Dr. — table the only difference was the non-statistical increase in number of rats with grade 4 tumors. There was no increase in mean histological grade and no increase in number of tumors per group or mean number of tumors per animal. Therefore, he concluded that there was no adequate evidence to indicate that RS-zop is a carcinogen.

- Dr. — concluded that in absence of genotoxicity, changes in tumor distribution in hormone-dependent and endocrine organs do not increase cancer risk in humans.
- Chronic elevation in 17beta estradiol has been shown to increase the proportion of mammary gland tumors induced by radiation.
- Rodent mammary tumors are not to be extrapolated to humans because estrogens have not been demonstrated to be causally related to mammary tumors in humans (the statement made in 1986).

Sponsor's Conclusion:
The sponsor heavily relied on the relationship between the elevation in 17beta estradiol and the increased incidence of mammary gland tumors in HDf dosed with RS zop. The sponsor concluded that RS-zop affect mammary glands through elevations in 17beta estradiol. This was based on "the increased incidence of mammary gland tumors at doses that caused an elevation in 17beta estradiol and absence of these tumors at RS-zop doses that did not cause an increase in this hormone level. **Absence of mammary tumors in the 18 month rat study at even higher doses of zop, underscores the requirement for both long term and high exposure to the cpd.** The sponsor also pointed out that these types of rodent tumors are irrelevant and can not be extrapolated to humans because the "role of estrogens in human mammary carcinogenesis is not yet proven".

See Reviewer Conclusions on thyroid and mammary tumors in Section 3.4.8 special Toxicology Studies.

3.4.6 REPRODUCTIVE TOXICOLOGY:

Study title: Oral rat developmental tox dose range finder of S-zopiclone.
Study No: Sepracor# 190-812/ — #ZCP/008
Site and testing facility:
Start and Completion Dates: Feb and March 1999 respectively.
GRP compliance and QA reports: Yes/OECD; report was NOT QA

Methods:
- Species/strain: Time-mated female Sprague-Dawley rats
- Age/B. wt: 8-10wks/200-225g
- Doses employed: **10, 50, 100, 200mg/kg/d**
- Route of Administration: oral gavage.
- Vehicle: **2.5% hydroxy propyl methyl cellulose.**
- Study Design: there were 5 time-mated femalesf per group dosed orally with S-zopiclone on days 6-17 of gestation (dose volume 10ml/kg). Pregnant rats were killed on gd20.
- Number of animals/sex/dosing group: 5females/dose group.
- Parameters and endpoints evaluated: clinical signs, mortality, B.wt, food intake, gross necropsy on all rats. Orgnas/tissues with abnormalities were examined microscopically and for pregnant rats that were killed on gd20 the following was determined: # corpora lutea, # implantation sites, early, late, and dead/live fetuses. Fetal wt, sex, external abs, and placental wts were determined
- Statistical evaluations: ANOVA, Kruskal-Wallis, and Fisher's Exact test. No analyses done on parameters with less than 5 values per group.

Results:
- Mortality: none
- Clinical signs: all dose group females experienced incoordination, abnormal gait, and hypoactivity on 1 or upto 5 days of dosing.
- Body weight/Food consumption: no drug effect.
- Toxicokinetics: not done.
- Fertility and Early Embryonic Development in Females: table below from sponsor:

Group mean uterine / implantation data

Group	:	1	2	3	4	5
Treatment	:	Control			(S)-zopiclone	
Dosage (mg/kg/day)	:	0	10	50	100	200

	Group 1	Group 2	Group 3	Group 4	Group 5
Number of females with implantations at scheduled kill	4	5	4	5	5
Number of corpora lutea	61	77	67	78	82
Mean number per female	15.3	15.4	16.8	15.6	16.4
Standard deviation	3.4	1.5	2.8	1.5	2.5
Number of implantations	41	65	50	56	64
Mean number per female	10.3	13.0	12.5	11.2	12.8
Standard deviation	4.9	1.0	1.7	3.4	1.9
Mean % pre-implantation loss	32.1	15.0	24.5	26.4	21.6
Number of early embryo/foetal deaths	2	6	4	11	4
Number of late embryo/foetal deaths	0	0	0	0	0
Number of dead foetuses	0	0	0	0	0
Mean % post-implantation loss	10.1	9.4	8.1	24.3	6.3
Number of live foetuses	39	59	46	45	60
Mean number per female	9.8	11.8	11.5	9.0	12.0
Standard deviation	5.2	2.5	1.7	5.7	2.2
Mean % of implantations	89.9	90.6	91.9	75.7	93.7

Group mean litter weights (g) / foetal data

Group	:	1	2	3	4	5
Treatment	:	Control		(S)-zopiclone		
Dosage (mg/kg/day)	:	0	10	50	100	200

	Group 1	Group 2	Group 3	Group 4	Group 5
Number of females with live foetuses at scheduled kill	4	5	4	4	5
Number of live foetuses	39	59	46	45	60
Mean number per female	9.8	11.8	11.5	11.3	12.0
Standard deviation	5.2	2.5	1.7	3.1	2.2
Number of male foetuses	17	29	26	26	33
Number of female foetuses	22	30	20	19	27
Mean % male foetuses	34.1	47.3	56.4	61.7	55.0
Mean litter weight	36.4	43.1	40.6	45.5	43.2
Standard deviation	17.6	13.3	14.4	13.7	7.0
Mean foetal weight	4.02	3.61	3.47	4.02	3.62
Standard deviation	0.75	0.47	0.85	0.15	0.19
Mean foetal weight - males only	3.80	3.77	3.57	4.08	3.74
Standard deviation	0.25	0.52	0.93	0.17	0.24
Mean foetal weight - females only	3.91	3.49	3.36	3.84	3.45
Standard deviation	0.84	0.42	0.73	0.29	0.16
Mean placental weight	0.52	0.51	0.61	0.56	0.57
Standard deviation	0.05	0.04	0.08	0.08	0.09

Summary and Conclusion: doses upto 200mg/kg/d of S-zaleplon to pregnant female rats administered orally during gd6-17 were well tolerated with no deaths. In general, there was no drug effect on fetal development however, the following were recorded: number of early embryo-fetal deaths was increased non-dose dependently in all drug groups, mean percent post-implantation loss was increased >2x in 100mg/kg group. These 2 findings led to a dcrease in mean % of implantations in 100mg/kg group. In all dose groups, clinical signs were seen and included abnormal gait, hypoactivity, piloerection, and cold to touch; severity was dose related.

Study title: Oral gavage rabbit MTD of S-zopiclone/Sepracor# not reported by Sepracor — ♯ ZCP/006
Site and testing facility —
Start and Completion Dates: Feb and March 1999 respectively.
GRP compliance: Yes/EC
QA- Reports: Yes ( ) No (x)

Methods:
- Species/strain: New Zealand white rabbit
- Age/B. wt: sexually mature females about 4months old between 3-4kg.
- Doses employed: mg/kg/d: **12.5, 18, and 25mg/kg/d** see below for study design.
- Route of Administration: oral gavage.
- Vehicle: **2.5% hydroxy propyl methyl cellulose.**
- Study Design: there were 2 phases for the study. Phase I, 3 females were dosed 25mg/kg/d for 3d followed by 2d wash out. The dose was then reduced to 12.5mg/kg administered daily for 7d. in phase II, the 3 control female rabbits from phase I were dosed 18mg/kg/d for 7d. Dose volume was 2ml/kg. Rabbits that received the drug in phase I were killed and necropsied.
- Number of animals/sex/dosing group: 3females/dose group.

- Parameters and endpoints evaluated: clinical signs, mortality, B.wt, food intake, gross necropsy on all rabbits including those that were found dead or killed prior to schedule. Histopath done on orgnas/tissues with abnormalities

Results:
- Mortality: none for both phases.
- Clinical signs: one or more female in both phases showed abnormal gait, subdued behavior, hypoactivity and 1f phase II had slow breathing on d1 of dosing.
- Body weight/Food consumption: there seemed to be loss in mean B.wt and reduction in mean food intake in both phases.
- no other drug related findings.

It was concluded that oral administration of S-zopiclone to female rabbits was tolerated upto 25mg/kg/d without deaths. Clinical signs were seen in all drug groups tested.

Study title: Oral gavage rabbit developmental tox dose range finder of S-zopiclone.
Study No: Sepracor# 190-809, s# ZCP/007
Site and testing facility:
Start and Completion Dates: March & April 1999 respectively.
GLP compliance: Yes/OECD
QA- Reports Yes ( ) No (x)

Methods:
- Species/strain: New Zealand white rabbit
- Age/B. wt: sexually mature females about 4months old between 3-4kg.
- Doses employed: mg/kg/d: **4, 8, 16, and 24mg/kg/d.**
- Route of Administration: oral gavage.
- Vehicle: **2.5% hydroxy propyl methyl cellulose.**
- Study Design: time-mated females were orally dosed S-zopiclone from gd6-18.
- Number of animals/sex/dosing group: 5 time-mated females/dose group.
- Parameters and endpoints evaluated: clinical signs, mortality, B.wt, food intake, gross necropsy on all rabbits. Histopath done on orgnas/tissues with abnormalities.

Results:
- **Mortality:** none.
- **Clinical signs:** were seen throughout dosing at ≥8mg/kg. Signs included unsteady gait, subdued behavior, reduced feces; signs were dose related in severity and duration.
- **Body weight/Food consumption:** mean wt was significantly reduced in rabbits dosed ≥8mg/kg throughout the dosing period (6-18days). Similar findings reported for food intake. Mean wt loss was 50, 40, and 190g at 8, 16, and 24mg/kg/d doses respectively, relative to a gain of 110g in the control during the same period. Perhaps this decline was caused by the significant reduction noted during the period 6-9days. Food consumption was reduced as follows: 86, 67, and 57g/rabbit/d in 8, 16, and 24mg/kg/d doses respectively relative to 146g/rabbit/d consumed by the control over the 6-18day of dosing. Fetal toxicity was relatively small seen as a non significant 9% reduction in mean wt relative to the control at the high dose of 24mg/kg.

It was concluded that maternal toxicity seen as clinical signs and reduced mean B.wt, were observed at ≥8mg/kg/d and, fetal toxicity at 24mg/kg/d when zopiclone was orally dosed to rabbits during gd6-18.

Table below from sponsor presents repro parameters:

Group mean uterine / implantation data

Group	:	1	2	3	4	5
Treatment	:	Control		(S)-zopiclone		
Dosage (mg/kg/day)	:	0	4	8	16	24

	Group 1	Group 2	Group 3	Group 4	Group 5
Number of females with implantations at scheduled kill	5	4	5	4	5
Number of corpora lutea	47	40	53	43	57
Mean number per female	9.4	10.0	10.6	10.8	11.4
Standard deviation	1.7	1.4	1.1	1.3	1.7
Number of implantations	34	31	44	31	39
Mean number per female	6.8	7.8	8.8	7.8	7.8
Standard deviation	3.1	3.6	1.6	2.5	4.1
Mean % pre-implantation loss	24.8	21.3	16.9	28.1	32.0
Number of early embryo/foetal deaths	1	4	4	1	8
Number of late embryo/foetal deaths	1	3	2	6	1
Number of dead foetuses	0	0	0	0	0
Mean % post-implantation loss	6.2	18.1	15.4	17.2	15.8
Number of live foetuses	32	24	38	24	30
Mean number per female	6.4	6.0	7.6	6.0	6.0
Standard deviation	3.2	2.4	2.5	1.4	3.5
Mean % of implantations	93.8	81.9	84.6	82.8	84.2

Group mean litter weights (g) / foetal data

Group	:	1	2	3	4	5
Treatment	:	Control		(S)-zopiclone		
Dosage (mg/kg/day)	:	0	4	8	16	24

	Group 1	Group 2	Group 3	Group 4	Group 5
Number of females with live foetuses at scheduled kill	5	4	5	4	5
Number of live foetuses	32	24	38	24	30
Mean number per female	6.4	6.0	7.6	6.0	6.0
Standard deviation	3.2	2.4	2.5	1.4	3.5
Number of male foetuses	9	13	21	12	11
Number of female foetuses	23	11	17	12	19
Mean % male foetuses	42.0	62.5	53.3	48.1	49.2
Mean litter weight	240.2	241.3	283.3	234.5	205.9
Standard deviation	100.8	82.7	84.3	50.4	136.4
Mean foetal weight	39.4	41.2	38.2	39.3	35.7
Standard deviation	5.5	3.5	6.4	3.5	8.9
Mean foetal weight - males only	40.3	42.1	38.8	39.6	37.0
Standard deviation	5.8	2.6	7.2	3.9	7.6
Mean foetal weight - females only	37.9	38.9	36.9	39.1	31.1
Standard deviation	5.6	3.3	6.2	3.5	7.6

A study of the effects of S-zopiclone on fertility and early embryonic development to implantation in rats/Sepracor# 190-827' — study# 312066.
Conducting lab: —
Study Initiation/Termination Dates: Mar 2000/Jun 2000
GLP: Yes (x) Japanese & US FDA.
QA: Yes (x)
Drug Lot/Batch#/purity: for S-zop 0290005 — , for RS-zop 3265659 purity —
Species/Strain: sexually mature male and virgin female Sprague-Dawley rats.
B.wt/Age at study initiation: males: 278-404g; females: 208-290g/males 10wk old and females 10-11wk old for females at start of dosing.
Doses: **0, 60, 120, 180mg/kg/d S-zop and 120mg/kg/d RS-zop.** On d3 (4th dose), results from a subchronic tox study in rats, — 312047, showed adverse effects on spermatogenesis, therefore, dosing in males in the current study was suspended on d3-5 and resumed on d6 where **males** were dosed S-zop at **5, 15, and 45mg/kg/d and RS-zop at 15mg/kg/d**. Table below from sponsor summarizes the number of rats per group, doses and dose volume. Both S- and RS- zop were prepared in 0.5% aqueous CMC administered by oral gavage once daily.

Group Number	Test Article	Dosage Level (mg/kg/day) Males[a]	Females	Dosage Concentration (mg/mL)	Volume (ml/kg) Males[a]	Females	Number of Animals Male	Female
1	Vehicle	0	0	20	2.25	9	25	25
2	(S)-Zopiclone	5	60	20	0.25	3	25	25
3	(S)-Zopiclone	15	120	20	0.75	6	25	25
4	(S)-Zopiclone	45	180	20	2.25	9	25	25
5	(R,S)-Zopiclone	15	120	20	0.75	6	25	25

[a] = On study days 0-3, males were dosed at the same dosage levels/volumes as the females. Following a two-day recovery period (not dosed), males were administered the dosage levels/concentrations listed here.

Duration and dosing regimen: **males** were dosed for at least **28d premating, through mating**, and until 1d prior to sacrifice (about 62-67 doses). **Females** were dosed **14d premating, through mating, and through gd7** (about 22-36 doses), females with no evidence of mating were dosed till day prior to sacrifice. Males were killed when mating was confirmed in females and females were killed on gd15 (dosing was till gd7). Males and females were mated at 1:1 ratio for a maximum of 14d.

The following parameters were assesed:
Clinical signs/mortality: 2x daily observations at 1,2,&4hr postdose.
B.wt. & food intake: 2x weekly for both parameters and at specified times during mating and gestation.
Reproductive Parameters: standard parameters were assessed. Mating index, fertility index for each sex, # corpora lutea, implantation sites and #, resoptions, live/dead fetuses. For males, immediately after euthanasia, the male reproductive tract was exposed and epididymides excised and processed, sperm parameters were determined (motility, morphology, sperm production rate, and number).
Organ wts: testes, epididymides (total and cauda), ovaries, and brain from all parental rats killed at scheduled necropsy.

Macroscopic Exam: complete exam on each male and female was done this included all orifices, external surfaces, cranial, abdominal, thoracic, pelvic, and visceral cavities, and external surfaces of the brain and spinal cord. The following tissues/organs were removed and preserved in 10% formalin for future processing if needed: cervix, epididymides (2), ovaries & oviduct (2), pituitary, prostate, seminal vesicles (2), testes (2), uterus & vagina, vas deferens, and all gross lesions.

S-zop Segment I Fertility study in the rat/Sepracor#190-827 (Cont.)

Results:

Mortality & Clinical signs: no deaths in any group of either sex. Clinical signs were similar and observed in both sexes in all drug groups: stereotypic behavior (repetitive mouth and jaw movement, wiping face on cage surfaces, excessive pawing of cage surfaces), hyperactivity, salivation, reduced hind-limb extensor reflex and resistance, lethargy, rocking, lurching & swaying while ambulating. These signs observed immediately postdose and up to 4hr observation period postdose.

B.wt: Males: mean wt was not significantly reduced but a non significant decrease noted in 45mg/kg/d S-zop starting on study d20 through end of dosing (4-5% less than corresponding control), similar change noted in RS-zop. Mean wt gain on the other hand, was significantly ($p<0.05$ or 0.01), reduced in males dosed 15&45mg/kg/d S-zop and 15mg/kg/d RS-zop during premating study period 3-6d (this was the period prior to adjusting/lowering the doses and no dosing on days 4&5, dosing resumed on d6 at the lower doses). These decreases in mean wt gain were 55% in S-zop and 73% in RS-zop less than the control gains at this period. The only other period where mean wt gain in males was reduced ($p<0.05$) was during days 13-17 of dosing at 22% less than control in 45mg/kg/d S-zop and 15mg/kg/d RS-zop. It should be noted that when wt changes were calculated using d6 as start of dosing (day of resuming dosing at lower doses), there was no significant decrease in wt gain was observed in males except during days13-17 as noted above. Females: because no female was pregnant in the 180mg/kg/d, evaluation of B.wt was not done in this group during gestation period. During pre-mating period (study days 20-33), no drug effect in 60mg/kg/d on mean wt or wt gain, no effect on mean wt in 120mg/kg/d but a significant ($p<0.05$) *increase* in mean wt of 5.6% seen in the 180mg/kg/d group relative to the control. Mean wt gain, was significantly *increased* during pre-mating d17-20 in all drug groups ($p<0.05$ or 0.01), and in HD S-zop and 120mg/kg/d RS-zop at d20-24 (dosing started on d20). Significant increase in wt gain was also observed in 180mg/kg/d S-zop when dosing intervals compared to 1st day of dosing (d20 of study) i.e. d20-24, 20-27, 20-31, and 20-33. Mean B.wt during gestation was unaffected but wt gain was significantly reduced in 120mg/kg/d group relative to control during gd7-10 ($p<0.01$), and significantly increased during gd10-13 in 120mg/kg S-zop and RS-zop.

Food Intake: males: parallel to wt changes, mean food intake computed as either g/animal/d or g/kg/d, was reduced in all male drug groups prior to dose adjustment and through d6 when dosing resumed at the lowered doses (8-17% less than controls). Also noted that intake was *increased* ($p<0.05$) at few intervals in low and mid doses or high dose S-zop relative to the controls (in HD-zop these increases began on postmating days 48-52 through 62-66d based on g/animal/d and from d6-13 and d48-68). Females: during pre-mating, a small but significant *increase* in mean intake, g/animal/d, was seen in 180mg/kg/d S-zop relative to the control during study days 27-31 and 31-33, relative to the control values. Similar increase was seen when intake was calculated as g/kg/d. Throughout gestation, mean intake calculated as either g/animal/d or g/kg/d, was significantly *increased* in 120mg/kg/d S-zop and RS-zop relative to the control, the increase was up to 20% of the control.

Fertility Parameter: Males: **significant drug related decrease in fertility indices noted at the 15mg/kg/d (72%), and complete absence of fertility seen in 45mg/kg/d S-zop group** (i.e. zero) compared to the control (table from sponsor). There was no significant or drug related effects on male

mating index (84, 100, 92, 84, and 96% in cont., 5, 15, 45, and 15mg/kg/d doses respectively). There was also no drug related effect on # of days between pairing and coitus. Females: similar to the males, **fertility indices were significantly reduced (0%) in 180mg/kg/d** relative to controls, no drug effect on mating indices, but **mean length of estrus cycle was increased by approximately 1d in 120&180mg/kg/d S-zop and 120mg/kg/d RS-zop relative to the control.**

S-zop Segment I Fertility study in the rat/Sepracor#190-827 (Cont.)

SUMMARY OF MALE REPRODUCTIVE PERFORMANCE

DOSE GROUP :	1		2		3		4		5	
	NO.	%	NO.	%	NO.	%	NO.	%	NO.	%
MALES ON STUDY	25		25-A		25		25		25	
MALES THAT DIED DURING STUDY	0		0		0		0		0	
MALES WITH EVIDENCE OF MATING	21	84.0	24	100.0	22	88.0	21	84.0	24	96.0
NO. THAT SIRED A LITTER	21	100.0	21	87.5	17	77.3	0	0.0	21	87.5
NO. THAT DID NOT SIRE A LITTER	0	0.0	3	12.5	5	22.7	21	100.0	3	12.5
MALES WITH NO EVIDENCE OF MATING	4	16.0	0	0.0	3	12.0	4	16.0	1	4.0
NO. THAT SIRED A LITTER	0	0.0	0	0.0	1	33.3	0	0.0	0	0.0
NO. THAT DID NOT SIRE A LITTER	4	100.0	0	0.0	2	66.7	4	100.0	1	100.0
MALES THAT SIRED MORE THAN ONE LITTER	0	0.0	0	0.0	2	8.0	0	0.0	1	4.0
MALE MATING INDEX	21/25	84.0	24/24	100.0	23/25	92.0	21/25	84.0	24/25	96.0
MALE FERTILITY INDEX	21/25	84.0	21/24	87.5	18/25	72.0	0/25	0.0**	21/25	84.0
MEAN PRE-COITAL INTERVALS (DAYS)	2.4	NA	1.5	NA	3.1	NA	3.1	NA	2.1	NA
S.D.	1.07	NA	0.83	NA	3.44	NA	2.96	NA	2.87	NA
N	21		24		24		22		25	

1- 0 MG/KG/DAY 2- 5(S) MG/KG/DAY 3- 15(S) MG/KG/DAY 4- 45(S) MG/KG/DAY 5- 15(RS)MG/KG/DAY

NOTE: MALES WERE CONSIDERED TO HAVE SIRED A LITTER IF THE PAIRED FEMALE WAS GRAVID. NA = NOT APPLICABLE

MATING INDICES NOT SIGNIFICANTLY DIFFERENT FROM CONTROL GROUP USING CHI-SQUARE TEST

** = SIGNIFICANTLY DIFFERENT FROM CONTROL GROUP AT 0.01 USING CHI-SQUARE TEST

PRE-COITAL INTERVALS NOT SIGNIFICANTLY DIFFERENT FROM CONTROL GROUP USING DUNNETT'S TEST

A = MALE NO. 42365 WAS NOT PAIRED DUE TO EUTHANASIA OF FEMALE, NOT INCLUDED IN CALCULATIONS

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ON ORIGINAL

SUMMARY OF FEMALE REPRODUCTIVE PERFORMANCE

DOSE GROUP :	1		2		3		4		5	
	NO.	%	NO.	%	NO.	%	NO.	%	NO.	%
FEMALES ON STUDY	25		25		25		25		25	
FEMALES THAT DIED DURING STUDY	0		1-A		0		0		0	
FEMALES PAIRED FOR MATING	25		24		25		25		25	
FEMALES WITH EVIDENCE OF MATING	21	84.0	24	100.0	24	96.0	22	88.0	25	100.0
GRAVID	21	100.0	21	87.5	19	79.2	0	0.0	22	88.0
NONGRAVID	0	0.0	3	12.5	5	20.8	22	100.0	3	12.0
FEMALES WITH NO EVIDENCE OF MATING	4	16.0	0	0.0	1	4.0	3	12.0	0	0.0
GRAVID	0	0.0	0	0.0	1	100.0	0	0.0	0	0.0
NONGRAVID	4	100.0	0	0.0	0	0.0	3	100.0	0	0.0
FEMALE MATING INDEX	21/25	84.0	24/24	100.0	24/25	100.0	22/25	88.0	25/25	100.0
FEMALE FERTILITY INDEX	21/25	84.0	21/24	87.5	20/25	80.0	0/25	0.0**	22/25	88.0
MEAN PRE-COITAL INTERVALS (DAYS)	2.4	NA	1.5	NA	3.1	NA	3.1	NA	2.1	NA
S.D.	1.67	NA	0.83	NA	3.44	NA	1.96	NA	1.67	NA
N	21		24		24		22		25	

1- 0 MG/KG/DAY 2- 60(S) MG/KG/DAY 3- 120(S) MG/KG/DAY 4- 180(S) MG/KG/DAY 5- 120(RS) MG/KG/DAY

PRE-COITAL INTERVALS NOT SIGNIFICANTLY DIFFERENT FROM CONTROL GROUP 1 USING DUNNETT'S TEST NA = NOT APPLICABLE
MATING INDICES NOT SIGNIFICANTLY DIFFERENT FROM CONTROL GROUP USING CHI-SQUARE TEST
** = SIGNIFICANTLY DIFFERENT FROM CONTROL GROUP AT 0.01 USING CHI-SQUARE TEST

A = FEMALE NO. 41929 WAS EUTHANIZED PRIOR TO PAIRING, NOT INCLUDED IN CALCULATIONS

S-zop Segment I Fertility study in the rat/Sepracor#190-827 (Cont.)

Reproductive Parameters: Females: there were no changes in any parameter that reached statistical significance. However, the following were considered drug related though did not achieve statistical significance: pre-implantation loss was increased dose dependently in 60&120mg/kg/d S-zop and 120mg/kg/d RS-zop with the relative loss being outside the historical range for — , (15.4, 21.7, and 15.1% per litter for 60 and 120mg/kg/d S-zop and 120mg/kg/d RS-zop respectively (table from sponsor). There was also a small decrease in # of live embryos and implantation sites in 120mg/kg/d S-zop compared to the control, all these effects occurred in absence of maternal toxicity.

PROJECT NO.: — ,120666F SUMMARY OF MEAN EMBRYONIC DATA AT THE SCHEDULED NECROPSY

GROUP		VIABLE EMBRYOS	DEAD EMBRYOS	RESORPTIONS EARLY	RESORPTIONS LATE	POST IMPLANTATION LOSS	IMPLANTATION SITES	CORPORA LUTEA	PRE IMPLANTATION LOSS	NO. OF GRAVID FEMALES
1	TOTAL	311	0	28	0	28	339	377	38	21
	MEAN	14.8	0.0	1.3	0.0	1.3	16.1	18.0	1.8	
	S.D.	1.97	0.00	1.24	0.00	1.24	1.53	2.31	1.78	
2	TOTAL	293	0	27	0	27	320	375	55	21
	MEAN	14.0	0.0	1.3	0.0	1.3	15.2	17.9	2.6	
	S.D.	4.40	0.00	1.76	0.00	1.76	4.15	3.72	3.04	
3	TOTAL	257	0	18	0	18	275	341	66	19
	MEAN	13.5	0.0	0.9	0.0	0.9	14.5	17.9	3.5	
	S.D.	5.23	0.00	0.91	0.00	0.91	5.43	3.47	3.70	
4	THERE WERE NO GRAVID DAMS SURVIVING TO THE SCHEDULED NECROPSY IN THIS GROUP									
5	TOTAL	321	0	20	0	20	341	403	62	22
	MEAN	14.6	0.0	0.9	0.0	0.9	15.5	18.3	2.8	
	S.D.	3.53	0.00	1.06	0.00	1.06	3.64	2.59	3.20	

None significantly different from control group 1- 0 MG/KG/DAY 2- 60(S)MG/KG/DAY 3-120(S)MG/KG/DAY 4-180(S)MG/KG/DAY 5-120(RS)MG/KG/DAY
MEAN NUMBER OF VIABLE EMBRYOS, MEAN NUMBER OF IMPLANTATION SITES, MEAN NUMBER OF CORPORA LUTEA COMPARED USING DUNNETT'S TEST

SUMMARY OF MEAN EMBRYONIC DATA AT SCHEDULED NECROPSY (% PER LITTER)

GROUP NUMBER:	1	2	3	4	5
TOTAL RESORPTIONS (%)					
MEAN	8.4	8.4	6.3	A	5.6
S.D.	7.66	11.92	5.98		6.71
N	21	21	19		22
PRE-IMPLANTATION LOSS (%)					
MEAN	9.4	15.4	21.7	A	15.1
S.D.	8.16	20.81	25.56		17.32
N	21	21	19		22
POST-IMPLANTATION LOSS (%)					
MEAN	8.4	8.4	6.3	A	5.6
S.D.	7.66	11.92	5.98		6.71
N	21	21	19		22

1- 0 MG/KG/DAY 2- 60(S)MG/KG/DAY 3-120(S)MG/KG/DAY 4-180(S)MG/KG/DAY 5-120(RS)MG/KG/DAY

PROPORTIONAL (%) DATA COMPARED USING THE KRUSKAL-WALLIS TEST None significantly different from control group
CORPORA LUTEA AND IMPLANTATION SITES COMPARED USING DUNNETT'S TEST A = NO GRAVID DAMS SURVIVED TO THE SCHEDULED NECROPSY

S-zop Segment I Fertility study in the rat/Sepracor#190-827 (Cont.)

Spermatogenic Parameters: drug effects were observed on all parameters in 45mg/kg/d S-zop as follows ($p<0.05$ or 0.01): decrease in sperm #, motility (also in RS-zop), and morphology (tables from sponsor).

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(NO. OF SPERM IN MILLIONS/GRAM OF TISSUE) - SUMMARY OF MEANS HTM-IVOS

GROUP:	0 MG/KG/DAY	5(S) MG/KG/DAY	MALE 15(S) MG/KG/DAY	45(S) MG/KG/DAY	15(RS)MG/KG/DAY
LEFT TESTIS					
MEAN	91.0	90.4	85.2	64.3*	102.0
S.D.	18.59	26.97	41.26	43.88	26.15
N	25	25	25	25	25
LEFT EPIDIDYMIS					
MEAN	585.8	560.1	453.6*	307.2**	537.7
S.D.	175.8	133.90	209.07	111.18	176.37
N	25	25	25	25	25

* = Significantly different from control group at 0.05 using Dunnett's test
** = Significantly different from control group at 0.01 using Dunnett's test

(NO. OF SPERM IN MILLIONS/GRAM OF TISSUE/DAY) - SUMMARY OF MEANS HTM-IVOS

GROUP:	0 MG/KG/DAY	5(S) MG/KG/DAY	MALE 15(S) MG/KG/DAY	45(S) MG/KG/DAY	15(RS)MG/KG/DAY
LEFT TESTIS					
MEAN	14.9	14.8	13.5	10.5*	16.7
S.D.	3.05	6.41	6.33	7.19	4.29
N	25	25	25	25	25

* = Significantly different from the control group at 0.05 using Dunnett's test

A: SPERM PRODUCTION RATE = NUMBER OF SPERM PER GRAM OF TISSUE / 6.1 DAYS = 6.1 DAYS = THE RATE OF TURNOVER OF THE GERMINAL EPITHELIUM

SPERM MOTILITY ASSESSMENT (PERCENTAGE) SUMMARY OF MEANS HTM-IVOS

GROUP:	0 MG/KG/DAY	5(S) MG/KG/DAY	MALE 15(S) MG/KG/DAY	45(S) MG/KG/DAY	15(RS)MG/KG/DAY
MOTILE SPERM					
MEAN	84.2	77.2	71.3**	3.0-A	80.4*
S.D.	14.94	15.07	16.41	0.00	10.61
N	25	25	20	2	25

A = UNABLE TO PERFORM STATISTICAL ANALYSIS DUE TO INSUFFICIENT SAMPLE SIZE
* = Significantly different from the control group at 0.05 using Mann-Whitney U-test
** = Significantly different from the control group at 0.01 using Mann-Whitney U-test

Sperm production rate was significantly reduced in 45mg/kg/d S-zop relative to the control (10.5 vs. 15 million cells per gm tissue/d respectively). There were significantly less normal sperms in 15&45mg/kg/d S-zop than the control; no difference in RS-zop. Abnormal morphology included normal head with no flagellum (12.3% vs. 0.2% cont), and normal flagellum with no head (26.6% vs. 0.3% in control).

S-zop Segment I Fertility study in the rat/Sepracor#190-827 (Cont.)

SPERM MORPHOLOGY DIFFERENTIAL COUNT (PERCENTAGE) SUMMARY OF MEANS

GROUP:	0 MG/KG/DAY	5(S) MG/KG/DAY	MALE 15(S) MG/KG/DAY	45(S) MG/KG/DAY	15(RS)MG/KG/DAY
NORMAL					
MEAN	99.5	99.1	95.6**	61.1**	99.2
S.D.	0.56	1.04	4.39	31.29	0.79
N	25	25	20	9	25
NORMALLY SHAPED HEAD SEPARATED FROM FLAGELLUM					
MEAN	0.2	0.4	1.6	12.3	0.4
S.D.	0.28	0.70	1.45	10.45	0.55
N	25	25	20	9	25
HEAD ABSENT WITH NORMAL FLAGELLUM					
MEAN	0.3	0.5	2.9	26.6	0.4
S.D.	0.41	0.66	3.51	21.85	0.58
N	25	25	20	9	25
HEAD ABSENT WITH ABNORMAL FLAGELLUM					
MEAN	0.0	0.0	0.0	0.0	0.0
S.D.	0.00	0.00	0.00	0.00	0.00
N	25	25	20	9	25
MISSHAPEN HEAD WITH NORMAL FLAGELLUM					
MEAN	0.0	0.0	0.0	0.0	0.0
S.D.	0.00	0.00	0.00	0.00	0.00
N	25	25	20	9	25
MISSHAPEN HEAD WITH ABNORMAL FLAGELLUM					
MEAN	0.0	0.0	0.0	0.0	0.0
S.D.	0.00	0.00	0.00	0.00	0.00
N	25	25	20	9	25

** = PERCENT NORMAL SPERM SIGNIFICANTLY DIFFERENT FROM THE CONTROL GROUP AT 0.01 USING MANN-WHITNEY U-TEST

Necropsy: Females: at scheduled kill on gd15, there were 3, 5, 22, and 3 nongravid females in 60, 120, and 180mg/kg/d S-zop and 120mg/kg/d RS-zop respectively, compared to all females gravid in control. Males: small and soft testes were seen in all males dosed 45mg/kg/d S-zop and in 3/25 males dosed 15mg/kg/d. Raised yellow and white areas in epididymides were seen in all drug groups (19-22 rats affected per group), but not in the control.

Organ wts: Females: mean **absolute and relative ovary wts in RS-zop were significantly increased** relative to the wts in the control ($p<0.01$; 13% over the control). Males: mean absolute and relative wt of the testes and epididymides in 45mg/kg/d S-zop was significantly reduced compared to the control values. These reductions ranged between 10-17% of the control and up to 21% for the right cauda epididymides. There were no drug related changes in 5 and 15mg/kg/d groups except for increases in absolute and relative wt of epididymides in males dosed 5mg/kg/d that reached statistical significance (this is in contrast to the reduction in wt observed in 45mg/kg/d; see above).

Summary & Conclusion:

S-zop and RS-zop were administered by oral gavage to either male or female rats at pre-mating, during mating, and in females till gd7. Additional group was administered RS-zop at 15mg/kg/d in males and 120mg/kg/d in females. S-zop doses in females were 60, 120, and 180mg/kg/d and in males 5, 15, and 45mg/kg/d. Both drugs had no effect on survival in either sex but clinical signs were observed in all drug groups. Mean wt gain and food intake in females were generally increased in 120 and 180mg/kg/d S-zop and RS-zop. There was no decrease in wt gain in males after dose adjustment (see above). There was **100% infertility in males dosed 45mg/kg/d and in females dosed 180mg/kg/d S-zop** without an effect on mating index, relative to the controls. Also, mean length of estrus cycle was prolonged by about 1d in HD S-zop. Pre-implantation loss was increased dose dependently in 60&120mg/kg/d S-zop (no gravid females survived till end of study at 180mg/kg/d), and 120mg/kg/d RS-zop with the relative loss

being outside the historical range for — data (15.4, 21.7, and 15.1% per litter for 60 and 120mg/kg/d S-zop and 120mg/kg/d RS-zop respectively). There was also a small decrease in # of live embryos and implantation sites in 120mg/kg/d S-zop compared to the control. It should be noted that all these effects occurred in absence of maternal toxicity. NOEL for male reproductive/fertility effects is <5mg/kg/d because of the macroscopic findings and the increase in preimplantation loss in LD females mated with these males. The NOAEL in females could not be determined because of preimplantation loss in the 60mg/kg/d group and the nature of the study design where both sexes were treated with the drug. Therefore, a clear conclusion on the female reproductive effects could not be contributed solely to the male (reduction in sperm parameters).

Additional study was conducted to further investigate the effects of S-zop on female reproduction and fertility (Sepracor# 190-835). In this study only female rats were treated with the drug and later mated with untreated males. There were 2 phases for the study in phase 1 female SD rats (25/group), were administered oral gavage doses of S-zop at **0, 60, 120, and 180mg/kg/d and 120mg/kg/d RS-zop** starting 14d prior to mating, through mating, and until gd7 inclusive; females with no evidence of mating were euthanized. One female dosed 180mg/kg/d was found dead on gd6 clinical signs were observed 1wk prior to death included excessive salivation, lurching, swaying, and hypoactivity before dose; this death was considered drug related. Clinical signs of exaggerated pharmacology of the drug were also observed in all S-zop and RS-zop drug groups immediately postdose and up to 4hr postdose and some persisted till next day of dosing. Mating index (fertility) was reduced in all drug groups though not dose dependently and not significantly but consistently less than the control (92, 68, 83, 84, and 76% in cont., 60, 120, 180mg/kg/d S-zop and 120mg/kg/d RS-zop respectively), the — historical mean fertility index is 90.4%. There was no drug effect on mating indices (96% in cont., 100, 100, 92, and 92% respectively)(tables from sponsor). Mean estrus cycle lengths were increased in drug groups relative to control: 5,5, 7.0, 6.3, 5.8, and 6.7 days in cont., 60, 120, and 180mg/kg/d S-zop and 120mg/kg/d RS-zop respectively. It is noted that the mean value of the estrus length for the concurrent control of 5.5d was outside — ange historical value: 4.1-5.1 days but this effect still considered drug related. Moreover, the number of females where cycle length could not be determined due to absence of estrus, was also increased 5, 6, 3, 12, and 11 in the respective groups. Because of the death and findings in all drug groups, a 2nd phase was carried out following to determine a NOAEL using comparable design except as noted and lower doses: female SD (n=25/group), were dosed for 27d prior to mating through mating and through gd7 with 5, 15, and 25mg/kg/d S-zop and 25mg/kg/d RS-zop, the control group received 0.5% CMC. The reason for the prolonged premating dosing period was to ensure target concentrations were ingested because of inconsistent concentrations and changes in formulation procedures and stability data; according to the sponsor none of these affected study outcome. A laparotomy was done on gd15.

Results:
Phase 1: tables from sponsor

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DOSE GROUP :	1		2		3		4		5	
	NO.	%	NO.	%	NO.	%	NO.	%	NO.	%
FEMALES ON STUDY	25		25		25		25		25	
FEMALES THAT DIED DURING STUDY	0		0		2-A		0		0	
FEMALES PAIRED FOR MATING	25		25		23		25		25	
FEMALES WITH EVIDENCE OF MATING	24	96.0	25	100.0	23	100.0	21	84.0	23	92.0
GRAVID	23	95.8	17	68.0	19	82.6	19	90.5	19	82.6
NONGRAVID	1	4.2	8	32.0	4	17.4	2	9.5	4	17.4
FEMALES WITH NO EVIDENCE OF MATING	1	4.0	0	0.0	0	0.0	4	16.0	2	8.0
GRAVID	0	0.0	0	0.0	0	0.0	2	50.0	0	0.0
NONGRAVID	1	100.0	0	0.0	0	0.0	2	50.0	2	100.0
TOTAL FEMALES GRAVID	23	92.0	17	68.0	19	82.6	21	84.0	19	76.0
	NO.	%	NO.	%	NO.	%	NO.	%	NO.	%
FEMALE MATING INDEX	24/25	96.0	25/25	100.0	23/23	100.0	23/25	92.0	23/25	92.0
FEMALE FERTILITY INDEX	23/25	92.0	17/25	68.0	19/23	82.6	21/25	84.0	19/25	76.0
MEAN PRE-COITAL INTERVALS (DAYS)	3.1	NA	2.8	NA	3.1	NA	2.7	NA	2.7	NA
S.D.	2.32	NA	2.73	NA	1.41	NA	1.31	NA	2.72	NA
N	24		25		23		21		23	

1- 0 MG/KG/DAY 2- 60 MG/KG/DAY 3- 120 MG/KG/DAY 4- 180 MG/KG/DAY 5- 120(RS)MG/KG/DAY
A = FEMALE NOS. 66704 AND 66815 WERE EUTHANIZED PRIOR TO PAIRING, NOT INCLUDED IN CALCULATIONS

ncidence of pre-implantation loss was observed in all drug groups and exceeded both concurrent and historical data of 10.0 and 14.3% respectively (table from sponsor). Because of this increase, mean # of implantation sites and corpora lutea were also reduced (mean values for the former were outside historical data but the concurrent control was within these values). Mean number of viable embryos in all drug groups was also reduced compared to the control.

GROUP	VIABLE EMBRYOS	DEAD EMBRYOS	RESORPTIONS EARLY	RESORPTIONS LATE	POST IMPLANTATION LOSS	IMPLANTATION SITES	CORPORA LUTEA	PRE IMPLANTATION LOSS	NO. OF GRAVID FEMALES
1 TOTAL	313	0	22	0	22	335	371	36	23
MEAN	13.6	0.0	1.0	0.0	1.0	14.6	16.1	1.6	
S.D.	3.39	0.00	1.02	0.00	1.02	3.23	2.05	2.31	
2 TOTAL	195	0	19	0	19	214	290	76	17
MEAN	11.5	0.0	1.1	0.0	1.1	12.6	17.1	4.5	
S.D.	5.92	0.00	0.93	0.00	0.93	6.22	3.57	4.69	
3 TOTAL	232	0	28	0	28	260	324	64	19
MEAN	12.2	0.0	1.5	0.0	1.5	13.7	17.1	3.4	
S.D.	5.65	0.00	1.07	0.00	1.07	5.71	3.73	4.48	
4 TOTAL	227	0	11	0	11	238	285	47	18
MEAN	12.6	0.0	0.6	0.0	0.6	13.2	15.8	2.6	
S.D.	5.34	0.00	0.85	0.00	0.85	5.42	2.18	3.71	
5 TOTAL	255	0	18	0	18	273	321	48	19
MEAN	13.4	0.0	0.9	0.0	0.9	14.4	16.9	2.5	
S.D.	4.22	0.00	0.91	0.00	0.91	4.09	3.14	3.24	

None significantly different from control group
MEAN NUMBER OF VIABLE EMBRYOS, MEAN NUMBER OF IMPLANTATION SITES, MEAN NUMBER OF CORPORA LUTEA COMPARED USING DUNNETT'S TEST

1- 0 MG/KG/DAY 2- 60 MG/KG/DAY 3- 120 MG/KG/DAY 4- 180 MG/KG/DAY 5-120(RS)MG/KG/DAY

In summary, 1f dosed 180mg/kg/d S-zop was dead and both drugs increased pre-implantation loss and decreased fertility in all drug groups compared to concurrent control and tc historical data. Based on these findings, new doses were selected and phase 2 of study began (see above). There were no drug related deaths in any group and no reproductive findings in 5mg/kg/d group. Clinical signs were seen in all drug groups including low dose, mean B.wt and food intake were decreased in 15&25mg/kg/d groups including RS-zop compared to control; no effect on gross exam, or ovary and pituitary wts. Fertility indices were reduced in ≥15mg/kg/d and RS-zop relative to control (tables from sponsor). Because of decreased fertility and pre-implantation loss at ≥15mg/kg/d the NOEL for female reproduction is 5mg/kg/d and the NOAEL for embryofetal development is 25mg/kg/d.

DOSE GROUP :	1		2		3		4		5	
	NO.	%	NO.	%	NO.	%	NO.	%	NO.	%
FEMALES ON STUDY	25		25		25		25		25	
FEMALES THAT DIED DURING STUDY	0		0		0		0		0	
FEMALES PAIRED FOR MATING	25		25		25		25		25	
FEMALES WITH EVIDENCE OF MATING	24	96.0	25	100.0	23	92.0	25	100.0	25	100.0
GRAVID	23	95.8	24	96.0	18	78.3	20	80.0	21	84.0
NONGRAVID	1	4.2	1	4.0	5	21.7	5	20.0	4	16.0
FEMALES WITH NO EVIDENCE OF MATING	1	4.0	0	0.0	2	8.0	0	0.0	0	0.0
GRAVID	1	100.0	0	0.0	2	100.0	0	0.0	0	0.0
NONGRAVID	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0
TOTAL FEMALES GRAVID	24	96.0	24	96.0	20	80.0	20	80.0	21	84.0
	NO.	%	NO.	%	NO.	%	NO.	%	NO.	%
FEMALE MATING INDEX	25/25	100.0	25/25	100.0	25/25	100.0	25/25	100.0	25/25	100.0
FEMALE FERTILITY INDEX	24/25	96.0	24/25	96.0	20/25	80.0	20/25	80.0	21/25	84.0
MEAN PRE-COITAL INTERVALS (DAYS)	3.0	NA	2.8	NA	3.4	NA	2.9	NA	3.1	NA
S.D.	0.69	NA	1.09	NA	2.68	NA	2.75	NA	1.13	NA
N	24		25		23		25		25	

1- 0 MG/KG/DAY 2- 5 MG/KG/DAY 3- 15 MG/KG/DAY 4- 25 MG/KG/DAY 5- 25 (RS)MG/KG/DAY

GROUP	VIABLE EMBRYOS	DEAD EMBRYOS	RESORPTIONS EARLY	RESORPTIONS LATE	POST IMPLANTATION LOSS	IMPLANTATION SITES	CORPORA LUTEA	PRE IMPLANTATION LOSS	NO. OF GRAVID FEMALES
1 TOTAL	351	0	32	0	32	383	409	26	23
MEAN	15.3	0.0	1.4	0.0	1.4	16.7	17.8	1.1	
S.D.	2.86	0.00	2.06	0.00	2.06	1.90	2.88	2.18	
2 TOTAL	383	0	16	0	16	399	426	27	24
MEAN	16.0	0.0	0.7	0.0	0.7	16.6	17.8	1.1	
S.D.	1.92	0.00	0.76	0.00	0.76	1.88	2.89	1.83	
3 TOTAL	272	0	11	0	11	283	303	20	18
MEAN	15.1	0.0	0.6	0.0	0.6	15.7	16.8	1.1	
S.D.	2.08	0.00	0.85	0.00	0.85	2.02	1.54	1.18	
4 TOTAL	307	0	18	0	18	325	344	19	20
MEAN	15.4	0.0	0.9	0.0	0.9	16.3	17.2	0.9	
S.D.	4.02	0.00	1.02	0.00	1.02	4.10	3.35	1.73	
5 TOTAL	343	0	13	0	13	356	391	35	21
MEAN	16.3	0.0	0.6	0.0	0.6	17.0	18.6	1.7	
S.D.	2.48	0.00	0.80	0.00	0.80	2.33	3.53	2.01	

Mons significantly different from control group
MEAN NUMBER OF VIABLE EMBRYOS, MEAN NUMBER OF IMPLANTATION SITES, MEAN NUMBER OF CORPORA LUTEA COMPARED USING DUNNETT'S TEST

1- 0 MG/KG/DAY 2- 5 MG/KG/DAY 3- 15 MG/KG/DAY 4- 25 MG/KG/DAY 5-25 (RS)MG/KG/DAY

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A study of the effects of S-zopiclone on embryo-fetal development in rats/Sepracor# 190-821; — Study# — 312058

Conducting lab: —

Study Initiation/Termination Dates: Feb 2000/Mar 2000

GLP: Yes (x) Japanese and US FDA.

QA: Yes (x)

Drug Lot/Batch#/purity: for S-zop 0290005/ —, for RS-zop Z9910002 purity —

Species/Strain/: sexually mature virgin female Sprague-Dawley rats from —

B.wt/Age at study initiation: females: minimum 220g/males were sexually mature and used exclusively for breeding/females were 12wk old when mated.

Doses: **0, 62.5, 122, 250mg/kg/d S-zop and 125mg/kg/d RS-zop**. Table below from sponsor summarizes the number of rats per group, doses and dose volume. Both S- and RS- zop were prepared in 0.5% aqueous CMC administered by oral gavage once daily at 20ml/kg.

Group Number	Test Article	Dosage Level (mg/kg/day)	Dose Concentration (mg/ml)	Dosage Volume (ml/kg)	Number of Females
1	0.5% CMC (Control Article)	0	0	20	25
2	(S)-Zopiclone	62.5	12.5	5	25
3	(S)-Zopiclone	125	12.5	10	25
4	(S)-Zopiclone	250	12.5	20	25
5	(R,S)-Zopiclone	125	12.5	10	25

Drug administration started on **gd6 through gd17 and dams were killed on gd20.**

The following parameters were determined in this study:

Mortality & Clinical Signs: 2x daily (am and pm), detailed exam done from gd0-20 predose during treatment period.

Body wt & Gravid Uterine wt: maternal B.wts were recorded on gd0, 6-18 (daily), and gd20. Mean wt changes were calculated for given intervals (6-9, 9-12, 12-18, 6-18, and 0-20). Gravid uterine wt was determined and net B.wt (B.wt gd20 minus wt of uterus + content), and net B.wt change, were calculated and reported for each gravid female at time of laparohysterectomy.

Food Intake: reported as g/rat/d and as g/kg/d for the corresponding B.wt change intervals.

Reproductive Parameters: all surviving females were killed by CO_2 inhalation on gd20. Thoracic, abdominal, and pelvic cavities were examined. Uteri and ovaries were isolated and weighed and standard reproductive parameters were evaluated i.e. # & distribution of implantation sites, # corpora lutea, early/late resorptions, live/dead fetuses, etc. The sponsor indicated that maternal tissues from treated females were preserved in 10% formalin for possible future histopath exam, however, unlike what the protocol specified, control tissues/organs were not preserved and retained. This deviation was indicated not to have an effect on quality or integrity of the study.

S-zop Seg II study in the rat/Sepracor#190-821 (Cont.)

Fetal Parameters: fetal sex, weight, and number were determined. Complete external exam was done and included: eyes, palate, and external orifices. Heads from ½ of the fetuses in each litter were sectioned for soft tissue evaluation (Wilson Method; Bouin's sol.), and the other ½ heads were examined by mid-coronal slices and all fetal carcasses were prepared and stained with Alizarin Red S for skeletal exams. All and any external visceral, and skeletal findings were recorded as "developmental variations

(alterations in anatomic structures representing slight deviations from normal and have no significant biological effects on animal health) or as "malformations" (structural anomalies that alter body conformity, interfere with function, or can be incompatible with life).

Results:

Mortality & Clinical Signs: 1f dosed 250mg/kg/d S-zop was killed in moribund on gd9 due to gavage accident confirmed by necropsy; no other deaths in any group. Clinical signs were observed in all drug groups: dose related stereotypy (excessive pawing and/or wiping of face on cage postdose), up to 4hr postdose, salivation frequency and severity were dose related (not seen in control), dose related swaying while walking, rocking, and lurching 1hr postdose on gd6-14, and dose related increase in repetitive movement (stereotypy), of mouth and jaw when rats were handled up to 4hr postdose during gd10-17. Rales were infrequent but drug related and occurred in 250mg/kg/d S-zop and 125mg/kg/d RS-zop up to 4hr postdose on gd8, 11, and/or 16. Also cool to touch and moderate amount of wet red material around mouth were seen in 1HD S-zop 1hr postdose on gd8&9 respectively.

B.wt: no drug effect on mean wt except for a 6% decrease in RS-zop on gd20 relative to the control ($p<0.05$). However, mean **wt gain was reduced dose dependently** during the entire gestation period (gd0-20; note that dosing period ended on gd18)($p<0.01$)(17, 18, and 19% in 125&250mg/kg/d S-zop and 125mg/kg/d RS-zop respectively). Mean wt gain was also reduced during few gestation intervals: gd9-12 (57% of control; $p<0.01$) in 125&250mg/kg/d S-zop and gd18-20 in all 4 drug groups (34&75% in 62.5&125mg/kg/d and a loss of -5 and -1g in 250mg/kg/d S-zop and 125mg/kg/d RS-zop respectively; $p<0.05$ or 0.01). These latter reductions occurred after dosing was ended. There was no significant drug effect on gravid uterine wt but net B.wt in all but low dose drug groups and net B.wt gain in all drug groups were significantly reduced compared to the control (6-8% for net B.wt and 21-47% for net B.wt change; $p<0.05$ or 0.01).

Food Intake: mean intake in 250mg/kg/d S-zop was transiently but significantly reduced ($p<0.05$&0.01) during the 1st 3 days of dosing i.e gd6-9 and intake was comparable to the control throughout the rest of dosing i.e. gd9-12 and 12-18. However, intake computed for the entire gestation period (d0-20) showed a small (-4g/kg), but significant reduction in 250mg/kg/d S-zop compared to the control. During postdose gestation period (gd18-20) food intake expressed as g/rat/d or g/kg/d, was reduced relative to the control ($p<0.01$) in drug groups. Mean wt gains were also reduced during postdose period in the 2 highest doses of S-zop and RS-zop, these reductions were attributed to drug termination.

Necropsy: mean *female* fetal wt at necropsy was reduced in 125&250mg/kg/d S-zop ($p<0.05$ for the 250mg/kg/d), compared to the control. Mean fetal wt in males was comparable in drug groups and the control. The 3.2g mean wt reduction in 250mg/kg/d group was below the minimum historical value of 3.4g in [redacted] (compared to 3.5g in control). No drug effect on resorptions, implantation sites, or post-implantation loss. The small and not significant decrease in fetal wt at 125mg/kg/d was considered by the sponsor to be drug related because of effects on development such as bent ribs, unossified sternebrae etc., (see below).

SUMMARY OF MEAN FETAL DATA AT SCHEDULED NECROPSY (% PER LITTER)

GROUP NUMBER:	1	2	3	4	5
FEMALE FETAL WEIGHTS (g)					
MEAN	3.4	3.5	3.2	3.1**	3.3
S.D.	0.16	0.19	0.36	0.21	0.43
N	22	23	22	23	25
COMBINED FETAL WEIGHTS (g)					
MEAN	3.5	3.6	3.3	3.2*	3.4
S.D.	0.15	0.18	0.36	0.26	0.43
N	22	23	22	23	25

1- 0 MG/KG/DAY 2-62.5(S)MG/KG/DAY 3-125(S)MG/KG/DAY 4-250(S)MG/KG/DAY 5-125(RS)MG/KG/DAY

PROPORTIONAL (%) DATA COMPARED USING THE KRUSKAL-WALLIS TEST
FETAL WEIGHTS COMPARED USING DUNNETT'S TEST

* = Significantly different from the control group at 0.05
** = Significantly different from the control group at 0.01

The effects of S-zopiclone on embryo-fetal development in rats (Cont.)

Fetal Morphology: fetuses and (litters) examined were as follows: 342(22), 364(23), 339(22), 382(23), and 413(25) in cont, 62.5, 125, 250, and 125mg/kg/d S-zop and RS-zop respectively. Malformations in these dose groups were as follows: 0(0), 1(1), 1(1), 1(1), and 1(1) respectively, these malformations presented as % per litter were 0.0, 0.3, 0.3, 0.2, and 0.2% respectively, none of these differences were statistically significant. Drug related variations were seen in 125&250mg/kg/d S-zop and included unossified sternebrae #5 and/or 6 and/or bent ribs, also drug related reductions in mean litter proportions of fetuses with ossified cervical centrum #1 was seen 125&250mg/kg/d S-zop (table from sponsor). These findings reflect developmental delay which was also seen as smaller fetal wts in these groups. Other findings were within historical range of the lab and/or small and not dose dependent.

PROJECT NO.: 112056 NUMBER OF FETUSES AND LITTERS WITH VARIATIONS - SUMMARY

DAY 20	FETUSES					LITTERS				
DOSE GROUP:	1	2	3	4	5	1	2	3	4	5
NUMBER EXAMINED EXTERNALLY	342	364	339	382	413	22	23	22	23	25
NUMBER WITH FINDINGS	0	0	0	0	0	0	0	0	0	0
NUMBER EXAMINED VISCERALLY	342	364	339	382	413	22	23	22	23	25
HEMORRHAGIC RING AROUND THE IRIS	0	0	1	1	0	0	0	1	1	0
MAJOR BLOOD VESSEL VARIATION	1	0	0	0	0	1	0	0	0	0
NUMBER EXAMINED SKELETALLY	342	364	339	382	413	22	23	22	23	25
CERVICAL CENTRUM #1 OSSIFIED	54	67	29	25	49	17	14	7*	11	16
14TH RUDIMENTARY RIB(S)	39	25	35	39	38	13	9	12	15	10
14TH FULL RIB(S)	3	5	1	3	2	1	2	1	2	1
STERNEBRA(E) #5 AND/OR #6 UNOSSIFIED	23	27	47	78	36	6	10	11	16*	8
HYOID UNOSSIFIED	6	8	4	8	2	5	5	3	3	1
REDUCED OSSIFICATION OF THE 13TH RIB(S)	3	0	2	0	3	2	0	2	0	2
7TH STERNEBRA	0	0	0	0	1	0	0	0	0	1
7TH CERVICAL RIB(S)	2	3	2	1	3	2	3	2	5	2
27 PRESACRAL VERTEBRAE	3	2	0	0	2	1	2	0	0	1
STERNEBRA(E) #1,#2,#3 AND/OR #4 UNOSSIFIED	0	0	0	0	1	0	0	0	0	1
STERNEBRA(E) MALALIGNED(SLIGHT OR MODERATE)	0	0	1	1	1	0	0	1	1	1
BENT RIB(S)	0	0	4	4	1	0	0	3	3	1
25 PRESACRAL VERTEBRAE	1	0	0	0	1	1	0	0	0	1

1- 0 MG/KG/DAY 2-62.5(S)MG/KG/DAY 3-125(S)MG/KG/DAY 4-250(S)MG/KG/DAY 5-125(RS)MG/KG/DAY

* = Significantly different from the control group at 0.05 using Fisher's Exact test

Summary and Conclusion:

Oral gavage administration of S-zop up to 250mg/kg/d and RS-zop at 125mg/kg/d to pregnant female rat during organogenesis did not cause death in any group and clinical signs were those observed in previous rat studies and were extension of the pharmacology of the drug. There was maternal drug toxicity in 125&250mg/kg/d S-zop and RS-zop doses as seen by reduction in mean wt gain and food intake particularly postdose (gd18-20), and reduction in net mean B.wt and net mean wt gain. Fetal developmental delay was observed in 125&250mg/kg/d doses of S-zop reflected by mean fetal wt reductions and increase in unossified sternebrae and/or bent ribs and small incidences of ossified cervical centrum#1. The maternal NOAEL could not be determined due to maternal toxicity observed at the lowest dose of 62.5mg/kg/d and the developmental NOAEL is 62.5mg/kg/d since growth delay was observed at higher doses. There were no malformations in either the S- or RS- zop and that the 2 drugs are comparable in terms of their fetal effects at 62.5mg/kg/d S-zop to 125mg/kg/d RS-zop.

A study of the effects of S-zopiclone on embryo-fetal development in rabbits/Sepracor# 190-822/ — Study# 312059

Conducting lab —

Study Initiation/Termination Dates: Apr 2000/May 2000

GLP: Yes (x) Japanese and US FDA; ICH guidance on reproductive studies Sep 94/Apr 96.

QA: Yes (x)

Drug Lot/Batch#/purity: for S-zop 0290005/ — ; for RS-zop 3265659 purity —

Species/Strain/# per dose/sex: 22 sexually mature virgin female New Zealand White rabbits/group were received from — ,see below for #/group.

B.wt/Age at study initiation: females: 3058-3719g on d0 of gestation and 6months old at time of insemination.

Doses: **0, 4, 8, 16mg/kg/d S-zop and 32mg/kg/d RS-zop.** Table below from sponsor summarizes the number of rabbits per group, doses and dose volume. Both S- and RS- zop were prepared in 0.5% aqueous CMC administered by oral gavage once daily at 3.2ml/kg.

Drug administration started on **gd7 through gd20 and dams were killed on gd29.**

Group Number	Test Article	Dosage Level (mg/kg/day)	Dosage Concentration (mg/ml)	Dosage Volume (ml/kg)	Number of Females
1	0.5% CMC (Control Article)	0	0	3.2	22
2	(S)-Zopiclone	4	10	0.4	22
3	(S)-Zopiclone	8	10	0.8	22
4	(S)-Zopiclone	16	10	1.6	22
5	(R,S)-Zopiclone	32	10	3.2	22

Female rabbits in this study were artificially inseminated. Semen was collected from 16 resident males of the same strain and from the same supplier as the females. Diluted semen from 1m inseminated an equal # of females in each group.

The following parameters were determined in this study:

Mortality & Clinical Signs: 2x daily, detailed exam done from gd0-29(predose during treatment).

Body wt & Gravid Uterine wt: maternal B.wts were recorded on gd0, 7-21 (daily), 24 and gd29. Mean wt changes were calculated for given intervals (7-10, 10-13, 13-21, 7-21, 21-29 and 0-29). Gravid uterine wt was determined and net B.wt (B.wt gd29 minus wt of uterus + content), and net B.wt change, were calculated and reported for each gravid female at time of laparohysterectomy.

Food Intake: reported as g/rabbit/d and as g/kg/d for the corresponding B.wt change intervals.

Reproductive Parameters: all surviving females were killed by i.v. injection of Na-pentobarb via the marginal ear vein on gd29. Thoracic, abdominal, and pelvic cavities were examined. Uteri and ovaries were isolated and weighed and standard reproductive parameters were evaluated i.e. # & distribution of implantation sites, # corpora lutea, early/late resorptions, live/dead fetuses, etc. The sponsor indicated that maternal tissues from treated females were preserved in 10% formalin for possible future histopath exam; control tissues/organ sections were also preserved and retained for comparison.

Fetal Parameters: fetal sex, weight, and number were determined. Complete external exam was done and included: eyes, palate, and external orifices. Heads from each fetus in each litter were examined by mid coronal slice. All fetal carcasses were prepared and stained with Alizarin Red S for skeletal exams. All and any external, visceral, and skeletal findings were recorded as "developmental variations (alterations in anatomic structures representing slight deviations from normal and have no significant

biological effects on animal health) or as "malformations" structural anomalies that alter body conformity, interfere with function, or can be incompatible with life).
Effects of S-zopiclone on embryo-fetal development in rabbits/Sepracor# 190-822 (Cont.)

Results:

Mortality & Clinical Signs: 1f dosed 32mg/kg/d RS-zop aborted on gd19. This abortion was considered spontaneous and not drug related since it was within the historical range of spontaneous abortions a[illegible] (the historical control data is 29% or 12/42 studies). It should be noted however, that drug related clinical signs were observed in this female prior to abortion and were similar to those seen in other drug groups (see below). There were no other deaths in any group. Drug related clinical signs were observed mainly in the 2 higher doses of S-zop primarily during gd7-13 and in RS-zop during gd7-20: lethargy, hypoactivity, rocking, lurching or swaying while ambulating, delayed righting reflex, and rabbits lying on their side with heads down on cage floor. These signs were seen up to 4hr postdose. At 4mg/kg/d the only signs observed were hypoactivity and/or rocking, lurching or swaying while ambulating in 6 rabbits during gd7-12. Other signs in the 2 higher doses of S-zop included rales, prostration, and rapid respiration during gd7-11 at time of dosing and up to 2hr postdose. Decreased defecation was noted in all drug groups as well as the control but occurred earlier in the former (gd8-till end of study compared to gd21-29 in control). This effect was considered to be drug related since decreased food intake (see below) was seen concurrently with this finding.

B.wt: mean wt, wt gain, and, net B.wt gain were affected in all drug groups. Mean B.wt was significantly reduced ($p<0.05$ or 0.01) in 8&16mg/kg/d S-zop and RS-zop starting on gd11 through gd21 (5-8% less than control), though non-significant reductions were seen on gd9-10. Mean wts in these groups were comparable to the control values during gd24&29. Mean B.wt was also decreased in 4mg/kg/d (5-6% less than control; $p<0.05$) from gd13 through gd18 and comparable to the control thereafter. Mean wt gain was significantly reduced ($p<0.05$ or 0.01), in 8&16mg/kg/d S-zop and RS-zop starting on gd8-9 through gd10-11 with maximum loss of 50g in RS-zop gd10-11 and 38g in HD S-zop gd7. However, significant reductions in wt gain were seen during gd7-10 and 10-13 in all drug groups including RS-zop relative to the control as well as during the *entire dosing* period of gd7-21 (91% less than control in 8mg/kg/d S-zop and 89% less than control in RS-zop). However, when B.wt was calculated based on the *whole study duration of gd0-29*, there were ***no*** differences in mean wt cahnges between drug and control groups. There was however, a significant reduction during postdose period, gd21-29, in the 2

MEAN BODY WEIGHT CHANGES (GRAMS) DURING GESTATION

GROUP :	1	2	3	4	5
DAY 16- 17 MEAN	13.	35.	29.	17.	21.
S.D./N	27.8/21	36.1/19	47.2/21	34.8/19	46.5/19
DAY 24- 29 MEAN	-8.	7.	80.*	73.	73.
S.D./N	149.4/21	126.5/19	102.0/21	92.4/19	76.9/18
DAY 7- 10 MEAN	51.	-15.*	-63.**	-73.**	-113.**
S.D./N	39.7/21	96.3/19	82.0/21	80.1/19	78.5/19
DAY 10- 13 MEAN	38.	-42.**	-75.**	-39.**	-69.**
S.D./N	44.3/21	91.6/19	77.7/21	75.9/19	78.4/19
DAY 13- 21 MEAN	162.	170.	162.	153.	211.
S.D./N	95.8/21	92.7/19	75.5/21	155.1/19	109.2/18
DAY 7- 21 MEAN	270.	113.**	24.**	41.**	30.**
S.D./N	118.4/21	219.8/19	126.5/21	160.5/19	128.2/18
DAY 21- 29 MEAN	38.	103.	175.**	151.*	156.*
S.D./N	148.5/21	166.8/19	108.1/21	103.7/19	93.3/18
DAY 0- 29 MEAN	580.	620.	637.	628.	632.
S.D./N	250.6/21	230.1/19	185.1/21	258.8/19	116.3/18

1- 0 MG/KG/DAY 2- 4 MG/KG/DAY 3- 8 MG/KG/DAY 4- 16 MG/KG/DAY 5- 32 MG/KG/DAY

* = Significantly different from the control group at 0.05 using Dunnett's test
** = Significantly different from the control group at 0.01 using Dunnett's test
MEAN DIFFERENCES CALCULATED FROM INDIVIDUAL DIFFERENCES
NONGRAVID WEIGHT(S) NOT INCLUDED IN CALCULATION OF MEAN

highest doses of S-zop and RS-zop, relative to the control (table from sponsor):
Effects of S-zopiclone on embryo-fetal development in rabbits/Sepracor# 190-822 (Cont.)

There were no significant drug related effects on mean gravid uterine wts in any group or in the net mean B.wt. However, mean net B.wt *change* was significantly reduced in all S-zop groups and RS-zop relative to the control, noted was the large s.d. indicative of the large intra-animal variability (means±s.d.: 195±278.7 cont., -15±201 4mg/kg/d, 10±165g 8mg/kg/d, -4.6±236g 16mg/kg/d S-zop ($p<0.05$), and 59±181g RS-zop (not significant)).

Food Intake: was significantly reduced in all drug groups including RS-zop relative to the control intake throughout dosing period (7-10, 10-13, 13-21, and 7-21). Mean intake was also significantly and dose dependently reduced throughout pregnancy period (study period), 0-29 in all drug groups relative to the control ($p<0.01$).

Necropsy: no drug related effect.

Fetal parameters: although the sponsor indicated no drug effects, early/late resorptions and implantation loss were non significantly increased in 8mg/kg/d S-zop relative to the control (table from sponsor); though findings were within historical range for the lab.

SUMMARY OF MEAN FETAL DATA AT THE SCHEDULED NECROPSY

GROUP	SEX M	SEX F	VIABLE FETUSES	DEAD FETUSES	RESORPTIONS EARLY	RESORPTIONS LATE	POST IMPLANTATION LOSS	IMPLANTATION SITES	CORPORA LUTEA	PRE IMPLANTATION LOSS	FETAL WEIGHTS IN GRAMS	NO. OF GRAVID FEMALES
1 TOTAL	72	64	136	0	4	1	5	141	195	54	NA	21
MEAN	3.4	3.0	6.5	0.0	0.2	0.0	0.2	6.7	9.3	2.6	45.3	
S.D.	2.25	2.27	3.64	0.00	0.40	0.22	0.44	3.49	4.11	2.40	7.79	
2 TOTAL	73	64	137	0	11	1	12	149	200	51	NA	19
MEAN	3.8	3.4	7.2	0.0	0.6	0.1	0.6	7.8	10.5	2.7	43.7	
S.D.	1.95	1.74	2.04	0.00	0.69	0.23	0.76	2.09	3.15	3.00	4.63	
3 TOTAL	75	72	147	0	30	7	37	184	219	35	NA	21
MEAN	3.6	3.4	7.0	0.0	1.4	0.3	1.8	8.8	10.4	1.7	42.9	
S.D.	2.06	1.94	2.14	0.00	2.66	0.80	2.64	1.95	2.11	1.53	4.25	
4 TOTAL	75	62	137	0	6	3	9	146	195	49	NA	19
MEAN	3.9	3.3	7.2	0.0	0.3	0.2	0.5	7.7	10.3	2.6	42.8	
S.D.	2.01	1.52	2.04	0.00	0.67	0.37	0.70	2.03	3.16	2.71	4.72	
5 TOTAL	65	46	111	0	5	2	7	118	189	71	NA	18
MEAN	3.6	2.6	6.2	0.0	0.3	0.1	0.4	6.6	10.5	3.9	46.2	
S.D.	1.06	1.76	3.17	0.00	0.46	0.47	0.61	3.03	2.33	2.86	7.17	

None significantly different from control group
NA = NOT APPLICABLE
MEAN NUMBER OF VIABLE FETUSES, MEAN NUMBER OF IMPLANTATION SITES, MEAN NUMBER OF CORPORA LUTEA, FETAL WEIGHTS COMPARED USING DUNNETT'S TEST

Fetal Morphology: the following number of fetuses (litters) was evaluated: 136(19), 137(19), 147(21), 137(19), and 111(17) in cont., S-zop 4, 8, and 16mg/kg/d and 32mg/kg/d RS-zop respectively. There were 2(2), 3(3), 7(5), 6(4), and 0(0)% per litter malformations in these fetuses(litters) respectively, and 74, 74.5, 79, and 83% per litter variations respectively. Fetal soft tissue, external, and skeletal malformations were considered spontaneous in nature. As indicated, there was no significant difference in the total percent per litter with malformation or developmental variations between control and drug groups.

Summary and Conclusion:

Oral gavage administration of 4, 6, 16mg/kg/d S-zop and 32mg/kg/d RS-zop to pregnant New Zealand white rabbits during gd7-21 caused no death in any group and no fetal toxicities and no malformations or variations. Maternal NOAEL may be <4mg/kg/d S-zop due to effects on mean B.wt and food intake at all doses. However, animals seemed to recover with time and weights were comparable or higher than the

controls. Therefore, in absence of other maternal toxicities and fetal effects the maternal NOAEL may be 16mg/kg/d. The NOAEL for fetal parameters/development is 16mg/kg/d S-zop and 32mg/kg/d RS-zop.

APPEARS THIS WAY
ON ORIGINAL

Pre- and postnatal development study of (S)-ZOP in rats (Sepracor No. 190-828 — Study No. — -312067, 2001, GLP, NDA Section 5) This study was reviewed and evaluated by Dr. Edward Fisher.

Methods

Pregnant rats (25/group) were treated with 0 (vehicle: 0.5% CMC), 60, 120, or 180 mg/kg/day S-zop orally (gavage) from gestation day 6 through lactation day 20. A comparator control group (25 females) was administered 120 mg/kg/day RS-zop during this period. Maternal clinical observations, body weights, and food consumption were recorded and dams were allowed to deliver and rear offspring. Offspring (25/sex/group) were evaluated for survival, growth, physical and behavioral development, and reproductive performance. No basis for dose selection was provided.

Strain: Sprague Dawley — CD(SD)IGS BR)
Drug lot #: 029 0005

Results

a. Effects on the dam and reproductive parameters

i. The only deaths considered drug related in the report were in the RS-zop group: 2 females (#s 44524 and 44520) in this group died, and 2 (44603 and 44615) were euthanized in moribund condition, all on gestation day 22. However, 1 female (44571) in the LD (S) group was also euthanized on gestation day (gd)23. Clinical signs noted in these females prior to death or euthanasia were the same as in survivors, and included hypoactivity in animal #s 44520, 44603 and 44571; rocking, lurching or swaying while walking, unkempt appearance, and hunched appearance in female #44615; and prostration, paleness in color, and noticeable difficulty with parturition in female #44603. All other females survived to the scheduled necropsy on lactation day (LD) 21.

ii. Clinical signs consisted primarily of stereotypical behavior (mouth wiping and excessive pawing in the cage bedding), other neuromotor changes (hypoactivity, rocking, lurching or swaying) attributed to exaggerated pharmacology, which occurred in all treated groups, with a D-R incidence in the S-zop groups. Incidences in the RS-zop group appeared to be generally similar to those in the MD S-zop group (Table 1). These findings were observed for up to four hours following dosing. Salivation was also noted in S-zop groups at all doses.

iii. Weight gain during the gestational dosing period was decreased (21%, Stat Sig) in the RS-zop group, but there were no drug related or statistically significant changes in the S-zop groups (mean BW gains of 117, 107, 110, 110, and 93 grams over gds6-20 in C, LD, MD, HD S-zop and RS-zop groups, respectively). Food consumption during the gestational dosing period was significantly decreased only in the HD S-zop group (means of 67, 67, 65, 62, and 64 gm/kg/day, respectively, over gds6-20). BW gain during the lactational dosing period was dose dependently increased in all treatment groups (mean gains of 26, 39, 45, 56, and 48 gm in the respective groups over LD 1-21). Statistically significant (but not dose related) decreases in food consumption were seen during the lactational dosing period in all treated groups (means of 173, 159, 154, 161, and 148 gm/kg/day, respectively, over LDs 1-21).

iv. Pregnancy rates were 96.0%, 100%, 100%, 100% and 100% in C, LD, MD, HD S-zop and RS-zop groups, respectively. Gestation length was increased statistically significantly in all S-zop groups compared to the concurrent control (means of 21.8, 22.2, 22.2 and 22.3 in C, LD, MD, and

HD, respectively) and historical control (21.8 days). While there was no significant increase in the RS-zop group (22.0 days), 3 dams in this group died or were euthanized on gd22, prior to parturition. According to the report, "no signs of dystocia were noted in any of these animals prior to death or euthanasia," but clinical signs in these animals prior to death "suggested that the test articles may have interfered with the initiation of parturition." Numbers of implantation sites were similar among groups, but there was a dose related increase in the difference between the number of pups born and the number of implantation sites counted at necropsy (ie, postimplantation loss) in all treated groups (means of 0.5, 0.8, 1.4, 1.9, 1.3, respectively), reaching statistical signficance in the HD S-zop group. 0, 2, 1, 3 and 2 females in the C, LD, MD, HD S-zop and RS-zop groups, respectively, had total litter losses between LDs 1 and 8. There were no apparent drug related gross findings at dam necropsy on LD 21.

b. Offspring evaluations

i. Live litter size at birth and pup survival (primarily PND 0-4) was decreased in treated groups (**Tables 2** and **3**). An apparent dose related decrease in % male offspring was seen. No gross findings that could be attributed to treatment were noted in pups that were found dead.

ii. Pup weights were decreased in treated litters at birth, and this deficit persisted into the postweaning period (**Table 4**).

iii. There were no apparent drug related effects on the attainment of sexual developmental landmarks: mean days of acquisition of balanopreputial sepearation (PND 44.3, 43.6, 44.2, 43.9 and 44.0 in C, LD, MD, and HD S-zop and RS-zop groups, respectively) and vaginal patency (PND 33.7, 33.6, 33.7, 33.3 and 33.6) were similar among groups.

iv. Behavioral evaluations consisted of auditory startle (10/sex/group on PNDs 20 and 60 using an automated startle response device; same animals tested at each interval), locomotor activity (10/sex/group on PNDs 21 and 61 using a photobeam device; same animals tested at each interval), and Biel maze testing (10/sex/group on PNDs 22 and 62 using water-filled eight-unit T-maze; animals not tested twice). No T-R effects on locomotor activity were seen at either testing age, and no adverse effects on learning and memory were evident in the Biel maze data. However, in the auditory startle test, statistically significant increases in the mean Vmax and Vave values (peak and average response amplitude) were observed in the MD (S) and (R,S) group males, and reductions (generally statistically significant) in Tmax (latency to peak) values were observed in males and females in all S-zop and RS-zop groups during the PND 60 testing interval (**Table 5**). These differences appeared to be drug related, although, according to the study report, "the concurrent control group values for Vmax and Vave were uncharacteristically low, and the concurrent control group values for Tmax were uncharacteristically high when compared with values in the ~ nistorical control data."

v. Offspring reproductive function did not appear to be affected by maternal treatment. Female mating and fertility indices were 100%, 100%, 96.0%, 100% and 100% in the C, LD, MD, and HD S-zop and RS-zop groups, respectively, and male mating and fertility indices were 100%, 95.8%, 95.2%, 95.8% and 100% in the respective groups. Maternal BWs were reduced in all treated groups throughout gestation, but gravid uterine weights and overall BW gains were similar between C and treated groups. There were no clear treatment effects on F2 fetal development. Slightly decreased (but statistically significant) numbers of corpora lutea and implantation sites in

LD and MD S-zop groups were not considered drug related (in report) since there was no change in the HD S-zop group.

TABLE 1 CLINICAL OBSERVATIONS IN PREGNANT FEMALES (F0) 1-HOUR POST-DOSING

GROUP:		1	2	3	4	5
BEHAVIOR/CNS						
-ROCKS, LURCHES OR SWAYS AS IT WALKS		0/ 0	25/19	26/22	31/25	33/25
-HYPOACTIVE	1/ 1	34/16	32/18	40/22	39/23	
-WIPES MOUTH IN CAGE BEDDING UPON HANDLING		0/ 0	3/ 3	34/15	68/22	24/13
-EXCESSIVE PAWING IN CAGE BEDDING UPON HANDLING	0/ 0	5/ 4	6/ 5	17/ 9	7/ 5	

1- 0 mg/kg/day 2- 60(S)mg/kg/day 3-120(S)mg/kg/day 4-180(S)mg/kg/day 5-120(RS)mg/kg/day

TABLE 2 SUMMARY OF PND 0 LITTER (F1) DATA

GROUP :	1	2	3	4	5	
N	24	24	25	25	21	
NUMBER BORN	16.5	15.9	15.8	15.0	15.2	
SEX AT BIRTH (% MALES PER LITTER)	52.0	51.9	46.9	43.5	46.0	
LIVE LITTER SIZE (PND 0)		16.0	14.3	15.0	12.9**	12.9**

1- 0 mg/kg/day 2- 60(S)mg/kg/day 3-120(S)mg/kg/day 4-180(S)mg/kg/day 5-120(RS)mg/kg/day

TABLE 3 SUMMARY OF POSTNATAL SURVIVAL - % PER LITTER

GROUP :		1	2	3	4	5
PND 0 (RELATIVE TO NUMBER BORN)		97.3	89.9	95.0	86.0	84.7
PND 0 TO PND 1	98.4	88.7**	91.1**	83.7**	84.3**	
PND 1 TO PND 4 (PRE-SELECTION)		100.0	91.8**	88.2**	89.5**	88.1**
PND 4 (POST-SELECTION) TO PND 7		100.0	92.7*	98.5	99.4	92.9**
PND 7 TO PND 14		100.0	94.6	100.0	100.0	95.0
PND 14 TO PND 21		100.0	100.0	99.0	100.0	100.0
BIRTH TO PND 4 (PRE-SELECTION)		95.9	75.7**	78.1**	72.5**	67.2**
PND 4 (POST-SELECTION) TO PND 21		100.0	89.6**	97.5*	99.4	91.3**

1- 0 mg/kg/day 2- 60(S)mg/kg/day 3-120(S)mg/kg/day 4-180(S)mg/kg/day 5-120(RS)mg/kg/day

PND = POSTNATAL DAY
* = Significantly different from the control group at 0.05
** = Significantly different from the control group at 0.01

TABLE 4 SUMMARY OF MEAN OFFSPRING WEIGHTS (GRAMS)

DOSE GROUP:		1	2	3	4	5
PND 1						
MALES		7.1	6.4**	6.5*	6.2**	6.5*
FEMALES		6.7	6.1**	6.0**	5.8**	6.2*
PND 4 (BEFORE SELECTION)						
MALES		9.7	8.5**	8.9*	8.7*	8.8*
FEMALES		9.2	7.9**	8.3*	8.3*	8.4
PND 7						
MALES		16.1	13.0**	13.9**	13.5**	13.9**
FEMALES		15.2	12.4**	13.1**	13.0**	12.9**
PND 14						
MALES	34.4	29.2**	29.6**	29.1**	29.5**	
FEMALES		32.8	27.7**	28.2**	28.3**	28.5**
PND 21						
MALES	54.4	48.4**	48.5**	46.9**	47.5**	
FEMALES		52.1	46.0**	46.3**	45.7**	45.4**
PND 70						
MALES	421.0	401.4	385.8**	380.8**	401.8	
FEMALES		267.8	244.5**	243.4**	235.5**	231.1**

1- 0 mg/kg/day 2- 60(S)mg/kg/day 3-120(S)mg/kg/day 4-180(S)mg/kg/day 5-120(RS)mg/kg/day

* = Significantly different from the control group at 0.05 using Dunnett's test
** = Significantly different from the control group at 0.01 using Dunnett's test

TABLE 5 SUMMARY OF STARTLE RESPONSE DATA

GROUP:		1	2	3	4	5
		---- MALE ----				
PND 20						
Vmax (Millivolts)	158.8	138.5	181.7	156.0	127.0	
Tmax (Milliseconds)		24.0	25.0	24.2	24.7	30.0
Vave (Millivolts)		34.8	30.2	37.0	32.4	27.8
N		10	10	10	10	10
PND 60						
Vmax (Millivolts)	87.2	209.8*	172.0	156.3	229.4**	
Tmax (Milliseconds)		38.9	30.4**	28.8**	30.6**	30.4**
Vave (Millivolts)		21.2	44.7*	37.7	34.8	49.4**
N		10	10	10	10	10
		---- FEMALE ----				
PND 20						
Vmax (Millivolts)	149.7	166.8	163.7	163.6	159.2	
Tmax (Milliseconds)		23.9	25.8	24.4	23.1	24.8
Vave (Millivolts)		32.4	35.7	33.2	33.5	32.9
N		10	10	10	10	10
PND 60						
Vmax (Millivolts)	82.6	98.0	151.9	125.6	91.6	
Tmax (Milliseconds)		37.1	32.1	29.7**	30.0**	32.5
Vave (Millivolts)		18.1	20.5	29.8	23.5	19.0
N		10	10	10	10	10

* = Significantly different from the control group at 0.05 using Dunnett's test
** = Significantly different from the control group at 0.01 using Dunnett's test

3. Summary and Conclusions

In a study that can be considered adequate for regulatory purposes, administration of S-zop to rats during pregnancy and lactation resulted in developmental toxicity (decreased offspring growth and survival pre- and postnatally, and pup behavioral alterations) in the absence of significant maternally toxicity (a similar statement should appear in labeling). Although clinical signs thought to represent exaggerated pharmacological effects (stereotypic behavior, hypoactivity, effects on motor function) were seen in dams treated with both S- and RS-zop, treatment-related mortality and decreased body weight gain were only seen with RS-zop. Decreased food consumption in S-zop groups during lactation was likely a result of reduced litter sizes in these groups.

There was no NOEL for developmental toxicity following exposure to S-zop. Although the increase in postimplantation loss and decrease in live litter size were statistically significant only at

the HD, similar changes were seen at all doses; and the effects on postnatal survival, offspring body weight, and PND 60 startle response were statistically significant in all dose groups. Qualitatively similar effects on offspring development were seen in the (R,S)-zopiclone group. An effect on rat offspring survival has been reported previously with zopiclone (Esaki et al., Preclin Rep Cent Inst Anim 9:127-156,1983).

Other GABAmimetics have been reported to produce developmental toxicity in animals and humans. Benzodiazepines, while not generally considered teratogenic, have been associated with lasting neurochemical, immunological, and behavioral alterations in animals exposed prenatally or neonatally (see diazepam listing in REPROTOX or TERIS for references). Studies by Olney and colleagues indicate that drugs such as BDZs and barbiturates that activate $GABA_A$ receptors may trigger apoptotic neurodegeneration when administered to immature rodents during the period of synaptogenesis (Proc Natl Acad Sci 99:15089-94,2002). In humans, the use of BZDs during pregnancy is associated with impaired intrauterine growth and an increased frequency of pre- and perinatal adverse events. Although many of these effects have been attributed to acute pharmacological actions in the neonate (neurological effects due to intoxication and withdrawal symptoms), possible long-term neurobehavioral changes have not been well studied (REPROTOX, TERIS). Barbiturates are also considered potential human developmental toxicants (see phenobarbital listing in REPROTOX or TERIS).

Overall Reproductive/Developmental Summary:

In a dose range finding study, S-zop was administered by oral gavage to pregnant rats during gd6-17 upto 200mg/kg/d was well tolerated with no deaths, clinical signs were observed in all drug groups. In general, there were no drug effects on fetal development but some changes exceeded those in negative control such as the number of early embryo-fetal deaths was increased non-dose dependently in all drug groups, mean percent post-implantation loss was increased >2x in 100mg/kg group consequently the mean % of implantations loss in 100mg/kg group was increased. In a dose range finding study in pregnant rabbits, oral gavage doses of S-zopiclone during gd 6-18 caused maternal toxicity seen as clinical signs, reduced mean B.wt and, mean food intake at ≥8mg/kg/d as well as fetal toxicity seen as non-significant 9% reduction in mean body weight at 24mg/kg/d.

Reproductive and developmental assessment was previously studied with RS-zop. The drug in rat fertility study caused sperm death, clinical signs, and wt loss at ≥50mg/kg in rats. In another study on male reproductive parameters, RS-zop caused sperm death and infertility at ≥50mg/kg doses where copulation rates were reduced and pregnancy rates were zero. Histopathologically, this was seen as arrest of spermatogenesis and dilation of epididymal ducts. In a Segment II study in rats, mean maternal and fetal wts were reduced at 250mg/kg dose. RS-zop caused cannibalization of pups, death, and emaciation at ≥50mg/kg doses, postnatal development was also affected seen as reduced fetal wts; litter size was also reduced. In a cynomolgus study, RS-zop did not cause developmental toxicity when dosed upto 8mg/kg during gd23-35 but caused anorexia in dams. Therefore, RS-zop has several adverse effects on male and female reproductive and fetal parameters when orally dosed at ≥50mg/kg/d.

S-zop effects on fertility and reproduction were studied in the rat at S-zop doses up to 180mg/kg/d in females and up to 45mg/kg/d in males (RS-zop dose in males 15mg/kg/d and 120mg/kg/d in females), administered to both sexes premating, during mating and through gd7. Clinical signs were seen in all drug

groups and were dose dependent in severity; no drug related deaths in any group. No drug effect on B.wt, wt gain, or food intake. S-zop caused complete infertility in males dosed 45mg/kg/d and females dosed 180mg/kg/d without affecting mating index. Mean estrus cycle length was slightly increased in high dose females, pre-implantation loss increased in 60&120mg/kg/d and RS-zop group in addition, number of implantation sites and live embryos was decreased in the higher dose groups. In males various sperm parameters were adeversely affected and a NOEL for male fertility could not be determined. In females, a NOAEL could not be determined partly because of the study design (both sexes were treated with the drug), therefore, additional study was conducted where only females were treated and mated with untreated males. In this study the 1st part of the study tested S-zop at doses up to 180mg/kg/d where 1f was found dead at this dose and both S- and RS-zop increased pre-implantation loss and decreased fertility in all drug groups compared to concurrent control and to — .iistorical data (doses were 60, 120, and 180mg/kg/d S-zop and 120mg/kg/d RS-zop). The 2nd phase of the study attempted to find a NOAEL and tested the following doses 5, 15, 25mg/kg/d S-zop and 15mg/kg/d RS-zop. Clinical signs were seen in all drug groups including low dose, mean B.wt and food intake were decreased in 15&25mg/kg/d groups and in RS-zop compared to control; no effect on gross exam, or ovary and pituitary wts. Fertility indices were reduced at ≥15mg/kg/d and RS-zop relative to control.. Because of decreased fertility and pre-implantation loss at ≥15mg/kg/d the NOEL for female reproduction is 5mg/kg/d and the NOAEL for embryofetal development is 25mg/kg/d.

In Segment II rat reproductive developmental study, no deaths up to 250mg/kg/d S-zop and 125mg/kg/d RS-zop administered by oral gavage throughout the period of organogenesis. Clinical signs observed in all drug groups, maternal tox manifested as reduced B.wt gain and food intake occurred in 125&250mg/kg/d S-zop and RS-zop groups. Fetal developmental delay was seen at 125&250mg/kg/d S-zop and RS-zop groups reflected as decreased wt and increased incidence of skeletal variations. Maternal NOEL could not be determined and developmental NOAEL is 62.5mg/kg/d. Neither drug caused teratogenicity and both drugs had comparable effects on fetuses at the low dose tested. In rabbits, no maternal deaths and no fetal toxicity or teratogenicity up to 18mg/kg/d S-zop and 32mg/kg/d RS-zop administered by oral gavage during organogenesis. Maternal NOAEL as well as fetal NOAEL is 16mg/kg/d for S-zop and 32mg/kg/d for RS-zop. S-zop caused developmental toxicities (decreased pup growth and survival, and bpu behavioral changes), in absence of significant maternal toxicity when administered to female rats during pregnancy and lactation up to 180mg/kg/d S-zop and 120mg/kg/d RS-zop. A NOEL for developmental toxicity could not be determined for S-zop. Similar effects were observed for RS-zop at 120mg/kg/d dose.

3.4.7. LOCAL TOLERANCE

NA

APPEARS THIS WAY
ON ORIGINAL

3.4.8. SPECIAL TOXICOLOGY STUDIES:

Several studies were conducted to investigate the effects of RS-zop on thyroid and mammary gland following the findings of proliferation and tumors in these organs observed in 18month (only thyroid tumors), and 2 year carcinogenicity studies in rats. Rhone Poulenc Rorer (RPR) in France conducted a number of these studies starting in the early 1980s. Study titles and study#s are as follows:

1. Thyroid changes induced in rats by RP 27267 zopiclone. Sepracor# 190-866

2. Evaluation of the 18month oral tox study in the rat of cpd 27267RP. [redacted] report 1981. Sepracor# 190-858

3. RP 27267 zopiclone thyroid function study in rats/Sepracor# 190-853

4. zopiclone, RP 27267, mechanism of action on thyroid hormones in the rat/Sepracor# 190-845

5. RP 27267 the study of mechanism of thyroid effects of zopiclone after 10wks treatment in the rat by oral route/Sepracor# 190-846

6. zopiclone, RP 27267 action on hormonal system in the rat/Sepracor# 190-850

7. zopiclone, RP 27267, action on hormonal system in the rat (additional study)/Sepracor# 190-849

8. A 28d oral gavage endocrine function study of esopiclone and RS-zopiclone in male rats, amendment and final report/Sepracor# 190-837A1

9. A 3month dietary endocrine function study of RS-zopiclone in rats/Sepracor# 190-870F

10. A 3month dietary endocrine function study of RS-zopiclone in rats/Sepracor# 190-870A1

11. An endocrine function study of RS-zopiclone following single day and 3d oral exposure in ovarectomized female rats/Sepracor# 190-879A1

12. An endocrine function study of RS-zop, S-zop, zolpidem, and zaleplon following single and/or 3d oral exposure in ovariectomized rats/Sepracor# 190-884

13. Evidence of neuroendocrine disturbance in SD rats treated with RS-zopiclone/Sepracor# 190-878

14. Effects of muscimol, KCl, and RS-zop on basal and naloxone-induced GnRh secretion by the rat hypothalamus in vitro/ex vivo/Sepracor# 190-425

15. A uterotrophic assay of RS-zop and S-zop administered orally yo ovariectomized rats/Sepracor# 190-883

16. Rat early reproductive senescence study with S-zop and RS-zop/Sepracor# 190-882I*

* 6month interim report, study will be completed in Feb 04.

1. Thyroid changes induced in rats by RP 27267 zopiclone. Sepracor# 190-866.

This is a joint report dated March 8th 1982, by 2 expert pathologists, Dr [redacted], and Dr [redacted]. These pathologists were consulted by RPR the sponsor of the drug at that time, to evaluate the thyroid tumors observed in an 18month rat carcinogenicity study conducted by the [redacted] (report date Feb 24th 1981). RPR provided the 2 pathologists with a total of 103 H&E stained histopathology slides from 103 rats. There were 35 slides from the control (gr.I), 34 slides from mid dose **20mg/kg/d** (gr.II), and 34 slides from high dose **200mg/kg/d** (gr.VI). Rats were dosed daily with RS-zop by dietary administration for 18months. There were 50/sex/group, with a 6month interim kill of 15/sex/group. All slides contained sections from 2 thyroid lobes (only 1 slide from 20mg/kg group could not be evaluated because tissue was decomposed); not mentioned how many sections were prepared from each rat. There was agreement between the 2 pathologists on criteria and nomenclature of pathology except for 1 where one pathologist considered the finding to be follicular cell adenoma and the other pathologist considered it to be hyperplasia. The thyroid findings in the *original* carcinogenicity report were as follows:

Dose (mg/kg/d)		0	2	20	200
	Sex				
# thyroids examined	m	35	34	34	34
	f	35	0	0	35
cystic acinar ectasis/ cystic follicles	m	3	0	0	1
	f	1	-	-	0
follicular hyperplasia	m	0	0	0	2
	f	1	-	-	0
follicular cell adenoma	m	1	1	5	9
	f	0	-	-	0

Both consultants concluded that the follicular adenomas diagnosed by — pathologist (with 1 possible exception), **do not fulfill the criteria for rat thyroid adenomas** as set forth by IARC and WHO. They found focal hyperplasia in 1/35 control, 2/34 in 20mg/kg/d, and in 5/34 200mg/kg/d male drug groups. Epithelia of normal thyroid tissue in 200mg/kg/d male group were frequently cuboidal whereas, in control and 20mg/kg/d RS-zop male groups, it was flat to cuboidal depending on the size of the follicles. Parafollicular hyperplasia and adenomas were observed in drug and control groups at comparable frequency. Based on these findings, the 2 consultants concluded that the thyroid results in male rats in the 18month study **are NOT of neoplastic nature but rather a result of modest thyroid stimulation.** This conclusion was also supported by results of a thyroid function study provided by RPR to the 2 consultants — 1981; Sepracor study# 190-853; see below for study detail). It should be noted that this study *was specifically designed to assess thyroid hormones*. Male and female rats (15/sex/group), were administered aqueous solution of RS-zop in gum Arabic, at **0.2, 2, 20, and 200mg/kg/d** by oral gavage for 1, 2, or 4wks (5/sex/group killed per time point). Serum T3, free and total T4, and TSH (^{125}I labeling), were measured; histopath as well as macroscopic exam of pituitary and thyroid were also done. Results showed **NO** drug effect on B.wt, pituitary or thyroid wts, no macroscopical findings, and no histopathology of the pituitary. However, in males dosed 20 and 200mg/kg/d, RS-zop caused dose related thyroid hyperactivity described histopathologically as "follicles with little colloid and tall columnar epithelium containing a protrusion of apical cytoplasm";
Thyroid changes in rats by zopiclone. Sepracor# 190-866 (Cont.)

no effect in females. Serum TSH in 200mg/kg/d males was significantly increased 2.3 fold the control during 4wk of dosing but only small increase in T3 observed and only at wk4. Small though significant decreases were measured in free and/or total T4 in HD males but only during 1st wk. The only drug related effect in female hormones was a significant increase in T3 in 20&200mg/kg/d groups at all 3 time points but female thyroids showed no drug related changes. This study **concluded that RS-zop administered orally by gavage caused at 200mg/kg/d an increase in serum TSH and what appears to be histologically, stimulation or hyperactivity of the thyroid tissue. No clear drug effects on serum T4 or T3 though a decrease that reached statistical significance was recorded in HDm at end of study wk4.** No findings in females except for increase in serum T3 in 20&200mg/kg at all 3 time points of measurements.

2. Evaluation of the 18month oral tox study in the rat of cpd 27267RP. Dr. — report 1981. Sepracor# 190-858.

This is a report by — MD who was consulted by RPR in 1983 to comment on the thyroid tumors observed in the 18month rat study. His report was dated Jan 12th, 1983, he reviewed the data but did not re-read the slides. He also had access to Drs. — — reports and/or conclusions (Sepracor# 190-866). Dr — was in general agreement with these pathologists that the thyroid tumors are possibly due to thyroid proliferation. The latter effect he continued, maybe due to direct effect of the drug on the thyroid tissue or suppression of thyroid function that can cause a negative feed back via the pituitary with a consequent increase in serum TSH (as demonstrated in Sepracor# 190-853 below). The results of the thyroid function study below (made available to him too), supported this proposal and he concluded that RS-zop effects on the thyroid gland if persisted for a prolonged period and was adequate, may eventually causes thyroid stimulation and proliferation that leads to tumor formation i.e. not a direct carcinogen.

3. RP 27267 zopiclone thyroid function study in rats/Sepracor# 190-853. This was the 1st study done to investigate RS-zop effects on the thyroid because of the thyroid tumors observed in 18 month rat chronic toxicity study. It was proposed that the drug acts indirectly affecting peripheral metabolism of thyroid hormones where increase in T4 metabolism leads to positive feedback on TSH levels i.e. RS-zop administration causes a decrease in serum levels of the former and increase in levels of the latter hormone in the rat. This study was conducted on November 16 1981 by — — at the request of RPR. The study results were QA (no signatures were present). This study was *specifically designed to assess thyroid hormones*. Male and female SD rats — (15/sex/group; 8wks old at start of study), were administered aqueous solution of RS-zop in *gum Arabic*, at **0.2, 2, 20, and 200mg/kg/d** by oral **gavage** for 1, 2, or 4wks (5/sex/group killed per time point). Serum T3, free and total T4, and TSH (^{125}I labeling), were measured; histopath as well as macroscopic exam of pituitary and thyroid were also done. Four types of thyroid lesions were descried (see PAS Hematoxylin micrographs from sponsor for reference):

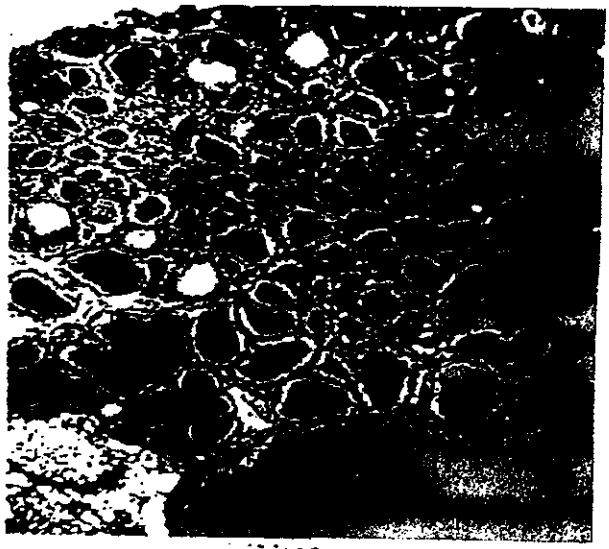
Type I / slightly active thyroid: many large follicles filled with profuse colloid and slightly flattened cubic epithelium, few smaller follicles with thicker epithelium generally cubic with small basal nuclei regularly arranged in the center of the gland.

Type II / moderately active thyroid: few large follicles filled with profuse colloid in the periphery of the gland, but many small follicles with cubic, tall columnar epithelium with clear nuclei irregularly arranged in the center and most parts of the gland.

Type III / marked activity: Very few sporadic follicles containing profuse colloid and many with nearly collapsed lumina and tall columnar epithelium with protrusion of apical cytoplasm containing fair number of droplets of PAS positive colloid.

Type IV: no follicles with profuse colloid. Follicles with very little colloid with collapsed lumina and tall columnar epithelium with protrusion of apical cytoplasm containing fair number of droplets of PAS positive colloid.

Type I thyroid

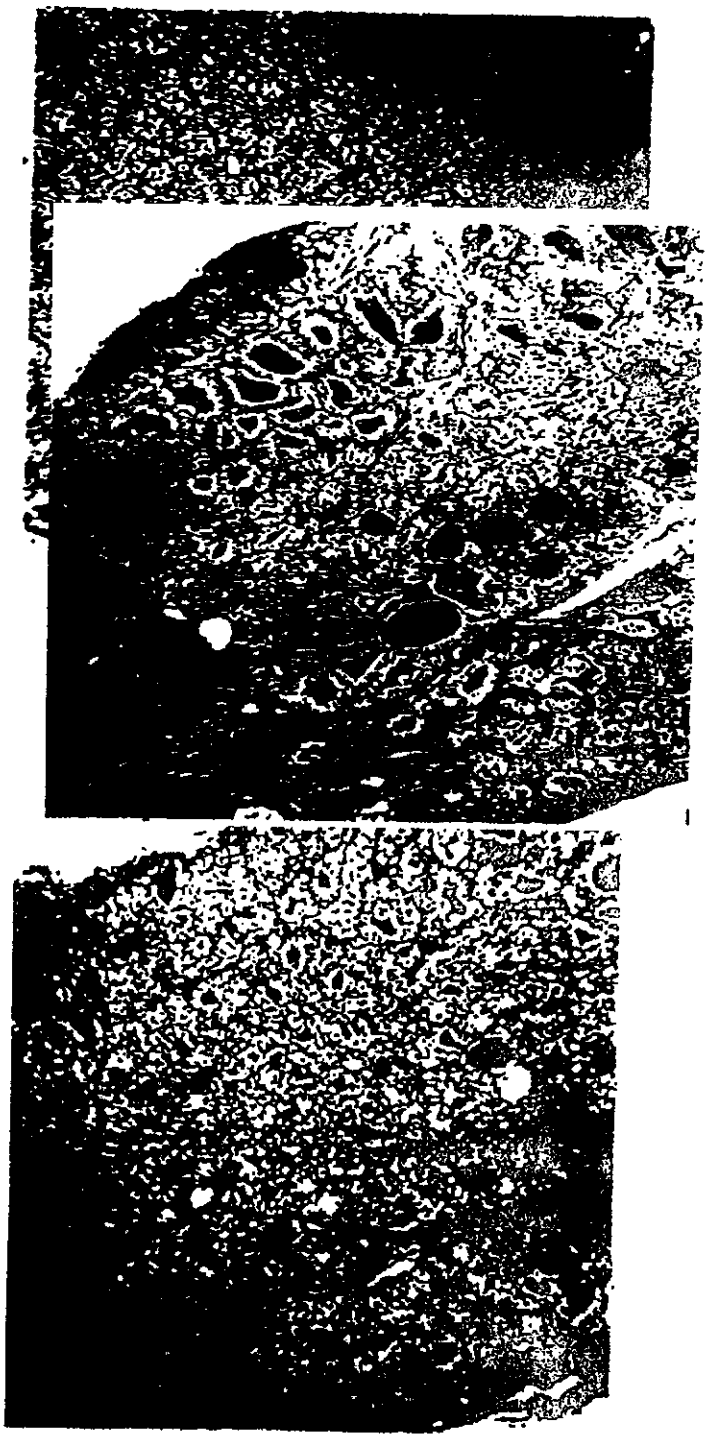


Type II thyroid

Thyroid function study in rats/Sepracor# 190-853 (Cont.)

Type III thyroid

Type IV thyroid



Thyroid function study in rats/Sepracor# 190-853 (Cont.)

Results showed no drug effect on B.wt, pituitary or thyroid wts, no macroscopical findings, and no histopathology of the pituitary. However, in males dosed 20 and 200mg/kg/d, the sponsor stated that RS-zop caused dose related thyroid "**hyperplasia**" characteristic of gland hyperactivity described histopathologically as "follicles with little or no colloid and tall columnar epithelium containing a protrusion of apical cytoplasm". There were no drug effects on thyroid in female rats. The following are thyroid findings according to type expressed as % for wks 1, 2, and 4 of sacrifice (15 rats per sex were examined):

	MALE RATS					FEMALE RATS			
	Type					Type			
DOSE	I	II	III	IV	DOSE	I	II	III	IV
CONTROLS	20	80	0	0	CONTROLS	40	40	20	0
0,2 mg/kg	40	40	20	0	0,2 mg/kg	40	60	0	0
2 mg/kg	0	100	0	0	2 mg/kg	40	40	20	0
20 mg/kg	0	60	40	0	20 mg/kg	40	60	0	0
200 mg/kg	0	60	40	0	200 mg/kg	0	60	40	0

In the reviewer's opinion, percentage of animals affected is not meaningful. Data better expressed to show whether the same rat had *progression* in the thyroid findings with the repeated administration of the drug i.e. from hypoactive to hyperactive gland tissue and if such finding correlated with the appropriate changes in serum thyroid levels. Combining percentage for the 3 time periods does indicate that thyroids of male rats administered the higher dose of RS-zop were in a hyperactive state compared to the control rats however, as indicated above, this representation may not reflect an accurate assessment of the results. Moreover, looking at each time point shows inconsistency: on wk1 20% of male rats each in 20&200mg/kg/d groups had thyroids in Type III vs. 0% in control yet with repeated dosing to wk2, no difference in thyroid status between control and any drug group. On wk4 however, all RS-zop treated male groups had thyroids with Type III stage vs. 0% in control (20, 20, 40, 40% in 0.2, 2, 20, and 200mg/kg/d respectively).

Serum TSH in 200mg/kg/d males was moderately and significantly increased after 4wk of dosing (2.3 fold the control), compared to control. However, the drug had minimal effect on T3 (small but significant increases in T3 levels measured on wk2 in males dosed 20mg/kg/d and on wk4 in males dosed 200mg/kg/d), and T4 where small but significant decreases were measured only during the 1st wk of dosing (28 & 37% less than corresponding control values for free and total T4 respectively)(table from sponsor). The only drug related effect in females was a significant increase in T3 in rats dosed 20 and 200mg/kg/d at all 3 time points (not significant on wk2 in HDf; table from sponsor).

Thyroid function study in rats/Sepracor# 190-853 (Cont.)

DOSE	Week	MALES TSH (µg/ml)	T3 (ng/100 ml)	free T4 (ng/100 ml)	Total T4 (µg/100 ml)	FEMALES TSH (µg/ml)	T3 (ng/100 ml)	free T4 (ng/100 ml)	Total T4 (µg/100 ml)
CONTROLS	1	0,49	78	2,78	7,38	0,39	74	2,54	6,62
	2	0,62	62	3,00	7,84	0,43	71	2,23	6,30
	4	0,58	58	3,15	7,92	0,40	61	2,77	7,16
0,2 mg/kg	1	0,70	74	2,55	6,32	0,44	91	2,60	6,42
	2	0,62	67	2,88	7,54	0,30	78	2,74	7,02
	4	0,82	58	3,14	8,00	0,40	80	2,76	7,50
2 mg/kg	1	0,71	84	3,05	7,70	0,39	90	2,48	6,54
	2	0,64	68	3,14	8,10	0,41	80	2,19	6,46
	4	1,08	59	3,15	7,94	0,38	63	2,44	6,10
20 mg/kg	1	0,59	88	3,22	8,50	0,50	115**++	2,37	6,34
	2	0,62	83*+	3,43	9,02	0,46	97*	2,64	6,98
	4	1,14	70	3,20	8,66	0,40	83*	2,58	6,26
200 mg/kg	1	0,87*	83	2,06*+	4,64**++	0,54	123**++	2,98	7,40
	2	1,81	84	2,19	6,30	0,53	92	2,02	5,00
	4	1,34*	87**+	2,96	7,90	0,50	85*	2,42	5,17

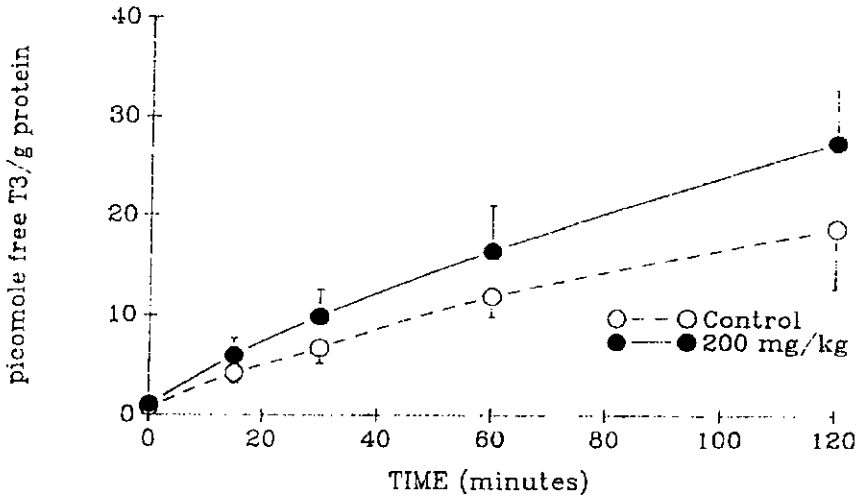
In conclusion, oral daily gavage dosing of RS-zop to male and female SD rats for 1, 2, or 4wk at 0.2, 2, 20, or 200mg/kg/d did not cause death, clinical signs or changes in B.wt, or thyroid or pituitary wts. There were no histopath findings in the pituitary up to 200mg/kg/d. Serum TSH was significantly increased in male rats dosed 200mg/kg/d on wks1&4. With the exception of wk1, there were no corresponding changes in T4 and only a small but significant increase in T3 was seen on wk4. Histopath changes indicative of hyperactive thyroid seem to correspond to hormone changes on wk4 in HDm as well as to hormone changes on wk1 (increase in TSH, decrease in free and total T4), but not on wk2. There were little drug related effects in females except for a significant increase in T3 at 20&200mg/kg/d dose groups without histopath correlate.

4. Zopiclone, RP 27267, mechanism of action on thyroid hormones in the rat/Sepracor# 190-845. Study conducted at — , no QA statement and no mention of GLP; RS-zop batch 7 (CA 84 10103). The study was conducted in July 1987 to further address RS-zop effects on thyroid hormones specially since at this time, results of the 2yr rat carcinogenicity study showed increased incidence of thyroid carcinomas in males dosed 100mg/kg/d via the diet. Male and female SD rats (17/sex/group from — were 11wks old when RS-zop was administered in the diet at **200mg/kg/d** (control group received basal diet with no drug). Rats were treated daily for 5, 8, or 10wks, 6/sex were killed at 5&8wks and 5/sex/ killed at 10wks for determination of conversion of T4 to T3 (wks5&8) and ^{125}I uptake into the thyroid and thyroglobulin binding. Livers were removed and homogenized, T4 was dissolved in buffer and added in vitro to liver homogenates. Free T3 was measured after 15, 30, 60, and 120min of incubation using commercial kit. Concentrations of free T3 reflected activity of 5'-deiodinase which converts T4 to T3 i.e. enzyme activity was measured *indirectly*, and activity expressed as pmol free T3/min/g protein using a regression line. For the iodine uptake and thyroglobulin study, control and drug treated rats were injected 3uCi ^{125}I 24hr before kill. Thyroids were removed and radioactivity of the whole gland was counted and thyroid uptake of iodine was measured when total radioactivity was expressed as total cpm and cpm per mg of thyroid. For the thyroglobulin uptake, the thyroids were homogenized, centrifuged, and aliquots of supernatant were

taken and subjected to chromatography. Radioactivity per cm of migration lane was counted. Percent of free ^{125}I and thyroglobulin bound ^{125}I were calculated based on the "fact" that free iodine will migrate 5-7cm whereas, bound iodine will not. Clinical signs, B.wt. and food intake were also monitored and recorded.

Results: there were no clinical signs, death, or changes in B.wt or food intake. Conversion of T4 to T3 no change at 5wks however at 8wks, free T3 levels were significantly increased in males indirectly reflecting an increase in 5'-deiodinase activity; no effect in females. After 10wks of dosing of RS-zop at 200mg/kg/d, no effect on uptake of ^{125}I and no change in ^{125}I thyroglobulin binding relative to controls in either sex (figure and tables from sponsor).

Male rats/Day 56 conversion of T4 to T3 by liver homogenates.



Mean values - day 56 of above figure (conversion of T4 to T3 in liver homogenate)

KINETICS OF THE APPEARANCE OF FREE T_3 IN MALE RAT LIVER HOMOGENATES

Treatment group	pmole free T3/g protein at each incubation time (Mean ± SD)				
	0 min	15 min	30 min	60 min	120 min
Controls (n=6)	0.8 ± 0.1	4.2 ± 0.4	6.7 ± 1.0	11.9 ± 2.1	18.8 ± 2.6
200 mg/kg (n=6)	1.1 ± 0.3	6.0 ± 1.7	9.9 ± 2.7	16.5 ± 4.5	27.5 ± 5.3
Significance	*	*	*	*	**

HEPATIC 5'-DEIODINASE ACTIVITY IN THE MALE RAT

Treatment group	pmole free T_3/min/g protein (Mean ± SD)
Controls (n=6)	0.18 ± 0.03
200 mg/kg (n=6)	0.25 ± 0.07

n Number of animals
* Significant for $p \leq 0.05$
** Significant for $p \leq 0.01$

Summary and Conclusion:

RS-zop at high dose of 200mg/kg/d administered to male rats in the diet caused thyroid proliferation in 18month chronic toxicity study and, in the 2yr carcinogenicity study at 100mg/kg/d by diet caused thyroid follicular carcinomas in male rats. Some of the results from mechanistic studies showed RS-zop affects thyroid hormones causing an increase in serum TSH and decrease in T4 with or without an increase in T3. Increase in TSH may result from action on one or more of the following sites:

1. direct effect on the hypothalamic-pituitary axis,
2. direct effect on the thyroid or,
3. indirect through effect on peripheral metabolism mainly in the liver

A central effect on the hypothalamic-pituitary axis was not supported by the results that showed an increase in TSH and a decrease in T4. A central effect on TSH or TRH would cause an increase in serum T4 and this has not been observed. Therefore, increase in TSH may result from a feedback mechanism that causes serum T4 to go down. The latter

zopiclone mechanism of action on thyroid hormones in the rat/Sepracor# 190-845 (Cont.)

may occur as a result of one or more of the following:

1. increase metabolism of T4 in the liver specifically enhanced 5'-deiodinase activity as well as increased Cl of T4.
2. deficient thyroid hormone biosynthesis which can affect iodine uptake or iodination.

The current study tested in vitro conversion of T4 to T3 after 5 and 8wks of RS-zop dosing and thyroid uptake of ^{125}I as well as thyroglobulin binding of ^{125}I after 10wk of RS-zop dosing. RS-zop 200mg/kg/d administered in the diet caused a significant increase in free T3 in males indicative of increase in 5'-deiodinase activity in the liver (expressed as pmol free T3/g protein) but no drug effect on thyroid uptake of iodine or thyroglobulin uptake of iodine. Therefore, this study supports the proposal that increase in TSH recorded after high doses of RS-zop is a result of peripheral action on hormone metabolism rather than a direct effect on thyroid biosynthesis and/or uptake. *It should be noted however, that the dose of RS-zop that caused thyroid tumors/carcinoma is 100mg/kg/d, the 200mg/kg/d caused thyroid proliferation in 18month chronic tox study in the rat. Therefore, the 100mg/kg/d dose should have been tested in addition to the 200mg/kg/d.*

5. RP 27267 the study of mechanism of thyroid effects of zopiclone after 10wks treatment in the rat by oral route/Sepracor# 190-846. Study conducted at — QA & GLP statements provided and signed. The study was conducted in March 1991, RS-zop batch 7 (CA 84 10103). This study was done to "complete the exploration" of the mechanism of RS-zop effects on thyroid hormones. Male and female SD rats (30/sex/group from — , were 10wks old when RS-zop was administered in the diet at **200mg/kg/d** (control group received the basal diet). Animals were treated daily for 10wks, for each control and drug groups, the 30/sex rats were divided into 15 and 9 per sex for hormone measurements (subgroups 1&2), and 6 per sex for [^{125}I] thyroxine clearance. Parameters assessed included: mortality, clinical signs, B.wt, food intake, ^{125}I T4 clearance, and the following blood hormones were measured using RIA: FT3 (free T3), FT4 (free T4), and TSH. In addition, the following PK parameters were determined: C_{max}, AUC_{inf} (from time 0 till time of last quantifiable sample then extrapolated to infinity), $T_{1/2}$, k (elimination rate constant), V_d, and Cl (total plasma Cl). Subgroups 1&2 of control and drug treated were killed by decapitation and necropsied on days70&71 for gross exam of liver, thyroids, and pituitary; weight of livers were determined from 11 or 12 rats/sex of subgroup 1; histopath was not done since no gross findings were observed. Transmission EM was done on livers from 17/sex/group and thyroids from 6/sex/group on days 71&72.

Results: there were no drug related effects on mortality, clinical signs, or gross exam. Mean B.wt in males and females started to decline from the 1st and 2nd wk of dosing respectively, till end of study with mean wt on d68 being 16 & 14% less than corresponding control weights respectively (figures from sponsor). A small decrease in mean daily food intake in both sexes of drug groups occurred in parallel to decrease in B.wt. (26&5% less daily intake in males and females respectively on d67).

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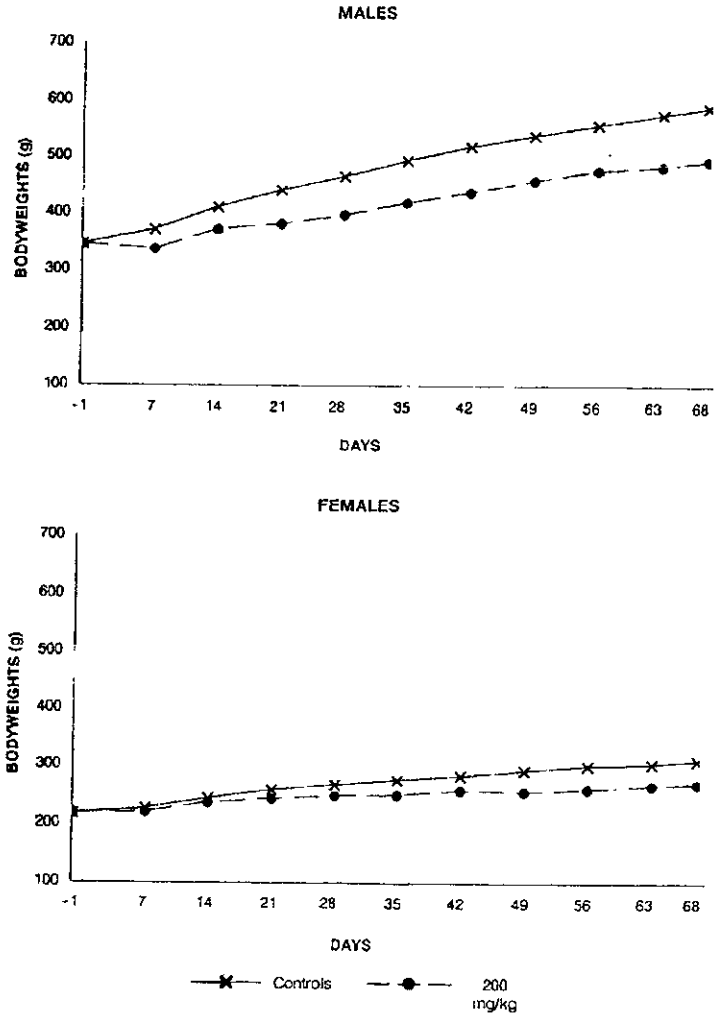
Mechanism of thyroid effects of zopiclone after 10wks treatment in the rat/Sepracor# 190-846 (Cont.)



Mean relative liver wt was only slightly increased (1.2 to 1.5x), in both sexes relative to corresponding controls (4.0 vs. 3.3% in males and 5.0 vs. 3.3% in females). Mean serum FT4 was significantly decreased in males (20%) and females (25%), relative to control values and mean serum TSH on the other hand was significantly increased (70% in males and 200% in females)(table below). Only in females, a small 10% increase in FT3 was recorded (table below).

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Mechanism of thyroid effects of zopiclone after 10wks treatment in the rat/Sepracor# 190-846 (Cont.)

Mean±s.d., FT4 and FT3 values are in ug/ml; TSH values are ng/ml. n= 24m for cont. and drug group; n= 23f cont. and 24f drug group.

	FT4	TSH	FT3
		MALES	
Control	16.7±3.0	9.6±4.6	5.1±0.8
200mg/kg/d	13.5±3.6**	16.4±8.7**	5.0±0.7
		FEMALES	
Control	14.0±2.4	5.2±1.5	6.0±0.8
200mg/kg/d	10.5±2.4***	15.3±7.4***	6.5±1.0*

* $p<0.05$ ** $p<0.01$ $p<0.001$ using Student t-test

^{125}I T4 i.v. injection, caused a biphasic decay curve. In drug groups, mean C_{max} (cpm/ml), and $AUC_{0\text{-}inf}$ (cpm.hr/ml), of labeled T4 were decreased relative to control values whereas, mean V_d (ml/kg) and Cl (ml/hr/kg) were significantly increased relative to control. Mean $T_{1/2}$ in drug groups was slightly increased relative to that in control. The sponsor indicated that increased V_d may explain the unexpected increase in T4 half life. Significant increases in UDPGT-T4 activity was recorded in both sexes of drug groups when activity expressed in pmol T4-G formed/min/**mg microsomal protein** (60% in males and 52% in females over the control), and as pmol of T4-G formed/min/**g liver** (104% in males and 95% in females over the control). Liver enzyme activity of 5'-deiodinase was unaffected in males or females when FT3 was expressed per mg protein but when expressed per g liver, a small but statistically significant increase of 25% over the control was seen in females, in males the increase was 29% but not statistically significant.

Summary and Conclusion: daily dietary administration of RS-zop at 200mg/kg/d to rats for 10wks caused a significant decrease in serum T4 and a significant increase in serum TSH, no change in FT3 in males but a small increase in females. Unlike the previous study (Sepracor# 190-845), no correlating increase in 5'deiodinase activity was observed in males when expressed as either per mg protein or gm liver, a small though statistically significant increase noted in females only when expressed per g liver. However,

activity of UDP-GT for T4 was significantly increased in both sexes. Mean concentration and exposure of ^{125}I T4 were decreased and its Cl and V_d were significantly increased. These changes collectively are likely to reflect a peripheral mechanism where RS-zop modifies thyroid hormone metabolism in the liver.

6. Zopiclone, RP 27267 action on hormonal system in the rat/Sepracor# 190-850. Study conducted at — study was QA with no signatures, no mention of GLP; RS-zop batch 3 (CA 83 336 00). The study was conducted in Dec 1984 three years after the 1st study that investigated RS-zop effects on thyroid (Sepracor# 190-853). This study investigated the potential drug effects on a number of serum hormones: **TSH, LH, FSH, GH, prolactin, progesterone, and 17β estradiol.** Note the maximum dose of RS-zop in this study was 100mg/kg/d, however, 200mg/kg/d was the maximum dose tested in the above study and in the 18month rat toxicity study that caused thyroid lesions. Male and female SD rats — 9wks old on study initiation), 40/sex/group were administered RS-zop in the **diet** at daily doses of **1, 10, or 100mg/kg/d** for 2, 4, or 9wks. Clinical signs, mortality, B.wt, and food intake were determined. Blood was collected from the abdominal aorta and serum hormones were measured in 10/sex/group at day0 and after 2, 4, and 9wks of dosing *[it is noted that killings were time-scheduled between 9 and 10:30am]*. The following organs were removed and processed for histology from all groups on wks 2, 4, and 9 of dosing: pituitary, adrenals, thyroid, ovaries, mammary glands, and liver (after 9wks only). The following organs were weighed: pituitary, adrenals, ovaries, and liver. Histopathology exam was done after 9wk on control and HD however, the 2 remaining drug groups killed at 9wks, were included later because of thyroid findings in HD. After 9wks of dosing, samples of livers from 5/sex/group were processed for Electron microscopy (although samples were processed for EM there was no indication that EM exam was done). Also activity of liver enzymes was determined in 6/sex/dose after 9wks. Microsomal protein content was determined as well as the activity of P450, aniline hydroxylase, and aminopyrine N-demethylase. Plasma RS-zop were measured at 9wks in 5/sex/drug group using HPLC with spectrometry detection.

Results: there were no drug related effects on mortality, clinical signs, B.wt, or food intake at any dose. There were **NO drug effects on serum FSH, LH, or 17-ß estradiol**. It should be noted that data were highly variable as reflected by the large s.d. Serum **TSH** was significantly increased at 9wks in ***females*** dosed 100mg/kg/d (359±100 vs. 195±113ng/ml in control), however, the sponsor stated that this change may not be drug related since the value of the control on wk9 was less than that at the other time periods (301, 260, 336ng/ml at predose, 2, and 4, respectively, vs. 195ng/ml on wk9). *Again accurate conclusion with respect to hormone levels can not be made because of the marked inter-animal variability of the data for all hormones.* Serum **progesterone** was significantly decreased after 2wks in 100mg/kg/d males (however, note that predose values in all 3 male drug groups, were significantly *higher* than the control), and in all 3 female drug groups relative to corresponding controls (non-dose dependent). This decline persisted till 9wks only in HDf (19±11ng/ml vs. 29±13ng/ml in control); values were comparable to control in other female groups and HDm. Serum **prolactin** was significantly decreased in HDm after 4&9wks (43&26% less than control), noted that NO change in 17β estradiol at any dose in either sex. Serum **GH** was significantly increased after 9wks in males dosed 10&100mg/kg/d (128±169 and 65±60ng/ml respectively, vs. 17±9ng/ml control). No drug effect on organ wts except for a 30&40% increase in *absolute* liver wt in males and females dosed 100mg/kg/d respectively, however, no change in relative wt and no histopath correlate were found in either sex up to 100mg/kg/d. No histopath findings were found in adrenals, ovaries, pituitary, or mammary glands. There were no changes in thyroids of any group at 9wks however at wk4, thyroids in males dosed 100mg/kg/d showed slight activity with follicles

containing little colloid and cylindrical tall epithelium often with many PAS positive colloid droplets. Based on thyroid findings in HD, thyroids from mid and low dose rats were examined; no drug changes were noted (table below). The sponsor stated that in general, thyroids in male rats seemed more active than those in females.

Zopiclone action on hormonal system in the rat/Sepracor# 190-850 (Cont.)

Number of rats out of 10 for each type of activity; values in () are for females (see study# 190-853 for definition of thyroid types):

Dose	Thyroid findings after 4wks			
(mg/kg/d)	Type 1	Type 2	Type 3	Type 4
control	0 (7)	7 (3)	3 (0)	0 (0)
1	0 (6)	7 (4)	3 (0)	0 (0)
10	0 (5)	7 (5)	3 (0)	0 (0)
100	0 (5*)	4 (3*)	5 (1*)	1 (0*)
	After 9wks			
Control	3 (8)	7 (2)	0 (0)	0 (0)
100	0 (6)	10 (4)	0 (0)	0 (0)

* only 9f were examined after 4wks at 100mg/kg/d, slide from the 10th rat could not be assessed.

It can be seen from the above table that type 3 activity was seen in 5/10 males in 100mg/kg/d vs. 3/10 in control males and type 4 seen in 1/10 male rats dosed 100mg/kg/d vs. none in control. No change in thyroid stimulation at 9wk of dosing in any group in either sex.

There were no drug related effects on liver enzymes or microsomal protein content. Mean plasma RS-zop at 9wks was higher in females than males and increased with dose linearly between 1&10mg/kg/d but more than proportional between 10&100 and 1&100mg/kg/d.

Plasma concentration in ng/ml wk9

Dose (mg/kg/d)	Males	Females
1	<2	4±2.7
10	17±8	36±16
100	336±119	840±231

In summary, results of this study **failed to support the findings** from study# Sepracor 190-853 above i.e. no drug related changes in serum TSH in either sex. The only drug related findings in this study were an increase in serum GH in males and decrease in progesterone in females and in the reviewer's opinion, there was no drug effect on thyroid activity in males up to 100mg/kg/d.

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7. Zopiclone, RP 27267, action on hormonal system in the rat (additional study)/Sepracor# 190-849. Study conducted at —, study was QA, no mention of GLP status. The study was conducted in Oct 1985, RS-zop batch 3 (CA 83 336 00). Study objective was to address RS-zop effect on hormones since the 1st hormone study conducted in 1981 (#190-853) showed increase in serum TSH with some histopath but the 2nd study (#190-850) conducted in 1984 failed to reproduce these findings. There were differences between these 2 studies: different maximum dose: 100 or 200mg/kg/d, and mode of administration i.e. diet vs. gavage as well as other technical issues that aimed at minimizing the variability in the data. In this study, both routes of administration were tested, diet and gavage, blood was collected at 2 times of the day noon and 9pm, to account for potential diurnal variations of hormones and, to reduce busy workload, the study was divided into 2 parts: study in males protocol# 372 conducted Oct 1985 and study in females protocol 382 conducted Nov 1985. It is noted however, that though rats were purchased from — *males were obtained from the supplier* — *and females from the supplier* —, could not supply females on time). Male and female SD rats ages 10-11wks at start of dosing, were orally administered daily doses of RS-zop at **1, 10, 100, 200mg/kg/d by gavage** (CMC for the control)**, 100 and 200mg/kg/d via diet for 4 or 10wks.** There were 40/sex/group, parameters assessed: mortality, clinical signs, B.wt, and food intake. The following hormones were measured in serum at wk4 & 9 in 20/sex/group with 10/sex/group killed at noon and the remaining 10 rats killed at night (9pm): TSH, T3, rT3, T4, fT4, GH, Prolactin, FSH, LH, progesterone, and 17-ß estradiol. Hormones were analyzed at a radioimmunology lab in — Stage of estrus in females was determined by a vaginal smear. The following organs were removed and processed for H&E staining: pituitary, thyroids, adrenals, liver, ovaries, and mammary glands (in females only). Absolute and relative wt of the pituitary, thyroid, adrenals, ovaries, and liver were recorded. Histopath was done on control and HD (200mg/kg/d) killed at 10wks, if any drug related findings are observed, lower doses and if necessary those at wk4, were also processed. The sponsor indicated that the *2 lobes of the thyroid were embedded to thoroughly examine the largest surface of the parenchyma due to differences in morphology of the follicles located in the center and those at the border of the gland.* Samples of livers from 5/sex/group at 10wks were processed for EM but not examined due to absence of any drug related findings by standard histopath exam. Plasma RS-zop were determined on wks 4 and 9/10 from 5/sex/dose 2hr postdose via gavage and 2hr post light on-set in animal room for rats dosed via diet. Plasma drug levels were analyzed by RPR in France using an HPLC with spectrophotometric detector.

Results: 4 males dosed 200mg/kg/d via gavage, died on days 5, 9, 32, and 68 of study cause of death considered by the sponsor to be drug related however individual animal history/data was not provided therefore, exact cause of death remains unknown. Clinical signs as reported by the sponsor, were seen only in rats dosed via **gavage** at 100 and 200mg/kg/d starting at wk4 onward they included: stereotypic

movements such as "prancing and licking", transient ptyalism and convulsion in 1m dosed 200mg/kg/d on d58 and 1f dosed 100mg/kg/d d56. *No clinical signs were observed in rats dosed via the diet up to 200mg/kg/d.* No drug effects on food intake. At both wks4&10, mean relative liver wt in males dosed 200mg/kg/d was increased 18% after gavage and 31% after dietary dosing over the control means. Similarly in females dosed 200mg/kg/d wks 4&10 both gavage and diet routes, relative liver wt was increased up to 55&72% respectively relative to control. Mean relative thyroid wt was increased 26% after gavage and 50% after dietary dosing in males dosed 200mg/kg/d relative to wts in controls; no marked changes in females. Mean relative ovary wt was increased 23&10% in 200mg/kg/d dietary and gavage dosing respectively; none of the organ wt changes reached statistical significance. Mean B.wt in males dosed 100&200mg/kg/d via gavage, was significantly reduced 4-13% less than controls, and 10-14% via the diet at 200mg/kg/d dose. These B.wt losses began during the 1st wk of dosing via both routes and continued till end of study d63. In females, mean B.wt was significantly reduced 7-12% relative to control wt, in 200mg/kg/d diet starting 1st wk of dosing and continuing till end of dosing d63.

RS-zop action on hormonal system in the rat (additional study)/Sepracor# 190-849 (Cont.)

However, mean B.wt was significantly *increased* 5-8% over the control wts in 200mg/kg/d females dosed by gavage. Mean serum **TSH was significantly increased in both sexes at 200mg/kg/d of both routes, there seem to be no effect of time on hormone measurement**. Note the large variability in the data reflected by the marked s.d. (table from sponsor; values are means±s.d.). Clearly such variability makes interpretation and clear conclusions difficult to make.

Serum TSH (ng/ml), MALES

		After 4 weeks		After 10 weeks	
		(12 H)	(21 H)	(12 H)	(21 H)
CONTROLS (gavage) (10)	MN	339.3	310.7	389.6	275.7
	SD	350.7	202.7	182.6	116.4
1 MG/KG (10)	MN	380.7	211.1	356.2	266.8
	SD	254.5	69.9	113.2	116.0
10 MG/KG (10)	MN	536.7	202.6	443.6	245.2
	SD	339.6	103.4	291.1	154.2
		+			
100 MG/KG (10)	MN	520.6	464.4	293.5	322.7
	SD	206.2	243.2	154.1	158.3
		+			
200 MG/KG (10)	MN	754.4	840.3	535.3	900.1
	SD	339.5	440.0	430.4	487.7
		**	***		***
		++	++		+++
		(9)		(8)	(9)
CONTROLS (diet) (10)	MN	499.1	319.5	308.9	238.7
	SD	253.0	116.6	103.4	91.9
100 MG/KG (10)	MN	509.1	309.9	480.3	373.3
	SD	129.2	85.0	160.9	190.2
				(++)	(+)
200 MG/KG (10)	MN	1163.7	712.8	769.9	593.2
	SD	725.4	336.7	350.4	259.1
		(**)	(***)	(***)	(***)
		(+)	(++)	(+++)	(+++)

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Zopiclone action on hormonal system in the rat (additional study)/Sepracor# 190-849 (Cont.)

Serum TSH (ng/ml), FEMALES

		After 4 weeks		After 10 weeks	
		(12 H)	(21 H)	(12 H)	(21 H)
CONTROLS (gavage) (10)	MN	334.1	151.4	224.3	129.0
	SD	149.3	67.8	128.7	104.5
1 MG/KG (10)	MN	235.3	164.2	233.3	107.8
	SD	57.8	95.1	100.6	34.3
10 MG/KG (10)	MN	287.0	182.9	189.3	115.5
	SD	106.4	99.7	58.7	34.6
					(8)
100 MG/KG (10)	MN	224.2	186.9	209.8	137.0
	SD	74.0	86.4	86.8	59.0
200 MG/KG (10)	MN	269.4	242.5	421.3	123.6
	SD	115.8	192.6	281.0 **	72.6
CONTROLS (diet) (10)	MN	312.4	100.3	159.4	103.2
	SD	179.6	55.7	106.6	67.9
100 MG/KG (10)	MN	311.1	167.0	388.2	132.0
	SD	137.0	75.3	231.6	65.0
				(**)	
			(+)	(++)	
200 MG/KG (10)	MN	1153.5	672.1	480.7	219.9
	SD	428.3	565.2	182.4	108 1
		(***)	(***)	(***)	(**)
		(+++)	(+++)	(+++)	(+)

The only change in TSH at 100mg/kg/d occurred in males/wk4 dosed via gavage and males/wk10 dosed via diet and in females/wk10 via diet (these changes reached statistical significance relative to control

values and ranged between 54% in males both times to 144% in females/wk10 diet). Serum **T3** in males dosed 200mg/kg/d via gavage and diet, was significantly increased after 10wks of both time points (noon & 12pm)(23-36% over control), T3 levels were also increased at 100mg/kg/d via diet on wk10 (not dose dependently; table from sponsor). **Reverse T3** levels on the other hand, were not changed at wk10 in males but significantly and dose dependently decreased at 100&200mg/kg/d wk4 after gavage and dietary dosing (table from sponsor). Female T3 levels were significantly but not dose dependently increased in 100&200mg/kg/d gavage/wk4 and wk9 at 200mg/kg/d dietary dose (25-33% over control). Reverse T3 levels in females were significantly decreased only after dietary dosing on wks4&9; all compared to corresponding control (table from sponsor). Mean **T4** levels in males were significantly reduced at both time points after gavage as well as dietary dosing (table from sponsor). T4 levels in females were significantly decreased in 200mg/kg/d at both time points and after gavage and dietary dosing. Parallel decreases in free T4 in males were seen at 100&200mg/kg/d dietary and gavage administrations at both

time points and in females, levels were significantly reduced only in 200mg/kg/d at both time points relative to control (table from sponsor). **GH** levels were significantly reduced in males dosed via gavage on wk10 compared to control but it should be noted that the data were extremely variable making accurate conclusion difficult; no change in females. **Prolactin** was significantly reduced in females on wk10 after gavage dosing, **FSH** levels were also significantly reduced in 200mg/kg/d gavage as well as dietary at 10wks compared to control. Serum **LH** level was significantly reduced at $\geq$10mg/kg/d gavage dosing but data were highly variable. Serum **estradiol** in females was significantly and dose dependently increased at 100&200mg/kg/d gavage and diet wk4 and wk10 relative to control (table); no drug effect on **progesterone** levels.

T3 in males (mean$\pm$s.d.; values in ng/dl):

		After 4 weeks		After 10 weeks	
		(12 H)	(21 H)	(12 H)	(21 H)
CONTROLS (gavage) (10)	MN	101.7	80.4	88.1	73.2
	SD	20.3	13.8	9.1	7.1
1 MG/KG (10)	MN	84.6	68.1	91.2	84.3
	SD	16.4	10.6	17.4	14.0
		(9)			
10 MG/KG (10)	MN	95.5	76.7	72.6	61.9
	SD	25.8	12.3	17.7 * +	8.4 +
100 MG/KG (10)	MN	92.1	87.9	84.1	77.3
	SD	34.1	27.0	11.2	7.4
200 MG/KG (10)	MN	93.9	88.3	110.7	99.2
	SD	17.7	17.4	22.2 ** +	35.8 **
		(9)		(8)	(9)
CONTROLS (diet) (10)	MN	75.3	58.8	67.9	55.8
	SD	9.7	8.7	8.2	8.4
100 MG/KG (10)	MN	78.1	63.5	79.6	71.7
	SD	19.8 (*) (+)	8.6	18.6	11.3 (**) (++)
200 MG/KG (10)	MN	77.0	76.6	75.3	69.3
	SD	6.2	19.3 (**)	9.8	13.5 (*) (+)

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Zopiclone action on hormonal system in the rat (additional study)/Sepracor# 190-849 (Cont.)

T3 in females (mean±s.d.; values in ng/dl):

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		After 4 weeks (12 H)	After 4 weeks (21 H)	After 10 weeks (12 H)	After 10 weeks (21 H)
CONTROLS (gavage) (10)	MN	103.7	77.4	123.9	90.5
	SD	25.7	16.6	29.3	20.9
1 MG/KG (10)	MN	89.9	91.8	102.9	92.3
	SD	18.5	32.3	15.3 *	15.3
10 MG/KG (10)	MN	100.0	86.9	109.6	94.1
	SD	20.2	15.9	12.0	28.6 (8)
100 MG/KG (10)	MN	119.8	103.0	139.6	106.0
	SD	17.0	21.0 ++	15.9	17.6
200 MG/KG (10)	MN	123.8	98.8	132.7	107.1
	SD	24.9 *	20.6 +	27.1	25.7
CONTROLS (diet) (10)	MN	106.6	103.3	129.7	101.6
	SD	19.3	23.1	18.6	18.6
100 MG/KG (10)	MN	128.5	105.3	139.3	117.0
	SD	26.9	21.6	15.1	24.6
200 MG/KG (10)	MN	120.2	126.2	167.0	126.1 (*) (++)
	SD	23.7	30.7	53.7	18.3

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Zopiclone action on hormonal system in the rat (additional study)/Sepracor# 190-849 (Cont.)

rT3 in males (mean±s.d.; values in ng/dl):

		After 4 weeks		After 10 weeks	
		(12 H)	(21 H)	(12 H)	(21 H)
CONTROLS (gavage) (10)	MN	28.9	24.2	20.8	23.2
	SD	16.0	9.6	6.3	7.3
1 MG/KG (10)	MN	24.9	21.3	23.9	25.0
	SD	6.9	3.6	5.2	5.3
10 MG/KG (10)	MN	24.3	26.5	23.0	21.2
	SD	7.7	7.8	9.1	6.5
100 MG/KG (10)	MN	15.0	18.8	20.1	19.0
	SD	5.1	2.8	5.3	4.1
		**			
		++			
200 MG/KG (10)	MN	14.7	18.9	16.5	19.3
	SD	3.6	4.5	3.0	6.4
		**			
		+++			
		(9)		(8)	(9)
CONTROLS (diet) (10)	MN	26.9	22.7	23.5	17.9
	SD	6.6	5.1	5.5	5.1
100 MG/KG (10)	MN	20.6	17.4	21.1	19.5
	SD	7.0	3.0	7.5	2.4
		(*)	(**)		
		(-)	(+)		
200 MG/KG (10)	MN	16.0	13.7	21.6	21.8
	SD	4.8	2.7	6.1	9.6
		(***)	(***)		
		(+++)	(+++)		

Zopiclone action on hormonal system in the rat (additional study)/Sepracor# 190-849 (Cont.)

rT3 in females (mean±s.d.; values in ng/dl):

		After 4 weeks		After 10 weeks	
		(12 H)	(21 H)	(12 H)	(21 H)
CONTROLS (gavage) (10)	MN	38.7	26.2	29.3	28.6
	SD	23.6	8.8	8.0	8.7
			(9)		
1 MG/KG (10)	MN	34.2	31.5	31.3	33.5
	SD	7.6	10.0	9.1	8.0
10 MG/KG (10)	MN	39.2	31.8	32.6	30.5
	SD	13.9	6.8	9.9	15.5
				(9)	(8)
100 MG/KG (10)	MN	30.7	26.6	25.9	23.8
	SD	5.5	8.5	5.4	6.0
200 MG/KG (10)	MN	28.4	22.3	29.8	23.4
	SD	7.2	6.9	7.4	6.8
					(5)
CONTROLS (diet) (10)	MN	26.8	31.4	25.0	20.9
	SD	9.9	13.0	4.4	4.7
100 MG/KG (10)	MN	21.6	20.8	19.9	17.3
	SD	5.5	7.1	3.7	2.2
			(*)	(**)	
			(+)	(+)	
200 MG/KG (10)	MN	22.9	21.6	18.9	20.9
	SD	8.2	6.9	3.2	7.3
			(*)	(**)	
			(++)	(++)	

T4 in males (mean±s.d.; values in ng/dl):

		After 4 weeks (12 H)	After 4 weeks (21 H)	After 10 weeks (12 H)	After 10 weeks (21 H)
CONTROLS (gavage) (10)	MN	61.8	52.4	56.5	51.7
	SD	7.3	8.3	13.5	6.3
		(9)			
1 MG/KG (10)	MN	53.9	44.0	56.0	57.0
	SD	7.8	7.8 * +	7.7	12.0
		(9)		(9)	
10 MG/KG (10)	MN	62.3	56.4	53.0	56.2
	SD	14.3	7.2	10.7	12.0
100 MG/KG (10)	MN	41.0	42.8	37.5	46.7
	SD	14.8 *** ++	8.1 ** ++	9.0 *** ++	10.9
200 MG/KG (10)	MN	33.7	39.9	40.5	37.7
	SD	10.8 *** +++	7.5 *** ++	8.8 ** +	9.8 ** ++
		(9)		(8)	(9)
CONTROLS (diet) (10)	MN	60.1	41.0	60.6	54.5
	SD	10.2	12.0	11.2	7.2
		(9)			
100 MG/KG (10)	MN	40.8	37.2	47.1	49.6
	SD	15.8 (**) (+)	3.7	6.7 (***) (++)	8.5
200 MG/KG (10)	MN	26.2	22.9	32.3	35.3
	SD	5.6 (***) (+++)	10.1 (***) (++)	5.0 (***) (+++)	10.2 (***) (+++)

T4 in females (mean±s.d.; values in ng/dl):

		After 4 weeks (12 H)	After 4 weeks (21 H)	After 10 weeks (12 H)	After 10 weeks (21 H)
CONTROLS (gavage) (10)	MN	45.0	33.1	37.2	36.3
	SD	10.0	7.1	12.4	11.6
		(6)	(7)	(5)	(4)
1 MG/KG (10)	MN	37.3	39.6	33.5	24.2
	SD	6.9	7.2	8.6	3.7 *
			(5)	(8)	(6)
10 MG/KG (10)	MN	34.6	27.8	32.8	28.7
	SD	7.9	8.0	9.5	7.3
			(6)	(6)	(7)
100 MG/KG (10)	MN	33.1	26.7	30.0	29.6
	SD	6.8	7.7	7.5	6.9
200 MG/KG (10)	MN	36.6	24.6	29.7	23.3
	SD	8.3	6.6 * +	9.7	7.3 **
					(9)
CONTROLS (diet) (10)	MN	38.8	32.0	35.3	27.3
	SD	11.3	7.3	3.5	8.0
		(9)	(8)	(6)	(6)
100 MG/KG (10)	MN	33.1	28.6	35.6	21.7
	SD	8.2	12.1	5.6	3.7
		(8)	(8)	(9)	(7)
200 MG/KG (10)	MN	28.3	22.8	27.7	23.5
	SD	4.2 (*)	5.1	7.4 (*) (+)	12.4
			(9)		

Free T4 in males (mean±s.d.; values in pmol/l):

		After 4 weeks		After 10 weeks	
		(12 H)	(21 H)	(12 H)	(21 H)
CONTROLS (gavage) (10)	MN	59.3	55.9	53.7	47.0
	SD	8.3	5.1	5.1	6.0
1 MG/KG (10)	MN	51.4	52.0	50.9	45.6
	SD	7.2	3.3	5.5	5.5
		+			
10 MG/KG (10)	MN	48.0	51.8	48.8	49.7
	SD	9.7	7.6	8.9	4.4
		*			
		+			
100 MG/KG (10)	MN	47.9	52.0	44.1	52.3
	SD	12.6	5.0	8.4	5.2
		*		**	*
				+	+
200 MG/KG (10)	MN	32.4	39.0	34.5	26.5
	SD	8.9	10.1	7.2	7.7
		***	***	***	***
		+++	+++	+++	+++
		(9)		(8)	(9)
CONTROLS (diet) (10)	MN	49.6	46.3	59.2	55.4
	SD	6.1	11.2	4.8	5.5
100 MG/KG (10)	MN	50.1	53.4	51.7	53.4
	SD	9.3	6.6	4.1	4.2
				(**)	
				(++)	
200 MG/KG (10)	MN	27.6	28.5	40.4	41.9
	SD	6.9	8.1	7.7	8.0
		(***)	(***)	(***)	(***)
		(+++)	(++)	(+++)	(++)

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Zopiclone action on hormonal system in the rat (additional study)/Sepracor# 190-849 (Cont.)

Free T4 in females (mean±s.d.; values in pmol/l):

		After 4 weeks		After 10 weeks	
		(12 H)	(21 H)	(12 H)	(21 H)
CONTROLS (gavage) (10)	MN	49.2	37.2	43.8	32.4
	SD	11.0	9.8	8.6	10.3
1 MG/KG (10)	MN	38.8	37.8	34.8	39.8
	SD	11.8	12.1	6.4	11.2
				*	
				+	
10 MG/KG (10)	MN	48.1	32.9	40.8	37.7
	SD	7.3	10.8	8.8	10.0
					(8)
100 MG/KG (10)	MN	40.3	32.3	33.4	31.4
	SD	8.5	14.4	7.9	6.5
				**	
				+	
200 MG/KG (10)	MN	42.5	31.6	40.6	25.3
	SD	6.6	6.8	6.8	6.3
CONTROLS (diet) (10)	MN	51.4	47.5	53.4	41.8
	SD	6.9	12.0	6.8	9.0
100 MG/KG (10)	MN	53.5	49.0	46.6	32.0
	SD	8.2	15.3	5.7	9.6
				(*)	
				(+)	(+)
200 MG/KG (10)	MN	36.2	31.5	39.8	30.8
	SD	8.1	7.8	6.1	12.5
		(***)	(**)	(***)	
		(++)	(++)	(+)	(+)

Zopiclone action on hormonal system in the rat (additional study)/Sepracor# 190-849 (Cont.)

FSH in females (mean±s.d.; values in ng/ml)

		After 4 weeks		After 10 weeks	
		(12 H)	(21 H)	(12 H)	(21 H)
CONTROLS (gavage) (10)	MN	130.1	174.5	157.3	187.6
	SD	40.7	59.8	66.0	90.0
			(9)	(9)	(9)
1 MG/KG (10)	MN	128.5	186.8	150.8	185.1
	SD	54.9	120.2	80.3	114.8
			(9)		
10 MG/KG (10)	MN	131.9	158.5	111.1	107.3
	SD	57.6	161.3	27.9	29.5 +
				(9)	(8)
100 MG/KG (10)	MN	143.5	167.5	149.3	214.9
	SD	50.9	57.3	47.1	107.4
		(9)			
200 MG/KG (10)	MN	138.4	156.0	118.9	119.6
	SD	81.5	53.8	51.9	19.4 +
					(9)
CONTROLS (diet) (10)	MN	164.9	221.9	159.6	191.2
	SD	64.4	111.5	29.1	95.1
		(9)		(9)	
100 MG/KG (10)	MN	180.1	205.3	113.2	118.4
	SD	63.5	127.1	35.7 (**) (++)	47.2
		(9)			(9)
200 MG/KG (10)	MN	186.8	236.5	133.8	199.6
	SD	84.5	97.0	41.6 (+)	119.5
		(9)			

RS-zop action on hormonal system in the rat (additional study)/Sepracor# 190-849 (Cont.)

17 ß estradiol in females (mean±s.d.; values in pg/ml)

		After 4 weeks		After 10 weeks	
		(12 H)	(21 H)	(12 H)	(21 H)
CONTROLS (gavage) (10)	MN	72.5	64.2	80.0	80.9
	SD	29.5	16.1	34.5	39.6
1 MG/KG (10)	MN	78.9	63.9	58.3	69.3
	SD	26.9	14.0	27.5	20.1
10 MG/KG (10)	MN	85.8	83.7	86.6	80.7
	SD	27.2	19.7 +	32.7	31.1 (8)
100 MG/KG (10)	MN	76.7	117.7 *** +++	93.5	98.8 +
	SD	14.6	50.8	24.2	22.9
200 MG/KG (10)	MN	120.4	132.1	99.3	121.3
	SD	31.8 *** +	38.3 *** +++	17.5	33.5 ** ++
CONTROLS (diet) (10)	MN	45.4	37.4	48.3	49.5
	SD	15.4	24.9	15.7	8.3
100 MG/KG (10)	MN	86.0	81.9	114.8	75.7
	SD	22.2 (*) (++)	19.5 (*) (+)	37.4 (**) (+++)	18.7 (*)
200 MG/KG (10)	MN	135.8	92.6	132.9	90.1
	SD	64.1 (***) (+++)	19.5 (**) (++)	73.7 (***) (+++)	26.9 (**) (+++)

No histopathology found in any organ/tissue except in the liver and thyroids. Thyroid hyperactivity (types 3&4) was observed in both sexes dosed 200mg/kg/d via both routes for both wks4&10 and, in 100mg/kg/d males dosed via gavage and dietary on wk10 (table below). There was no difference in thyroid activity types 1+2 between drug and control groups at any dose. As noted earlier, livers were examined only after 9wks of dosing by both routes at 100 and 200mg/kg/d doses. Mild to moderate liver hypertrophy was seen on wk10 in males dosed 200mg/kg/d via gavage and females of both routes (histopathology data were not presented, this is taken from sponsor's statement in report).

RS-zop action on hormonal system in the rat (additional study)/Sepracor# 190-849

Incidence of animals with type 3+4 thyroid hyperactivity (number of animals out of total examined). Number of animals examined excluded dead rats and those with thyroids irrelevant for rating due to technical reasons.

	Week 4		Week 10	
	Males	Females	Males	Females
GAVAGE				
Cont	5/17	1/20	3/19	3/20
100mg/kg/d	5/20	2/19	14/20*	3/20
200mg/kg/d	15/18*	9/20*	12/17*	12/20*
DIET				
Cont	9/20	2/20	5/20	2/20
100mg/kg/d	9/20	4/20	14/20*	3/20
200mg/kg/d	14/20	10/18*	17/20*	10/20*

* Fishers exact test or Chi square test.

Mean plasma RS-zop (ug/ml) increased proportional to dose between 1&10mg/kg/d but no change in levels between 100&200mg/kg/d (table from sponsor). Levels were 2-5 fold higher after gavage than after dietary at the same doses. There was no sex difference.

		MALES		FEMALES	
		After 4 weeks	After 10 weeks	After 4 weeks	After 9 weeks
1 MG/KG (gavage) (5)	MN	0.094	0.077	0.115	0.157
	SD	0.005	0.008	0.045	0.050
10 MG/KG (gavage) (5)	MN	0.435	1.130	1.017	1.113
	SD	0.100	0.235	0.207 (4)	0.100 (4)
100 MG/KG (gavage) (5)	MN	7.815	10.940	7.400	10.170
	SD	2.441	3.960	1.880	3.714
200 MG/KG (gavage) (5)	MN	8.472	8.806	7.670	13.771
	SD	3.668 (4)	3.157 (4)	2.205	3.308
100 MG/KG (diet) (5)	MN	1.875	2.311	3.397	3.400
	SD	0.738	0.527	1.145	0.754
200 MG/KG (diet) (5)	MN	1.591	2.382	2.634	3.438
	SD	1.003	0.554	0.659	0.589

RS-zop action on hormonal system in the rat (additional study)/Sepracor# 190-849 (Cont.)

Summary and Conclusion:

This study re-examined the effects of RS-zop on thyroid and other hormones following gavage and dietary administration at doses that caused thyroid tumors in previous 18month chronic toxicity study in the rat. RS-zop in this study was administered to male and female SD rats at **1, 10, 100, 200mg/kg/d by gavage** and at **100 and 200mg/kg/d via diet for 4 or 10wks**. The following hormones were measured in serum at wks4&9 in 20/sex/group with 10/sex/group killed at noon and the remaining 10 rats killed at night (9pm): TSH, T3, rT3, T4, fT4, GH, Prolactin, FSH, LH, progesterone, and 17-ß estradiol. The 2 time point measurements of the day were done to see if diurnal variations affect serum hormone levels. RS-zop administered via gavage caused 4 deaths in males dosed 200mg/kg/d therefore, this dose exceeded the MTD. However, the drug was well tolerated up to 200mg/kg/d via the diet in both sexes. Unlike previous studies, clinical signs were observed in this study and included stereotypy, clinical signs occurred in the 100&200mg/kg/d doses via gavage and none seen when drug administered via the diet. RS-zop caused up to 13-14% decrease in mean B.wt in males dosed 100&200mg/kg/d via gavage and 200mg/kg/d via diet compared to controls. These wt changes started during the 1st wk of dosing and continued till end of study. Mean wt was also reduced throughout the study starting from wk1, in females dosed 200mg/kg/d via diet (up to 12% relative to control), however, mean wt was significantly *increased* up to 8% in females dosed 200mg/kg/d via gavage. Mean serum TSH was significantly increased in both sexes at 200mg/kg/d via both routes and time of blood collection had no effect on hormone levels. Serum T4 and rT3 levels were decreased and T3 were increased in both sexes at 200mg/kg/d and some in 100mg/kg/d doses via one or both routes of administration. Together with these hormone changes mean relative thyroid and liver wts were also increased in both sexes at both routes at 200 but not 100mg/kg/d dose. Thyroid histopathology described as hyperactivity of the gland tissue was seen in both sexes at 200mg/kg/d at both wks4&10 and only in males dosed 100mg/kg/d at wk10. These changes in thyroid hormones and histopathology indicated an indirect mechanism of the drug rather than direct effect on hypothalamic-pituitary axis. This is because a direct effect on TRH or TSH would cause an increase in serum T4 which did not occur and a direct inhibition of hormone synthesis by RS-zop would lead to decrease in not only T4 but also T3 which was not observed. Therefore, an indirect mechanism of RS-zop may be responsible for the thyroid changes. RS-zop may be inducing liver enzyme activity specifically 5'-deiodinase leading to accelerated breakdown of T4 to T3 and rT3 to T2 with consequent stimulation of TSH that in turn causes thyroid proliferation. In support of such proposition, liver hypertrophy was observed as stated by the sponsor. Other drug related effects included an increase in mean relative ovary wt in females dosed 200mg/kg/d of both routes; without histopathology. RS-zop seem to affect other endocrine hormones: a significnt decrease in prolactin and FSH levels in females dosed 200mg/kg/d, serum **LH was also significantly reduced** at ≥10mg/kg/d. Mean **17-β estradiol was significantly and dose dependently increased** in females dosed 100&200mg/kg/d via both routes and both wks4&10; no drug effect on progesterone. Drug effect on estradiol may also be peripheral and indirect since a central role is not supported by an increase in FSH (in fact levels of FSH were decreased).

8. A 28d oral gavage endocrine function study of esopiclone and RS-zopiclone in male rats, amendment and final report/Sepr.# 190-837A1, — -312087, 2001

Conducting laboratory and location: —

Date of study initiation: April 19th 2001; final report date Jan 8th 2002

GLP compliance: yes: FDA, Japanese & OECD

QA report: yes (x)

Study Objectives: primary objective was to evaluate the potential effects of S-zop and RS-zop On thyroid hormone homeostasis and liver enzyme induction and secondary objective was to investigate drug effects on spermatogenic parameters based on findings in previous toxicity and reproductive studies in the male SD rat. Reversibility of any of these findings was assessed by allowing rats 28d drug free recovery period.

Methods:

RS-zopiclone (lot 326-7226; purity —, and S-zopiclone (lot 029-0014 purity — were administered daily for 7, 14, or 28days by oral **gavage at 200 and 100mg/kg/d** respectively to 12wk old male Sprague Dawley rats. Rats were purchased from — The control group received the vehicle 0.5% carboxymethylcellulose (CMC) on a comparable regimen; drug or control were administered at 10ml/kg volume. There were 60 rats/group, each 15/group were killed at 7, 14, and 28days postdose, remaining 15/group were kept for additional 28d recovery period. At each necropsy, liver and prostate samples from each rat were frozen for potential analysis and spermatogenic parameters evaluated on 8 males at each necropsy. Blood for hormone analyses was collected from the vena cava of all *non-fasted* anesthetized rats at each necropsy. Samples were collected in the morning between 7:30-10:30am and the following hormones analyzed: TSH (by RIA), T4 & T3 —, rT3 (by RIA), FSH & LH (by RIA), testosterone and beta estradiol (by —

Parameters Studied:

- Clinical signs and mortality were checked 2x daily (during recovery 1x daily), detailed exam, food intake and, B.wt checked weekly,
- Serum hormones days 7, 14, 28, and 56 (recovery). FSH, LH, beta estradiol, testosterone, TSH, T3, rT3, and T4.
- Complete standard gross exam on all rats at each necrospy and on any rat in extremis. The following were preserved in buffered formalin: all gross lesions, liver sections from both lobes, pituitary, thyroids, adrenals, testes, epididymides, and prostate.
- The following organs from all rats were weighed: adrenals, brain, epididymides, liver, pituitary, prostate, testes, and thyroid/parathyroid (paired organs weighed separately).
- Microscopical exam of H&E prepared slides from the above tissues/organs was done. The pituitary glands were sectioned longitudinally, both halves processed and special immunohistochemical staining was done using rat TSH antibody. All tissues were evaluated by — , — senior pathologist at —
- Spermatogenic assessment: immediately after euthanasia, the reproductive tract of 8 males per group at each necropsy was exposed and epididymides removed according to SOPs. Sperm motility was determined by computer-assisted sperm analysis system
  on at least 200 motile and nonmotile spermatozoa/rat (actual numbers of spermatozoa/rat ranged between 0-384). Sperm morphology was assessed by a modified wet-mount technique and abnormal

sperms recorded from a differential count of 200 spermatozoa/rat as applicable. Also sperm production rate were determined from the left testes and epididymides of the 1st 8 rats per group at each necropsy.

- Liver enzyme induction was evaluated from microsomes from the 1st 8 males per group at final necropsy (analysis done at —). Samples were analyzed for P450 content and UDP-glucuronyltransferase (UDP-GT). The following tissues were frozen for potential future analyses: testes, epididymides (for rats not receiving spermatogenic evaluation), adrenals, and prostate. [enzyme induction was not done under GLP].

Results:

Mortality and Clinical Signs: no drug related deaths. Clinical signs occurred in both drugs and were consistent with the pharmacology of the drug: hypoactivity, sleeping, and impaired muscle coordination.

B.wt, wt gain, Food intake: no drug effect.

Hormone Effects: there were **NO drug related effects on serum estradiol, FSH, or LH.** Mean **TSH** was significantly increased after 2wks in both drugs and only in RS-zop after 4wks (2.6&2x for RS-zop wks2&4 respectively, and 1.6x for S-zop over the control). Mean **T4** levels were significantly decreased 32&20% relative to control in RS-zop on wks1&2 but not different on wk4 (end of dosing); no effect in S-zop. Mean **T3** was 14% increased over the control in S-zop on wk1 only; no other changes in either drug at any time. Mean **rT3** was significantly reduced 11&33% in S- and RS-zop respectively only on wk1 relative to control. Mean **testosterone** was significantly reduced on wk1 in RS-zop (61% less than control) and in both S- and RS-zop at recovery wk8 (61 and 54% less than control respectively).

Organ wts: Mean absolute and relative **liver wt** was significantly increased over the control in RS-zop in all interim necrospsies as well as recovery period d56 these increases ranged between 7.5% on d7 to 19% on d28 (11% on d57*), no liver wt changes in S-zop.* Mean absolute and relative **thyroid wts** were increased (rel.wt 17% over control), wk4 in RS-zop only. Mean absolute and relative wt of **adrenals** (both or one adrenal), were significantly increased in RS-zop on all necropsies but comparable to control on recovery d57 (increases in rel.wt up to 29% d7 to 14% d28). Both drugs caused significant increase in mean relative **testes and epididymides wts** relative to control on days 7,14, and 28 (epididymides wt not affected on d28 by either drug). These increases ranged between 16 to 41% (up to 108% for epididymides on d7 in RS-zop and up to 93% for S-zop); weights were comparable to control during recovery. These changes corresponded to histopath findings.

Gross morphology: both drugs caused changes in epididymides and testes as early as wk1 necropsy; compared to nil changes in control. These effects included raised yellow areas, white areas and enlarged epididymides and enlarged testes. Changes in epididymides persisted till d56 recovery period in both drugs whereas enlarged and soft testes were observed only in RS-zop during recovery period.

Histopathology: **Adrenal** cortical hyperplasia was seen in 3/15 rats in S-zop on wk1; no findings in other groups. **Liver** wt changes correlated with *minimal* hepatocellular hypertrophy found in 2/15 wk1 RS-zop, 4/15 each on wks 2,4, and 8 (recovery period), with no incidence in control or S-zop groups. **Thyroid** changes in RS-zop included thyroid follicular hypertrophy and decreased colloid from wk1 through wk4 but no effect at end of recovery. The thyroid changes were seen in S-zop only on wk4.

Immunohistochemical staining in the **pituitary** was seen in drug and control groups at all times but the rating of "severe" was seen only in RS-zop on wks2&4 and S-zop wk2. "Moderate" rating was seen in

both drugs on wk4 (8/15 each drug vs. 3/15 control); no changes in pituitary at end of recovery in either drug. Minimal hypertrophy of the pars distalis of the pituitary was seen on wk4 in 3/15 RS-zop group vs. none in S-zop and control. Histopathology of the **epididymides** occurred in both drugs of the

28d oral gavage endocrine function study of S-zop & RS-zop in male rats/Sepracor#190-837A1 (Cont.)

majority of rats starting on wk1 and through recovery period they included sperm granuloma (mild to severe), interstitial edema, degenerative changes, cytoplasmic vacuolation, and subacute inflammation. Changes in **testes** seen in both drugs and included seminiferous dilatation, and degeneration observed throughout study and end of recovery in both drugs; none of these seen in control. **Prostate** changes observed in both drugs and included epithelial hyperplasia and decreased secretion from wk1 through end of dosing wk4 (only decreased secretion remained on recovery wk8 in RS-zop).
Spermatogenic parameters: drug related effects were observed at all 3 time periods with both drugs. Mean testicular and epididymal sperm count, production rate, and motility were significantly reduced in both drugs relative to control ($p<0.05$ or 0.01). This reduction was pronounced in some animals that evaluation was not possible due to insufficient number of sperms available. Incidence of abnormal sperms was increased with both drugs and consisted of normally shaped heads separated from flagellum and absent heads with normal flagellum. At recovery period, sperm motility remained reduced in both drugs compared to control ($p<0.01$), all other parameters were comparable to control.
Liver Enzymes: total protein was unaffected by either drug and P450 content was unaffected by S-zop and only mildly increased by RS-zop over the control value (1.3x control, $p<0.05$). Cytochrome B5 was not affected by S-zop and increased only 1.4x control in RS-zop ($p<0.05$). Mean thyroxine glucuronidation was slightly but significantly elevated in both drugs 1.3x and 1.6x control ($p<0.05$). A moderate and significant 4x increase was measured in 4-methylumbelliferone glucuronidation only in RS-zop, relative to control (table from sponsor).

Samples from Sponsor[a] - Male

TREATMENT	Protein yield (mg protein/g liver)	Cytochrome b_5 (nmol/mg protein)	Cytochrome P450 (nmol/mg protein)	4-Methylumbelliferone glucuronidation (nmol/mg protein/min)	Thyroxine glucuronidation (pmol/mg protein/min)
Vehicle Control	14.0±3.6	0.541±0.059	0.768±0.096	136±32	27.3±2.7
100 mg/kg/day esopiclone	16.2±2.1 (1.2)	0.581±0.075 (1.1)	0.815±0.130 (1.1)	223±50 (1.6)	35.6±2.3* (1.3)
200 mg/kg/day (RS)-zopiclone	14.4±4.2 (1.0)	0.743±0.131* (1.4)	0.979±0.182* (1.3)	530±99* (3.9)	43.5±4.8* (1.6)

* = $p<0.05$ [a] = Values are mean ± standard deviation of eight rats.
Values in parentheses indicate the fold increase over control or the fraction of the control (0 mg/kg/day vehicle control)

Summary and Conclusion:
Oral gavage daily administration of 100mg/kg S-zop and 200mg/kg RS-zop to male rats for 1, 2, and 4wks caused no drug related deaths and no effects on B.wt, wt gain, or food intake. Clinical signs were extension of the pharmacology and observed in both drugs. Both drugs affected some serum hormones, organ wts, liver enzymes, and induced histopathology. Sperm parameters were also affected by both drugs but more so by S-zop. There were NO drug related effects on serum estradiol, LH, or FSH at any time period. Mean serum TSH was significantly increased as early as 2wk of dosing by both drugs (2.6x and 1.6x for RS-zop and S-zop respectively, relative to control). Serum T4 was significantly reduced only

in RS-zop on wks1&2 but comparable to control by end of dosing wk4. Serum T3 was significantly increased 14% over the control only in S-zop and only wk1; no effect in RS-zop. Serum rT3 on the other hand, was significantly decreased 11&13% in S-zop and RS-zop respectively, but again, only on wk1. Only RS-zop caused a significant decrease in serum testosterone on wk1 (61% less than control) but both drugs caused 54-61% reduction relative to control at recovery wk8. Only RS-zop caused a significant

28d oral gavage endocrine function study of S-zop & RS-zop in male rats/Sepracor#190-837A1 (Cont.)

increase in absolute and relative weights of the liver, thyroid, and adrenals at 2 or all 3 time periods relative to control. Both drugs caused a significant increase in weights of epididymides and testes at all 3 time points but comparable to control at end of recovery. Gross exam of these 2 organs revealed them to be enlarged with raised yellow areas. Histopathology showed thyroid follicular cell hypertrophy and decreased colloid after 1wk and through wk4 of dosing but no effect at end of recovery period. Thyroid changes were seen in S-zop only after 4wks of dosing. Immunohistochemical staining of the pituitary was seen in both drug groups and control group at all times but the rating of "severe" was seen only in RS-zop on wks2&4 and S-zop wk2 and moderate rating was seen in both drugs on wk4; no changes in pituitary at end of recovery in either drug. Minimal hypertrophy of the pars distalis was seen on wk4 in 3/15 RS-zop group vs. none in S-zop and control. Minimal liver hypertrophy was found in RS-zop on wks2, 4, and 8 with no incidence in control or S-zop. RS-zop caused a mild induction of liver enzymes at 1.3-1.6x control with a moderate 4x increase in 4-methylumbelliferone glucuronidation. Liver enzyme induction was less impressive with S-zop without an effect on P450 content; neither drug affected total proteins. Both drugs exerted toxic effects on the epididymides in the majority of rats starting on wk1 and through recovery period such effects included sperm granuloma, interstitial edema, degenerative changes, cytoplasmic vacuolation, and subacute inflammation. In the testes, seminiferous dilatation and degeneration observed throughout study and end of recovery in both drugs; none of these seen in control. Prostate changes observed in both drugs and included epithelial hyperplasia and decreased secretion from wk1 through end of dosing wk4. Testicular and epididymal sperm count, production rate, and motility were significantly reduced in both drugs relative to control and percentage of abnormal sperms was increased with both drugs. At recovery period, sperm motility remained reduced in both drugs compared to control.

In conclusion, RS-zop at 200mg/kg/d administered via gavage had the following effects on the thyroid and liver (see summary table from sponsor):

- Increased serum TSH and decreased serum T4,
- Increased relative liver and thyroid wts,
- Increased thyroid UDP-GT and P450 content,
- Caused thyroid follicular cell hypertrophy and mild liver hypertrophy,
- Immunohistochemical staining in the pituitary for TSH was observed equally in control and drug group however, rating of severe and moderate were observed only in drug group.

These changes seem to be consistent with an indirect secondary effect of the drug on the thyroid tissue however, some of these changes occurred after 1 or 2wks but were not observed at end of study wk4.

S-zop at 100mg/kg/d also affected these 2 organs but not to the same extent as did RS-zop.
Both drugs adversely affected male reproductive parameters with S-zop more so than the RS:

- Significant decrease in serum testosterone only in wk1 and recovery wk8,
- Macroscopic changes in testes and epididymides,
- Increase in testes and epididymides relative wts,

- Histopathological changes included sperm granulomas and degeneration, obstruction, dilation and other inflammatory changes in epididymides,
- Tubule dilation in testes and decreased secretory function in the prostate,
- Decreased sperm motility, production rate, and count as well as increase in percent of abnormal malformed sperms.
- **Noted that neither drug affected serum estradiol, LH, or FSH levels.**

28d oral gavage endocrine function study of S-zop & RS-zop in male rats/Sepracor#190-837A1 (Cont.)

Note that organ wts in the table are absolute wts.

SUMMARY OF LIVER DATA		0 mg/kg/day	Esopiclone	(RS)-zopiclone
Mean Liver Weights (Grams)	Week 1	14.45	14.37	15.75**
	Week 2	14.12	14.71	16.79**
	Week 4	14.89	15.84	17.86**
	Week 8 (R)	16.51	16.77	18.08**
Hepatocellular Hypertrophy (% Incidence)	Week 1	0	0	13
	Week 2	0	0	27
	Week 4	0	0	27
	Week 8 (R)	0	0	29
Rat Liver Microsome: UDP-Glucuronosyltransferase Activity at Week 4				
Thyroxine (pmol/mg protein/min)		27.3	35.6*	43.5*
4-Methylumbelliferone (nmol/mg protein/min)		136	223	530*
SUMMARY OF THYROID DATA		0 mg/kg/day	Esopiclone	(RS)-zopiclone
Mean Thyroid/Parathyroid Weights (Grams)	Week 1	0.0224	0.0224	0.0213
	Week 2	0.0219	0.0238	0.0236
	Week 4	0.0226	0.0241	0.0274**
	Week 8 (R)	0.0216	0.0237	0.0233
Follicular Cell Hypertrophy (% Incidence)	Week 1	7	7	33
	Week 2	7	7	27
	Week 4	0	47	40
	Week 8 (R)	7	7	7
Decreased Colloid (% Incidence)	Week 1	7	7	33
	Week 2	14	7	27
	Week 4	0	47	40
	Week 8 (R)	7	14	14
Total T_4 (µg/dL)	Week 1	4.91	4.73	3.40**
	Week 2	4.99	5.22	3.81**
	Week 4	4.89	5.42	4.51
	Week 8 (R)	4.83	5.61*	5.14
TSH (ng/mL)	Week 1	11.37	11.65	13.49
	Week 2	9.72	16.56*	26.05**
	Week 4	11.83	13.27	23.19**
	Week 8 (R)	9.99	8.82	8.39

* = statistically significant at 0.05 ** = statistically significant at 0.01, R= Recovery

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9. A 3month dietary endocrine function study of RS-zopiclone in rats/Sepr.# 190-870F/ -312109, 2002

Conducting laboratory and location:

Date of study initiation: Dec 14th 2001; final report date Nov14th 2002

GLP compliance: yes: FDA, Japanese & OECD

QA reports: yes (x)

Study Objectives: this is a mechanistic study to evaluate the proposed theory that RS-zop causes endocrine disruption in male rat (thyroid hormones) and female rat (reproductive hormones).

Methods:

RS-zopiclone (lot ZP0010102; purity was administered daily for 92days at **100 and 200mg/kg/d** via the **diet** to male and female Sprague Dawley rats purchased from . The control group received basal diet on a comparable regimen. There were 90 rats/sex/group and additional 15/sex/group for TK. Rats were approximately 11wk old at start of dosing and weighed 297-358g males and 197-249g females. Animals were killed at 3 time points for hormone analyses: the first 30/sex/group were killed after 2wks of dosing, the next 30/sex/group were killed after 4wk (28d) of dosing and the remaining 30/sex/group were killed at end of 3month (d84 for males and 88-92 for females). Blood was collected from the TK animals on days 28&84 at several time points from 3/sex/group/time point from the retro orbital sinus. B.wt and food intake was determined for these groups. Blood samples for both periods were collected from the same 12 rats with each rat sampled up to 2x in 24hr. After blood collection, TK rats were discarded without further examination or assessment; samples were shipped to , for analysis. Liver samples from all *male* rats at end of study were evaluated for enzyme induction (total P450 content, UDP-GT), by

Parameters Studied:

- Clinical signs and mortality checked 2x daily, detailed exam, food intake and, B.wt checked weekly,
- Serum hormones days 14, 28, and 84 (males)/days 88-92 (females),
- Vaginal smears were examined daily for each female to determine stage of estrus from days –7 through 14 for necropsy females on d14, from d8-28 for necropsy females d28, and from d61 through 84 for necropsy between days 88-92. Mean cycle length was calculated. Because of results on d14 and 28 (see result section), the sponsor attempted to kill each female at final necropsy at the estrus phase of her cycle. Therefore, necropsy was reassigned from d84 to 88-92. Criteria for day of necropsy for each female: those that had a normal cycle were assigned a necropsy day based on their mean estrus cycle length so that they were killed on expected day of estrus, those that had an abnormal cycle were assigned necropsy day based on evaluation of cycle data by beginning with the last evidence of cycling (i.e. occurrence of estrus, metestrus, or proestrus), and counting in increments of 5 days (normal cycle).
- Liver samples from all *male* rats at end of study were evaluated for enzyme induction (total P450 content, UDP-glucuronyltransferase), by
- The following organs were isolated and weighed at end of study from all animals: liver, adrenals, ovaries/oviducts, pituitary, uterus, and thyroid/parathyroid.

- All microscopic exams were done by Dr ___ a consultant pathologist. These exams were done to determine the stage of estrus at euthanasia.
  H&E stained slides were examined microscopically for the following tissues/organs: ovaries, uterus, vagina, and mammary tissue from all females at end of study. The inguinal pair of mammary gland were examined as follows: 3 sections of each pair were examined with 1 section cut through the nipple (when it can be identified), and remaining 2 sections cut lateral to the nipple but on opposite sides.
- TK parameters were determined for S-zop, R-zop, and S- & R- desmethyl zop using ___ with quantitation limit of ___ Sampling period was between 0-24hr

Results:

Clinical Signs and Mortality: no drug related signs or deaths in either drug group. It is noted however, that in previous oral *gavage* study in the rat (Sep# 190-837F), clinical signs reflective of extended pharmacology of the drug were observed at 100mg/kg/d. The sponsor suggested absence of any clinical signs in the current study may be related to diet vs. gavage administration however, signs have been observed in animals dosed via diet in previous studies.

B.wt: (done only on tox groups), mean wt in both sexes at both dose groups was reduced compared to the control animals throughout the study generally reaching statistical significance. Mean wt gain was significantly and dose dependently reduced in both sexes throughout the study relative to wt gain in controls. The decrease in *wt. gain* was as follows at 100 and 200mg/kg/d:

Wk 0-4 m: 20 and 40% f: 20* and 53% (* not statistically significant)
Wk 0-8 m: 14 and 33% f: 23 and 45%
Wk 0-12 m: 15 and 32% f: 23 and 43%
Wk 0-13 not measured in males; f: 28 and 44%
Values are % less than corresponding mean value in control groups. $p<0.05$ or 0.01

Food Intake: no drug related effects. Mean intake during some weeks was reduced and reached statistical significance however, the change was small and not dose dependent.

Organ wts: mean absolute and relative liver and thyroid wts in both sexes and both doses were significantly and dose dependently increased over the corresponding control wts. At end of dosing, mean relative liver wt increased 16&25% in males and 31&57% in females over the control wts in 100and 200mg/kg/d respectively. Mean absolute and relative liver wt in males on d28 was significantly increased only in 200mg/kg/d relative to the control (12&14%). Mean relative thyroid wt increased in males 20&40% and in females 13&25% over the control wts in 100&200mg/kg/d respectively. Mean relative wt of the left adrenal in males and right adrenal in females were significantly increased in both doses relative to the control wts (8-17% more than control). No changes in uterus or ovary wts except for a 16&24% increase ($p<0.01$) in 100mg/kg/d over the control but no change in 200mg/kg/d group therefore, this effect was not considered drug related.

Macroscopy: at end of study, 7-8 males out of 30 males dosed 200mg/kg/d had soft or small testes and 2/30 males dosed 200mg/kg/d had small epididymides. In females, 15/30 dosed 200mg/kg/d had clear fluid in uterus (6 and 8 of 30 each in control and 100mg/kg/d RS-zopiclone respectively). None of these findings in either sex corresponded to histopathological changes nevertheless, they were considered to be drug related.

Histopathology: the following is the pathology report written by consultant pathologist Dr ___ .. As indicated earlier, vaginal cytology was done up to d63-84 and was not done from d85-92 (terminal necropsy). Cycle stage was therefore, estimated for these final days (see method section above). The expert pathologist concluded the following: **mammary gland changes develop**

over time in response to sustained increase in estradiol level. Such prolonged elevation in estradiol can trigger earlier onset of reproductive senescence and over 1-2 year, mammary tumors can develop *early* and occurs at higher incidence than the control. The persistent increase in E2 sensitizes the gland to progesterone that consequently leads to lobular/acinar proliferation. These hormonal changes in E2 and progesterone will cause prolonged cycle lengths. The following table from the sponsor lists length of estrus cycle for each rat during days 63-84 (as stated above, data for terminal necropsy were not done). Since normal cycle length is 4-5 days in the rat, this reviewer used cycle lengths ≥6 days to represent abnormally long cycles. Based on this, abnormally long cycles were seen in 3/30 rats in control, 9/30 in 100mg/kg/d, and 3/30 rats in 200mg/kg/d groups (table to follow). These rats spent the length of the cycle mostly in diestrus. The mean cycle length was significantly increased in 100mg/kg/d at 6.2 days compared to 4.9 day in control. The variability was so large at s.d. of 3.31 days which was contributed to 2 rats out of the 9 with cycle lengths of 19 and 16 days (rats #82686 & 82706 respectively). Also, noted absence of dose response with respect to prolonged cycle length as seen from the comparable mean cycle length at 200mg/kg/d RS-zop and the mean cycle length in the control.

DIPLOMATE
AMERICAN COLLEGE
OF
VETERINARY PATHOLOGISTS

TO:

RE: PROJECT NO. 312109Z. A 3-MONTH DIETARY STUDY OF (RS)-ZOPICLONE IN RATS.

Thirty females each from GROUP 1 - 0 mg/kg/day, GROUP 2 - 100 mg/kg/day, and GROUP 3 - 200 mg/kg/day were killed at study termination and blood collected for hormonal analysis. Prior to termination estrous cycle data was collected by daily vaginal lavage for 22 to 23 days. The rats were bled at presumed peak estradiol level by correlating the vaginal cytology with the afternoon of proestrus. After a 2 to 4 day lag period the rats were necropsied and protocol specified tissues collected.

The ovary, uterus, vagina, and mammary gland were evaluated separately to determine either normal or abnormal estrous cycles by histomorphological determinants.

Review of the individual estrous cycle data derived from vaginal cytology revealed many rats from the controls, 100, and 200 mg/kg/day groups with abnormally long estrous cycles. The histomorphological evaluation of the ovary, uterus, and vagina also indicated many rats with increased diestrus intervals. Those with abnormal estrous cycles included 14/30 - Group 1, 19/30 - Group 2, and 22/30 - Group 3 rats. The irregular cycles were consistent with an increased diestrus interval which is the first estrous cycle irregularity associated with initial reproductive senescence. The tissue changes in the ovaries, uterus, and vagina indicated a slightly increased time exposure to corpora luteal progesterone (which causes vaginal mucification) and then a slowly rising E_2 level but increased exposure under the E_2 curve prior to the next E_2 spike and ovulation. Nearly all rats of all dose groups were cycling but irregularly at the time of necropsy.

Cycle irregularities may have been partially influenced by treatment and appeared dose responsive. The treatment influence on the estrous cycle was subtle but tended to exaggerate irregular cycles associated with the onset of reproductive senescence.

The mammary gland is an important indicator of repeated exaggerated exposure to E_2, P_3, or PRL. Estradiol sensitizes the gland to P_3 stimulation which results in acinar/lobular development. Under normal estrus cycle conditions the mammary gland remains static. If lengthened cycles with increased P_3 exposure occur, varying degrees of lobular development occurs. If E_2 levels are sustained, as in most early abnormal cycles associated with reproductive senescence, then there may be associated mammary gland alterations over time.

There was no treatment associated advancement of abnormal mammary gland changes in test rats when compared to controls at this time and age interval. However, if treatment initiates the earlier onset of changes related to reproductive senescence as suggested by the number of abnormal estrous cycles in treated rats when compared to controls then over the long term (1 - 2 years) the onset time and number of mammary neoplasms could be affected.

TABLE 32 (DAY 62 TO 84 EVALUATION) A 3-MONTH DIETARY STUDY OF (RS)-ZOPICLONE IN RATS

INDIVIDUAL ESTROUS CYCLE DATA

FEMALES FROM GROUP 1: 0 MG/KG/DAY

FEMALE NUMBER	DETERMINATION DAY: 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23	INDIVIDUAL MEAN LENGTH OF ESTROUS CYCLE (DAYS)
82668	P E D D D P E D D D P E D D D P E D D D P E	5.0
82670	D D P D D P E M D D P D P E D D D P E D D D	4.0
82671	M D D D P E D D D P E D D D P M D D D D M D	4.5
82672	D D P E E D E D E E D D D D D D D D D D D D	2.5
82678	D P E D D D E E D D D D D D D D D D D D D D	5.0
82679	M D D D P M D D D P E D D D P M D D D P D D	5.0
82684	D D D P M D D D E M D D D E E D D P M D D D	4.7
82688	M D D D E D D D D P E D D D E E D D D M D D	5.5
82696	E D D D E E D D D E M D D D D D D D E D D	6.3
82697	D D P E D D P E E D D P M D D D E D D P E D	4.3
82707	D D D D D D D E D D D D E M D D D P E D D D	5.5
82708	M D D D E M D D D D D E D D D D D D D D D D	7.0
82715	E D D D E M D D D P E D D D E E D D D M D D	5.0
82717	P E D D D E E D D D P E D D D E E D D D P E	5.0
82718	D D D P D D D D D E D D D D E E D D D P E D D	5.3
82725	P M D D E D E D D D E M D D D D P D D D P E D	4.2
82732	D D E E D D D E E D D D E D D P E M D D P E M	4.5
82736	D D D P M D D D E E D D D E E D D D P M D D D	5.0
82738	D D D D D D D D D D D D D D D M D D E D D D E	A
82739	P E D D D E D D P E D D D E D D D E M D P E D	4.0
82742	M D D D D P E D D D D D D D D D D D D D D D D	A
82743	D P E D D D P E D D D D M D D D P E D D D E M	6.3
82744	D D D P E D E D P D D D D E E D D D P E D D D	3.8
82745	D D P E D D D P E D D D P E D D D E E D D D P	5.0
82747	E D D D P E D D D P M D D D P E M D D M D D D	5.0
82751	D D P E D D D E D D D E E D D D P E D D D E E	4.7
82752	D P M D D D E M D D D P E D D P E M D D E E D	5.0
82756	D D D P M D D D E D D D D E D D D D E M D D D	5.0
82761	E D D D E E D D D E E D D D E E D D D P E D D	5.0
82765	D D D E E D D D E M D D D P E D D D E E D D D	5.0

ESTROUS STAGE CODE: E = ESTRUS, D = DIESTRUS, M = METESTRUS, P = PROESTRUS

A = UNABLE TO DETERMINE LENGTH OF ESTROUS CYCLE

MEAN	4.9
S.D.	0.85
N	28

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TABLE 32 (DAY 63 TO 84 EVALUATION)
A 3-MONTH DIETARY STUDY OF (RS)-ZOPICLONE IN RATS
INDIVIDUAL ESTROUS CYCLE DATA

FEMALES FROM GROUP 2: 100 MG/KG/DAY

FEMALE NUMBER	DETERMINATION DAY: 1 2 3 4 5 6 7 8 9 0 1 2 3 4 5 6 7 8 9 0 1 2 3	INDIVIDUAL MEAN LENGTH OF ESTROUS CYCLE (DAYS)
82657	D D P M D D D P E D D D D D D D D D D D D	6.0
82673	E D D D P E D D D P M D D D E D D D D P D D	4.8
82674	D P M D D D D D D D D D D D E D D P E D D	9.0
82675	D E M D D D E E D D D D D D D D D D E D D	9.0
82681	M D D D E M D D D D E D D D E E D D P M D D	4.7
82682	D D P M D D D P E D D D D P E D D D E M D D D	5.0
82686	D P M D D D D D D D D D D D D D D D P E M	19.0
82690	D M D D D P M D D D P M D D D P M D D D P M D D	4.3
82693	E M D D P E E D D P E M D P E E D D P E D D	4.8
82694	D D D P D D D D D D D D D E D D P E M D D D	7.0
82695	D D M D D D E M D D P E M D D D E E D D D P	5.5
82699	M D D D P E D D D P E D D D E E D D D P M D	4.7
82702	D E M D D D E M D D P E E D D D P M D D M E	5.0
82704	D E M D D D P M D D D E M D D D P E D D D P	5.1
82706	D D P D D D D D D D D D D D D D D E P M D D	16.0
82714	D D D E M D D D E M D D P E D D P M M D D D D	4.3
82716	E D D P E E D D P E E D D D E E D D P E D D D	4.8
82720	P E D D D D E M D D E E E D D P E D D D E E D	5.0
82722	D P E D D D P M D D P E D D D D D D D D E D D	6.0
82723	D E M D D D E M D D D D E D D P D D D D D E E	4.7
82727	D D E M D D D P E D D D P E D D D E M D D P E	5.0
82728	D P D D D D D D D P E D D D E M D D P M D D E	5.7
82730	D P E M D D D D P E M D D D D D P M D D D D M	7.0
82735	D D E M D D D E E D D E M D D P E E D D D E M	4.8
82740	E M D D D E M D D D E E M D D P M D D D P E D	5.3
82749	P M D D E D D D E D D P E D D D D E E D D D E	4.5
82755	E D D D E E D D P E D D P E M D D P M D D D E	4.3
82758	M D D D P M D D D E E D D P E D D D E M D D D	4.7
82759	D D D D D D D P D D P E D D D D M D D D E M D	6.5
82764	P E M D D D P M D D D D D D D D D D D D M D D	5.0

MEAN	6.2
S.D.	3.31
N	30

FEMALES FROM GROUP 3: 200 MG/KG/DAY

FEMALE NUMBER	DETERMINATION DAY: 1 2 3 4 5 6 7 8 9 0 1 2 3 4 5 6 7 8 9 0 1 2 3	INDIVIDUAL MEAN LENGTH OF ESTROUS CYCLE (DAYS)
82660	D D D P M D D D E M D D P E M D D D P E M D	5.3
82665	M D D P E D D D P M D D D E E D D D M D D D	5.0
82669	D D D D D D D D D D D D D P E M D D P E M D	5.0
82676	D E M D D D D P E D D D P E D D D E M D D D	5.3
82677	D D P E D D D D E D D P E E D D D E M D D D	4.7
82683	D P E M D D P M E M E E E E E E E M M E E	5.0
82689	D D D E M D D D E M D D D E E D D D E M D D	5.0
82692	D D D E M D D P E M D D D E M D D D E M D D	5.0
82698	P E M D D D E E M D D P E E D D P E M D D D	5.3
82700	E D D P E E D D D P E D D D E E D D D E M D	4.8
82701	D E M D D P E M D D P E M D D P E E D D P E	5.3
82703	D D D M D D D E M D D P E E D D D P E D D D	5.5
82705	D P M D D D P M D D D E E D D D E D D D P M	4.3
82711	E M D D D D E E D D D D E E D D D P E M D D	6.0
82713	E D D D E M D D D E E D D E M M M D D D E E	4.3
82719	P E D D D P E D D D E E D D D E E D D D D E M	5.0
82721	D D P E M D D D E M D D D E E D D D P E M D D	5.3
82724	E D D D E D D P E E D D D E D D D P E M D D E	4.5
82726	D D E E D D D E M D D D D D D D D E E M D D D	7.5
82729	D D D E M D D P E M D D D E D D D E M D D D P	4.7
82731	D P E D D D D D D D D D D D D D D D D D D D D	A
82733	E D D D P E D D D P E D D D E E D D D P E M D	5.0
82734	E D D D P E D D D D D E D D D D D D M D D P E	5.5
82737	D D P E D D P E D D D E M D D D E M D D D E M	4.5
72741	E D D D P E D D D E E D D D P E E D D P M D D	4.8
82750	D E D D D D D P M D D P E D D P E D D P E M D	4.8
82753	D D E E D D D D D P E E D D D D E D D D E E D	6.0
82754	E D D D P M D D D P E M D D D E M D D D P M D	5.0
82757	E E E D D D P E D D D D P E D D D E E D D D P	5.3
82763	D P M M P E M M E E M M E E E E E E M M M E E	5.3

MEAN 5.2 S.D. 0.60 N 29

3mo dietary endocrine function study of RS-zopiclone in rats/Sepr.# 190-870F (Cont.)

Summary of estrus cycle data days 61-84:

Group	**0**	**100**	**200mg/kg/d**
Estrus cycle length (days)			
Mean±s.d.	4.9±0.85	**6.2±3.31**	5.2±0.60
N	28	30	29

When estradiol levels for the *individual rats with long cycles* were compared, there was no correlation between length of estrus cycle and estradiol levels in these animals with cycle length ≥6 days. In addition, "mean" estradiol levels were comparable between drug groups and control. The sponsor however, determined estradiol and other hormone levels at each estrus stage for days 14, 28, and 84. *[In all these analyses it should be kept in mind the large inter-/intra-animal variability reflected by the marked s.d.]*. Statistics should not be performed on these data since n was as low as 1 rat (tables from sponsor). Estradiol level at estrus seemed elevated in 200mg/kg/d on d14 (62±30pg/ml vs. 46±18pg/ml cont), d28 55±12 vs. 39±11pg/ml cont at estrus, but inconclusive on d84 because only 1 rat was in estrus and in fact mean value was "significantly" reduced in both dose groups relative to the control during proestrus (n at this stage ranged between 20-28)(table from sponsor).

PROJECT NO. WIL-312109F

TABLE 10 — A 3-MONTH DIETARY STUDY OF (RS)-ZOPICLONE IN RATS
SUMMARY OF SERUM HORMONE VALUES [EVALUATED BY ESTROUS STAGE]

ANALYSIS	GROUP:	-----F E M A L E ----- 0 MG/KG/DAY	100 MG/KG/DAY	200 MG/KG/DAY
ESTRADIOL (pg/ml)				
DAY 14				
ESTRUS	MEAN	45.9	43.7	62.2
	S.D.	18.09	16.28	29.73
	S.E.	5.72	6.65	11.24
	N	10	6	7
DIESTRUS	MEAN	79.1	68.5	70.6
	S.D.	37.56	28.35	36.86
	S.E.	9.70	8.55	9.52
	N	15	11	15
METESTRUS	MEAN	38.3	28.8	43.1
	S.D.	11.44	8.57	18.77
	S.E.	5.72	3.24	7.09
	N	4	7	7
PROESTRUS	MEAN	60.8	97.4	297.0
	S.D	0.00	92.96	0.00
	S.E	0.00	37.95	0.00
	N	1	6	1
DAY 28				
ESTRUS	MEAN	38.5	66.3	54.9
	S.D.	11.45	26.45	12.42
	S.E.	6.61	18.70	4.69
	N	3	2	7
DIESTRUS	MEAN	64.7	62.4	61.3
	S.D.	30.40	24.47	23.51
	S.E.	6.48	5.10	5.54
	N	22	23	18
METESTRUS	MEAN	26.5	53.1	47.6
	S.D.	1.56	20.52	18.40
	S.E.	0.90	11.85	10.62
	N	3	3	3
PROESTRUS	MEAN	81.6	77.8	45.1
	S.D.	61.33	31.40	2.05
	S.E.	43.35	22.20	1.45
	N	2	2	2

3mo dietary endocrine function study of RS-zopiclone in rats/Sepr.# 190-870F (Cont.)

Other Serum Hormones in females: in addition to estradiol, FSH, LH, prolactin and progesterone were measured and similar to estradiol, inter- and intra-animal variability was large (table below for **Day 84**; values are mean±s.d. in ng/ml; n = 30 unless indicated otherwise; data from table 9 page 97 of 1172 of sponsor report):

	0	100	200mg/kg/d
FSH[$]	1.1±2.2	1.4±2.3	3.2±3.7*
LH	12±19	9±12	27±30**
Prolactin	185±140	134±96	214±177
Progesterone	105±87	141±111	201±155*
Estradiol/FSH ratio[$]	697±568	510±413	398±499
Prolactin/estradiol ratio	2.6±2.3	2.2±2	3±2.4

$ mean includes estimated FSH values * p=0.05 from control ** p=0.01 from control

Because no drug effect was observed on serum hormones when mean values were calculated, data were re-analyzed in relationship to **estrus stage.** [it should be noted that statistics are meaningless for small "n" in this case as small as n=1], therefore, this reviewer did not consider them in data evaluation but are reported. Mean **FSH** values seem to show an increasing trend: on d14 during *estrus,* on d28 in *metestrus* and, on d84 at *proestrus* compared to control mean values (table below; values are mean±s.d; ( ) number of rats)(from report table# 10 page 106 of 1176):

	0	100	200mg/kg/d
Day 14 Estrus	3.6±4.4 (10)	6±4 (5)	7±4 (7)
Day 28 Metestrus	1.4±0.5 (3)	4.6±2.5 (3)	4±2.3 (3)
Day 84 Proestrus	1.6±2.5 (21)	2±2.6 (20)	3±4 (28)

Note that none were significantly different from control and the **marked s.d.**

Mean **LH** value at 200mg/kg/d was **increased** on d84 at proestrus (p=0.05) but no effect at 100mg/kg/d; conclusion could not be made at the other time points. Mean **prolactin** levels were **reduced** on d14 and d28 reaching statistical significance on d14 at both dose groups relative to control during *diestrus* (p=0.01); however, mean levels *increased* dose dependently but not significantly, on d84 at *proestrus* (table below). Mean **progesterone** exhibited an increasing trend during *estrus* d14 and d28 and, at *proestrus* on d84 (values in table are mean±s.d.; number of rats is in ( )). **Again, note the large s.d.**

	0	100	200mg/kg/d
LH Day 84 Proestrus	15.6±21.5 (21)	11±13.6 (20)	29±30* (28)
Prolactin Day 14 Diestrus	173±98 (15)	85±31** (11)	84±33** (15)
Prolactin Day 84 Proestrus	151±115 (21)	138±99 (20)	224±179 (28)
Progesterone Day 14 Estrus	145±111 (10)	193±220 (6)	297±200 (7)
Progesterone Day 28 Estrus	164±60 (3)	172±223 (2)	205±166 (7)
Progesterone Day 84 Proestrus	111±98 (21)	168±116 (20)	193±138 (28)

* significantly different from control at p=0.05
** significantly different from control at p=0.001

3mo dietary endocrine function study of RS-zopiclone in rats/Sepr.# 190-870F (Cont.)

Serum Hormones in males: mean TSH, T4, T3, and rT3 (reverse T3) were determined in males on study days 14, 28, and 84. Mean TSH was increased dose dependently and significantly at the 3 time points. Such increase in TSH corresponded to the expected decline in serum T4 levels that were significantly and dose dependently reduced at the 3 times points (table from sponsor). No drug effect on T3 but rT3 was slightly though significantly reduced at both doses on d14 and only in 200mg/kg/d on d84. These thyroid hormonal changes correlated with increase in thyroid wts of rats in drug groups compared to those in control but no corresponding histopathology. RS-zopiclone-induced changes in TSH and T4 were also observed in previous rat study (~ , 312087). The sponsor indicated that these changes together with increases in liver enzymes (see below), and liver wts, support the theory that thyroid hormone changes are secondary to liver enzyme induction.

A 3-MONTH DIETARY STUDY OF (RS)-ZOPICLONE IN RATS

ANALYSIS	GROUP	0 MG/KG/DAY	100 MG/KG/DAY	200 MG/KG/DAY
		----- M A L E -----		
TOTAL T3 (ng/dL)				
DAY 14	MEAN	129.8	130.5	130.8
	S.D.	12.05	14.75	15.94
	N	30	30	30
DAY 28	MEAN	120.6	123.2	120.3
	S.D.	18.04	20.89	18.08
	N	30	30	30
DAY 84	MEAN	127.5	123.0	121.7
	S.D.	14 26	13.76	15.93
	N	30	30	30
TOTAL T4 (uG/dL)				
DAY 14	MEAN	2.9	2.6**	1.9**
	S.D.	0.49	0.50	0.35
	N	30	30	30
DAY 28	MEAN	3.5	3.2	2.4**
	S.D.	0.57	0.53	0.51
	N	30	30	30
DAY 84	MEAN	2.2	1.7**	1.4**
	S.D.	0.67	0.56	0.56
	N	30	30	30

MODIFIED STATISTICS USED. * INDICATES PARAMETRIC ANALYSIS AND ** = Significantly different from the control group at 0.01

A 3-MONTH DIETARY STUDY OF (RS)-ZOPICLONE IN RATS

ANALYSIS	GROUP:	0 MG/KG/DAY	100 MG/KG/DAY	200 MG/KG/DAY
		----- M A L E -----		
TSH (ng/ml)				
DAY 14	MEAN	14.8	17.5	23.5**
	S.D.	7.85	6.73	9.31
	N	30	30	30
DAY 28	MEAN	14.4	21.0++	27.0++
	S.D.	5.21	7.82	12.71
	N	30	30	30
DAY 84	MEAN	13.6	15.5	21.7++
	S.D.	5.74	5.57	9.84
	N	30	30	30
REVERSE T3 (ng/ml)				
DAY 14	MEAN	0.05	0.05*	0.04**
	S.D.	0.010	0.009	0.011
	N	30	30	30
DAY 28	MEAN	0.06	0.07	0.05
	S.D.	0.013	0.082	0.012
	N	30	30	30
DAY 84	MEAN	0.04	0.03	0.03*
	S.D.	0.011	0.010	0.008
	N	30	30	30

MODIFIED STATISTICS USED. * INDICATES PARAMETRIC ANALYSIS AND + INDICATES NON-PARAMETRIC ANALYSIS.
* = Significantly different from the control group at 0.05
**, ++ = Significantly different from the control group at 0.01

3mo dietary endocrine function study of RS-zopiclone in rats/Sepr.# 190-870F (Cont.)

Liver Enzymes: RS-zop caused a significant but small (except the 3x in 4-MUF), increase in P450 content, thyroxine glucuronidation, and 4-MUF glucuronidation*. Table below presents fold increases over the control (n=30 male rats per group):

Dose (mg/kg/d)	P450 Content nmol/mg protein	4-MUF glucuronidation nmol/mg protein/min	T4 glucuronidation pmol/mg protein/min
100	1.1	1.8	1.5
200	1.3	3.1	1.8

* 4-methylumbeliferone has been shown to be catalyzed by UGT1A6/7 in rat, and UGT1A6 and UGT2B8 in human liver microsomes.

For comparative purposes, protein content of P450 in historic controls at XenoTech labs in male SD rats treated with Aroclor or beta-naphthoflavone caused 5.0 and 3.0 fold increase respectively, in P450 content, phenobarb caused 3x increase. Similarly, glucuronidation of 4-MUF using historical male SD rats showed 4.3 and 5.6x increases respectively and phenobarb 2x in 4-MUF glucuronidation. Lastly, for T4 glucuronidation, historical data for Arochlor and beta-naphthoflavone showed 7.3 and 12x increases respectively, and phenobarb 1.5x increase in T4 glucuronidation using male SD. It is clear that quantitative comparison is inappropriate based on differences in animals, duration of treatment, differences in exposure, and other factors however, qualitatively, it may be stated that RS-zop induces UDP-glucuronosyltransferase activity slightly better than phenobarb but less than beta-naphthoflavone.

TK: rats were exposed to all 4 cpds measured. The highest plasma levels were seen for S-zop and R-desmethyl zop in both sexes. Females had 2-3 fold higher S-zop and R-zop levels than males however, plasma levels of R-desmethyl and S-desmethyl were comparable between the sexes (the former was slightly higher in males than females (table from sponsor). Note that **t_{max} was relatively long for all cpds ranging between 12-20hr** (one at 8hr), some of this may reflect route of administration (dietary vs. gavage).

(RS)-Zopiclone

TOXICOKINETIC RESULTS

Gender/Analyte	Zopiclone Results* AUC_{0-24h} (ng•h/mL) Day 28	AUC_{0-24h} (ng•h/mL) Day 84	C_{max} (ng/mL) Day 28	C_{max} (ng/mL) Day 84	t_{max} (h)** Day 28	t_{max} (h)** Day 84
100 mg/kg/day						
Males						
(S)-(+)-zopiclone	12997	13250			20	12
(R)-(-)-zopiclone	2739	3813			12	12
(S)-(+)-desmethyl zopiclone	7398	7752			20	16
(R)-(-)-desmethyl zopiclone	12717	14380			20	16
Females						
(S)-(+)-zopiclone	38728	37862			20	20
(R)-(-)-zopiclone	10070	12182			16	12
(S)-(+)-desmethyl zopiclone	7643	7284			20	20
(R)-(-)-desmethyl zopiclone	11995	12207			20	20
200 mg/kg/day						
Males						
(S)-(+)-zopiclone	23765	20053			16	12
(R)-(-)-zopiclone	6029	6003			8	12
(S)-(+)-desmethyl zopiclone	16276	13516			16	12
(R)-(-)-desmethyl zopiclone	27709	26399			16	12
Females						
(S)-(-)-zopiclone	48183	51803			20	0
(R)-(-)-zopiclone	13301	14089			12	0
(S)-(+)-desmethyl zopiclone	12556	13593			20	0
(R)-(-)-desmethyl zopiclone	19528	21926			20	0

* The values are presented as the indicated analyte.

** Time relative to 6:30 am on the day indicated.

Summary and Conclusion:

This study was conducted in the rat to investigate the proposed effects of RS-zop on endocrine hormones in males (thyroid), and females (reproductive). Such hormonal disruption could explain the thyroid and mammary tumors observed in life-time bioassays. RS-zop was fed daily to male and female SD rats for 13wk at 100 or 200mg/kg/d; additional rats were used to assess TK. There were no deaths or clinical signs or effect on food intake. Mean B.wt and wt gain were significantly reduced in both sexes at both doses, overall mean wt gain reduction relative to control in males ranged between 15 and 32% and in females 28 and 44% at 100 & 200mg/kg/d respectively. Drug related effects on organ wts included a significant and dose related increase in mean liver and thyroid wts in both sexes with mean relative liver wt increases of 16&25% in males and 31&34% in females over the control in both doses respectively. The corresponding increases in mean relative thyroid wts were 20&40% in males and 13&25% in females in both doses respectively. None of the organ wt changes corresponded to histopathology in either sex. The only other change in organ wts were a 16&24% increase ($p<0.01$) in mean uterus and ovary wts in the 100mg/kg/d females but not in the 200mg/kg/d group; again without corresponding histopathology; this

change was not considered by the sponsor to be drug related due to absence of dose response. However, drug effects on reproduction were seen in rat and rabbit Segment I and II studies therefore, a drug effect in this case can not be ruled out (see section on reproduction and development).
3mo dietary endocrine function study of RS-zopiclone in rats/Sepr.# 190-870F (Cont.)

Drug related macroscopical findings included soft or small testes in 7-8 males out of 30 dosed 200mg/kg/d and small epididymides in 2/30 males, half of the females in 200mg/kg/d group had fluid in their uteri. Though none of these macroscopical findings correlated with histopathology, they were considered drug related in both sexes since drug effects on fertility were observed in rats (see section on reproduction and development). A consultant pathologist carried out the histomorphological exam of the uterus, ovaries, vagina, and mammary glands for all females. In general, a normal cycle length in SD rats is 4-5 days long, cycles equal to or longer than 6 days were observed in 3/30 controls, 9/30 rats in 100mg/kg/d and in 3/30 rats dosed 200mg/kg/d. Clearly this prolonged cycle length was *NOT* dose dependent based on number of rats and mean cycle lengths of 4.9, 6.2, and 5.2 days in control, 100, and 200mg/kg/d respectively. The length of the cycle was spent mostly in diestrus i.e. diestrus interval was increased. It should be noted that intra-animal variability was very large with s.d. of 3.3 in the 100mg/kg/d (where the mean reached statistical significance from the control). This variability was contributed to 2 out of the 9 rats that had cycle lengths $\geq$6 days, (19 and 16 days). Mean serum estradiol levels were not increased in drug groups compared to the control values and this hormone level in rats with prolonged cycle was not elevated as the hypothesis proposes. However, when the sponsor analyzed the hormone levels as function of estrus phase (estimated for end of study), estradiol level at estrus was elevated in 200mg/kg/d on d14 (62$\pm$30pg/ml vs. 46$\pm$18pg/ml cont), d28 55$\pm$12 vs. 39$\pm$11pg/ml cont at estrus, but inconclusive on d84 because only 1 rat was in estrus and in fact mean value was "significantly" reduced and not increased in both dose groups relative to the control during proestrus. Similarly, serum LH, FSH, prolactin, and progesterone did not differ from the corresponding controls (very large intra-animal variability), however, when data analyzed as a function of estrus phases mean FSH was increased at the 3 time points and during various stages of estrus relative to control level in both doses. Mean LH was increased at 200mg/kg/d on day 84 at proestrus without any effect at 100mg/kg/d and inconclusive at other time points. This is not in support of the early senescence hypothesis where levels of LH should decrease leading to persistent elevation or plateau of estradiol. Mean prolactin levels were *reduced* on days 14&28 with statistical significance on d14 at diestrus, but mean levels *increased* dose dependently but not significantly, on d84 at *proestrus*. Mean progesterone exhibited an increasing trend during *estrus* d14 and d28 and, at *proestrus* on d84. These hormonal changes in females did not correspond to histopathological changes in mammary glands or other reproductive organs except for mucification of the vagina indicative of changes in progesterone. In males, mean TSH was increased dose dependently and significantly at the 3 time points. Such increase in TSH corresponded to the expected decline in serum T4 levels that were significantly and dose dependently reduced at the 3 times points. No drug effect on T3 but rT3 was slightly though significantly reduced at both doses on d14 and only in 200mg/kg/d on d84. These thyroid hormonal changes correlated with increase in thyroid wts of rats in drug groups compared to those in control but no corresponding histopathology. RS-zop-induced changes in TSH and T4 were also observed in previous rat study. In addition, mean liver wt and liver P450 content and glucuronidation were increased in males. Taken together, these changes support the hypothesis that RS-zop effect on the thyroid is peripheral through affecting thyroid hormone metabolism in the liver.

3mo dietary endocrine function study of RS-zopiclone in rats/Sepr.# 190-870F (Cont.)

The following can be concluded from this mechanistic study:

- RS-zopiclone-induced thyroid changes in long-term studies in male rats may be secondary to induction of liver enzymes. However, liver enzyme induction occurred to the same extent in both sexes yet, thyroid tumors were observed only in male rats. Therefore, such conclusion does not fully explain the tumor findings and other factors may be involved.
- None of the changes in liver or thyroid weights, enzyme induction, or hormone changes correlated with any histopathology in these organs in either sex.
- RS-zopiclone seems to have an overall effect on female hormones specifically, FSH where mean levels were increased during the 3 time points and at various stages of estrus. However, mean LH was increased during d84 at 200mg/kg/d but unaffected at any other time point nor at 100mg/kg/d. A pattern opposite to what expected for early onset of reproductive senescence (i.e. $\downarrow$ in LH level). Trend for prolactin was inconsistent and progesterone seemed to increase over the 3 time points. However, changes in estradiol are inconclusive because only 1 rat could be analyzed at end of study.
- The sponsor concluded that RS-zopiclone increased estrus cycle length specifically diestrus. A clear and accurate conclusion can not be made because of the large noise in the data (intra-animal variability), and the effect on mean cycle length was not dose-dependent (mean values: 4.9, 6.2, and 5.2 days in control, 100, and 200mg/kg/d). Moreover, 2 rats in 100mg/kg/d group had cycle lenghts of 16 and 19 days that contributed to the large mean as well as large s.d. in this group. Also, the number of rats with cycle lengths $\geq$6 days was 3, 9, and 3 out 30 rats in each control, 100, and 200mg/kg/d i.e. no dose dependency. Also a correlation between estradiol level and those rats with prolonged estrus cycle length could not be established.
- None of the hormonal changes in females correlated with histopathology in mammary, vagina, uterus, or ovaries except for increase in mucification of the vagina indicative of elevated progesterone level.
- RS-zopiclone caused a significant reduction in mean body weight and body weight gain in both sexes without an effect on food intake.
- RS-zopiclone did not affect survival and did not cause any clinical signs up to 200mg/kg/d in this study.
- Rats were adequately exposed to all 4 cpds, S- and R-zopiclone and, S- and R-desmethyl zopiclone. It is noted that t_{max} was relatively long for all cpds ranging between 12-20hr (one at 8hr), some of this may reflect route of administration, dietary vs. gavage.

10. 3 month dietary endocrine function study of RS-zop in the rat/Sepracor study# 190-870A1

GLP: Yes QA: Yes
Report date: March 2003
Conducting lab:

This is an addendum to the previously conducted and reviewed study. After the final report was issued, the sponsor decided to analyze samples of omental and peri-renal if needed, adipose tissue from females at end of 3month study, for CYP19 (aromatase enzyme) activity. This enzyme converts androgens to estrogens therefore, a potential source of elevated estradiol. At end of 3month study, 2g of omental adipose tissue from 30 female rats were removed, frozen, appropriately stored and sent to for analysis. The aromatase protein activity was analyzed by Western immunoblotting to determine the levels of immunoreactive aromatase with mouse anti-human P450 aromatase antibody from

Results and Conclusion:

RS-zop at 100 or 200mg/kg/d did not increase the levels of omental adipose tissue immunoreactive aromatase enzyme activity relative to that in the vehicle control. Mean values were 2.61, 2.49, and 1.28pmol/mg protein in vehicle, 100 and 200mg/kg RS-zop respectively. In fact, the level at the high dose was 50% suppressed compared to the control vehicle or low dose RS-zop (unclear biological significance for this suppression). Additional control CYP19 cDNA was used and stained consistently across the Western immunoblott. Failure of RS-zop to increase aromatase activity in female adipose tissue excludes the potential mechanism where by elevated estradiol results from increased conversion of testosterone and such elevated and persistent E2 levels could have caused the mammary tumors

11. An endocrine function study of (RS)-zopiclone following single-day and three-day oral exposure in ovariectomized female rats/Sepr.# 190-879A1 ~ -312122, 2002
Conducting laboratory and location: —
Date of study initiation: final report date Dec 19th 2002
GLP compliance: yes: FDA & OECD
QA reports: yes (x)

Methods:
Sexually mature SD females were purchased from — Study design was as follows (table from sponsor):

Group Number	Test Article	Dosage Level (mg/kg/day)	Dosage Concentration (mg/mL)	Dosage Volume (mL/kg)	Number of Females 1-Day Treatment	Number of Females 3-Day Treatment
1	Vehicle Control	0	0	5	10	12
2	Positive Control	30	6	5	10	10
3	Racemic Zopiclone	100	20	5	10	12
4	Racemic Zopiclone	250	50	5	10	12

Two regiments were tested: (1) the vehicle, drug, and positive control were dosed once (single dose), on the 3rd day after overiectomy, the (2) vehicle and drug were dosed once daily for 3 days starting 1d after overiectomy and silastic capsule implantation. To validate the procedure, a preliminary study was done where a positive control, Na pentobarb, and the drug were tested. LH surge was seen in the vehicle control females whereas, this response was blocked by pentobarb and 250mg/kg RS-zop following a single dose on the 3rd day after ovariectomy (data not provided).

Rats for the 1d study were 14-15wk old and weighed 198-237g and those for the 3d study were 13wks old and weighed 211-259g on first day of dosing [noted rats weighed more at younger age than older rats?]. Estradiol benzoate was prepared in *sesame oil* and the implants prepared at — Estradiol benzoate at 4mg/ml in sesame oil filled the 12-14mm silastic tubing. Ovariectomy was done under anesthesia and the femoral vein was catheterized for multiple blood collection. The silastic tubing containing estradiol was implanted s.c. in the right flank region. This implant maintained an estradiol level comparable to that seen in intact proestrus females. The implants also produced a daily afternoon LH surge in the females to allow a synchronized cohort of rats where the timing and amplitude of hormones are evaluated. Following overiectomy and implants, rats were allowed to recover and health was monitored daily. The drug was administered via oral gavage and the positive control, pentobarb, was administered via i.p. injection.

Observations and times:
Rats were observed 2x daily for mortality and morbidity, B.wt was recorded weekly starting on 1st day of vaginal lavage (including day of overiectomy), and gross exam done; no tissues were preserved for histopathology. Blood was collected at 4 time points from the femoral vein of all rats 3d after overiectomy. Blood was collected from the trunk of all rats after decapitation at 2100hr. From all samples the following hormones were analyzed: **LH and prolactin** (RIA), and **estradiol** (chemiluminescent).

Endocrine function study of RS-zop following single- & 3d oral exposure in ovariectomized female rats/Sepracor# 190-879A1 (Cont.)

Results:

Single DAY

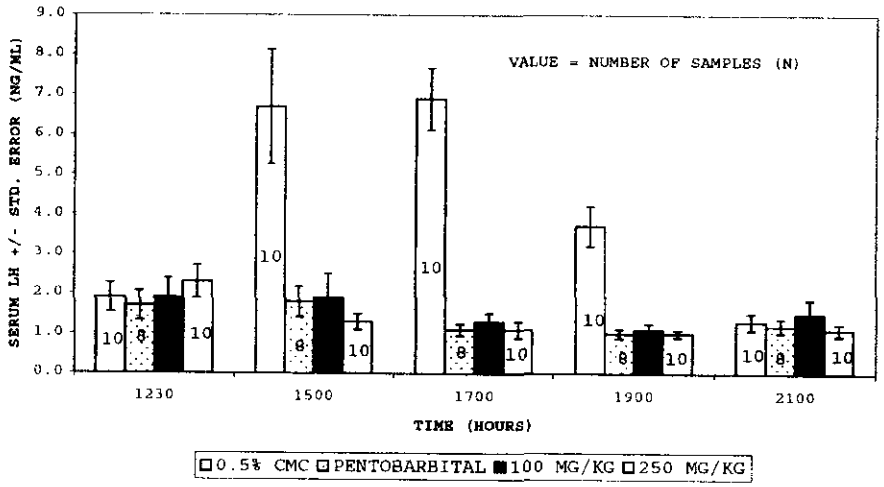
Estradiol levels were consistent in all groups as expected. Mean LH surge in the vehicle control peaked at 3&5pm and progressively declined thereafter. Mean LH levels were significantly reduced in positive control and both doses of RS-zop compared to vehicle control values at all 3 time points measured (3, 5, and 7pm)(figures and tables from sponsor). Mean prolactin levels were 95 & 96% less than the vehicle control at 3pm (or 25 & 30 fold less than vehicle), 77 & 83% less at 5pm and, 60 & 87% less at 7pm in the 2 dose groups respectively; no effect of positive control values at any time point.

TABLE 7 (SINGLE-DAY REGIMEN)

PROJECT NO. 121229 ENDOCRINE STUDY OF (RS)-ZOPICLONE IN OVARIECTOMIZED FEMALE RATS PAGE 2
SPONSOR:SEPRACOR INC. SUMMARY OF SERUM HORMONE LEVELS DAY 3

ANALYSIS	GROUP	FEMALE 0.5% CMC	FEMALE PENTOBARBITAL	FEMALE 100 MG/KG	FEMALE 250 MG/KG
1230 LH (ng/ml)	MEAN	1.9-A	1.7-A	1.9-A	2.3-A
	S.D.	1.16	1.05	1.51	1.42
	N	10	8	10	10
1500 LH (ng/ml)	MEAN	6.7-A	1.8-A	1.9-A##	1.3-A##
	S.D.	4.54	1.07	1.90	0.62
	N	10	8	10	10
1700 LH (ng/ml)	MEAN	6.9	1.1-A	1.3-A##	1.1-A##
	S.D.	2.43	0.41	0.67	0.65
	N	10	8	10	10
1900 LH (ng/ml)	MEAN	3.7-A	1.0-A	1.1-A##	1.0-A##
	S.D.	1.57	0.12	0.49	0.30
	N	10	8	10	10
2100 LH (ng/ml)	MEAN	1.3-A	1.2-A	1.5-A	1.1-A
	S.D.	0.67	0.50	1.11	0.48
	N	10	8	10	10

ng/ml = NANOGRAMS/MILLILITER
A SIGNIFICANT INTERACTION EFFECT OF TREATMENT VS. TIME WAS OBSERVED (p<0.001); REFER TO APPENDIX G FOR FURTHER DETAILS
= SIGNIFICANTLY DIFFERENT FROM THE CONTROL GROUP AT p<0.01 USING DUNNETT'S TEST
A = FOR VALUES THAT WERE BELOW INSTRUMENT RANGE, THE LOWEST STANDARD VALUE OF 0.8 NG/ML WAS USED TO CALCULATE GROUP MEAN
NOTE:STATISTICS ONLY PERFORMED FOR THE (RS)-ZOPICLONE GROUPS



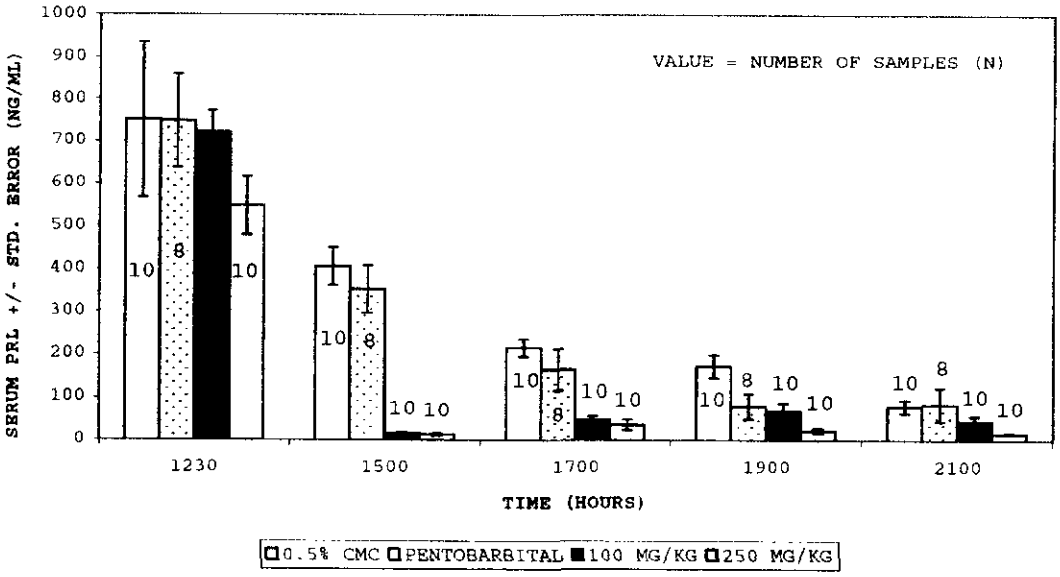
Endocrine function study of RS-zop following single- & 3d oral exposure in ovariectomized female rats/Sepracor# 190-879A1 (Cont.)

TABLE 7 (SINGLE-DAY REGIMEN)

PROJECT NO 12122B — ENDOCRINE STUDY OF (RS)-ZOPICLONE IN OVARIECTOMIZED FEMALE RATS — PAGE 3
SPONSOR:SEPRACOR INC. — SUMMARY OF SERUM HORMONE LEVELS — DAY 1

ANALYSIS GROUP:	0.5% CMC	-----F E M A L E ----- PENTOBARBITAL	100 MG/KG	250 MG/KG
1230 PRL (ng/ml)				
MEAN	751.4	748.1	721.8	548.9
S.D.	582.31	312.73	163.97	216.87
N	10	8	10	10
1500 PRL (ng/ml)				
MEAN	406.4	352.7	16.5##	13.4##
S.D.	139.67	158.42	9.26	9.47
N	10	8	10	10
1700 PRL (ng/ml)				
MEAN	216.1	165.4	48.8##	36.6##
S.D.	64.55	136.65	25.10	38.70
N	10	8	10	10
1900 PRL (ng/ml)				
MEAN	172.4	79.5	68.8##	23.1##
S.D.	83.74	84.35	60.35	18.24
N	10	8	10	10
2100 PRL (ng/ml)				
MEAN	79.4	84.3	45.1	16.6##
S.D.	48.97	112.30	37.72	5.90
N	10	8	10	10

ng/ml = NANOGRAMS/MILLILITER
A SIGNIFICANT INTERACTION EFFECT OF TREATMENT VS. TIME WAS OBSERVED (p<0.001). REFER TO APPENDIX G FOR FURTHER DETAILS
= SIGNIFICANTLY DIFFERENT FROM THE CONTROL GROUP AT p<0.01 USING DUNNETT'S TEST
NOTE:STATISTICS ONLY PERFORMED FOR THE (RS)-ZOPICLONE GROUPS



3 DAY

Similar to the 1d, estradiol levels were consistent in all groups as expected. Mean LH surge was significantly but not dose dependently reduced in both drug groups relative to vehicle value but only at 1 time point, 3pm. The extent of this reduction was of much less magnitude than that observed following the single dose above. LH values were also reduced though not significantly at 5pm but comparable to control

vehicle at other time points (table and figure from sponsor). Similar to the single dose regimen, LH surge in control vehicle occurred at 1500 and 1700hrs. The sponsor analyzed the individual data for Endocrine function study of RS-zop following single- & 3d oral exposure in ovariectomized female rats/Sepracor# 190-879A1 (Cont.)

females that had LH surge and those that did not, with the conclusion that 4 females in both drug groups had LH surges similar to or greater than the vehicle control but the remaining rats had suppressed LH surge at 1500 and 1700hr. The sponsor suggested that these data (minus the 4 females) demonstrated in the majority of rats, LH blockade (figure from sponsor).

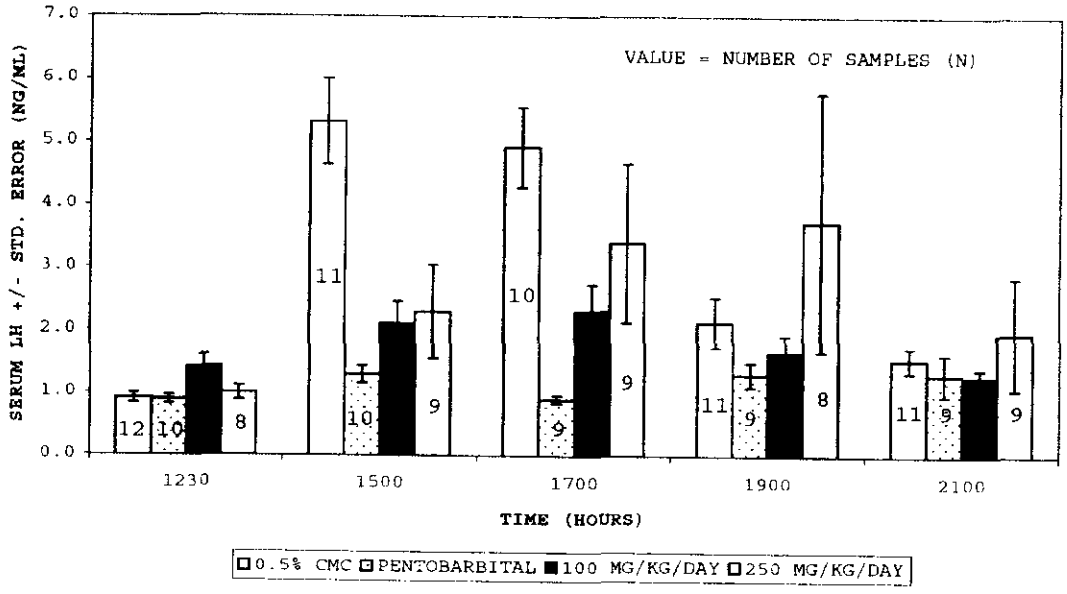
TABLE 3 (THREE-DAY REGIMEN)

PROJECT NO. 12122D — ENDOCRINE STUDY OF (RS)-ZOPICLONE IN OVARIECTOMIZED FEMALE RATS — PAGE 2

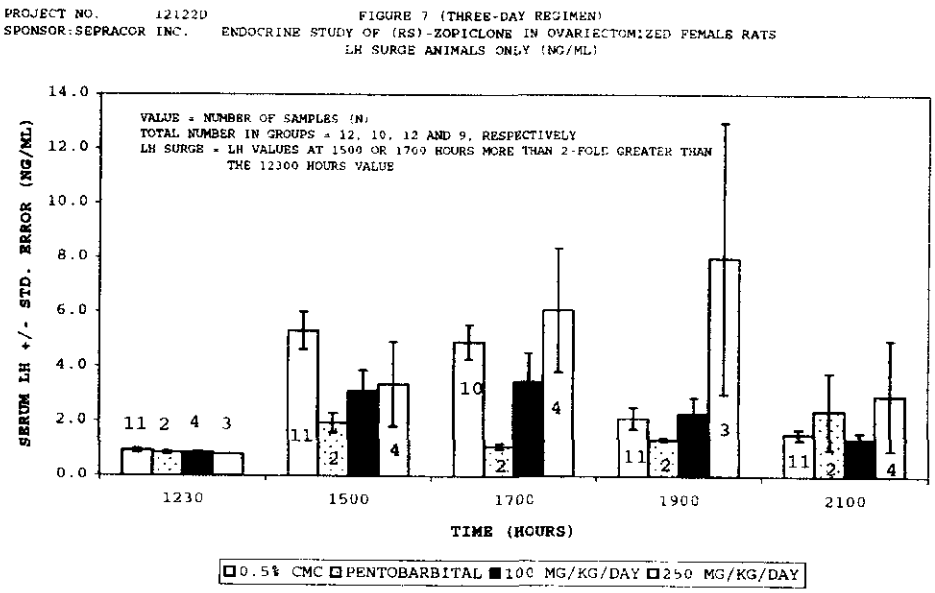
SPONSOR:SE... INC. — SUMMARY OF SERUM HORMONE LEVELS — DAY 3

ANALYSIS GROUP:	0.5% CMC	FEMALE PENTOBARBITAL	100 MG/KG/DAY	250 MG/KG/DAY
1230 LH (ng/ml)				
MEAN	0.9-A	0.9-A	1.4-A#	1.6-A
S.D	0.27	0.22	0.68	0.33
N	12	10	12	8
1500 LH (ng/ml)				
MEAN	5.3	1.3-A	2.1##	2.3 A##
S.D	2.27	0.44	1.21	2.24
N	11	10	12	9
1700 LH (ng/ml)				
MEAN	4.9	0.9-A	2.3#	3.4-A
S.D	1.99	0.19	1.56	3.80
N	10	9	12	9
1900 LH (ng/ml)				
MEAN	2.1-A	1.3-A	1.7-A	3.7 A
S.D.	1.33	0.58	0.94	5.82
N	11	9	12	8
2100 LH (ng/ml)				
MEAN	1.5-A	1.3-A	1.3-A	2.0-A
S.D.	0.66	0.98	0.43	2.67
N	11	9	12	9

ng/ml = NANOGRAMS/MILLILITER
A SIGNIFICANT INTERACTION EFFECT OF TREATMENT VS. TIME WAS OBSERVED ($p<0.001$), REFER TO APPENDIX G FOR FURTHER DETAILS
= SIGNIFICANTLY DIFFERENT FROM THE CONTROL GROUP AT $p<0.01$ USING DUNNETT'S TEST
# = SIGNIFICANTLY DIFFERENT FROM THE CONTROL GROUP AT $p<0.05$ USING DUNNETT'S TEST
A = FOR VALUES THAT WERE BELOW INSTRUMENT RANGE, THE LOWEST STANDARD VALUE OF 0.8 NG/ML WAS USED TO CALCULATE GROUP MEAN
NOTE:STATISTICS PERFORMED ONLY FOR THE (RS)-ZOPICLONE GROUPS

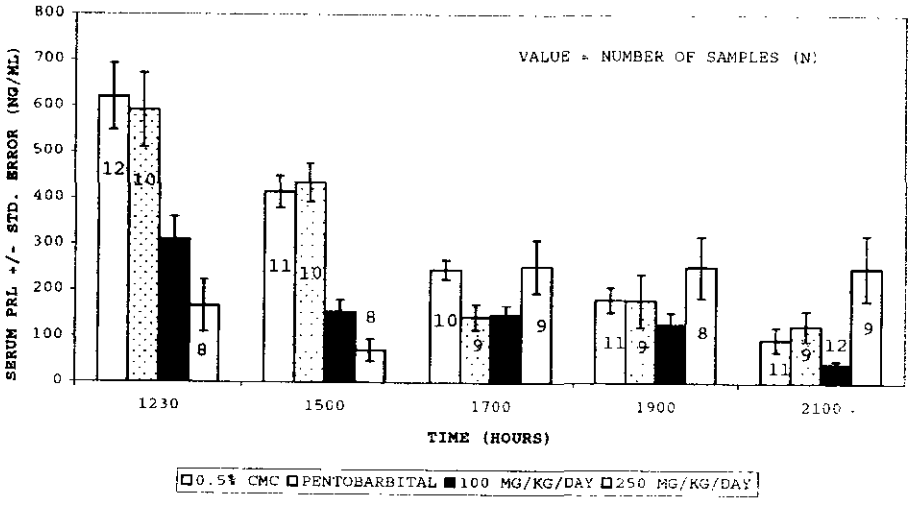


Endocrine function study of RS-zop following single- & 3d oral exposure in ovariectomized female rats/Sepracor# 190-879A1 (Cont.)



There were no drug related effects on B.wt, no deaths in the 100mg/kg dose however, 3f dosed 250mg/kg were killed in moribund after 2-3doses* of RS-zop in the 3d regimen. Clinical signs in these females included lethargy, prostration, paleness, cold to touch, shallow respiration, lacrimation, and drooping eyelids.

* statements made in the report including those on page 31 of 295 indicated that these females were killed in extremis "prior to dose administration 2 or 3days after surgery." This gave the impression that these animals were not dosed however, on page 38 of 295 3rd paragraph, it was stated that these animals were moribund and killed in extremis after "1 or 2 doses". This created confusion and was misleading.
Mean prolactin level was dose dependently and significantly reduced at 12:30 and 3pm but comparable to vehicle control at the other time points and even statistically *increased* in HD at 9pm. This reduction in prolactin occurred at an earlier time point than that following single administration of the drug and did not last as long. Pentobarb had no effect.



Endocrine function study of RS-zop following single- & 3d oral exposure in ovariectomized female rats/Sepracor# 190-879A1 (Cont.)

TABLE 8 (THREE-DAY REGIMEN)
PROJECT NO 112122D ENDOCRINE STUDY OF (RS)-ZOPICLONE IN OVARIECTOMIZED FEMALE RATS PAGE 3
SPONSOR:SEPRACOR INC. SUMMARY OF SERUM HORMONE LEVELS DAY 3

ANALYSIS	GROUP:	0.5% CMC	-----F E M A L E ----- PENTOBARBITAL	100 MG/KG/DAY	250 MG/KG/DAY
1230 PRL (ng/ml)					
	MEAN	619.6	590.6	399.9##	166.7##
	S.D.	248.87	256.17	172.03	160.13
	N	12	10	12	8
1500 PRL (ng/ml)					
	MEAN	413.8	433.8	153.7##	71.0##
	S.D.	114.67	131.44	90.44	72.53
	N	11	10	12	9
1700 PRL (ng/ml)					
	MEAN	245.7	142.5	147.4	253.1
	S.D.	65.01	82.15	72.47	173.43
	N	10	9	12	9
1900 PRL (ng/ml)					
	MEAN	180.7	178.8	128.1	252.1
	S.D.	88.94	171.55	85.12	188.14
	N	11	9	12	8
2100 PRL (ng/ml)					
	MEAN	94.7	124.7	41.6	250.4#
	S.D.	89.23	99.52	28.00	213.48
	N	11	9	12	9

ng/ml - NANOGRAMS/MILLILITER
A SIGNIFICANT INTERACTION EFFECT OF TREATMENT VS. TIME WAS OBSERVED (p<0.001); REFER TO APPENDIX G FOR FURTHER DETAILS
- SIGNIFICANTLY DIFFERENT FROM THE CONTROL GROUP AT p<0.01 USING DUNNETT'S TEST
- SIGNIFICANTLY DIFFERENT FROM THE CONTROL GROUP AT p<0.05 USING DUNNETT'S TEST
NOTE-STATISTICS PERFORMED ONLY FOR THE (RS)-ZOPICLONE GROUPS

Summary and Conclusion:

Following single oral administration of RS-zop to ovariectomized female rats, mean LH surge was blocked significantly at 3 time points (3, 7, and 9pm), relative to vehicle control. However, such block was of less magnitude and observed only after 1 time point following 3 daily dosing in overiectomized rats. Prolactin levels were blocked significantly at 3 time points (3, 7, and 9pm), following single administration but, such decrease noted earlier at 12:30 and 3pm after 3 daily dosing and did not last as was observed after single administration of RS-zop. Some of these inconsistencies in hormone responses were contributed to a subset of females, the sponsor re-analyzed the data for females that had an LH surge and those that did not. Doing so, showed that the majority of rats did block LH surge. In general, this study following single RS-zop oral administration showed blockade of LH surge and decrease in prolactin levels but this profile did not persist, for whatever reason, when the drug was administered daily for 3days. There were 3 females in 250mg/kg in the 3d design that were killed in moribund following 1 or 2 doses of RS-zop. No deaths in either dose in the single dose design or in the 100mg/kg of the 3d design. Clinical signs observed in the females included lethargy, prostration, cold to touch, shallow respiration, and drooping eyes.

12. An endocrine function study of RS-zop, S-zop, zolpidem, and zalplon following single and/or 3day oral exposure in ovariectomized rats/Sepracor study# 190-884f

GLP: Yes but signatures not provided*
QA: No*
RS-zop lot# Not Reported (NR) S-zop lot# NR
Study initiation date: Jan 2003
Conducting lab: —

* This is an interim report of the study, data analyses and statistics are still ongoing. The report has not yet been QA by —.
Objectives: to investigate the proposed mechanism of RS-zop and S-zop early onset of reproductive senescence in the rat and to see if it is a class effect. Zaleplon and zolpidem, 2 known GABA agonists were used to test the latter assumption; pentobarb was tested as the positive control.

Methods:
SD — ovariectomized female rats with normal 4-5day estrus cycles were used. A femoral vein catheter with access port was implanted and a silastic capsule with 4mg/ml estradiol benzoate in sesame oil was implanted in the right flank region. This maintained a blood estradiol level comparable to normal proestrus female. The implant also provided a daily afternoon LH surge and prolactin so that a synchronized cohort of rats was obtained in which timing and amplitude of each hormone could be evaluated. There were 2 parts for the study, a single day and a 3 day regimens. S-zop was administered by oral gavage at **4, 16, and 50mg/kg for the single** day and **2, 4, 8, and 16mg/kg/d for the 3d** at 10ml/kg. There were 10 and 12 rats for the 1 and 3d studies respectively, and 2 comparable control groups that received the vehicle control 0.5% CMC. The single day regimen, 10f per group dosed the drug once after the 3rd day of ovariectomy and for the 3d regimen, 12f per group were dosed daily for 3d one day after ovariectomy. Two positive controls, one for each regimen were also used, 10 and 12f were injected i.p. with pentobarb 30 and 20mg/kg respectively. Zolpidem at 80 and 200mg/kg and zaleplon at 20 and 50mg/kg were administered to female rats using the single day regimen only. RS-zop was also studied in a group of ovariectomized females at **100mg/kg** administered at 10ml/kg by oral gavage following the single day regimen. All doses were administered at 1330hr ±10min.

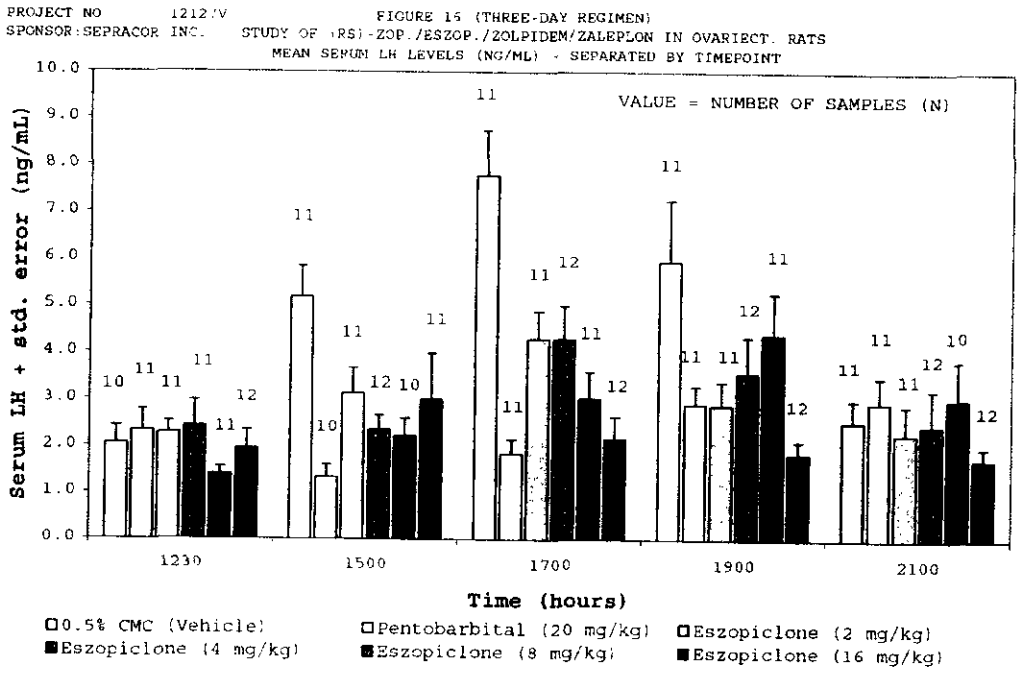
Results (see figures and table from sponsor):
S-zop as well as RS-zop caused a significant decrease/attenuation in LH amplitude and decrease in serum prolactin so did zaleplon and zolpidem 2 GABA agonists, indicating that this response of neuroendocrine disruption at high doses may be a class effect. At S-zop of 4mg/kg single dose no drug effect was observed therefore, this dose was considered the NOEL. In the single day regimen,
S-zop at 50mg/kg and RS-zop at 100mg/kg caused a comparable reduction in LH surge and prolactin at 1500, 1700, and 1900hr. At 16mg/kg S-zop, decline in LH amplitude was also comparable to that of the 50mg/kg S-zop and 100mg/kg RS-zop however, decline in prolactin was only evident at the 1500hr. In the 3d regimen, S-zop caused a decrease in maximum amplitude of LH surge and a dose dependent shift in time of day at which the LH peaked (table below from sponsor). The decrease in mean amplitude of peak LH by S-zop was comparable at 2,4, and 8mg/kg but greater reduction observed at 16mg/kg which was comparable to that observed at this dose following single administration. The shift in time to peak for LH surge observed in the 3d regimen had no effect on serum prolactin up to 8mg/kg S-zop dose. Serum prolactin levels were does-dependently reduced following S-zop at the peak of prolactin level i.e. 1500hr; so did the positive control pentobarb at this time; all relative to vehicle control. Also noted, the number of

rats that surged in the 3d was as follows: 10/11, 8/11, 7/12, 10/11 and 6/12 in control, 2,4,8,and 16mg/kg S-zop respectively, and 6/11 in pentobarb (table below). The lower number of animals surging and lower amplitude of the surge indicate reduction in serum LH levels therefore, supporting the early reproductive senescence mechanism of mammary gland tumors.

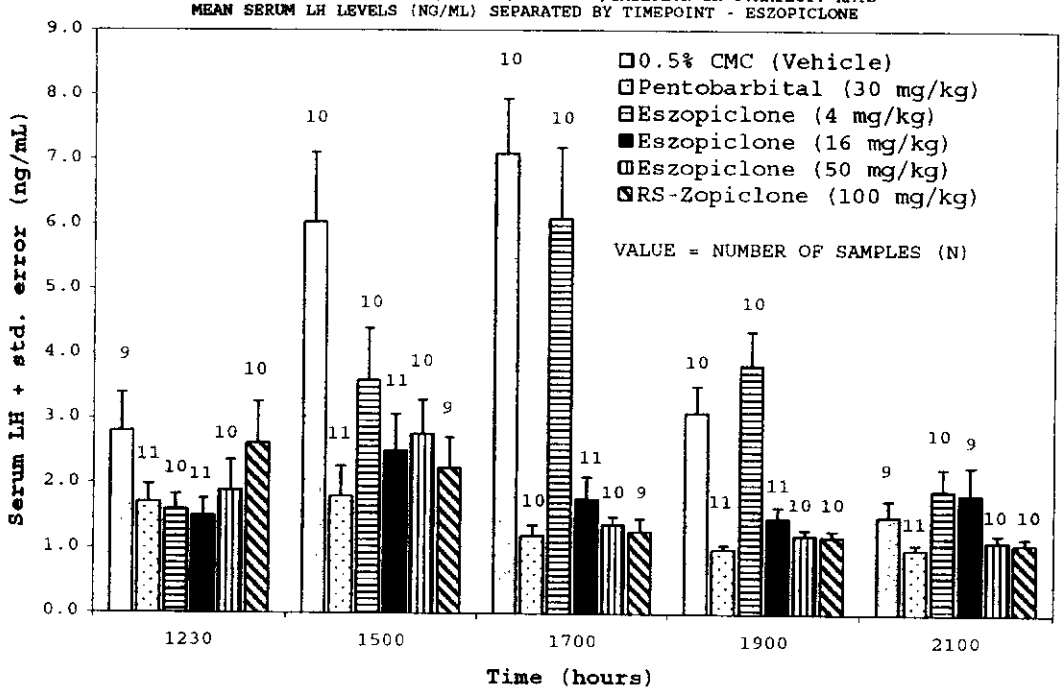
Effect of Eszopiclone on Serum Luteinizing Hormone Levels Following Three-Day Exposure in Ovariectomized Female Rats

	Vehicle Control	20 mg/kg Pentobarbital	2 mg/kg Eszopiclone	4 mg/kg Eszopiclone	8 mg/kg Eszopiclone	16 mg/kg Eszopiclone
Number of Animals that Surged/Total Number of Animals	10/11 (91%)	6/11 (55%)	8/11 (73%)	7/12 (58%)	10/11 (91%)	6/12 (50%)
Mean Amplitude of Peak LH Among Animals that Surged (ng/mL ± S.E.M.)	9.36 ± 0.90	3.87 ± 0.78**	5.81 ± 0.60*	6.76 ± 0.89	5.45 ± 0.83**	5.00 ± 1.37**
Mean Amplitude of Peak LH for all Animals (ng/mL ± S.E.M.)	8.87 ± 0.95	3.89 ± 0.46**	5.22 ± 0.53**	5.63 ± 0.78*	5.19 ± 0.79**	3.56 ± 0.82**
Number of Animals that Surged with LH T_{max} at Each Timepoint						
1500	2	0	2	0	2	2
1700	6	0	4	4	2	0
1900	2	3	1	1	4	1
2100	0	3	1	2	2	3

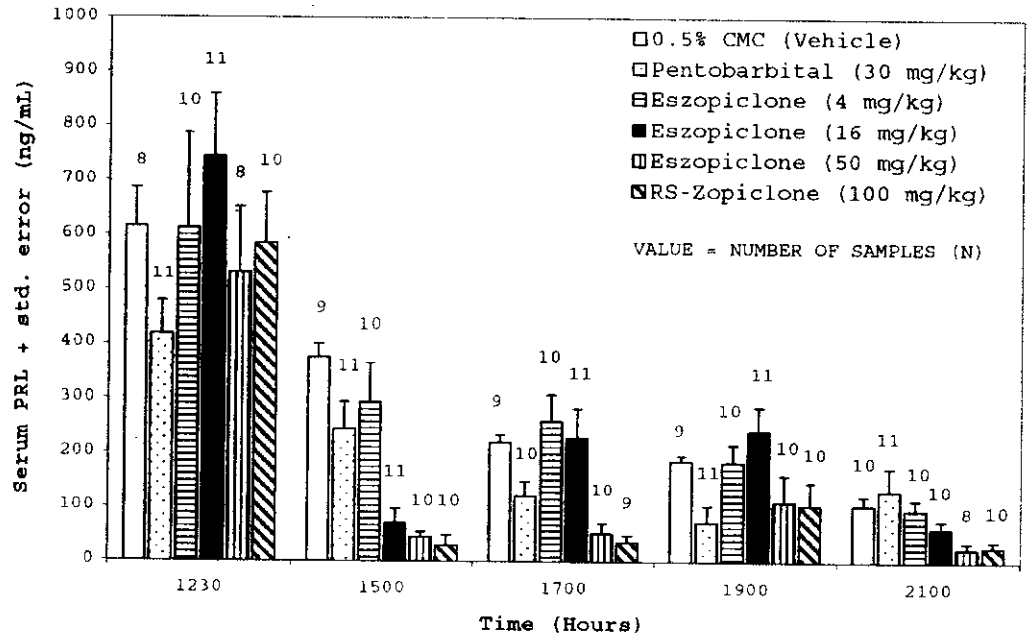
*Significantly lower than vehicle control ($p<0.05$).
**Significantly lower than vehicle control ($p<0.01$).



PROJECT NO. 312127C FIGURE 3 (SINGLE-DAY REGIMEN)
SPONSOR:SEPRACOR INC. STUDY OF (RS)-ZOP./ESZOP./ZOLPIDEM/ZALEPLON IN OVARIECT. RATS
MEAN SERUM LH LEVELS (NG/ML) SEPARATED BY TIMEPOINT - ESZOPICLONE

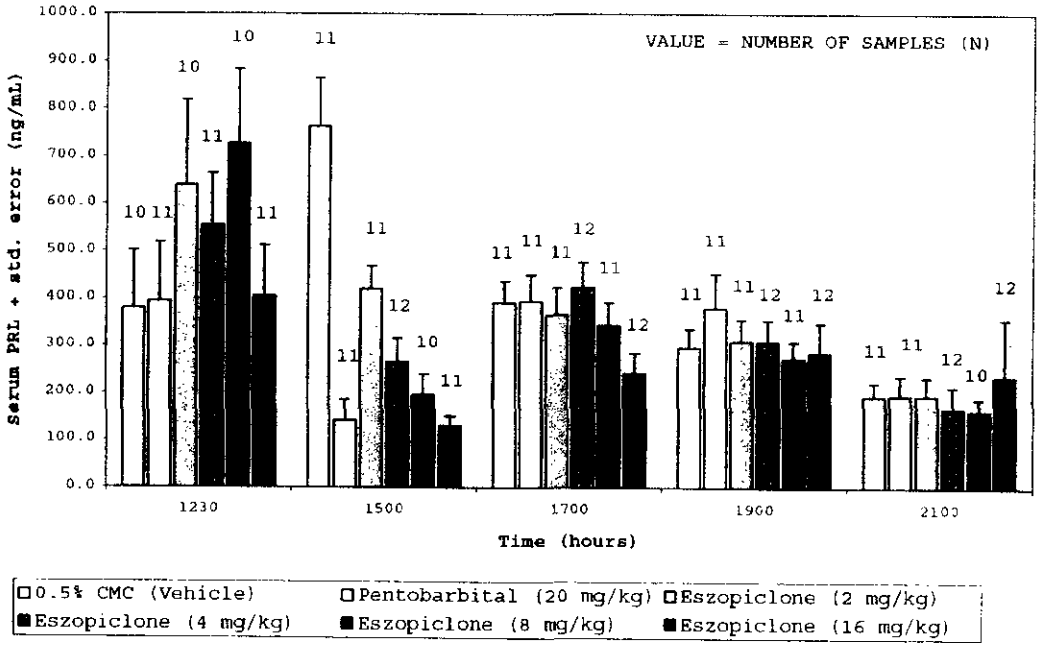


PROJECT NO. 312127C FIGURE 8 (SINGLE-DAY REGIMEN)
SPONSOR:SEPRACOR INC. STUDY OF (RS)-ZOP./ESZOP./ZOLPIDEM/ZALEPLON IN OVARIECT. RATS
MEAN SERUM PRL LEVELS (NG/ML) - ESZOPICLONE

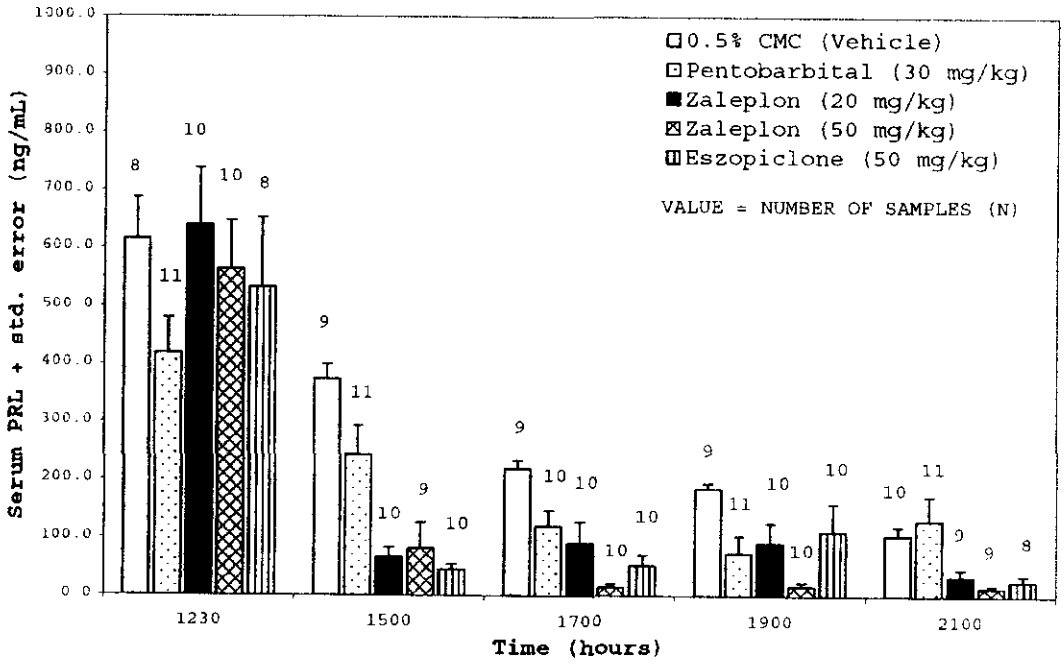


Endocrine function study of RS-zop, S-zop, zolpidem, and zalplon following single and/or 3day oral exposure in ovariectomized rats (Cont.)

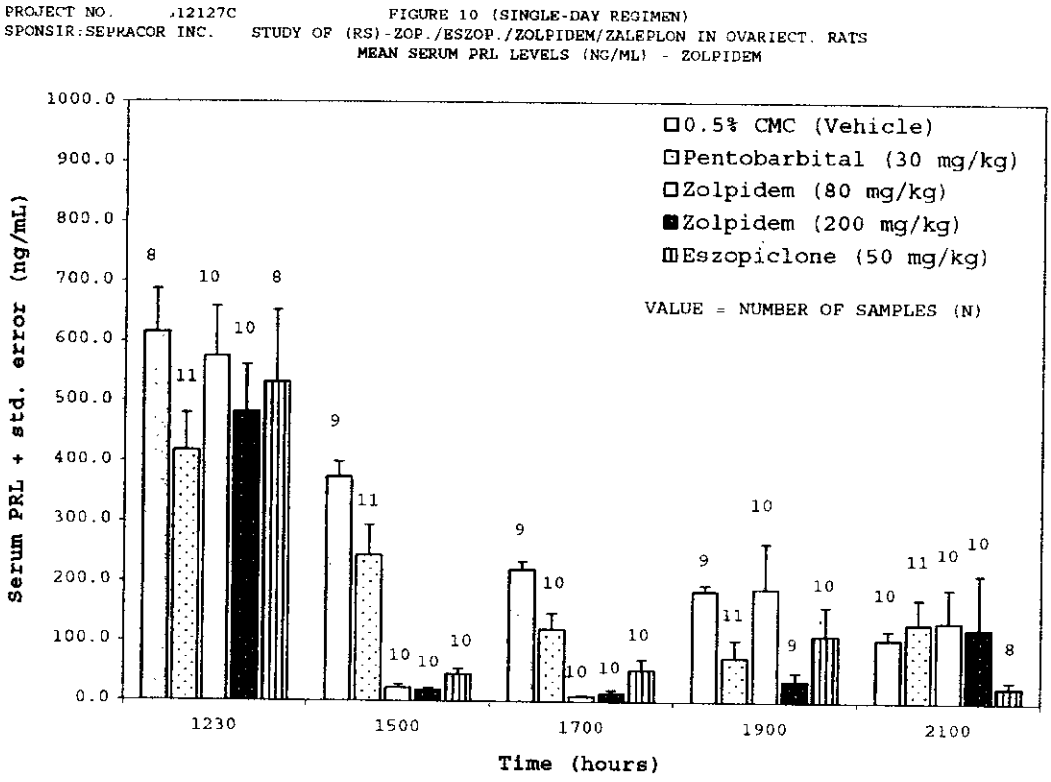
PROJECT NO. ,12127V FIGURE 18 (THREE-DAY REGIMEN)
SPONSOR:SEPRACOR INC STUDY OF (RS)-ZOP./ESZOP./ZOLPIDEM/ZALEPLON IN OVARIECT. RATS
MEAN SERUM PRL LEVELS (NG/ML) - SEPARATED BY TIMEPOINT



PROJECT NO. ,12127C FIGURE 12 (SINGLE-DAY REGIMEN)
SPONSOR:SEPRACOR INC. STUDY OF (RS)-ZOP./ESZOP./ZOLPIDEM/ZALEPLON IN OVARIECT. RATS
MEAN SERUM PRL LEVELS (NG/ML) - ZALEPLON



Endocrine function study of RS-zop, S-zop, zolpidem, and zalplon following single and/or 3day oral exposure in ovariectomized rats (Cont.)

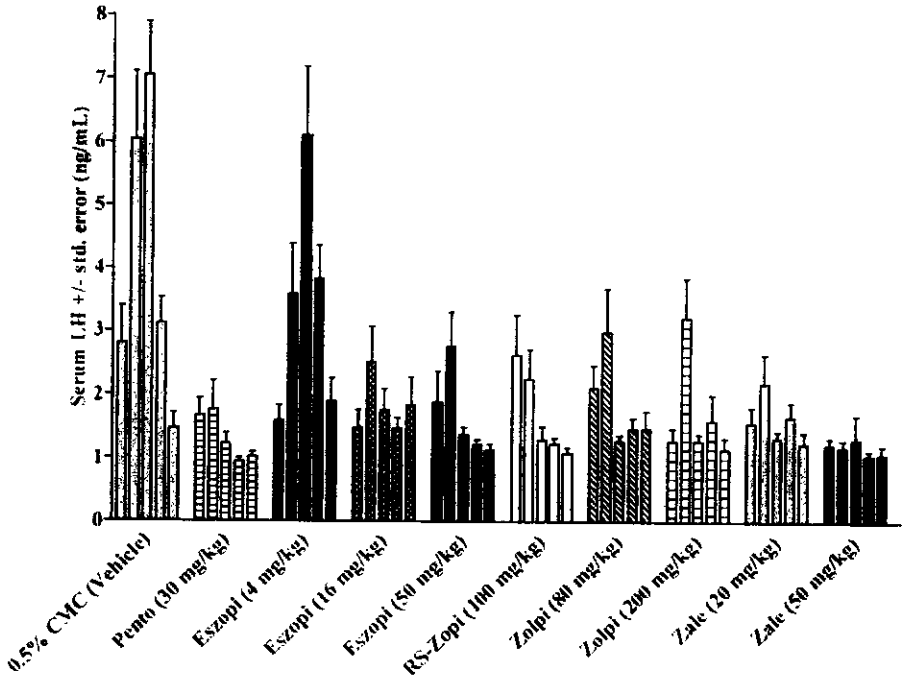


APPEARS THIS WAY
ON ORIGINAL

Endocrine function study of RS-zop, S-zop, zolpidem, and zalplon following single and/or 3day oral exposure in ovariectomized rats (Cont.)

This figure presents the effect of all drugs on LH level:

Figure 9.2.1.2-1 **Effect of Eszopiclone, (RS)-Zopiclone, Zolpidem, and Zaleplon on Serum Luteinizing Hormone Levels following Single-Day Exposure in Ovariectomized Female SD Rats**



CMC = Carboxymethylcellulose, Pento = Pentobarbital, Eszopi = Eszopiclone, RS-Zopi = (RS)-Zopiclone, Zolpi = Zolpidem, Zale = Zaleplon.
Bars for each treatment correspond to blood collection times of 1230 (predose), 1500, 1700, 1900, and 2100, respectively.

13. Evidence of neuroendocrine disturbance in SD rats treated with RS-zopiclone – Dr. RL Cooper report/Sepracor# 190-878

This report was presented in serial 109 dated Nov. 17th 2002 and was reviewed. Dr. RL Cooper, a neuroendocrinologist with NIEHS, EPA, reviewed studies from RS-zop and S-zop and all other relevant information provided to him by Sepracor. Dr. Cooper expanded the hypothesis on endocrine disruption by RS-zop and proposed an in vivo mechanistic study. The following is a brief summary of his report and conclusions (report date Nov 18th 2002). Dr. Cooper concluded that all data reviewed indicated that no single hormone change such as estradiol or prolactin, is responsible for the rat mammary gland tumors observed in the 2yr rat carcinogenicity study as previously suggested. However, results indicate that a **central disturbance in neural control at the hypothalamic/pituitary axis of GnRH and LH, is the site of action of RS-zop. Changes in LH specifically decrease, lead to early onset of reproductive senescence with persistent estrus and increase in serum estradiol levels eventually leading to mammary tumors. He continued that this mechanism of tumorigenesis in the rat is irrelevant to humans.** He also cited results from reproductive studies and general tox studies in support of this theory. To further support this mechanism, Dr. Cooper proposed conducting an *in vivo* rat study that is used by EPA to prove or disprove this mechanism and he stated that definitive results from this study should provide adequate evidence that the rat mammary tumors occurred as a result of disturbance in LH surge. The following are Dr. Cooper's arguments in support of the proposed mechanism:

1. GABA activity in the hypothalamus of the rat is affected by age and reproductive status. During and before LH surge, GABA secretion is decreased in the preoptic area leading to increase in GnRH and LH levels during the estrus cycle. Because of these changes in GABA during the estrus cycle as well as its decline with aging, GABAergic cpds can affect reproductive activity in the rat. Zaleplon and zolpidem are non-bzd cpds with GABAergic activity used in insomnia. High dose of 100mg/kg/d of zolpidem in the rat led to irregular estrus, zaleplon at 100mg/kg/d decreased fertility in rats and zaleplon label states that decreased fertility is due to an effect on females. Since RS-zop is a non-bzd with GABAergic activity, these effects on female reproductive activity may be a class effect.

2. In a 3mo endocrine study, RS-zop administered in the diet at 100 and 200mg/kg/d showed prolongation in estrus cycle length in the 200mg/kg/d* compared with the control (Sepracor# 190-870). Such an effect is indicative of onset of reproductive senescence.
*Reviewer Comment: according to results in Sepracor190-870 study, mean estrus cycle length was increased only in 100mg/kg/d not 200mg/kg/d as reported by Dr. Cooper (see review and tables for detail).

3. In 3mo study (Sepracor#190-849), serum estradiol was increased and FSH decreased in female rats after 100 and 200mg/kg/d RS-zop. Changes in these hormones* were also seen at 200mg/kg/d RS-zop dosed for 2 or 4wks but no changes at end of 3mo (Sepracor#190-870). Also in the latter study, changes in serum FSH, progesterone, and LH were also observed*. These hormone changes support central drug related effect on GnRH.
* Reviewer Comment: an increase was observed only in estradiol, no change in FSH, LH, or progesterone (see review and tables of study 190-870 for detail).

4. Both RS- and S-zop have effects on male fertility. S-zop at 45mg/kg/d reduced spermatogenesis (Sepracor#190-827) and sperm motility was reduced in RS-zop administered at 15mg/kg/d. Decreased spermatogenesis was also seen in 13wk RS- and S-zop tox study (Sepracor#190-819) where these changes correlated with marked decrease in testosterone hydroxylase activity and the effect was reversible after cessation of treatment. In female rats, S-zop at 120&180mg/kg/d decreased implantation sites and at 180mg/kg implantation sites were absent. In a 2nd Segment I study (Sepracor#190-835I), untreated males and treated females had decreased fertility and implantation losses at equivalent dose of S-zop of 60mg/kg/d and 120mg/kg/d RS-zop. All these effects indicate changes in estrus cycle and decrease in LH in female rats.

5. Gestation length was increased in SD rats dosed 120mg/kg/d RS-zop and 60 & 120mg/kg/d S-zop by oral gavage (Sepracor#190-828). Also in this same study, dams neglected their pups. These effects are indicative of disruption of the neuroendocrine system.

6. Dr. Cooper referred to the genetic toxicology evaluation of S-zop and RS-zop. He mentioned the positive findings in the *in vitro* cytogenetic assays with RS- and S-zop but dismissed them and concludes "with no apparent *in vivo* relevance". He also referred to the negative P53 transgenic studies with the S-zop and its active metabolite S-desmethyl, taken together, concludes that the tumor findings with RS-zop are not the result of a genotoxic mechanism.

Reviewer Comment: this reviewer disagrees with this conclusion which is also made by the sponsor. RS-zop was clastogenic *in vitro* mammalian assays, having a negative P53 and *in vivo* MN does not over-ride the positive findings in the clastogenicity *in vitro* assays (see genetic toxicology section for detail).

7. Dr. Cooper discussed some of the clinical data from other hypnotics and their effects on endocrine hormones. He also mentioned the increase in prolactin levels without an effect on ACTH or LH after 7.5mg/d RS-zop. It is noted that there is not adequate information or testing in humans regarding this issue.

Dr. Cooper as indicated above, proposed one *in vivo* and 2 in vitro studies to show if RS-zop triggers the neuroendocrine effects necessary for the induction of early reproductive senescence as a mechanism for mammary tumors. The following are the study titles, they were conducted by Sepracor and reviewed (see below):

1. Endocrine function study of RS-zop after 1 and 3-day oral expeosure in ovariectomized female rat.
2. Effects of RS-zop on basal and estradiol-induced GnRH secretion by the rat hypothalamus *in vitro*/ex-vivo.
3. *in vitro* effect of DA, GABA, and RS-zop on basal and GnRH induced prolactin and LH hormone secretion by cultured rat anterior pituitary cells.
[this study could not be done due to technical difficulties with the cell cultures].

The following is the in vivo study proposed by Dr. Cooper, it is ongoing. The study objective is to demonstrate if RS-zop and/or S-zop administered chronically will accelerate the time to onset of reproductive senescence in female rats. An interim report for the completed 6months of the 1yr study was submitted electronically on Aug 28th 03, the following describes study design and brief summary of the results (full and final report will be submitted in 2004).

14. Effects of muscimol, KCl, and RS-zop on basal and naloxone-induced GnRH secretion by the rat hypothalamus in vitro/ex vivo/Sepracor study# 190-425

GLP & QA: Yes
RS-zop lot# 0022400(IN-0693)
Study initiation date: Aug 2002
Conducting lab:

Objectives: to determine whether RS-zop affect hypoythalamus GnRH secretion. Originally, the objectives were to compare the effects of GABA, RS-zop, and KCl- on basal and estradiol-induced release of GnRH by rat hypothalamus in vitro/ex vivo. Preliminary experiments **failed** because neither estradiol nor GABA stimulated hormone release. Later, norepinephrine was tested since data indicate that it enhances GnRH secretion by rat median eminence *in vitro*, however, **this also failed** since the results were inconsistent. Another stimulator, naloxone, in preliminary data caused a reproducible increase in GnRH secretion therefore, naloxone (1mg/ml), was selected to induce GnRH secretion from the hypothalamus explants. Also originally, effects of RS-zop were to be compared to those of GABA however, the latter was ineffective in stimulating hormone release in the preparation. Based on literature, GABA may stimulate, inhibit, or has no effect on GnRH secretion depending on the hypothalamic tissue and experimental conditions. Therefore, muscimol, a GABA agonist, was selected as the positive control since it has a reliable and consistent response in inducing GnRH secretion in vitro. It is noted that though the effects of $GABA_A$ agonists are inconsistent on the hypothalamus in vitro, they do weaken LH surge in vivo.

Methods:
Seventy seven female Hsd rats 2-4months old weighed 200-270g purchased from were housed 4/cage. Estrus cycle for each rat was monitored by examining changes in vaginal cytology through vaginal lavage. Vaginal wash was placed on a slide to dry and processed for microscopical exam. Animals were considered acyclic if they stopped cycling and remained in one stage through the study duration. Rats were killed on day of proestrus by decapitation, basal hypothalamic explants were dissected away from the hypothalamus. Tissues processed and placed in oxygenated buffer and incubated according to SOPs. At final incubation period, 56mM KCl was added to ensure release of GnRH from tissues after exposure to K^+-induced depolarization. Supernatants and digested hypothalamus were then frozen awaiting analysis of GnRH by ELISA or protein determination.

Results & Conclusion:
Both RS-zop and muscimol in a concentration-dependent manner increased the secretion of basal and naloxone-induced secretion of GnRH from hypothalmic explants (figures from sponsor). Therefore, RS-zop acted like a typical $GABA_A$ agonist on GnRH secretion by the hypothalamus in vitro. Also as expected, KCl induced the release of GnRH from the preparation.

Figure 8: Effect of muscimol on naloxone induced GnRH secretion (as % increase in GnRH secretion)

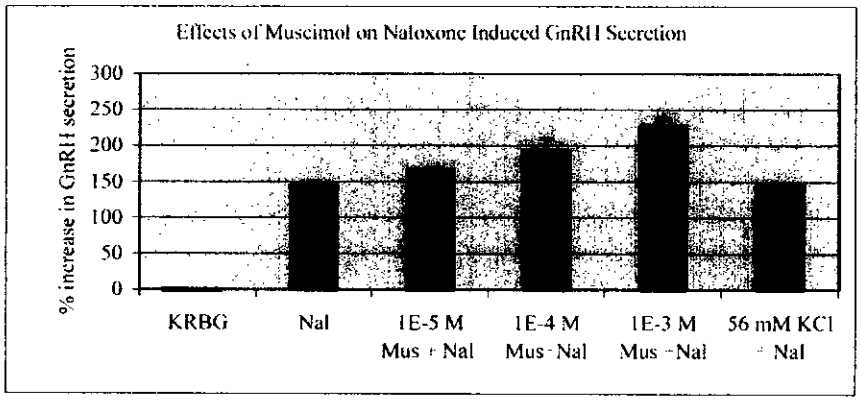
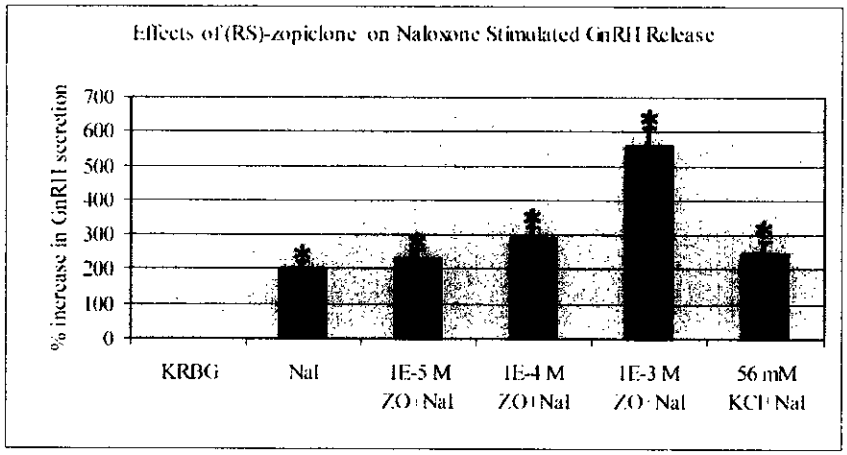


Figure 10: Effect of (RS)-zopiclone on naloxone induced GnRH secretion (as % increase in GnRH secretion)



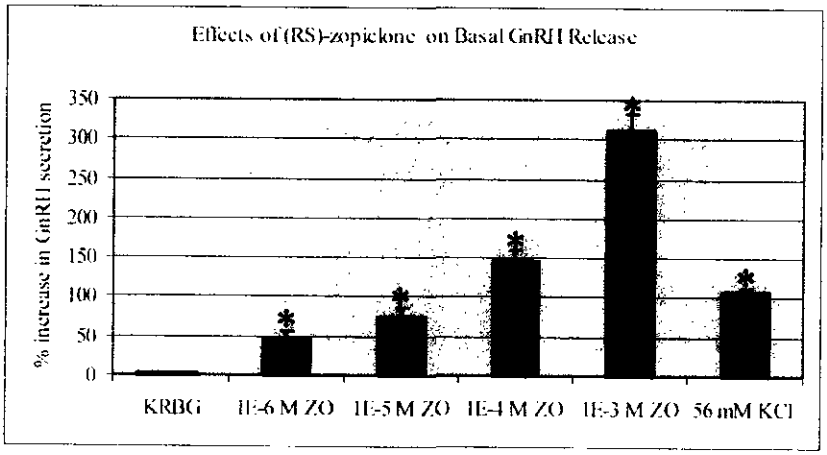
15. A uterotrophic assay of RS-zop and S-zop administered orally to ovariectomized rats/Sepracor study# 190-883

GLP & QA: Yes
RS-zop lot# ZP0010102 S-zop lot# 0290013 code 029
Study initiation date: Jan 2003
Conducting lab:

Objectives: to determine if RS-zop and S-zop possess any estrogenic activity that may explain the mammary tumors observed in RS-zop 2yr rat carcinogenicity study. A uterotrophic assay was used to address this issue. This assay is conducted in immature or ovariectomized rats to identify cpds that mimic biologic activity comparable to that of natural estrogens. Compounds with estrogenic activities cause an increase in uterine wt following a minimum of 3d exposure. It is an accepted and recommended assay by the USEPA as part of Tier 1 screening for endocrine disruptor activity.

Methods:

Figure 14: Effect of (RS)-zopiclone on basal GnRH secretion (as % increase in GnRH secretion)



SD ovariectamized female rats from were 65-68d old at start of dosing and weighed 246-291g. There were 8 groups of 10/group; animals were housed individually. Compounds were administered daily for 3 consecutive days via oral gavage. RS-zop and S-zop were prepared in 0.5% CMC and additional 10 rats were administered CMC and served as the control for these drugs. **RS-zop** was administered at **1, 10, and 100mg/kg/d** and **S-zop at 16 and 50mg/kg/d.** The positive control was injected s.c. 17α-ethinylestradiol 0.001mg/kg/d prepared in corn oil, and additional 10 rats were administered corn oil served as the vehicle control for this group. All animals were killed 24hr after the last dose and blotted as well as wet uterine wts were recorded. Luminal fluid was measured by subtracting blotted uterine wt from the wet wt. Parameters assessed included clinical signs, mortality, B.wt, and uterine wts.

Results and Conclusion:
There were no mortalities. Clinical signs observed in both drug groups and were extension of the pharmacology. Within 1hr postdose hypoactivity recorded for both drugs, in RS-zop 100mg/kg/d and 1-2 rats in the S-zop groups showed hypoactivity and/or rocking, lurching or swaying while ambulatory. Mean B.wt was significantly decreased in 100mg/kg/d RS-zop and 50mg/kg/d S-zop during the 1-2d period and

in RS-zop 100mg/kg/d wt loss was seen over the entire dosing period of 0-2. Mean wt gain was also reduced in the positive control group on d1-2 and 0-2 ($p<0.01$ relative to corn oil control values). Neither drug had an effect on wet or blotted uterine wts, the positive control 17α ethinylestradiol caused a significant increase in wet and blotted uterine wt as well as mean luminal fluid wt. (7, 3, and 20x increase respectively, relative to control corn oil wts). Therefore, this study demonstrated that RS-zop does not have an estrogenic activity (indirectly via uterine wt changes), that may have played a role in induction of mammary tumors observed in the 2yr rat carcinogenicity study.

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16. Rat early reproductive senescence study with S-zop and RS-zop/Sepracor.# 190-882I — 312125, 2003
Conducting laboratory and location: —
Date of study initiation: Jan 6th 2003
GLP & QA: yes however, signature page will be presented with final report..

Methods:
S-zop was administered daily by oral gavage to — female SD rats according to the experimental design below (from sponsor):

Design for Phase 1

Group Number	Treatment	Dose Level (mg/kg/day)	Dosage Concentration (mg/mL)	Dosage Volume (mL/kg)	Number of Females
1	Vehicle Control	0	0	10	40
2	Atrazine	40	4	10	40
3	Eszopiclone	4	0.4	10	40
4	Eszopiclone	16	1.6	10	40
5	Eszopiclone	50	5	10	40
6	(RS)-Zopiclone	100	10	10	40

Design for Phase 2

Group Number	Treatment	Dose Level (mg/kg/day)	Dosage Concentration (mg/mL)	Dosage Volume (mL/kg)	Number of Females
7	Vehicle Control	0	0	10	11
8	Eszopiclone	50	5	10	11
9	(RS)-Zopiclone	100	10	10	11

Female rats for phase I were 9-10wks old and for phase 2 were 11-12wks old at study initiation. First day and 1st wk of dosing were designated as 0. Animals were checked 2x daily for mortality and morbidity and after 134d of study detailed weekly exam was also incorporated; B.wts were recorded weekly. Following assignment, vaginal lavages were done 1x daily between 8-11am to determine stage of estrus for a 2wk interval each month. Prior to dose, vaginal lavage for phase 1 was done 2wk before and for phase 2 4wks before dosing. Rats with normal 4-5d cycles (typically including 1d of estrus) prior to dosing, were assigned to phase 1 groups. For comparison, rats displaying irregular cycles with 2 or more days of estrus in a cycle, were assigned to phase 2. Rats in the latter groups were included to test if a sub-population of rats that is in estrus at higher frequency than normal prior to dosing would be more likely to have estrus cycle disruption with RS-zop and/or S-zop than rats with normal cycle (phase 1 rats). Only 33 females of 411 or 8% were identified as having greater than normal frequency of days in estrus and those were distributed equally among the phase 2 groups (11/group).

APPEARS THIS WAY
ON ORIGINAL

The following is example of normal regular and irregular cycles (from sponsor):

4-5 Day Estrous Cycle (assigned to Phase 1)

Animal No. 18197 (WTDMS™ Protocol WIL-312125R), Weeks -2 to -1

P E |D D P E| |D D D E| |D D D E| D D

2 or More Consecutive Days of Estrus (assigned to Phase 2)

Animal No. 18114 (WTDMS™ Protocol WIL-312125R), Weeks -2 to -1

D| E E| D D D| E E| D D P| E E| D

where E = Estrus, M = Metestrus, D = Diestrus and P = Proestrus

Dose Selection: RS-zop dose of 100mg/kg/d is the dose used in the 2yr carcinogenicity study where mammary gland tumors were observed. The 50mg/kg/d S-zop is equivalent as an isomer, to the racemate dose of 100mg/kg/d and the 4&16mg/kg/d are the low and high doses used in the 2yr carcinogenicity study with the S-zop. Atrazine was used as a *positive* control since it has been shown to cause at 40mg/kg/d, estrus cycle disruption and increased incidence *or* early onset of mammary tumors in *Charles River* SD rats (Eldrige et al., 1999; Wetzel et al. 1994). As noted, most of the atrazine studies used SD from Charles River, Sepracor used SD from [redacted] because this was the source for the carcinogenicity study. [redacted]), used SD from [redacted] and it seemed that these rats are less sensitive to atrazine effects than Charles River SD rats. In that study, atrazine at 75mg/kg/d administered orally for 21d disrupted the cycle but without an effect on the percent of days spent in

diestrus or estrus, 150mg/kg/d significantly increased the percent of days in diestrus and, 300mg/kg/d both increased percent of days in diestrus and decreased the days spent in estrus. However, the MTD in chronically fed SD rats was 40mg/kg/d (Eldrige et al., 1999), therefore, Sepracor used this dose here since the study is long term.

Stages of Estrus Cycle: there is a good correlation between estrus cycle changes in cell types present in the vagina and, changes in plasma levels of estradiol and progesterone secreted by the ovary (Cooper and Goldman 1999). — s divide estrus cycle changes into 4 stages:

Estrus: the majority of cells are the cornified epithelial cells (indicative of estrogen secretion), with no leuckocytes present. Estrus usually lasts 10-15hrs

Metestrus: in the early phase of metestrus, the majority of cells are nucleated epithelial cells though some leuckocytes and cornified epithelial cells can be present. This stage looks similar to estrus except the cells are nucleated and dark in color. End stage metestrus, majority of cells are leuckocytes surrounding groups of nucleated epithelial cells. Metestrus lasts 6-14hr.

Diestrus: in any group of rats, many of the females will be in this stage of the cycle. In early diestrus, usually, all 3 cell types (leuckocytes, cornified epithelial, and nucleated epithelial cells), are found and toward late diestrus, nucleated epithelial cells become more spherical and all other cells become more less populated. Rats spend 2-3d or 60-70hr is diestrus.

Proestrus: this stage usually follows diestrus and is the prep stage for estrus therefore, 50% of the cells are cornified epithelial cells. this lasts 8-12hrs.

Regularly cycling rats have 4-5 estrus cycle. In this study irregular rats are those that either have >4d consecutive days in diestrus (persistent diestrus), or >3 consecutive days in persistent estrus (metestrus, proestrus or estrus). The sponsor presented example for each as follows:

Persistent Diestrus

Phase 1 Animal No. 17893 ' Protocol -312125B), Group 1, Weeks 0-1

M | D D D D D D D D D | M D D E

Persistent Estrus

Phase 1 Animal No. 17845 (Protocol ' 312125D), Group 4, Weeks 8-9

| M M M M | D | M M M M | D D E E M

and

Phase 1 Animal No. 18007 ' Protocol 312125E), Group 4, Weeks 12-13

M M D | P M M E E M M M M M E |

Determination of Repro Senescence: in aging rats (8-15month old depending on strain) regular cycles will be replaced with persistent estrus where the ovarian follicles are fully developed but, no ovulation (Cooper et al.1986; Meites 1982). Some but not all rats will have irregular cycles during the period of transition from regular to persistent estrus. The constant estrus state may continue for several months which is often followed by a state of pseudopregnancy. At this time of persistent estrus, ovulation ceases and the animals are said to be in reproductive senescence. The sponsor therefore, used this as its definition for onset of reproductive senescence i.e. constant estrus.

Interim Results:
The following are tables from the sponsor showing the interim results for the 1st 6mo of the study. These results in this reviewer's opinion as well as opinions of reproductive toxicology experts in the Division, are **NOT in full support** of the theory that RS-zop and S-zop induces early onset of repro senescence in the female rat which in turn is responsible for mammary tumor induction as a result of persistent increase in serum estradiol and other hormones. This is demonstrated by the following negative results:

- Absence of dose response in the S-zop in any of the measured parameters (tables from sponsor),
- No difference in percent of rats in persistent diestrus vs. the control (table 5A) in fact fewer rats were in this stage in 16&50mg/kg/d S-zop than in control at end of 6mo (2/40 vs. 14/40 in 50mg/kg/d and control respectively); positive control failed,
- Failure of the positive control, atrazine, to cause the expected changes in all measured parameters,
- Rats in phase 2 were not more responsive to the effects of RS-zop or S-zop as was anticipated. Although any conclusions from this group should be considered with caution because of the inherent noise in such group of rats as well as the small number of rats tested per group,
- The positive responses when observed, generally showed a stronger signial with the S-zop than with the RS-zop. Yet, mammary tumors were seen in the latter and not in the formwe i.e. even the positive findings don't seem to support the proposed mechanism.

A convincing finding would be to see if either drug will cause mammary tumors at the end of the 1yr study and/or the appropriate changes in serum hormones (see Sepracor#190-870 Dr report).

The following results were in support of the proposed mechanism:

- Table 1A - the cumulative number of rats that reached senescence was higher in drug groups than in control. By end of 6mo, S-zop 16mg/kg/d had 78% of rats vs. 5% in control that reached senescence and 50 and 20% in 50mg/kg/d S-zop and 100mg/kg/d RS-zop respectively (no dose response). Atrazine positive control failed to show any difference.
- Mean percent of days in estrus was higher in S-zop 16&50mg/kg/d though not dose dependent, and RS-zop (table 2A),
- At 16&50mg/kg/d S-zop, statistical significance was observed in percent of regularly/irregularly cycling rats vs. the control starting on wk8 onwards (table 3A). However by the same token, there was NO difference in RS-zop as of wk16 onward vs. control; atrazine failed to show a favorable response.

- Percent of rats in persistent estrus was increased in drug groups vs. control (table 4A) though not dose dependently and the positive control also failed.

Conclusion:

In conclusion, so far results from this study are not in full support of the early senescence theory of mammary gland tumor induction. There were negative as well as positive findings and generally, the positive findings with the S-zop showed stronger signal than those with the RS-zop (tables 1A, 2A, 4A), and in some parameters, the RS-zop failed to induce any effect (tables 3A & 5A). Based on the positive findings, mammary tumors should be observed (increased incidence and/or early onset per atrazine mechanism), by S-zop. However, no tumors were observed in the S-zop carcinogenicity studies in the rat.

Although not all parameters up to this point, support the early senescence theory of mammary gland-induced tumors, it remains to be seen if by the end of the 1yr the number of aging rats in drug groups with signs of early senenscence is much higher than those in the control and/or will develop mammary tumors early or with higher incidence than the controls. Also in support of this theory, would be to correlate changes in hormone levels or loss of cyclicity in these rats with vaginal cytology. However, it is unfortunate that this study is not planning to assess any of these parameters because its mainly and exclusively a cytology investigation of changes in estrus cycle.

In conclusion, this study up to the 6mo interim have shown some changes in vaginal cytology that supports the theory of early onset of reproductive senescence however, other parameters did not and the positive control failed to show any effect. Another important finding here is the weaker signal of the RS-zop (the drug that caused mammary tumors), relative to that of the S-zop which did not cause mammary tumors in a recent 2yr bioassay. It is unclear how meaningful the results will be at the end of 1yr in absence of assessment of palpation, mammary gland histopathology in search of tumors, and hormone measurements to show absence of cyclicity or elevated levels. These parameters were assessed in atrazine and their results supported its proposed mechanism of mammary gland tumor induction via early onset of senescence.

STUDY NO. 312125
SPONSOR:SEPRACOR INC.

SUMMARY OF TIME TO SENESCENCE
CUMULATIVE NUMBER (PERCENTAGE) OF ANIMALS TO REACH SENESCENCE BY END OF STUDY DAY INTERVAL

TABLE 1A - PHASE 1

GROUP	VEHICLE CONTROL (N=40)	40 MG/KG ATRAZINE (N=40)	4 MG/KG ESZOPICLONE* (N=40)	16 MG/KG ESZOPICLONE* (N=40)	50 MG/KG ESZOPICLONE* (N=40)	100 MG/KG (RS)-ZOPICLONE* (N=40)
STUDY DAYS						
PRETEST	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
0 - 27	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
28 - 55	0 (0.0)	0 (0.0)	0 (0.0)	2 (5.0)	6 (15.0)	1 (2.5)
56 - 83	0 (0.0)	0 (0.0)	0 (0.0)	10 (25.0)	8 (20.0)	2 (5.0)
84 - 111	0 (0.0)	0 (0.0)	0 (0.0)	14 (35.0)	8 (20.0)	3 (7.5)
112 - 139	2 (5.0)	2 (5.0)	2 (5.0)	24 (60.0)	16 (40.0)	7 (17.5)
140 - 167	2 (5.0)	5 (12.5)	9 (22.5)	31 (77.5)	20 (50.0)	8 (20.0)

* - Significantly different from vehicle control at p<0.05

TABLE 1B - PHASE 2

GROUP	VEHICLE CONTROL (N=11)	50 MG/KG ESZOPICLONE (N=11)	100 MG/KG (RS)-ZOPICLONE (N=11)
STUDY DAYS			
PRETEST	0 (0.0)	0 (0.0)	0 (0.0)
0 - 27	0 (0.0)	0 (0.0)	1 (9.1)
28 - 55	0 (0.0)	4 (36.4)	2 (18.2)
56 - 83	0 (0.0)	4 (36.4)	2 (18.2)
84 - 111	0 (0.0)	4 (36.4)	2 (18.2)
112 - 139	1 (9.1)	5 (45.5)	2 (18.2)
140 - 167	1 (9.1)	5 (45.5)	2 (18.2)

None significantly different from vehicle control

STUDY NO. 312125
SPONSOR:SEPRACOR INC.

MEAN PERCENTAGE OF DAYS IN ESTRUS OR METESTRUS

TABLE 2A - PHASE 1

GROUP	VEHICLE CONTROL (N=40)	40 MG/KG ATRAZINE (N=40)	4 MG/KG ESZOPICLONE (N=40)	16 MG/KG ESZOPICLONE (N=40)	50 MG/KG ESZOPICLONE (N=40)	100 MG/KG (RS)-ZOPICLONE (N=40)
STUDY WEEKS						
-2 - -1	30.4	30.4	30.0	31.4	29.8	30.8
0 - 1	29.8	26.6	28.4	28.9	33.9*	33.9*
4 - 5	26.8	22.0	24.1	40.0*	42.5*	37.7*
8 - 9	24.1	18.6	20.2	51.3*	44.6*	42.0*
12 - 13	24.8	22.0	18.9	47.5*	35.0*	33.9

STUDY NO. 312125
SPONSOR:SEPRACOR INC.

PERCENTAGES OF REGULARLY/IRREGULARLY CYCLING ANIMALS

TABLE 3A - PHASE 1

GROUP	VEHICLE CONTROL (N=40)	40 MG/KG ATRAZINE (N=40)	4 MG/KG ESZOPICLONE (N=40)	16 MG/KG ESZOPICLONE (N=40)	50 MG/KG ESZOPICLONE (N=40)	100 MG/KG (RS)-ZOPICLONE (N=40)
STUDY WEEKS						
-2 - -1	92.5/ 7.5	97.5/ 2.5	95.0/ 5.0	100.0/ 0.0	97.5/ 2.5	100.0/ 0.0
0 - 1	95.0/ 5.0	87.5/ 12.5	85.0/ 15.0	95.0/ 5.0	92.5/ 7.5	95.0/ 5.0
4 - 5	75.0/ 25.0	65.0/ 35.0	80.0/ 20.0	50.0/ 50.0	65.0/ 35.0	67.5/ 32.5
8 - 9	65.0/ 35.0	45.0/ 55.0	72.5/ 27.5	37.5/ 62.5*	50.0/ 50.0	50.0/ 50.0
12 - 13	72.5/ 27.5	65.0/ 35.0	70.0/ 30.0	17.5/ 82.5*	37.5/ 62.5*	47.5/ 52.5*
16 - 17	62.5/ 37.5	62.5/ 37.5	65.0/ 35.0	15.0/ 85.0*	27.5/ 72.5*	45.0/ 55.0
20 - 21	50.0/ 50.0	45.0/ 55.0	40.0/ 60.0	15.0/ 85.0*	17.5/82.5*	42.5/ 57.5

* - Significantly different from vehicle control at p<0.05

TABLE 3B - PHASE 2

GROUP	VEHICLE CONTROL (N=11)	50 MG/KG ESZOPICLONE (N=11)	100 MG/KG (RS)-ZOPICLONE (N=11)
STUDY WEEKS			
-2 - -1	100.0/ 0.0	100.0/ 0.0	100.0/ 0.0
0 - 1	100.0/ 0.0	90.9/ 9.1	81.8/ 18.2
4 - 5	36.4/ 63.6	18.2/ 81.8	9.1/ 90.9
8 - 9	63.6/ 36.4	45.5/ 54.5	36.4/ 63.6
12 - 13	72.7/ 27.3	27.3/ 72.7	63.6/ 36.4
16 - 17	63.6/ 36.4	9.1/ 90.9*	36.4/ 63.6
20 - 21	45.5/ 54.5	45.5/ 54.5	27.3/ 72.7

* - Significantly different from vehicle control at p<0.05

STUDY NO. 312125
SPONSOR:SEPRACOR INC.

PERCENTAGES OF ANIMALS IN PERSISTENT ESTRUS

TABLE 4A - PHASE 1

GROUP	VEHICLE CONTROL (N=40)	40 MG/KG ATRAZINE (N=40)	4 MG/KG ESZOPICLONE (N=40)	16 MG/KG ESZOPICLONE (N=40)	50 MG/KG ESZOPICLONE (N=40)	100 MG/KG (RS)-ZOPICLONE (N=40)
STUDY WEEKS						
-2 - -1	0.0	0.0	0.0	0.0	0.0	0.0
0-1	0.0	2.5	2.5	0.0	2.5	2.5
4-5	5.0	2.5	0.0	25.0*	30.0*	22.5*
8-9	12.5	7.5	2.5	45.0*	42.5*	32.5
12-13	7.5	2.5	0.0	50.0*	22.5	25.0
16-17	20.0	15.0	17.5	70.0*	57.5*	40.0
20-21	15.0	20.0	32.5	82.5*	77.5*	47.5*

* - Significantly different from vehicle control at p<0.05

TABLE 4B - PHASE 2

GROUP	VEHICLE CONTROL (N=11)	50 MG/KG ESZOPICLONE (N=11)	100 MG/KG (RS)-ZOPICLONE (N=11)
STUDY WEEKS			
-2 - -1	0.0	0.0	0.0
0-1	0.0	9.1	18.2
4-5	54.5	81.8	90.9
8-9	18.2	54.5	63.6
12-13	9.1	54.5	27.3
16-17	36.4	81.8	54.5
20-21	27.3	18.2	9.1

None significantly different from vehicle control

STUDY NO. 312125
SPONSOR:SEPRACOR INC.

PERCENTAGES OF ANIMALS IN PERSISTENT DIESTRUS

TABLE 5A - PHASE 1

GROUP	VEHICLE CONTROL (N=40)	40 MG/KG ATRAZINE (N=40)	4 MG/KG ESZOPICLONE (N=40)	16 MG/KG ESZOPICLONE (N=40)	50 MG/KG ESZOPICLONE (N=40)	100 MG/KG (RS)-ZOPICLONE (N=40)
STUDY WEEKS						
-2 - -1	7.5	2.5	5.0	0.0	2.5	0.0
0-1	5.0	10.0	12.5	5.0	5.0	2.5
4-5	20.0	32.5	20.0	25.0	5.0	10.0
8-9	27.5	50.0	25.0	17.5	7.5*	17.5
12-13	20.0	32.5	30.0	35.0	42.5	27.5
16-17	17.5	22.5	17.5	15.0	20.0	17.5
20-21	35.0	37.5	27.5	5.0*	5.0*	15.0

* - Significantly different from vehicle control at p<0.05

TABLE 5B - PHASE 2

GROUP	VEHICLE CONTROL (N=11)	50 MG/KG ESZOPICLONE (N=11)	100 MG/KG (RS)-ZOPICLONE (N=11)
STUDY WEEKS			
-2 - -1	0.0	0.0	0.0
0-1	0.0	0.0	0.0
4-5	9.1	0.0	0.0
8-9	18.2	0.0	0.0
12-13	18.2	18.2	9.1
16-17	0.0	9.1	9.1
20-21	27.3	45.5	63.6

None significantly different from vehicle control

OVERALL CONCLUSIONS AND RECOMMENDATIONS

OVERALL SUMMARY AND EVALUATION OF RS-ZOPICLONE EFFECTS ON THYROID GLAND AND THYROID TUMORS:

Thyroid proliferation and tumors were observed in 18month chronic toxicity study and 24month carcinogenicity study in the rat conducted in the early 1980s by — at the request of RPR — —. These findings prompted the sponsor to conduct a number of mechanistic studies to investigate whether the drug is acting centrally through the HPA or peripherally on thyroid hormone metabolism causing these thyroid lesions. In addition to conducting studies, experts in the field were consulted and provided with the specific slides for their review and evaluation. These experts prepared written reports and conclusions of their opinions. The sponsor as well as experts concluded that RS-zop acts peripherally and indirectly affect thyroid hormone metabolism through its direct action on the liver as follows:

- RS-zop causes liver enzyme induction specifically those enzymes responsible for thyroid hormone metabolism i.e. UDP-GT and 5' deiodinase.
- Increase in T4 metabolism and Cl will decrease its blood concentration which in turn through positive feed back increases TSH release from the pituitary.
- Increase in TSH stimulates protein synthesis in the thyroid and,
- Increase in thyroid synthetic capacity lead to tissue proliferation, increase in thyroid wt and if this stimulation continues for sometime, will lead to thyroid histopathology and tumors.

The results from the mechanistic studies were inconsistent and data showed marked inter- and intra-variability as reflected by the large s.d. values. However, <u>collectively</u>, the results from all the studies and other toxicity studies seem to support the proposed mechanism. The following are the results in favor of this indirect mechanism:

- Increase in liver weight relative to B.wt. sometimes with *minimal* hepatocyte hypertrophy indicative of enzyme induction (a finding also observed in mice and dogs).
- Increase in thyroid wt sometimes with follicular cell hypertrophy and hyperplasia and decrease in colloid.
- Increase in activity of UDP-GT for T4 and 5' deiodinase in the liver.
- Increase in serum TSH and decrease in serum T4.
- Inconsistent changes in T3 and rT3 serum levels.

In conclusion, the overall results from the mechanistic studies seem to support the mechanism where by RS-zop induces liver enzymes which in turn increases thyroid hormone metabolism and Cl. Decrease in serum T4 through a positive feed back, increases TSH release from the pituitary and stimulate protein synthesis in the thyroid to produce more T4. Continued thyroid proliferation can progress to tumors. RS-zop has not been shown to affect liver enzymes in humans and thyroid hormone physiology is different between humans and animals hence these tumors are irrelevant to human risk. As mentioned in the carcinogenicity section, S-zop in recently conducted study did not induce any thyroid tumors when administered daily by oral *gavage* to male and female rats for 2yrs up to 16mg/kg/d* and similar to RS-zop, does not seem to be a liver enzyme inducer in humans.

* this dose did not provide adequate exposure to S-zop compared to levels measured following RS-zop administration at the dose that did cause the thyroid tumors in the original study.

SUMMARY AND CONCLUSION FOR MAMMARY TUMORS IN FEMALE RATS:

Twelve investigative in addition to sections in the general toxicity studies, have been conducted to address the mechanism of RS-zop induced mammary tumors observed in female rats administered 100mg/kg/d RS-zop for 2yrs. These studies included in vivo, in vitro, and hypothalamic explants with the 1st study conducted in 1981 and the last study recently completed in November 2003. Over this long period of investigation (22 years), 2 theories were put forth for the mechanism of action of these tumors:

1. RS-zop induces an increase in 17β estradiol and other hormones such as LH and prolactin. Persistent elevation in serum estradiol is known to cause mammary proliferation in rats and consequent tumor formation. This theory would have human relevance.
2. RS-zop-induces early onset of reproductive senescence in female rat. Similar to the herbicide atrazine, where high doses in SD female rats over prolonged period were shown to enhance early onset of reproductive senescence characterized by constant secretion of estradiol and persistent estrus leading to mammary gland tumors. This mechanism would have no relevance to humans.

Two of the 4 studies that investigated the 1st theory showed an increase in serum estradiol and/or LH levels. The first study conducted in 1984 **failed** to show any drug effect on serum LH, FSH, prolactin, or estradiol at 100mg/kg/d RS-zop administered up to 9wks **(#190-850)**. One month oral gavage study done in *male* rats **failed** to show any changes in serum estradiol, LH, or FSH in male rats dosed up to 200mg/kg/d (**#190-837A1**)(*unclear why these hormones were measured in males).* A follow up study done in 1985 **did show** increase in serum estradiol in females dosed 100&200mg/kg/d RS-zop by gavage and dietary administration on both wks 4 and 10 of dosing **(#190-849)** with NO change in progesterone level. Also in this study, serum FSH, LH, and prolactin levels were affected/*reduced* in female rats compared to control (not dose dependent changes and change usually noted after gavage but not dietary administration). Mean relative ovary wt was increased in females dosed 200mg/kg/d by either gavage or dietary route without an effect at 100mg/kg/d dose (this is the dose where tumors were observed). This study was supportive of the theory and concluded that RS-zop may be acting indirectly to increase estradiol since a **central role was not** supported by the change in serum FSH (levels were decreased instead of increased). Study# **190-870F** conducted in 2001 was a 3-month mechanistic study with daily dietary administration of RS-zop at 100&200mg/kg/d where in addition to serum hormone measurements, vaginal smears were examined to assess stage of estrus. Mean estradiol and other hormones were **Not** affected by the drug however, hormone levels re-analyzed *as a function of estrus stage* showed **an increase** in estradiol level at wks 2 &4 but not at end of study d84. Serum FSH was increased at the 3 time points at different stages of estrus, so did progesterone levels, but *prolactin* levels seemed to *decrease* and ***LH* levels *increased*** (instead of a decrease). *It is important to point out that all of the serum hormone data were extremely variable and any conclusion should be considered cautiously).* Prolongation in diestrus was seen at 100mg/kg/d relative to control, 6.2days vs. 5days in control but with s.d. of 3.3days in the former. Dr. ____ — ____ a consultant, examined and reviewed the data and concluded that RS-zop may have caused cycle irregularities but the effect was subtle. He added, that absence of any histopathology in the mammary glands at this time could suggest that mammary gland changes may occur over prolonged period of exposure to the drug and in response to sustained increase in estradiol levels. For this same study, the sponsor decided to analyze samples of omental adipose tissue from females at end of the 3month study to assess CYP19 aromatase enzyme activity (**#190-870A1**).

Results showed that RS-zop at 100 and 200mg/kg/d did not increase the adipose tissue enzyme activity indicative that any potential elevation in estradiol did not occur from conversion of testosterone.

Five studies were conducted to investigate the 2nd theory. Study# **190-879A1** exposed ovariectomized rats to single or repeat 3d dose of RS-zop at 100 or 250mg/kg/d with Na-pentobarb used as the positive control. Animals were implanted with silastic estrogen capsules. LH levels were significantly decreased after the single administration at both doses and at all 3 time points compared to control, however, the response was much less obvious following 3d dosing and occurred only at 1 of the 3 time points. Re-analysis of the latter data however, showed 4 of the rats (out of 9 or 12 rats), had LH surge equal to or greater than the vehicle and the remaining rats showed LH suppression. Prolactin levels were decreased after the single and 3d dosing. These results seemed to **support RS-zop-induced decrease in LH surge but only after a single administration and less clear of an effect after 3days.** In another study with similar design to the above (ovariectomized rats, single and 3d regimen; **#190-884**), S-zop and RS-zop caused significant **attenuation in LH amplitude** and decrease in serum prolactin **so did zaleplon and zolpidem, 2 non-benzodiazepine GABA agonists**. The sponsor concluded that this response of neuroendocrine disruption at high doses may be a class effect. The **NOEL for S-zop was 4mg/kg following single administration**. In the 3d regimen, S-zop caused a decrease in maximum amplitude of LH surge and, a dose dependent shift in time of day at which LH peaked. The shift in time to peak for LH surge observed in the 3d regimen had no effect on serum prolactin up to 8mg/kg S-zop dose. Serum prolactin levels were does-dependently reduced following S-zop. In conclusion, the lower number of animals that surged and lower amplitude of the surge indicated reduction in serum LH levels therefore, **supporting the early reproductive senescence** mechanism. **However, it is of importance to note that zaleplon and zolepidem did not cause mammary tumors or any relevant tumors in the 2 year bioassays**. **Therefore, a cpd that causes estrus cycle irregularities i.e endocrine disruption, does not necessarily causes mammary tumors**. RS-zop caused a concentration-dependent increase in basal and naloxone-induced GnRH secretion from hypothalamic explants **supportive of "central" disruption of the endocrine hormones** (**#190-425**; *see above where a central role was ruled out as a mechanism for increase in serum estradiol).* Both RS-zop and S-zop have no estrogenic agonistic activity based on a single study where blotted or wet uterine from ovariectomized rats did not increase when the drugs administered up to 100&50mg/kg/d respectively (**#190-883**). In recently completed study **#190-882I (terminated in November 2003)** its objective is to show endocrine disruption and early onset of reproductive senescence evidenced by persistent estrus in drug groups. This was planned as a 1yr cytology study where — SD female rats administered S-zop at 4, 16, and 50mg/kg/d and RS-zop at 100mg/kg/d via oral gavage, the results of the 1st 6mo were submitted in August 2003. Atrazine, the prototype for this mechanism was included as the positive control at 40mg/kg/d oral dose. Interim results showed **PARTIAL support** for the proposed mechanism as evidenced by the **increase in cumulative number of rats that reached senescence** by end of study wk21-23 at: 50% in 50mg/kg/d S-zop and 20% in RS-zop 100mg/kg/d vs. 5% in vehicle control but 12.5% in atrazine group (the latter was not different from control). Moreover, the **percent of rats in persistent estrus was increased** in S-zop (16&50mg/kg/d not dose dependently), and RS-zop compared to control (positive control also failed to produce the anticipated response). However, the following were **not in support** of the mechanism: in none of the parameters measured was there a dose response in S-zop, the positive control failed to produce its effect, no difference in percent of number of rats in diestrus in fact, significantly lower percent of rats were in diestrus compared to control: 5, 5, and 15% in 16, 50mg/kg/d S-zop and 100mg/kg/d RS-zop respectively, vs. 35% in control (again, atrazine failed with 37%). Additionally phase 2 rats, selected specifically with irregular cycles prior to dosing, used by the sponsor with the expectation of a more

pronounced response than rats with regular cycles, failed to support this presumption. Although not all parameters up to this point, support the early senescence theory of mammary gland-induced tumors, it remains to be seen if by the end of the 1yr the number of aging rats in drug groups with signs of early senenscence is much higher than those in the control and/or will develop mammary tumors early or with higher incidence than the controls. Also in support of this theory, would be to correlate changes in hormone levels or loss of cyclicity in these rats with vaginal cytology. These parameters are the essence of the mechanism of early senescence proposed for atrazine and argued by Dr. Cooper. However, unfortunately there are no plans to assess any of these parameters in this study because its mainly and exclusively designed as a cytology study investigating changes in estrus cycle.

In conclusion, this mechanistic study up to the 6mo interim have shown some changes in vaginal cytology that supports the theory of early onset of reproductive senescence however, other parameters did not and the positive control failed to show any effect. Another important finding here is the weaker signal of the RS-zop (the drug that caused mammary tumors), relative to that of the S-zop which did not cause mammary tumors in a recent 2yr bioassay. It is unclear how meaningful the results will be at the end of 1yr in absence of assessment of palpation, mammary gland histopathology in search of tumors, and hormone measurements to show absence of cyclicity or elevated levels. These parameters were assessed in atrazine and their results supported its proposed mechanism of mammary gland tumor induction via early onset of senescence. Another important finding is the disturbance in estrus cycle evidenced by suppression of LH surge, by zalelpon and zolepidem, 2 drugs of the same class as zopiclone but neither drug cuased mammary tumors or any relevant tumors in long term bioassays. Therefore, it seems that not always disturbance in cyclicity can lead to mammary tumors unless the other factors observed with atrazine are fulfilled.

It is the opninon of this reviewer that although there are similarities between the atrazine-induced mechanism of mammary tumors and that proposed for RS-zop, there are also many differences (see below).

According to the literature, neuroendocrine disruption playes a significant role in the normal aging process of the rat as it applies to development of mammary and pituitary tumors (Eldridge et al., 1999; Lu et al.,1994; Meites 1982; Huang et al., 1978). The aging process begins at 10-12month of age (middle age), where irregular estrus cycles (prolonged) appears, followed by persistant estrus characterized by fully developed follicles but without ovulation (may last few months), then pseudopregnancy (ovaries with many corpora lutea but without ovulation), and finally anestrus where ovaries are atrophied with small undeveloped follicles. Prolactin levels seem to increase with age with highest levels occurring during anestrus. This elevation in blood prolactin occurs in presence of a parallel decline in estrogen secretion by the ovaries. Spontaneous mammary *fibroadenomas* in aging rats are seen in presence of elevated blood prolactin, incidence between 50-80% have been reported for SD and 25-40% for Long Evans. It is of note that both prolactin and estrogen are essential for the development of mammary tumors. In the specific case of atrazine and triazines in general, the proposed mechanism of compound-induced mammary tumors in female SD is that these cpds seem to accelerate the onset of senescence and the consequent persistant elevation in estrogen over a prolonged period leading to increased incidence and/or early onset of mammary tumors. The following was met (were observed) for the atrazine-induced tumors to qualify for the proposed mechanism of early senescence:

1. No intrinsic estrogenic activities,
2. Not mutagenic or genotoxic cpd,

3. Tumors develop only at very high doses close to or exceeding MTD,
4. In normally aging rats, tumors develop predominantely at ≥20 month of age, with many rats not developing tumors,
5. Only in female SD rats, a strain with high background spontaneous mammary tumors incidence (mean control incidence of 44% at 2yrs of age),
6. Histopathologically, these tumors are qualitatively identical to those in untreated rats,
7. Initial palpation of masses showed earlier onset of developing tumors "but not necessarily final higher incidence",
8. Female rats with high incidence of estrus tend to develop mammary tumors earlier than those with normal cycles that develop these tumors later in life,
9. Decline in LH surge and prolonged "elevation" in estrogen secretion i.e. loss of cyclicity in hormone secretions with age.

Of the above, RS-zop meets #s 1, 3, and 6 and part of #9 where LH levels were shown to decline with treatment but effects on estrogen were inconsistent. For #2, RS-zop is a clastogen so is S-zop and its active desmethyl metabolite, #5 can not be verified since RS-zop has been tested only in SD, #7 palpation incidence can not be verified but clearly, tumor incidence at end of study was significantly higher than in controls, #8 can not be validated at this time and, #9 though decline in LH levels have been demonstrated, elevation in estrogen have not.

In conclusion, results to date from all mechanistic studies, only partially support the theory of mammary gland tumors induced as a result of early onset of reproductive senescence. Therefore, we are left with the following profile of RS-zop:

- **Incompletely explained mammary tumors in the rat,**
- **Positive clastogenic response in *in vitro* mammalian assays for RS-zop, S-zop, and the active S-desmethyl zop,**
- **Marked reproductive toxicity and adeverse effects on male and female rat fertility,**
- **Zaleplon and zolepidem, 2 GABA agonistis of the same class as zopiclone, caused estrus cycle disturbances but neither drug induced mammary tumors or any relevant tumors in 2yr carcinogenicity studies.**

The original theory of drug-induced persistent elevation in estradiol could not be fully supported by the results from studies specifically designed to address this proposal with only 1 or 2 of the studies in support of this mechanism (this conclusion is agreed to by the sponsor). Data were highly variable and not all hormones were affected in the expected manner. It should be noted that this theory would have had a human relevance if proven. On the other hand, the 2nd mechanism of drug-induced mammary tumors seem to have more supportive findings for estrus cycle disturbances. Dr. Cooper of the EPA used the mechanism of early onset of reproductive senescence applicable to atrazine-induced mammary tumors, as the working hypothesis for RS-zop induced tumors. Similarly, studies specifically designed to address this effect were done and the 2 studies in ovariectomized rats showed marked decrease in LH surge and effect on prolactin level. However, not all of the results in these 2 studies agreed to this conclusion. The interim 6month results of the recently terminated 1yr study showed some reasonable evidence in support of the early senescence mechanism of action. Nevertheless, lack of dose response, failure of the positive control to induce its anticipated effect, and absence of correlation of serum estradiol in these rats (not done), made accurate conclusion

difficult. However, this study could provide adequate explanation if correlation between persistent estradiol elevation/absence of cyclicity in rats demonstrated to have early senescence and, early onset of mammary tumors is observed at end of 1yr. These 2 factors are the essences of the mechanism of atrazine-induced early onset of reproductive senescence with the consequent tumor promotion. It is however, unfortunate that this 1yr study is only a cytology investigation and the protocol does not include assessment of these parameters.

LIVER FINDINGS IN 18 MONTH RAT CHRONIC TOXICITY STUDY:

In addition to the thyroid findings in this study (Sepracor# 190-831f), liver hyperplastic nodules were also reported. RPR consulted in 1983 Dr — to review these liver findings in addition to the thyroid lesions (see Special Toxicology Studies Sepracor# 190-858). Unlike the thyroid report where he reviewed the data from the study, for the liver he was provided by RPR selected liver tissue slides from 12 males reported by — pathologist or Dr. — to have hepatic neoplasia. Dr. — re-reading and evaluation of these slides agreed with Dr. — conclusion that the few "foci of cellular alterations" were over diagnosed as adenoma/neoplastic nodules. The findings of 5 lesions in HDm vs. 1 in control male rats in his opinion, are sporadic variations of the spontaneous incidence of these tumors in aging rats. Moreover, and as shown by Dr. — , absence of increase in foci of cellular alteration in these rats supports the conclusion that RS-zop is not a true hepatocarcinogen.

Sepracor also requested the opinion of Dr. — on the liver findings (Sepracor# 190-859). He was provided with slides from 12 animals identified by the original pathologist as having liver lesions. Similar to Dr — above, he concluded that based on the data provided to him from the 18month study, RS-zop does not seem to be a liver tumor promoter. This conclusion was also based on his understanding that RS-zop was not a mutagen in the assays conducted at that time. He did however, recommend the drug be tested in the 2yr bioassay to further support this conclusion. Dr — in his report referred to some issues that made his assessment difficult where — processed different number of liver sections from rats in different groups with more slides in control and HD than in low and mid dose groups. He continued by stating that partly due to this, additional 3 standard slides per group were prepared from all rats killed at end of study. However, this created some inconsistencies he noted such as his doubts that the 3 additional slides processed from rat#62034came from the same animal since the findings reported in the original slides from this rat (infiltration by mononuclear cells indicative of adenoma), were completely absent from the current 3 sections.

<u>It is of note that hepatic tumor findings were not further addressed or investigated specially since no such tumors were observed in the subsequently conducted 2yr carcinogenicity study. Based on results from other toxicity and mechanistic studies, RS-zop as well as S-zop are found to be liver enzyme inducers in the rat.</u>

SUMMARY AND CONCLUSION FOR SKIN TUMORS IN MALE MICE

Incidence of skin sarcoma was significantly increased in male mice orally administered 100mg/kg/d RS-zop via the diet for 2yrs, compared to the control incidence. Re-evaluation of tumors by the Pathology

Working Group did not change the number of skin tumors in male mice reported in the original analysis. Therefore, the increase in skin sarcomas in high dose male mice remained statistically significant as agreed to by both the sponsor and our statistician. Consultants agreed that the incidence of s.c. sarcomas in the 100mg/kg/d RS-zop in male mice was unusually high and exceeded several historical data bases, that there was a good correlation between the incidence of dorsal tumors and incidence and duration of dorsal bites leading to the conclusion that fighting causes tumors in the subcutis of male B6C3F1 mice. This fighting behavior in RS-zop is paradoxical phenomenon of long term exposure to high dose of RS-zop. RS-zop seemed to modify the animal's fighting behavior increasing aggressiveness of these mice leading to biting and mutilation with consequent lesion formation. Therefore, the fibrosarcoma in male mice is not considered a direct effect of RS-zop but indirect mediated by increased aggressiveness and biting. The proposal that RS-zop increased aggressiveness in male B6C3F1 mice housed in a group setting was tested in a 2nd study were male mice were housed individually and others 4/cage and RS-zop administered at 100mg/kg/d RS-zop, appropriate control groups were also included. Results indeed showed absence of encrustations and skin tumors in single-housed males and their presence in group-housed mice. However, considering the incidence for both parameters in the control group housed mice vs. the RS-zop group housed mice, remained higher in the drug group compared to the control. This led to conclude that a drug effect can not be ruled out and that the drug may be acting as a promoter (i.e. in presence of wounds would RS-zop promote them to develop into tumors?). Having said all this, there are no reports in the literature to indicate that RS-zop increases aggressiveness in patients taking the drug therefore, the skin findings in male B6C3F1 mice may be considered irrelevant to humans though a promotr mechanism can not be ruled out.

SUMMARY AND CONCLUSION FOR PULMONARY TUMORS IN FEMALE MICE

Incidence of lung carcinoma was significantly increased in female mice orally administered 100mg/kg/d RS-zop via the diet for 2yrs, compared to the control incidence. Later re-analysis of these tumors reclassified the carcinomas reducing their number and, consequently the incidence fell within the historical range (2/52 vs. 0/104 in control originally the number in HD was 4/52). Statistical analysis by our statistician using pair-wise comparison of high dose and controls for combined tumors (adenomas and carcinomas) still showed statistical significance (p=0.0063); this finding is also reached by the sponsor. Although the statistics is still significant, this reviewer believes that these tumors are of no toxicological impact based on the following (but are drug related):

- Morphologically these tumors are similar to spontaneous tumors,
- They are usually common tumors,
- Incidence within historical range of NTP data base,
- Observed only in one sex,
- They were not fatal, and,
- Did not metastasize.

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/s/

Aisar Atrakchi
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Executive CAC
Date of Meeting: **November 25th 2003**

Committee: David Jacobson-Kram, Ph.D., HFD-024, Chair
Abby Jacobs, Ph.D., HFD-540, Alternate Member
James Farrelly, Ph.D., HFD-530, Alternate Member
Barry Rosloff, Ph.D., Team Leader
Aisar Atrakchi, Ph.D., Presenting Reviewer

Author of Minutes: Aisar Atrakchi, Ph.D.

The following information reflects a brief summary of the Committee discussion and its recommendations. Detailed study information can be found in the individual review.

NDA # **21-476**
Drug Name: **Estorra/Eszopiclone/S-isomer of RS-zopiclone**
Sponsor: **Sepracor Pharmaceuticals**
Marlborough, MA 01752

Background:

S-zopiclone is the pharmacologically active isomer of RS-zopiclone. It is a non-benzodiazepine $GABA_A$ agonist proposed as a short acting hypnotic. Rat and mouse dietary carcinogenicity studies have been previously conducted with RS-zopiclone at 1, 10, and 100mg/kg/d doses for both species. The results showed four tumor types at the high dose in both species and both sexes as follows: thyroid tumors in male rat, mammary gland tumors in female rat, lung tumors in female mice, and skin tumors in male mice. On October 2nd 2001 the Executive CAC reviewed these studies and recommended that lifetime bioassays be conducted with the S-zopiclone. These studies have recently been completed and final reports submitted to the Division. In addition, the sponsor conducted a P53 mouse alternative bioassay and provided the final report for evaluation.

Mouse Carcinogenicity Study:

S-zopiclone was administered daily via oral gavage to male and female CD-1 mice at 25, 50, and 100mg/kg/d for 97-104weeks and standard parameters were evaluated. In addition, TK of S-zopiclone and its active metabolite, S-desmethyl zopiclone was determined in satellite groups on wk25 of study. There were no drug related findings in any of the measured toxicological parameters. S-zopiclone did not cause a significant increase in tumors except for an increase in the incidence of malignant uterine leiomyosarcomas seen in females dosed at 100mg/kg/d. This finding was not considered to be drug related but rather a random occurrence. Exposure was measured for both the parent compound and the S-desmethyl zopiclone metabolite; the AUC for S-zopiclone was 17,608 and 17,095ng.hr/ml in males and females respectively. These values represent approximately 43 times the exposure in humans at the maximum recommended human dose (MRHD) of 3mg/d. In absence of any significant drug related finding, it is concluded that the MTD was not achieved in this study and the 25 fold human exposure multiples can not be used as basis for dose selection because S-zopiclone tested positive in *in vitro* mammalian mutagenicity assays.

Rat Carcinogenicity Study:

S-zopiclone was administered daily via oral gavage to male and female SD rats at 2, 4, 8, and 16mg/kg/d for 97-104weeks and standard parameters were evaluated. In addition, TK of S-zopiclone and its 2 metabolites S-desmethyl and N-oxide were determined on weeks 4, 24, and 56 of study and significant exposure to all three metabolites was demonstrated. The drug caused a significant decrease in survival in both sexes at the highest dose of 16mg/kg/d. There were no other significant drug related findings in any of the measured parameters and S-zopiclone did not induce a significant increase in any tumor type up to 16mg/kg/d. Exposure to S-zopiclone at 16mg/kg/d as measured by AUC on week 56 was 3980 and 17,000ng.hr/ml in males and females respectively, representing 10 and 42 times the exposure in humans at the MRHD of 3mg/d. It is concluded that the MTD was reached in this study based on decreased survival in both male and female rats at the highest dose of 16mg/kg/d and the study was considered as adequate.

p53 Mouse Carcinogenicity Study

S-zopiclone was administered daily via oral gavage to male and female p53 C57BL/6 haploinsufficient mice at 100, 200, and 300mg/kg/d for 26wks and standard parameters were evaluated. In addition TK analysis of S-zopiclone and its desmethyl metabolite were measured at end of study. Two positive control groups were included: p-cresidine in corn oil and p-cresidine in 0.5% CMC vehicle in addition to control vehicle and untreated control groups. S-zopiclone caused a 42-58% decrease in mean weight gain in male and 75-82% decrease in female mice in all 3 dose groups compared to vehicle control values. No other drug related findings observed in females. In males, changes were observed in the absolute and relative weight of the epididymides in all S-zopiclone groups in addition to histopathological findings in 300mg/kg/d group. There were no drug related tumors in either sex up to 300mg/kg/d, the positive control induced the expected tumors in the urinary bladder. Toxicokinetics revealed that mice were exposed to both the parent and desmethyl metabolite with exposures generally higher in females than males. It was concluded that oral administration of S-zopiclone to p53 haploinsufficient mice did not cause tumors up to 300mg/kg/d administered for 26weeks. This dose produced exposures of 9374 and 10,656ng.hr/ml in males and females respectively (equivalent to 23 and 26 times the MRHD of 3mg/d).

Executive CAC Recommendations and Conclusions:

The committee concluded that the rat carcinogenicity study is adequate based on MTD reached in both sexes. The mouse carcinogenicity study was found inadequate as the MTD was not achieved in either sex and exposure multiples could not be used as basis for dose selection because S-zopiclone was clastogenic in in vitro mammalian assays. However, the committee found the P53 mouse alternative assay to be adequate therefore, assessment of the drug's carcinogenic potential in the mouse has been fulfilled. For all three studies, the committee concurred that there were no drug-related findings up to the doses tested.

David Jacobson-Kram, Ph.D.
Chair, Executive CAC

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/Division File, HFD-120
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