CENTER FOR DRUG EVALUATION AND RESEARCH

APPROVAL PACKAGE FOR:

APPLICATION NUMBER
20-825

Pharmacology Review(s)

PHARMACOLOGY/TOXICOLOGY MEMORANDUM TO NDA 20-825

Date: 9/8/00
Reviewer: Lois M. Freed, Ph.D.
Drug: ziprasidone
Sponsor: Pfizer Inc
Indication: schizophrenia
Re: reconsideration of genotoxicity studies and related labeling.

It is Dr. DeGeorge's opinion that the Mutagenesis section of labeling should be revised to (1) remove discussion of the inadequacy of the genotoxicity battery and (2) strengthen the description of the positive findings. There is no serious objection to either of these recommendations, except regarding the *in vivo* micronucleus assay. The general inadequacy of the genotoxicity battery performed by the sponsor was discussed in detail in the original NDA review. In all but the *in vivo* micronucleus assay, the results were positive. Repeating the positive assays might provide additional information, particularly in the case of the Ames test; however, a repeat assay, if negative, would not negate the original positive result. The *in vivo* micronucleus assay was inadequate and negative; therefore, this study should be repeated as a Phase 4 commitment.

Based on these considerations, the Mutagenesis section should be revised as follows:

Mutagenesis

Draft

As a consequence of these revisions to labeling, the Pharmacology/Toxicology section of the Approval letter should be revised as follows:

___1___ pages redacted from this section of the approval package consisted of draft labeling

MEMORANDUM

DIVISION OF CARDIO-RENAL DRUG PRODUCTS CONSULTATION

To: Russell Katz, M.D., Director
Division of Neuropharmacological Drug Products, HFD-120

From: John Koerner, Ph.D., Pharmacologist
Division of Cardio-Renal Drug Products, HFD 110 JEK 8-23-00

Through: Charles Resnick, Ph.D., Supervisory Pharmacologist
Division of Cardio-Renal Drug Products, HFD 110 CAR 8-24-00

Raymond Lipicky, M.D., Director
Division of Cardio-Renal Drug Products, HFD 110 RL 8/24/00

Re: NDA #20825
Sponsor: Pfizer
Drug: Ziprasidone
Drug Class: Antipsychotic

Date of Consultation Request: 08/08/2000
Date Completed: 08/23/2000

Documents Evaluated
Section 19 of major amendment (dated 03/10/2000) provided in response to the nonapprovable letter (dated 06/17/1998).

Background
The present submission addresses effects of ziprasidone and its human metabolites (M1, M2, M9 and M10) on the delayed rectifier current (IKr) in mouse atrial tumor myocytes (AT-1 cells) and action potential duration in canine Purkinje fibers. These data were provided in response to questions (see nonapprovable letter dated 06/17/1998) regarding clinical findings of QT prolongation in clinical trials. The preclinical studies are summarized briefly below.

Preclinical Data Summary

IKr Inhibition

Ziprasidone and M10 were evaluated for effects on the delayed rectifier current (IKr) in mouse atrial tumor myocytes (AT-1 cells). Ziprasidone was evaluated at 10 and 100 nM, while M10 was evaluated at 0.32 and 3.2 μM. D-sotalol served as a positive control, and was evaluated at 10 and 100 μM. 1% DMSO in glacial acetic acid served as vehicle control. Additional comparators were the atypical antipsychotics, risperidone and olanzapine.

Ziprasidone and M10 inhibited IKr in AT-1 cells in a concentration-related manner. Concentration-related inhibition of IKr was also observed with risperidone, olanzapine and d-sotalol. The vehicle control did not inhibit IKr. Although ziprasidone inhibited IKr by less than 50% at the concentrations evaluated, the highest concentration evaluated was 100nM, which was at least 10-fold lower than the highest concentrations of the other drugs evaluated, and the complete concentration-response relationship was not characterized. Ziprasidone would likely inhibit IKr to a greater extent at higher concentrations. Ziprasidone appears more potent than risperidone and olanzapine on IKr.

Key:- Compound A = risperidone, Compound B = M10, Compound C = d-sotalol, Compound D = ziprasidone and Compound E = olanzapine.

Table 1. Inhibition of I_{Kr} by compounds A-E at the highest concentration tested. The amplitude of the tail current before and after compound is given for individual cells as well as individual percentage reduction values.

	Control I_{Kr} (pA/pF)	I_{Kr} after drug (pA/pF)	Reduction (%)	mean ± S.E.M. (%)
Compound A Risperidone 1μM	Cell #1 2.7 Cell #2 2.2 Cell #3 2.0	Cell #1 1.1 Cell #2 0.9 Cell #3 0.9	59.3 59.1 55.0	57.7±1.1
Compound B M10 3.2μM	Cell #1 2.8 Cell #2 3.4 Cell #3 2.0	Cell #1 1.8 Cell #2 2.6 Cell #3 1.5	35.7 23.5 25.0	28.1±3.1
Compound C D-Sotalol 100μM	Cell #1 6.1 Cell #2 3.2 Cell #3 3.5	Cell #1 1.4 Cell #2 1.0 Cell #3 0.7	77.0 68.8 80.0	75.3±2.7
Compound D Ziprasidone 100nM	Cell #1 6.4 Cell #2 2.8 Cell #3 4.0	Cell #1 3.9 Cell #2 2.2 Cell #3 3.4	39.1 21.4 15.0	25.2±5.9
Compound E Olanzapine 3.2μM	Cell #1 1.9 Cell #2 3.9 Cell #3 3.3	Cell #1 1.4 Cell #2 3.2 Cell #3 2.7	26.3 17.9 18.2	20.8±2.2

Table 2. Inhibition of I_{Kr} by compounds A-E at the lowest concentration tested. The amplitude of the tail current before and after compound is given for individual cells as well as individual percentage reduction values.

	Control I_{Kr} (pA/pF)	I_{Kr} after drug (pA/pF)	Reduction (%)	mean ± S.E.M. (%)
Compound A Risperidone 0.1μM	Cell #1 4.2 Cell #2 2.4 Cell #3 6.1	Cell #1 3.8 Cell #2 1.7 Cell #3 4.2	9.5 29.2 31.1	23.3±5.6
Compound B M10 0.32μM	Cell #1 6.1 Cell #2 5.2 Cell #3 4.4	Cell #1 4.9 Cell #2 4.4 Cell #3 2.4	19.6 15.4 45.4	26.8±9.4
Compound C D-Sotalol 10μM	Cell #1 2.4 Cell #2 3.6 Cell #3 3.5	Cell #1 1.8 Cell #2 2.5 Cell #3 3.1	25.0 30.6 11.4	22.3±4.7
Compound D Ziprasidone 10nM	Cell #1 5.3 Cell #2 3.2 Cell #3 3.6	Cell #1 4.1 Cell #2 2.7 Cell #3 3.3	22.6 15.6 8.3	15.5±3.4
Compound E Olanzapine 0.32μM	Cell #1 3.5 Cell #2 3.2 Cell #3 1.8	Cell #1 3.2 Cell #2 2.9 Cell #3 1.6	8.6 9.4 11.1	9.6±0.8

Table 3: Effects of 1% DMSO + glacial acetic acid (GAA)

Control I_{Kr} (pA, 0 min)	I_{Kr} after DMSO +GAA (pA, 30 min)	Reduction (%)	Mean ± SEM (%)
Cell#1 1741.7 Cell#2 280.6 Cell#3 2211.1	Cell#1 1224.4 Cell#2 323.8 Cell#3 2190.8	29.7 -15.4 0.9	5.1±13 %

Action potential duration

Effects of ziprasidone and its human metabolite, M10 on action potential duration (APD90) were determined in canine Purkinje fibers. Purkinje fibers were stimulated at 1 Hz and exposed sequentially to vehicle, low, middle and high drug concentrations after a 20 minute equilibration period (40 minutes per concentration, n=4/drug treatment group). D-sotalol served as a positive control.

Ziprasidone did not affect APD90 at the concentrations evaluated. In contrast, M10 prolonged APD90 in a concentration-related manner, similar to effects seen with d-sotalol. Neither ziprasidone, M10 nor d-sotalol affected other action potential characteristics (maximum upstroke velocity, action potential amplitude and resting membrane potential).

Although ziprasidone did not prolong APD90, the highest concentration evaluated was only 100nM, and a complete concentration response relationship was not determined. Consequently, lack of effect on APD90 is not reassuring. An additional limitation of this study is the lack of evaluation of drug effects at lower stimulation frequencies, since APD90 prolongation with IKr blocking drugs is generally greater at lower than at higher stimulation frequencies.

Effects of ziprasidone, M10 and d-sotalol on APD90. Results are mean ± S.E.M., percentage change from pre-dose values (n=4) taken 40 minutes after exposure to each concentration.

Concentration (nM)	Time matched vehicle	Ziprasidone	Time matched vehicle	M10	Time matched vehicle	D-Sotalol
10	1.0 ± 0.5	2.0 ± 1.4			-	-
32	-1.0 ± 1.3	2.9 ± 1.9			-	-
100	1.7 ± 1.9	6.9 ± 2.6			-	-
320	-	-	-1.8 ± 1.7	1.7 ± 0.9		
1000	-	-	-1.4 ± 3.3	3.7 ± 1.1	-0.4 ± 1.0	3.2 ± 1.6
3200	-	-	-0.5 ± 2.5	14.7 ± 1.3*	-	-
10000	-	-			1.6 ± 1.3	25.3 ± 3.8*
100000	-	-			2.4 ± 1.7	62.6 ± 5.7*

vehicle for ziprasidone and M10 =acidified 1% DMSO in physiological buffer
vehicle for d-sotalol = physiological buffer
* P<0.01 Student's t-test for unpaired data

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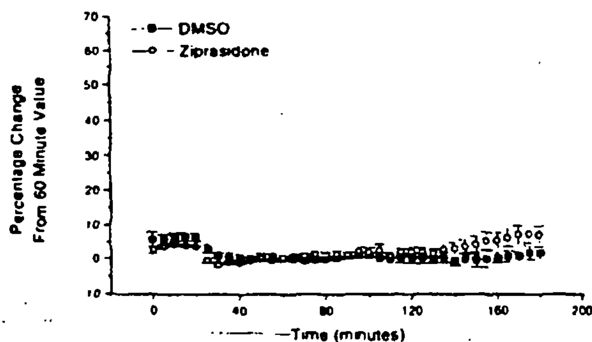

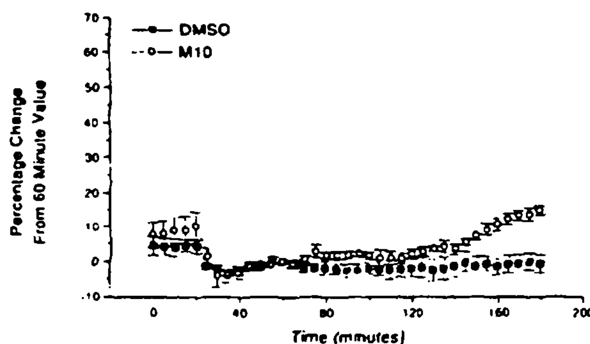

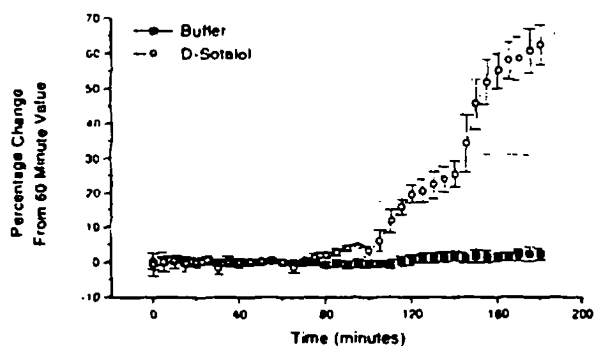

The effects of ziprasidone (upper panel), M10 (middle panel) and d-sotalol (lower panel) on APD90. Results are expressed as the percentage change from the 60 minute value against time. Each data point represents the mean ± SEM for the 4 separate preparations. Solution changes occur at 20, 60, 100 and 140 minutes as marked by the vertical lines. Compound is applied for the first time at 60 minutes (low concentration: 10, 320 and 1000 nM for each panel, respectively) then at 100 minutes (middle concentration: 32, 1000 and 10000 nM for each panel, respectively) and at 140 minutes (high concentration: 100, 3200 and 100000 nM for each panel, respectively).

Effects of risperidone, olanzapine and d-sotalol on action potential duration (APD90) were determined in canine Purkinje fibers. Effects of the human ziprasidone metabolites M1, M2 and M9, and the risperidone metabolite, 9-OH-risperidone were also evaluated for effects on APD90 in this assay system. Purkinje fibers were stimulated at 1 Hz and exposed sequentially to vehicle, low, middle and high drug concentrations after a 20 minute equilibration period (40 minutes per concentration, n=4/risperidone and olanzapine groups and n=3 for the d-sotalol group).

Risperidone prolonged APD90 in a concentration-related manner, similar to effects seen with d-sotalol. M9 and 9-OH-risperidone also prolonged APD90 in canine Purkinje fibers in a concentration-related way. Olanzapine did not affect APD90. None of the agents (with the exception of d-sotalol) affected other action potential characteristics (maximum upstroke velocity, action potential amplitude and resting membrane potential); d-sotalol at 100 μM reduced resting membrane potential slightly (data not shown).

Effects of risperidone, olanzapine and d-sotalol on the action potential duration at 90% repolarisation (APD_{90}). Results are mean ± S.E.M., percentage change from pre-dose values (n=4, n=3 for d-sotalol) taken 40 minutes after exposure to each concentration.

Concentration (nM)	Time matched vehicle	Risperidone	Time matched vehicle	Olanzapine	D-Sotalol
10	-	-			-
32	-	-			-
100	0.6 ± 0.9	8.1 ± 1.4			-
320	1.1 ± 0.9	26.1 ± 3.8*	0.6 ± 0.9	0.7 ± 0.5	
1000	1.7 ± 1.1	57.3 ± 11.0*	1.1 ± 0.9	2.2 ± 0.5	4.5 ± 1.1
3200	-	-	1.7 ± 1.1	6.8 ± 2.6	-
10000	-	-			26.5 ± 2.4
100000	-	-			73.6, 63§

vehicle for risperidone and olanzapine = 1% acidified DMSO in physiological buffer
vehicle for d-sotalol = physiological buffer
§ = individual values given as n=2 for 100 μM d-sotalol as impalement was lost before 40 minutes exposure to compound had occurred
* P<0.01 ANOVA vs time-matched vehicle

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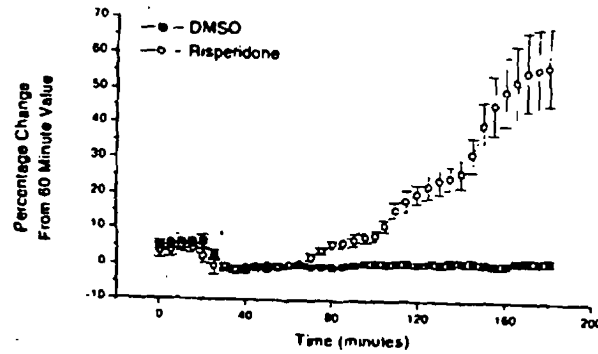
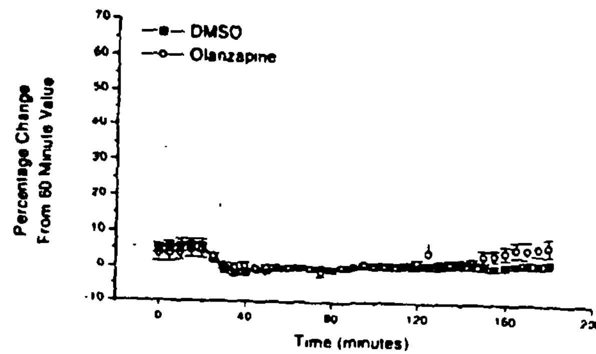
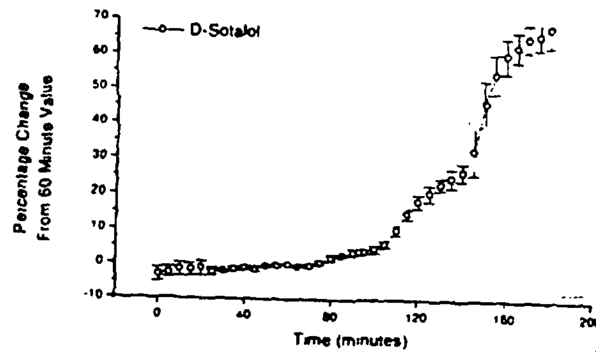




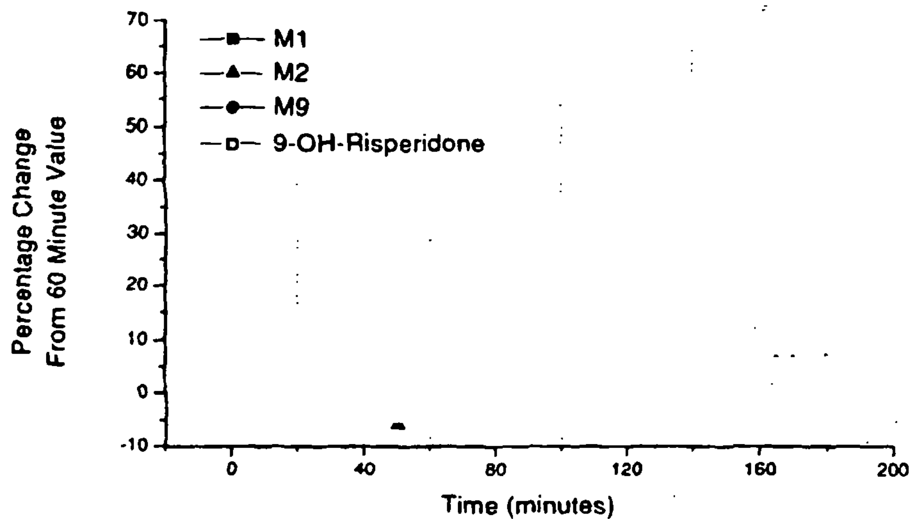


The effects of risperidone (upper panel), olanzapine (middle panel) and d-sotalol (lower panel) on APD90. Results are expressed as the percentage change from the 60 minute value against time. Each data point represents the mean ± SEM for the 4 separate preparations (n=3 for d-sotalol, n=2 for 100 μM). Solution changes occur at 20, 60, 100 and 140 minutes as marked by the vertical lines. Compound is applied for the first time at 60 minutes (low concentration: 100, 320 and 1000 nM for each panel, respectively) then at 100 minutes (middle concentration: 320, 1000 and 10000 nM for each panel, respectively) and at 140 minutes (high concentration: 1, 3.2 and 100 μM for each panel, respectively).

Effects of M1, M2, M9 and 9-OH-risperidone on the action potential duration at 90% repolarisation (APD_{90}). Results are percentage change from pre-dose values (n=1) taken 40 minutes after exposure to each concentration.

Concentration (nM)	M1	M2	M9	9-OH-Risperidone
320	0	2	7	14
1000	-1	3	20	41
3200	-1	1†	38	67

vehicle = 1% acidified DMSO in physiological buffer
† = data after 50 minutes exposure used owing to instability in recording at 40 minutes



The effects M1, M2, M9 and 9-OH-risperidone on APD90
Results are expressed as the percentage change from the 60 minute value against time. Each data point represents a measurement in a single preparation. Solution changes occur at 20, 60, 100 and 140 minutes as marked by the vertical lines. Compound is applied for the first time at 60 minutes (low concentration: 320 nM for each panel) then at 100 minutes (middle concentration: 1000 nM for each panel) and at 140 minutes (high concentration: 3200 nM for each panel). The final vertical line represents where the experiment is normally terminated.

Overall Summary and Evaluation

Ziprasidone and its human metabolites, M9 and M10, exhibited preclinical effects on IKr and action potential duration in cardiac tissues that are consistent with QT prolongation observed with ziprasidone in clinical trials.

Recommendations

None

CC: NDA 20-825

HFD-110 //120

HFD-110/JKoerner

HFD-110/CResnick

HFD-110/MGordon

HFD-110/DThrockmorton

HFD-120/Fitzgerald

HFD-120/Freed

HFD-120/Hardeman / Laughren

PHARMACOLOGY/TOXICOLOGY MEMORANDUM TO NDA 20-825

Date: 8/21/00
Reviewer: Lois M. Freed, Ph.D.
Drug: ziprasidone
Sponsor: Pfizer Inc
Indication: schizophrenia
Re: NDA Resubmission/Complete Response, submitted on 3/10/00.

The volume containing the sponsor's response to the pharmacology/toxicology deficiencies delineated in the Division's Non-Approvable letter (6/17/98) was received for review on 8/14/00. The Division's comments and the sponsor's response are provided below.

Division comments

"1. **Some of the assays that comprise the genotoxicity battery for ziprasidone appear to have had methodological limitations. Please refer to the following ICH Guidelines: Genotoxicity: A Standard Battery for Genotoxicity Testing of Pharmaceuticals and Specific Aspects of Regulatory Genotoxicity Tests for Pharmaceuticals.**

In the *in vitro* mammalian cell assays (mouse lymphoma gene mutation and human lymphocyte chromosomal aberration assays) there was test article precipitate at all doses. The nature of that precipitate needs to be defined, particularly given absence of precipitate in the Ames test. If this represents contamination or a methodological protlem, these tests may need to be repeated. In the *in vitro* chromosomal aberration assays, higher drug concentrations were needed in the presence of metabolic activation to produce the desired level of cytotoxicity (i.e., inhibition of mitotic index).

2. **Please submit full reports of the two *in vitro* studies which were conducted to examine potential cardiotoxicity [i.e., the canine Purkinje fiber assay and the rapidly activating delayed rectifier potassium channel assay, (IKr)] as soon as possible. Those reports should include methodology and data for all negative and positive controls and drug solubility data."**

Sponsor's response:

The sponsor addressed the two general issues, test article precipitation in the *in vitro* mammalian cell assays and the lack of cytotoxicity in the presence of metabolic activation in the *in vitro* chromosomal aberration assays.

The sponsor noted that the precipitate (ppt) detected in the *in vitro* mammalian cell assays was drug substance and not a contaminant based on (1) the low aqueous solubility of the compound and (2) the fact that a ppt was not detected in negative control samples. The sponsor pointed out that it is difficult to compare solubility among the various *in vitro* studies because of the differences in methodology, but did provide calculated data estimating a "...2-3 fold discrepancy in the minimum insoluble concentrations among the three *in vitro* tests conducted [i.e., Ames, *in vitro* gene mutation, *in vitro* chromosomal aberration assays].

In addressing the lack of cytotoxicity in the *in vitro* chromosomal aberration assay, the

sponsor noted that "...it is not uncommon to observe a modulatory role of metabolic activation systems in *in vitro* cytotoxicity assessments", but that "...upon re-examination of the data, there does not appear to be a notable difference in cytotoxicity profiles between the direct and indirect tests conducted using similar test article incubation times".

Reviewer comments: a comparison of the "information to the sponsor" as provided in the original NDA review (Review and Evaluation of Pharmacology/Toxicology Data, Original review of NDA 20-825, Lois M. Freed, Ph.D., 5/1/98) with that included in the Division's Non-Approvable letter (dated 6/17/98) indicates a marked discrepancy between the two versions. According to the NDA review, it was recommended that the following be relayed to the sponsor regarding the Genotoxicity studies:

> "The genotoxicity battery (cf. ICH Guideline, Genotoxicity: A Standard Battery for Genotoxicity Testing of Pharmaceuticals) should be repeated, addressing the methodological limitations of the original studies. The Ames test conducted was inadequate because (1) one of the following tester strains was not included: TA102, *E. coli* WP2uvrA, or *E. coli* WP2uvrA(pKM101) and (2) there was no evidence of cytotoxicity or insolubility at the high concentration for any tester strain tested. The mouse lymphoma gene mutation assay and the *in vitro* chromosomal aberration assays in human lymphocytes were inadequate due to the presence of precipitate at all concentrations tested. In addition, in the *in vitro* chromosomal aberration assays, higher drug concentrations should have been used in the presence of metabolic activation (the Mitotic Index was >50% of control at the high concentration tested). The *in vivo* chromosomal aberration assays were inadequate because (1) only one-half of the recommended number of metaphases per animal was analyzed and (2) only one dose level was used and that dose was not 2000 mg/kg (cf. OECD guidelines)."

The information actually relayed to the sponsor stated that the *in vitro* mammalian cell assays (i.e., mouse lymphoma gene mutation and human lymphocyte) might need to be repeated if the ppt detected was a contaminant or reflected a "methodological problem". The sponsor's response indicated that the ppt was not a contaminant, but a result of limited aqueous solubility. There was no documentation that attempts had been made to increase the solubility of the drug (e.g., by using other vehicles) in order to eliminate the ppt. Therefore, concerns regarding the presence of ppt in the *in vitro* mammalian cell assays have not been adequately addressed.

The wording of the Pharmacology section of the Non-Approvable did not accurately convey the concern regarding lack of cytotoxicity in the *in vitro* chromosomal aberration assays. The sponsor did address the statement made in the Division letter (i.e., the final sentence under Point #1, "In the *in vitro* chromosomal aberration assays, higher drug concentrations were needed in the presence of metabolic activation to produce the desired level of cytotoxicity..."), but, of course, not the actual issue of lack of cytotoxicity since this information was not conveyed. Similarly, the sponsor was not informed of the inadequacy of the Ames test or the *in vivo* chromosomal aberration assays.

In support of an NDA, the sponsor need only conduct a battery of genotoxicity tests as outlined in the ICH Guideline, "Genotoxicity: A Standard Battery for Genotoxicity Testing of Pharmaceuticals. Due to the methodological limitations of the genotoxicity assays conducted by the sponsor and described in the original information to the sponsor (NDA review), an adequate battery has not been conducted on ziprasidone. Therefore, the genotoxicity studies need to be repeated, but only to the extent that (1) the ICH recommended battery of tests be performed and (2) the sponsor is able to increase the solubility of ziprasidone. If it can be demonstrated that no

acceptable vehicle can be found to increase the solubility of ziprasidone, then that particular study need not be repeated.

2. The sponsor indicated that 4 reports, including the two requested, were submitted to NDA 20-825 on 5/20/99. Copies of these reports were also provided in the NDA resubmission.

Reviewer comments: these studies have been consulted to the Division of Cardio-Renal Drug Products for review.

A brief review of these studies indicated that ziprasidone and metabolite M10 were tested for I_{Kr} blocking potency in AT-1 cells (mouse atrial tumors) and ziprasidone and metabolites M1, M2, M9, and M10 were tested for effects on action potential duration (APD) using dog Purkinje fibers. In the I_{Kr} assay, ziprasidone (100 nM) and M10 (0.32-3.2 μM) inhibited I_{Kr} [IC_{50} = 25.2 ± 5.9 and 26.8 ± 9.4-28.1 ± 3.1%, respectively]. By comparison, risperidone (1 μM), olanzapine (3.2 μM), and d-sotalol (100 μM) inhibited I_{Kr} by 57.7 ± 1.1, 20.8 ± 2.2, and 75.3 ± 2.7%, respectively [the concentrations given represent the highest concentrations tested for each compound]. These data indicate that ziprasidone and one metabolite (M10) inhibited the I_{Kr} channel in this assay. It should be noted that neither compound was tested at sufficiently high concentrations. In one study in dog Purkinje fibers, ziprasidone (10-100 nM) had no effect on APD (APD_{50} or APD_{90}); however, metabolite M10 prolonged APD50 and APD_{90} by 15% at 3.2 μM, the highest concentration of M10 tested. As in the I_{Kr} assay, neither ziprasidone or M10 were tested at sufficiently high concentrations (or over a wide concentration range). In another study using dog Purkinje fibers, the effects of three human metabolites of ziprasidone (M1, M2, and M9) on APD were tested. M1 and M2 had no effect on APD at concentrations of 0.32-3.2 μM; M9 prolonged APD_{50} and APD_{90} in a concentration-dependent manner (17-20 and 30-38% at 1 and 3.2 μM, respectively). Ziprasidone and metabolites were not tested in the fourth *in vitro* study (conducted in dog Purkinje fibers)

The *in vitro* effects of ziprasidone and selected metabolites (M1, M2, M9 and/or M10) on I_{Kr} or APD were tested only to a limited extent, i.e., at a fairly low and narrow concentration range. Taken together, however, the data from the *in vitro* studies indicate that ziprasidone and certain metabolites (M9, M10) have the potential to prolong APD (QT) *in vivo*. M9 and M10 are S-methyl-dihydro and sulfoxide derivatives, respectively, of ziprasidone. [It should be noted that in the original NDA, M9 was identified as an oxide derivative, and it is unclear when the designation changed. The sponsor has been asked to clarify this apparent discrepancy and to verify that the S-methyl-dihydro derivative is present in the animal species/strains used for nonclinical testing.]

As noted previously, these data are under review by the Division of Cardio-Renal Drug Products. Based on a preliminary examination of the data, Dr. John Koerner (Pharmacologist, Division of Cardio-Renal Drug Products, HFD-110) indicated that these nonclinical data appear to be consistent with a prolongation of the QT interval in humans.

Recommendation: the sponsor should be asked to complete a standard battery of genotoxicity studies as a Phase 4 commitment, i.e., this is not a condition for NDA approval.

<u>The following information should be relayed to the sponsor</u>:

In the Division's Non-Approvable letter (6/17/98), the information regarding the genotoxicity data contained in the NDA was not complete. Each genotoxicity study provided in the NDA contained one or more deficiencies. Specifically,

(a) the Ames test did not include one of the tester strains, TA102, E. coli WP2uvrA, or E. coli WP2uvrA(pKM101), and there was no evidence of cytotoxicity or insolubility at the high concentration for any tester strain tested. You stated that concentrations "up to a concentration just below that which was insoluble in agar" were used. However, if insolubility is the concentration-limiting factor, some degree of precipitation needs to be demonstrated at the high-concentration for each tester strain, with and without metabolic activation.

(b) the mouse lymphoma gene mutation assay and the *in vitro* chromosomal aberrations assays were inadequate due to the presence of precipitate at all concentrations tested. You attributed this to the low aqueous solubility of ziprasidone under the conditions of each assay; however, there was no documentation that solubility could not have been increased by use of a different vehicle. You should explore additional vehicles in an attempt to increase the solubility of ziprasidone. If it can be documented that no appropriate vehicle can be found that will increase the solubility of ziprasidone under the specific conditions of the assay, the mouse lymphoma gene mutation assay (i.e., in which only the presence of precipitate was of concern) is acceptable.

(c) in the *in vitro* chromosomal aberration assays, higher drug concentrations should have been used in the presence of metabolic activation (the Mitotic Index was >50% of control at the high concentrations tested).

(d) the *in vivo* chromosomal aberration assays were inadequate because only one-half of the recommended number of metaphases per animal was analyzed and only one dose level was used (three dose levels are recommended if toxicity can be demonstrated, cf. OECD Guidelines).

Of the studies conducted, those comprising a standard battery of genotoxicity tests (cf. ICH Guideline, Genotoxicity: A Standard Battery for Genotoxicity Testing of Pharmaceuticals) should be repeated. They are not a condition for NDA approval, but need to be conducted as a Phase 4 commitment.

Lois M. Freed, Ph.D.

NDA orig (20-825)
div file
HFD-120
/GFitzgerald/LMFreed/SHardeman

GJF 8/22/00: agree that the standard genotoxicity battery must be completed as a Phase 4 commitment. Dr. Freed's comments should be included in the action letter.

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

DATE: May 12, 1998

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

TO: NDA 20-825
, ziprasidone hydrochloride
Sponsor: Pfizer, Inc.
20, 40, 60 and 100 mg capsules

SUBJECT: Approvability for Pharmacology and Toxicology

The pharmacology and toxicology studies submitted to this NDA for ziprasidone, indicated for the management of the manifestations of psychotic disorders, have been comprehensively reviewed by Dr. Lois Freed and are adequate to support an approvable action. This memo is intended only to highlight relevant nonclinical issues. Recommended labeling, which is a slightly modified version of Dr. Freed's recommended labeling, and comments for the letter to the sponsor are included.

As with other antipsychotic drugs, the mechanism of action of ziprasidone is unknown but it is proposed that it is mediated by its antagonist activity at serotonin type 2A and dopamine type 2 receptors.

Carcinogenicity

The results of two-year mouse and rat carcinogenicity studies were taken to the Carcinogenicity Assessment Committee - Executive Committee (CAC-EC) on April 28, 1998. The report of that meeting is not yet available. It was agreed that both studies were adequate and that there were dose-related increases in female mice in pituitary gland adenomas and carcinomas and mammary gland adenocarcinomas. The doses at which these tumors occurred were equal to or greater than the maximum recommended human dose of 200 mg/day on a surface area basis. A no-effect dose was not established. There was no increase in tumors in male mice or either sex of rat. Tumors of the mammary and pituitary glands (and also of the endocrine pancreas, not increased in these studies with ziprasidone) have been increased in studies with other

drugs of this class and are thought to be mediated by chronic exposure to elevated prolactin levels in rodents. The relevance to humans is unknown.

Reproduction Studies/Pregnancy Labeling

Although the sponsor has recommended Pregnancy Category C labeling based on increased numbers of rat pups born dead and decreased postnatal survival, there is no mention of teratogenic effects. However, it is the opinion of our reviewers[1] that there is sufficient evidence to consider ziprasidone to be a teratogen in rabbits even though none of the three teratogenicity studies conducted in that species would technically be considered a definitive one. In the first study (doses of 10, 30 and 60 mg/kg/day during organogenesis) there were not enough litters/fetuses available to evaluate due to a combination of dam mortality, reduced pregnancy rate and decreased viable litters. The study was repeated twice; once with two dose groups (10 and 30 mg/kg/day) and once with one dose group (30 mg/kg/day) rather than the usual three dose groups. However, in the first of those studies, ventricular septal defects associated with other cardiac defects occurred in 3 high dose fetuses from 3 different litters. In the second study, ectopic kidneys were observed, also in 3 fetuses from 3 different litters. These malformations were not associated with clear evidence for maternal toxicity, indicating a potentially direct effect on the fetus, and the incidences fell outside the historical control range for the particular strain/laboratory/time period. The fact that the findings were in each case from 3 separate litters adds strength to the signal. We have therefore concluded that ziprasidone is teratogenic in rabbits and have included the findings, which occurred at only 3 times the maximum recommended daily human dose on a surface area basis, in the pregnancy section of labeling, retaining the "C" category. Teratogenicity was not observed in the rat study.

Genotoxicity

The genotoxic potential of ziprasidone has not been well characterized. The sponsor has technically completed a full battery of assays, which include the following: 3 Ames tests, 3 *in vitro* mouse lymphoma mammalian cell mutation assays, 2 *in vitro* chromosomal aberration assays in human lymphocytes and 2 *in vivo* chromosomal aberration assays in mouse bone marrow. All of the assays fall short of current standards for methodology (see Dr. Freed's Summary and Evaluation section, pages 114 - 116, for a discussion of the flaws in each assay, which I shall not reiterate here). Given the limitations of the assays as they were conducted, it is difficult to draw conclusions about the mutagenicity or clastogenicity of ziprasidone. There are, however, some indications of a genotoxic effect. There was a repeatable positive response in one tester strain in the Ames test, and there were equivocally positive responses in the *in vitro* mammalian cell mutation assay and the *in vitro* chromosomal

1. Dr. J.E. Fisher examined the data as well as Dr. Freed

aberration assay. The *in vivo* assay for clastogenicity was not an adequate assay from which to draw any conclusions. Technically the sponsor has not fulfilled the ICH requirements for a genetic toxicology battery of studies to support an NDA. However, I agree with Dr. Freed's recommendation that the genetox studies should be repeated, but that this may be done in phase 4 (see her Recommendations, page 129 of her review). Although we will not be able to provide definitive labeling for the "Mutagenesis" section, the positive signals observed should be included until properly conducted studies are submitted. Also, we have the results of two lifetime carcinogenicity studies which provide reasonable assurance that ziprasidone is not a potent carcinogen, and that it is associated only with tumor types expected in rodents dosed with prolactin elevators, lessening the need for a more "definitive" genetox battery pre-marketing.

Cardiovascular Toxicity

No adverse effects on ECG (including QT interval) were reported for the 6 month and 1 year toxicology studies in dogs. When the measurements were taken in the 6 month study relative to dosing is not known; in the I year study, ECG's were taken pre-dose and at T_{max} (2 hours post-dose). Minimal safety pharmacology studies were conducted in 3 conscious dogs. An acute oral dose of 6 mg/kg was associated with up to a 60% increase in heart rate from 45 minutes post-dose on, with no effects on MAP or QA interval. QT interval was not measured. After reports of QTc prolongation in humans, two additional *in vitro* animal studies were requested by the Division of Cardio-Renal Drugs. A brief report of a canine Purkinjie fiber assay has been received, but no reports of an I_{kr} channel assay (rapidly activating delayed rectifier potassium channel) have been received in writing. Dr. Freed has requested full reports of both assays.

Recommendations

This NDA is approvable for Pharmacology and Toxicology. The following requests (see Dr. Freed's recommendations) should be transmitted to the sponsor in the approvable letter:

1. The assays which comprise the genotoxicity battery for ziprasidone are all inadequate because of methodological limitations. Please refer to the following ICH Guidelines: Genotoxicity: A Standard Battery for Genotoxicity Testing of Pharmaceuticals and Specific Aspects of Regulatory Genotoxicity Tests for Pharmaceuticals. We request that you commit to repeating those studies during phase 4, addressing the deficiencies of each, so that labeling may accurately reflect the genotoxic potential of ziprasidone. The problems with the conduct of the assays include, but are not necessarily limited to, the following points:

Ames test: A tester strain sensitive to cross-linking mutagens (TA 102 or E.coli) was not included in the assay; there was no evidence of cytotoxicity or insolubility at the high concentration for any strain tested and it is not clear why higher concentrations

were not tested.

In vitro mammalian cell assays (mouse lymphoma gene mutation and human lymphocyte chromosomal aberration assays): The effect of test article precipitate at all doses is unknown; higher drug concentrations were needed to produce the desired level of cytotoxicity/inhibition of mitotic index.

In vivo chromosomal aberration assay in mouse bone marrow: Only 50 rather than at least 100 metaphases per animal were analyzed; mitotic index should be determined in at least 1000 cells per animal; three dose levels, not one, should be used for the first sampling time which is 12 - 18 hours rather than 6 hours.

2. Please submit full reports of the two *in vitro* studies which were conducted to examine potential cardiotoxicity [i.e., the canine Purkinje fiber assay and the rapidly activating delayed rectifier potassium channel assay, (I_{Kr})] as soon as possible. Those reports should include methodology and data for all negative and positive controls and drug solubility data.

/S/

Glenna G. Fitzgerald, Ph.D.

NDA 20-825
c.c/ Div. File
Leber/Laughren/Freed//Fitzgerald/Fisher/Atrakchi/Hardeman

____4____ pages redacted from this section of the approval package consisted of draft labeling

REVIEW AND EVALUATION OF PHARMACOLOGY AND TOXICOLOGY DATA
Original Review of NDA

Date: April 30, 1998

Reviewer: Lois M. Freed, Ph.D.

NDA 20-825

Stamp Date: 3/19/97

PUDUFA Date:

Received: 3/21/97

Sponsor: Pfizer, Inc.
Eastern Point Rd
Groton, CT 06340

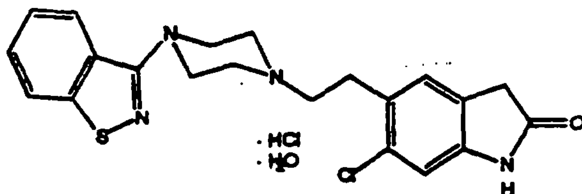
Drug: Ziprasidone hydrochloride

Code Name: CP-88,059-1

Pharmacologic Category: $5HT_{2A}$, D_2 receptor antagonist

Indication: psychosis

Structure:



Molecular weight: 467.42

Chemical Name: 5-[2-[4-(1,2-benzisothiazol-3-yl)-1-piperazinyl]ethyl]-6-chloro-1,3-dihydro-2H-indol-2-one, monohydrochloride, monohydrate

Drug Formulation: 20, 40, 60, and 80 mg capsules; inactive ingredients are lactose, pregelatinized starch, and magnesium stearate

Related IND: # [ ]

Previously Reviewed Studies:
Pharmacology (P/T review, J.J. DeGeorge, Ph.D., 4/13/90)
PK/ADME
rat, i.v., p.o. (P/T review, J.J. DeGeorge, Ph.D., 4/13/90)
dog, i.v., p.o. (P/T review, J.J. DeGeorge, Ph.D., 4/13/90)
tissue distribution (P/T review, J.J. DeGeorge, Ph.D., 4/13/90)

Toxicology

Acute: mouse, rat, p.o., i.p. (P/T review, J.J. DeGeorge, Ph.D., 4/13/90)

Subchronic:

- 2-wk i.v. study in Sprague-Dawley rats (P/T review, L.M.Freed, Ph.D., 12/31/92)
- 2-wk oral gavage study in Long-Evans rats (P/T review, L.M.Freed, Ph.D., 10/28/92)
- 1-mo gavage study in Long-Evans rats (P/T review, J.J. DeGeorge, Ph.D., 4/13/90)
- 2-wk i.v. study in Beagle dog (P/T review, L.M.Freed, Ph.D., 12/31/92)
- 1-mo gavage study in Beagle dog (P/T review, J.J. DeGeorge, Ph.D., 4/13/90)]

Chronic:

- 6-mo gavage study in Long-Evans (changed to Sprague-Dawley) rats (P/T review, L.M.Freed, Ph.D., 10/28/92)
- 6-mo gavage study in Beagle dog (P/T review, L.M.Freed, Ph.D., 2/16/93)
- 12-mo gavage study in Beagle dog (P/T review, L.M. Freed, Ph.D., 10/6/94)

Carcinogenicity

Summaries for range-finding studies (P/T review, L.M.Freed, Ph.D., 10/28/92)

- 3-wk, 1-mo in rat
- 1-mo, 3-mo in mice

Protocols for carcinogenicity studies in rat, mouse (P/T review, L.M.Freed, Ph.D., 10/29/92)

Reproduction

- Segment I in Sprague-Dawley rat (P/T review, L.M.Freed, Ph.D., 3/22/94)
- Segment II in Sprague-Dawley rat (P/T review, L.M.Freed, Ph.D., 3/22/94)
- Segment II in New Zealand white rabbit (P/T review, L.M.Freed, Ph.D., 3/22/94)

Genotoxicity

(P/T review, J.J. DeGeorge, Ph.D., 4/13/90)

- Ames test
- mouse lymphoma
- *in vitro* cytogenetic assay
- *in vitro* chromosomal aberration assay in human lymphocyte

<u>Reports</u>:

- Review and Evaluation of Pharmacology and Toxicology Data (original summary, J.J. DeGeorge, Ph.D., 4/13/90)
- Review and Evaluation of Pharmacology and Toxicology Data (L.M. Freed, Ph.D., 10/28/92)
- Review and Evaluation of Pharmacology and Toxicology Data (L.M. Freed, Ph.D., 12/31/92)
- Review and Evaluation of Pharmacology and Toxicology Data (L.M. Freed, Ph.D., 2/16/93)
- Pharmacology/Toxicology Memorandum to IND #[ ] (L.M. Freed, Ph.D., 8/17/93)
- Review and Evaluation of Pharmacology and Toxicology Data (L.M. Freed, Ph.D., 3/22/94)
- Review and Evaluation of Pharmacology and Toxicology Data (L.M. Freed, Ph.D., 10/6/94)

<u>Studies Reviewed (NDA submission)</u>:

Pharmacology (Vol 1.13)

PK/ADME (Vol 1.31-1.34)

Toxicology

subchronic:

- 2-wk oral gavage study in Beagle dog (Vol 1.14)

special:
antigenicity in guinea pigs (Vol 1.29)
dermal and ocular irritation studies (3) in rabbit (Vol 1.29)
exploratory prolactin study in rat (Vol 1.27)

Carcinogenicity
2-yr oral study in Long Evans rat (Vol1.23-1.26)
2-yr oral study in CD1 mice (Vol 1.18-1.21)

Reproduction
Preliminary Segment I study in Sprague-Dawley rat (Vol 1.27)
Maternal toxicity study in rat (Vol 1.28)
Maternal toxicity study in rabbit (Vol 1.28)
Segment II (Study III) in New Zealand White rabbit (Vol 1.28)
Teratology (Study III) in New Zealand White rabbit (Vol 1.29)
Reproductive study II prenatal and postnatal development in Sprague-Dawley rat (Vol 1.29)

Mutagenicity
Genetic Toxicology Report (Vol 1.30)

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ADDENDUM B: Statistical Review and Evaulation: Review of Carcinogenicity Data (Roswitha E. Kelly, Mathematical Statistician, HFD-710, 1/18/98)

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PHARMACOLOGY

Data on the pharmacology of ziprasidone were provided only in summary form; original reports were not provided. Therefore, the following summary is based solely on the sponsor's conclusions/interpretations and not on the review of original data.

Studies Related to Mechanism of Action

In vitro receptor binding

The *in vitro* receptor binding profile of ziprasidone was tested in membranes from a number of different species (rat, guinea pig, bovine, and cell lines). For the D_2 receptor, rat caudate and cloned human receptor in LTK cells were assayed. For the D_3 and D_4 receptor, cloned human receptors in CHO and COS-7 cell lines, respectively, were assayed. Serotonin receptor were tested using rat, guinea pig, and bovine brain tissue, a mouse NG-108 cell line ($5HT_3$), and rat recombinant membranes. Adrenergic receptors were tested using rat brain tissue and histamine receptors were tested in human cortex (H_1) and guinea pig brain tissue (H_2, H_3). Muscarinic receptors were tested in rat brain or cloned human receptors in CHO cells. Only the D_2 receptor was tested in more than one species (i.e., rat, human). The binding affinities (expressed as pK_i) for selected receptors (with data for comparaters) is provided in the following sponsor's tables:

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Table 1B: Receptor binding and neurotransmitter uptake inhibition profile for ziprasidone, risperidone and haloperidol

Receptor	pKi ± SEM (N) Ziprasidone	Risperidone	Haloperidol
Dopamine			
D1	6.28 ± 0.17(3)	6.37 ± 0.08(3)	6.67 ± 0.15(3)
D2	8.32 ± 0. 04(6)	8.43 ± 0.03(4)	9.15 ± 0.03(3)
D3*	8.14 ± 0.03(3)	8.02 ± 0.18(3)	8.61 ± 0.05(3)
D4*	7.49 ± 0.11(3)	8.07 ± 0.12(3)	8.48 ± 0.07(3)
Serotonin			
5HT2A	9.38 ± 0.03(5)	9.27 ± 0.04(4)	7.35 ± 0.13(5)
5HT1A	8.47 ± 0.05(4)	6.68 ± 0.07(3)	5.96 ± 0.05(3)
5HT2C	8.88 ± 0.05(6)	7.60 ± 0.06(7)	< 5 (3)
5HT1D	8.69 ± 0.04(6)	6.76 ± 0.05(5)	< 5 (3)
Adrenoceptors			
α1	7.98 ± 0.03(3)	9.13 ± 0.15(3)	8.20 ± 0.11(4)
α2	6.59 ± 0.08(5)	9.04 ± 0 14(4)	6.05 ± 0.05(4)
Histamine			
H1*	7.33 ± 0.07(3)	7.73 ± 0.09(3)	6.36 ± 0.09(3)

Values are mean pKi (-logKi) ± standard errors. Number of separate experiments in parenthesis.
*Denotes human receptor.
Assay conditions and species are described in Table 1A.

Table 1B (Continued): Receptor binding and neurotransmitter uptake inhibition profile for ziprasidone, risperidone, and haloperidol (Continued)

Neurotransmitter Reuptake Blockade:

Transmitter	pKi ± SEM (N) Ziprasidone	Risperidone	Haloperidol
Norepinephrine	7.30 ± 0.01(4)	< 5 (3)	5.33 ± 0.11 (3)
Serotonin	7.29 ± 0.06 (3)	5.88 ± 0.06 (3)	5.78 ± 0.02 (3)
Dopamine	6.58 ± 0.02 (3)	< 5 (3)	5.52 ± 0.04 (3)

Values are mean pKi (-logKi) ± standard errors. Number of separate experiments in parenthesis.

The affinity of ziprasidone for the D_2 receptor was similar to that of risperidone, but almost 7-fold less than that of haloperidol. The $5HT_2/D_2$ ratio (calculated as D_2 K_i)/$5HT_{2A}$ K_i) was ≈12. For comparison, the ratios for risperidone, clozapine, chlorpromazine, and haloperidol were 7.4, 5.5 (data not provided), 0.3 (data not provided), and 0.016, respectively.

The sponsor noted that "In preliminary studies...", ziprasidone was found to have "moderate" affinity for the $5HT_6$ and $5HT_7$ receptors (K_i = 130 and 23 nM, respectively). Ziprasidone was reported to have weak-to-no affinity (i.e., IC_{50} > 1μM) for other receptors/binding sites tested: "...ß-adrenergic,...5-HT3 and 5-HT4, acetylcholine muscarinic (m1, m2, m3, m4, m5 receptors) and nicotinic, opiate mu and sigma, benzodiazepine, adenosine A1, neurokinin-1 (NK-1), corticotrophin releasing hormone (CRH), cholecystokinin-A and B (CCK-A and CCK-B), and histamine H2 and H3 receptors".

Ziprasidone had moderate affinity for NE and 5HT reuptake sites (K_i ≈50 nM), and low affinity for the DA reuptake site (K_i = 260 nM).

The binding affinities of two ziprasidone metabolites, the sulfoxide and the sulfone, for selected receptors are summarized in the following sponsor's table:

Table 1C: Receptor binding studies with ziprasidone and ziprasidone metabolites at human dopamine and rat serotonin receptors

Compound	D2 pKi	D3 pKi	D4 pKi	5HT2A pKi
Ziprasidone	8.41	8.14	7.49	9.38
Ziprasidone sulfone	<5.40	<6	<5.29	5.97
Ziprasidone sulfoxide	6.11	<5.77	<5.29	6.82

Values are mean pKi (-logKi).

Neither metabolite had notable affinity for either the D_3 or D_4 receptors (i.e., K_i > 1 μM). The sulfone also had no appreciable affinity for the other receptors assayed. The sulfoxide exhibited weak affinity for the D2 receptor and moderate-to-low affinity for the $5HT_{2A}$ receptor (K_i = 780 and 150 nM, respectively). Ziprasidone exhibited 200-fold greater affinity for the D_2 receptor and 375-fold greater affinity for the $5HT_{2A}$ receptor compared to the sulfoxide metabolite.

In vitro functional assays

Monoamine reuptake and MAO activity: As noted in the previous section, ziprasidone was found to interact with monoamine reuptake sites. [Reuptake of NE, 5HT, and DA was tested in rat brain crude synaptosomal fractions.] Ziprasidone inhibited reuptake of all three monoamines, but was more potent at the NE and 5HT sites. By comparison, risperidone and haloperidol were inactive at all three uptake sites at concentrations up to 1 μM; amitriptyline (a tricyclic antidepressant) inhibited uptake of NE and 5HT with K_i's of ≈24 and 66 nM, respectively.

Ziprasidone did not inhibit MAO activity in rat brain or liver homogenates at concentrations up to 200 μM.

D_2 receptor: ziprasidone exhibited antagonist effects at the D_2 receptor as evidenced by inhibition of D_2 agonist (quinpirole)-induced attenuation of forskolin-induced stimulation of cAMP activity (K_i ≈ 1.3 nM). By comparison, risperidone also acted as an antagonist in this system, with a similar K_i (≈1.8 nM). Ziprasidone exhibited no agonist effects in this system.

D_1, $5HT_{1A}$, $5HT_{1D}$ receptors: activity at the D_1, $5HT_{1A}$, $5HT_{1D}$ receptors was tested in rat

striatum (D_1), in HeLa cells expressing human $5HT_{1A}$ receptors and in guinea pig hippocampus ($5HT_{1A}$), and in guinea pig substantia nigra ($5HT_{1D}$). Ziprasidone had the following effects:

1. ziprasidone ($\leq$10 μM) did not exhibit agonist activity at the D_1 receptor (i.e., no increases in adenylate cyclase activity), but did block dopamine-induced increases in adenylate cyclase activity ($K_i \approx$ 93 nM). Thus, ziprasidone exhibited antagonist effects at the D_1 receptor. Risperidone also exhibited antagonist effects at the D_1 receptor, with a $K_i \approx$ 140 nM.

2. ziprasidone exhibited agonist effects at the $5HT_{1A}$ receptor, inhibiting forskolin-induced increases in adenylate cyclase (i.e., agonist-induced decrease in adenylate cyclase activity at this receptor). The EC_{50} for this effect was ≈14-36 nM. Ziprasidone's effect was blocked by WAY-100,135, a $5HT_{1A}$ antagonist. Risperidone was inactive in this system at concentrations <1 μM. Ziprasidone exhibited similar potency at both the guinea pig and cloned human $5HT_{1A}$ receptors. Ziprasidone's maximal effect at this receptor was ≈80% of that of full agonist, 8-OH-DPAT at 10 μM.

3. ziprasidone exhibited no agonist activity at the $5HT_{1D}$ receptor (i.e., did not decrease forskolin-induced adenylate cyclase activity), but did inhibit serotonin-induced inhibition of forskolin-stimulated adenylate cyclase activity ($K_i \approx$ 0.3 nM). Thus, ziprasidone exhibited antagonist effects at this receptor.

$5HT_{2A}$, $5HT_{2C}$, and α_1 receptors: functional effects at the $5HT_{2A}$, $5HT_{2C}$, and α_1-receptors were tested in rat brain cortical slices ($5HT_{2A}$, α_1) or choroid plexus ($5HT_{2C}$) prelabeled with [^{3}H]-myo-inositol. Ziprasidone exhibited no agonist effects at the $5HT_{2A}$ or $5HT_{2C}$ receptors, but did inhibit 5HT-induced stimulation of [^{3}H]-IP_3 accumulation at both receptors ($K_i \approx$ 1.2 and 10 nM, respectively). By comparison, ketanserin, ritanserin, and risperidone antagonized this effect at the $5HT_{2A}$ receptor with K_i's of ≈0.7, 0.2, and 0.5 nM, respectively, and risperidone also antagonized this effect at the $5HT_{2C}$ receptor with a K_i of ≈79 nM. Ziprasidone inhibited NE-induced stimulation of [^{3}H]-IP_3 accumulation ($K_i \approx$ 7.9 nM). By comparison, prazosin and risperidone antagonized this effect with K_i's of ≈2.4 and 3.7 nM, respectively.

In vivo functional assays

Neurochemical studies: Ziprasidone was administered orally in two separate studies to determine its effect on dopamine and/or serotonin (and metabolite) levels in Sprague-Dawley rat brain. In the first study, male rats were given ziprasidone at doses of 0.32, 0, 3.2, and 10 mg/kg p.o. Ziprasidone produced a dose-dependent increase in forebrain levels of DOPAC ($\leq$3.2-fold) and HVA ($\leq$2.6-fold) at doses $\geq$3.2 mg/kg, with no changes in dopamine, serotonin, or 5-HIAA. In comparison, risperidone increased DOPAC and HVA levels a maximum of 5.6 and 3.7-fold, respectively, at 5.6 mg/kg and haloperidol increased these two metabolites by 4 and 5-fold, respectively, at a dose of 1 mg/kg p.o.

In the second study, ziprasidone was administered orally to freely-moving male rats at doses $\leq$10 mg/kg and dopamine release was measured in frontal cortex and striatum for up to 5 hr postdosing. At doses of 1, 3, and 10 mg/kg, ziprasidone increased dopamine release in the cortex by 60, 80, and 200%; in the striatum, dopamine was increased (40%) only at the highest dose. In both regions, the effect was rapid and in cortex, the increase in dopamine was noted throughout the measurement period. WAY 100,635 (a selective $5HT_{1A}$ antagonist) antagonized ziprasidone's effect in the cortex, but not the striatum. In the presence of WAY

100,635, ziprasidone increased dopamine release by 150% at all doses, suggesting a non-$5HT_{1A}$ component to the effect in the cortex. WAY 100,635 had no effect on dopamine release when given alone.

Electrophysiology: the effect of ziprasidone on the spontaneous firing rate of dorsal raphe 5HT neurons was tested in chloral hydrate-anesthetized Sprague-Dawley rats. $5HT_{1A}$ agonists and α_1 antagonists have been shown to decrease the spontaneous firing rate of these neurons. Ziprasidone inhibited spontaneous firing with an ID_{50} of 280 µg/kg i.v.; this effect was completely blocked by WAY 100,635, a $5HT_{1A}$ antagonist in 6 of 7 cells tested. The sponsor indicated that 8-OH-DPAT was tested in this system; however, data were not presented and results were not discussed for this compound.

Behavioral studies: all behavioral studies were conducted in male Sprague-Dawley rats. Ziprasidone, risperidone, and haloperidol were administered orally by gavage.

The data are summarized in the following sponsor's Table 2:

Table 2: Summary of the behavioral effects of ziprasidone in rats

Test	Ziprasidone	Risperidone	Haloperidol
Amphetamine Locomotor Activity ID_{50} (mg/kg)	1.53 (1.06 - 2.45)	0.44 (0.23 - 0.72)	0.14 (0.10 - 0.20)
Apomorphine Stereotypy ID_{50} (mg/kg)	2.43 (1.46 - 3.95)	1.80 (1.18 - 2.85)	0.25 (0.19 - 0.33)
Conditioned Avoidance ID_{50}(mg/kg)	2.59 (0.42 - 4.98)	1.58 (0.57 - 4.42)	0.93 (0.49 - 1.74)
Quipazine Head Twitch ID_{50} (mg/kg)	0.27 (0.12 - 0.42)	0.04 (0.03 - 0.06)	0.29 (0.18 - 0.55)
Spontaneous Locomotor Activity ID_{50} (mg/kg)	8.85 (5.26-19.05)	1.24 (.85 - 1.74)	0.68 (.27 - 1.41)
Catalepsy ED_{20sec} (mg/kg)	12.07 (9.69 - 15.06)	5.67 (3.67 - 8.76)	0.79 (0.43 - 1.46)

Values in parenthesis are 95% confidence limits.

Ziprasidone dose-dependently antagonized amphetamine- and apomorphine-induced behaviors. Both risperidone and haloperidol were more potent than ziprasidone in these paradigms. These compounds were also more potent than ziprasidone in inducing a minimal state of catalepsy (i.e., 20 secs of immobility).

In the CAR paradigm, ziprasidone inhibited avoidance responding, as did risperidone and

haloperidol. Unfortunately, effects on escape behavior were not tested, therefore, drug-effects on sensitivity to electric shock were not assessed. Ziprasidone did inhibit spontaneous motor activity, but at an ED_{50} almost 6-fold higher than that for antagonism of amphetamine-induced locomotor activity.

Ziprasidone dose-dependently inhibited quipazine-induced head twitches, a behavior thought to be mediated via $5HT_{2A}$ receptors. In this paradigm, ziprasidone was similar in potency to haloperidol, but almost 7-fold less potent than risperidone. Ziprasidone was ≈6-9 fold more potent in antagonizing $5HT_{2A}$-mediated behavior than D_2-mediated behavior *in vivo*.

General Pharmacology

In vitro studies: the effects of ziprasidone were tested in three isolated tissue preparations: guinea pig aorta (α_1; NE-induced contractions), guinea pig ileum (H_1; histamine-induced contractions), guinea pig atria (H_2; histamine-induced chronotropy), and rat uterus (oxytocin-induced contractions).

Ziprasidone exhibited concentration-dependent inhibition of NE-induced contractions in guinea pig aorta and histamine-induced contractions of the guinea pig ileum. Ziprasidone had no effect in the other paradigms at concentrations ≤10 μM.

In vivo studies: the effects of ziprasidone on overall behavior was tested in male CD-1 mice (3/grp) using the Irwin screen. Ziprasidone was administered orally (by gavage) at single doses of 0, 3.2, 10, 32, 100, 320, and 1000 mg/kg. Observations were made at 30, 60, and 120 min postdosing. Reduced respiration was noted at all doses. Ptosis was observed at doses ≥3.2 mg/kg. Inverted screen defect was noted at doses ≥10 mg/kg. Miosis, decreased urination, decreased spontaneous motor activity, and loss of tail pinch response were observed at doses ≥32 mg/kg. Reduced defecation was observed at doses ≥100 mg/kg, and complete immobility for 24 hr postdosing was noted in all animals at doses of 320 and 1000 mg/kg. Piloerection was noted only at the lower doses (1.0-32 mg/kg). There were no deaths at any dose.

Safety Pharmacology

Cardiovascular studies: the cardiovascular effects of ziprasidone were tested in 5 conscious mongrel dogs following a single oral dose of 6 mg/kg. Two male exhibited an emetic reaction upon dosing and were removed from the analyses. In the remaining 3 dogs, ziprasidone increased heart rate (≤60%) from ≈45 min postdosing on, but had no effect on MAP or QA-interval. According to the sponsor, plasma ziprasidone levels in these dogs were 0.5-0.8 μg/mL at 30-60 min postdosing.

Blood gas measurements: the effects of ziprasidone on acid-base balance were tested in male rats (strain not specified, 3 drug-treated, 2 control) at a single oral (gavage) dose of 12 mg/kg. Blood samples were collected at 15 min intervals for 5 collections. Ziprasidone had no effect on arterial blood pO_2, pCO_2, or pH.

Renal excretion: the effects of ziprasidone on renal function were tested in male Sprague-Dawley rats. Ziprasidone was administered orally (by gavage) at single doses of 1.6, 6. and 12 mg/kg. Two separate experiments were conducted. In one set of rats (n not specified), urinary volume, Na, K, and osmolarity were determined in urine samples collected over 5 hr postdosing. In another set (6/grp), 24-hr urine collections were obtained to determine 24-yr urine volume and electrolyte concentrations (Na, K).

In the 5-hr urine samples, there were decreases in the amount of Na excreted (44 and 61% and in the osmolality (25 and 33%) at 6 and 12 mg/kg, respectively. Urinary volume was

slightly (n.s.) increased at these doses (13 and 15%, respectively).

In the 24-hr urine samples, there were no differences among grps on any of the parameters assessed (same as for 5-hr study). Urinary volume was slightly reduced at all doses (not dose-related (13-29%).

GI transit time: the effects of ziprasidone on GI transit were assessed in rats (strain not specified, 8/grp) at doses of 1.6 and 6.0 mg/kg. An addition grp was treated with morphine sulfate (4.0 mg/kg), an agent known to inhibit GI motility. Charcoal was used as a marker to assess transit time.

Ziprasidone had no effect on GI transit time. In contrast, morphine reduced transit time by 44%.

Gastric acid secretion: the effects of ziprasidone on gastric acid secretion was assessed using the pylorus-ligated rat model (10/grp, strain not specified). Following ligation of the pylorus, ziprasidone (1.6 and 6.0 mg/kg), cimetidine (10 mg/kg, an H_2 antagonist) was injected intraduodenally. Gastric acid secretion was measured 2 hr postinjection.

Ziprasidone reduced gastric acid secretion by 65 and 58% at 1.6 and 6.0 mg/kg, respectively. In comparison, cimetidine reduced gastric acid secretion by 50%.

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Methods

1. **for CP-88,059-1 in dog plasma** (Study no. DM-89-128-39).

A method for assaying CP-88,059 in dog plasma was developed consisting of The limits of quantification were to ng/mL. Total recovery of spiked dog plasma was 68-92%. Accuracy was ≈94-101% at concentrations of ng/mL. The RSD (or CV) was 0.32-10.6% at concentrations of ng/mL. The stability of CP-88,059 in the autosampler was determined at room temperature. By ≈27 hr, the concentration had fallen from 1.0 μg/mL to 0.7 μg/m; even by 1 hr, the concentration had fallen by ≈10%. The decrease in concentration over the 27 hrs was more gradual after 1 hr.

Stability of CP 88,059 in dog plasma when stored at -20° C was tested for 63-64 days. [The data were provided in figure form only.] At the lowest concentration tested (0.2 μg/mL), there was an initial small, but rapid decrease in concentration (from ≈2.7 to 2.3 μg/mL), after which the concentration remained fairly stable (final concentration ≈2.1-2.2 μg/mL). At the higher concentrations (1, 3 μg/mL), the concentration fell ≈25 to 50% over the storage period. CP-88,059 was not stable in dog plasma at room temperature; sampling times were after 1, 2, and 7 days of storage. There was some variability in the first three measurements (Days 0-2), but the concentration appeared fairly stable; however, by Day 7 the concentration approached the LLOQ.

2. **Analysis of CP-88,059 in human serum by** (Study no. DM-91-128-23).

The method for separation and quantification of CP-88,059 in human serum involved an i Recovery of CP-88-059 and the internal standard were 91 ± 4 and 85 ± 15%, respectively (mean ± S.D). The CV for replicate injections was % for CP 88,059. The mean interassay accuracy was 95-103% over a concentration range of ng/mL. The limits of quantification were to ng/mL.

The stability of CP 88,059 in human serum was not tested.

3. **Analysis of CP-88,059 in human, dog, and rat serum by** (Study no. DM-93-128-24).

The method developed for separation and quantification of CP 88,059 in human, dog, and rat serum consisted of an initial solid phase extraction procedure followed by During the procedure, additional steps were taken to remove interfering endogenous compounds Autosampler stability was determined for up to 30 hr; clear degradation was observed after 72 hr. CP 88,059 was found to be stable in human serum after one freeze-thaw cycle. In human serum, recovery of CP 88,059 was found to be ≈67% at concentrations of ng/mL. The limits of quantitation of the method were to ng/mL.

Intra-assay precision and accuracy were acceptable. In human serum, attained concentrations were 91-109% of intended; attained concentrations were >100% of intended at all but the HC (ng/mL). The RSD over this concentration ranged was %. In dog serum, attained concentrations were 98-101% of intended and the RSD was 3.22-10.19% over a concentration range of ng/mL. In rat serum, the attained

concentrations were 93-96% of intended and the RSD was 6.6-12.3% over a concentration range of ι ng/mL. Interassay RSD in human serum was determined to be 4.2-6.3% over a concentration range of ng/mL.

4. **Synthesis and preparation of radiolabeled CP 88,059** (Study no. DM-93-128-22).

The methods for single-labeling CP-88,059 with either ^{3}H or ^{14}C were described and the *in vivo* stability of ^{3}H-labeled CP 88,059 was tested in 2 male Long-Evans rats following a single 1 mg/kg p.o. dose. A dual-labeled mixture was prepared by diluting ^{3}H and ^{14} CP 88,059 with unlabeled drug. Radiochemical purity was determined using .

The initial radiochemical purity of ^{14}C-CP 88,059 was <92% and the chemical purity was 65%. After further processing, the purities were increased to ≥98 and 80%, respectively. The radiochemical purity of ^{14}C CP 88,059 was 98.47%, with no additional processing; the chemical purity of the ^{14}C isotope was not determined.

The ^{3}H label was found to be stable *in vivo*; <0.5% of dose radioactivity was recovered as tritiated water in 0-24 hr urine samples. In the 1- and 24-hr plasma samples, 0.1 and 0.4% of the radioactivity, respectively, was associated with water. [Total recovery of radioactivity was 99-98% in urine and plasma samples.] In the dual-label mixture, the radio- and chemical purity was determined to be ≥98 and 79.97%, respectively.

Mice

1. **Pharmacokinetics and Oral bioavailability of CP-88,059 in CD-1 mice** (Study no. DM-94-128-1).

Methods: the absolute oral bioavailability of ziprasidone was determined by administration of single doses of 5 mg/kg i.v. and 10 and 100 mg/kg p.o. to male and female CD-1 mice (4/time point). Vehicles were 50% glycerol formal in physiological saline (i.v.) and 0.5% methylcellulose (p.o.). Blood samples were collected at 0.167, 0.333, 0.667, 1, 1.5, 2, 3, 5, and 6 hr postdosing following i.v. dosing and at 1, 2, 3, 4, 6, 8, 10, 12, 15, 18, 20 (HD females only), and 24 (HD females only) following p.o. dosing. Ziprasidone was quantitated in plasma using quantitation limits were ng/mL for i.v. and 100 mg/kg p.o., and ng/mL for 10 mg/kg p.o.

Results: the PK data are summarized in the following sponsor's Table 4:

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TABLE 4
PHARMACOKINETIC SUMMARY OF CP-88,059 IN MICE

IV DOSE

Dose (mg/kg)	Sex	AUC (0-inf) (ng-hr/ml)	AUMC(0-inf) (ng-hr*hr/ml)	Cl (ml/min-kg)	Vdss (L/kg)	Kel (1/hr)	T1/2 (hr)
5	M	1237	594	67.4	1.9	1.7	0.41
5	F†	1356	869	61.5	2.4	1.3	0.53

PO DOSE

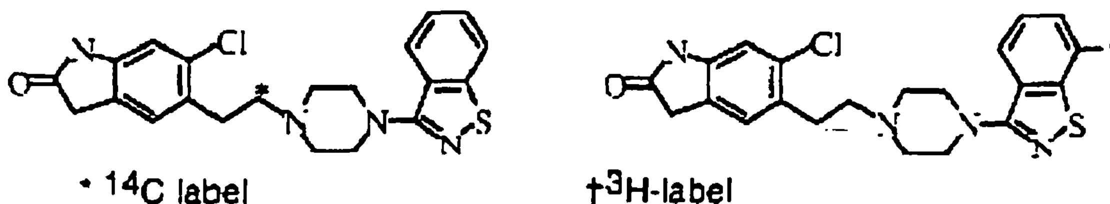
Dose (mg/kg)	Sex	AUC (0-inf) (ng-hr/ml)	Bioavailability (%)	Plasma Cmax (ng/ml)	Tmax (hr)
100	M	8093	25	917	1
100	F	15905	50	1663	1
10	M	678	27	221	1
10	F	2411	89	441	1

† - Mean plasma concentration data at 3 hr was omitted in the pharmacokinetic calculations.

Absolute oral bioavailability was markedly higher in females (particularly at the lower oral dose). [It is unfortunate that estimates of variability were not included in the summary table.] From the other data tables and figures, it is clear that interanimal variability was high, particularly at the C_{max}. CV ranged from % at peak plasma times.

2. **Radiolabel excretion of [^{3}H]- and [^{14}C] CP-88,059-1 in CD-1 mice** (Study no. DM-93-128-8)

Methods: double-radiolabelled ziprasidone (as illustrated in the following sponsor's figure) was administered to CD-1 mice (12/sex) as a single 10 mg/kg p.o. dose (vehicle: 0.5% methyl cellulose).



Urine and fecal samples were collected at the following intervals over a 7-day period: 0-6, 6-24, 24-48, 48-72, 72-96, 96-120, 120-144, and 144-168 hr postdosing. Quantitation of radioactivity was performed using .

Results: one female died at 96-120 hr. The data are summarized in the following table (data are given for each isotope as ^{3}H/^{14}C.

SEX	% OF DOSE RADIOACTIVITY		
	URINE	FECES	TOTAL
M	33.40/32.53	74.88/72.23	108.28/104.76
F	32.57/31.45	74.45/72.35	107.02/103.80

The data were similar between males and females and comparing isotopes. Fecal elimination accounted for the majority of drug-related material. The majority of dose radioactivity was eliminated during the first 24 hr postdosing. The data for total radioactivity recovered suggests some overestimation of drug levels.

3. **Comparison of total radioactivity versus unchanged drug level in plasma of CD-1 mice after oral administration of [^3H]- and [^{14}C] labeled CP-88,059** (Study no. DM-93-128-10)

Methods: double-radiolabelled ziprasidone was administered to CD-1 mice (56/sex) as a single 10 mg/kg dose (vehicle: 0.5% methylcellulose). Blood samples were collected (8/sex/time point) at 0.5, 1, 2, 4, 6, 8, and 12 hr postdosing. Plasma radioactivity was determined using plasma levels of ziprasidone were quantitated using

Results: the presentation of the data was confusing and inconsistent with the described methodology. This was compounded by the fact that individual data were not provided in the report. According to the methods section, 8/sex/time point (for a total of 56/sex) were used for analysis. However, according to the data tables, only 1/sex/time point was examined.

The data (C_{max} in ng/mL, AUC in ng•hr/mL; total radioactivity is expressed in ng-eq instead of ng) are summarized in the following table:

SEX	ZIPRASIDONE		TOTAL RADIOACTIVITY*		% OF TOTAL RAD**	
	C_{max}	AUC	C_{max}	AUC	C_{max}	AUC
M	269	605	1240/1281	7420/6552	22/21	8/9
F	302	708	2007/2032	9069/8175	15/15	8/9

*$^3H/^{14}C$

**ziprasidone as a percentage of total radioactivity for each radiolabel ($^3H/^{14}C$)

Clearly, ziprasidone administered orally is extensive metabolized in both male and female mice. This is not consistent with results of a previous study indicating up to 89% oral bioavailability in female mice.

4. **Identification of metabolites in urine and feces of CD-1 mouse after oral administration of [^3H]- and [^{14}C]-labeled CP-88,059-1** (Study no. DM-93-128-9)

Methods: double-radiolabelled ziprasidone was administered to CD-1 mice (12/sex) as a single 10 mg/kg p.o. dose (vehicle: 0.5% methylcellulose). Urine samples were collected for 24 hr postdosing; fecal samples were collected "...in the 24 hr interval following the dose..." Urine and fecal samples were pooled across sex for analysis of metabolites. Metabolites were isolated using quantitated using and identified using. Metabolite M4 was isolated by and derivatized (i.e., methylated) using

Results: in urine, identified metabolites accounted for 86% of total urinary radioactivity and ≈21% of dose radioactivity. Cleavage of the molecule at the piperazinylnitrogen accounted for ≈73% of urinary radioactivity and 24% of dose radioactivity in both males and females. Urinary metabolites were as follows:

Peak II (M1): cleaved product, detected only in ^3H chromatogram. Identified as BITP sulfone.

Peak III (M2): cleaved product, detected only in ^3H chromatogram. Identified as BITP sulfoxide.

Peak IV (M3): cleaved product, detected only in ^3H chromatogram. Identified as BITP sulfone-lactam.

Peak V (M4): cleaved product, detected only in ^{14}C chromatogram. Identified as 5-(2-carboxyethyl)-6-chloro-oxindole.

Peak VI (M5,M6): mixture of M5 and M6. M5 was identified as BITP; M6 was identified only as "...the intact molecule with the addition of two oxygen atoms at the benzisothiazole moiety. The exact sites for oxidation...were not established..." In a separate portion of the report, the identity of M6 was given as CP-88,059-S,S-dioxide

Peak VII (M7): chromatogram suggested monooxidation and hydration of parent compound. Identified as 6-chloro-5-[2-(4-(2-sulfinamido)benzoyl)-piperazinyl]thyl-oxindole.

Peak VIII (M8, M9): mixture of M8 and M9. M8 was identified as CP-88,059 sulfone, having undergone two oxidation steps. M9 was identified only as "...the intact molecule with the addition of one oxygen atom at the benzisothiazole moiety other than sulfur". The exact sites of oxidation could not be determined.

Peak IX (M13): this peak represented unchanged drug.

Fecal metabolites were identified as follows (the sponsor noted that there were no sex differences in metabolic profile): Peak I (M6), Peak II (M8), Peak III (M9), and Peak IV (M13, parent compound). The abundance of each identified metabolite and the parent compound in urine and feces are summarized in the following sponsor's table. The identified metabolites accounted for ≈84-96 and >99% of urinary and fecal radioactivity, respectively. In urine, the major metabolite was M4, 5-(2-carboxyethyl)-6-chloro-oxindole, accounting for ≈65 and 40% of urinary radioactivity in males and females, respectively. The sponsor indicated that unlike rats, there were no sex-related differences in the excretion of M4 in mice; however, according to the data, M4 was more abundant in male than in female urine. In feces, the parent compound accounted for the majority of dose radioactivity. The major fecal metabolites were M8 and M9, representing oxidation at the benzisothiazole moiety (not at the S atom). A minor metabolite, M6, was identified as CP-88,059-S,S-dioxide (structure confirmed by authentic standard).

Table 3. Percentages of metabolites of CP-88,059 in CD-1 male mice

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Metabolite	% of Dose[a]		
	Urine[b]	Feces[c]	Total
M1[c]	18.64	ND	18.64
M2[d]	3.51	ND	3.51
M3[d]	1.62	ND	1.62
M4[e]	20.96	ND	20.96
M5[d]	1.61	ND	1.61
M6	3.51	0.39	3.90
M7	0.82	ND	0.82
M8+M9[f]	3.08	38.96	42.04
M10	ND	ND	ND
M11	ND	ND	ND
M12	ND	ND	ND
M13	0.17	44.14	44.31

Table 4. Percentages of metabolites of CP-88,059 in CD-1 female mice

Metabolite	% of Dose[a]		
	Urine[b]	Feces[c]	Total
M1[d]	18.95	ND	18.95
M2[d]	4.20	ND	4.20
M3[d]	1.73	ND	1.73
M4[e]	12.04	ND	12.04
M5[d]	0.51	ND	0.51
M6	1.90	0.95	2.85
M7	0.63	ND	0.63
M8+M9[f]	2.46	37.52	39.98
M10	ND	ND	ND
M11	ND	ND	ND
M12	ND	ND	ND
M13	0.00	34.86	34.86

a: average of ^{3}H and ^{14}C
b; based on 33.40%(^{3}H) and 32.53% (^{14}C) recovery
c; based on 63.63% (^{3}H) and 62.21% (^{14}C) recovery
d; only ^{3}H label
e; only ^{14}C label
f; not separated on

These data suggest that metabolism of ziprasidone occurs in mice by the following pathways:

1. N-dealkylation of the ethyl side chain at the piperazinyl nitrogen.
2. oxidation at the sulfur atom, forming a sulfone.
3. oxidation of the benzisothiazole moiety at a site other than the sulfur atom.

According to the sponsor, N-dealkylation is similar to that observed for the "structurally-related drugs buspirone, tandospirone and tiospirone".

5. **Identification of metabolites in plasma of CD-1 mice after oral administration of [^{3}H]- and [^{14}C]-labeled CP-88,059-1** (Study No. DM-94-128-25)

Methods: double-labeled ziprasidone (lot # 20480-1, unlabeled) was administered to CD-1 mice as a single dose of 10 mg/kg p.o. (vehicle: 0.5% methylcellulose). Blood samples were

collected from 8/sex at 0.5, 1, 2, 4, 6, 8, and 12 hr postdosing. Drug-related compounds were analyzed following extraction from plasma using .
Identification of metabolites was performed using Plasma samples were pooled across sex for analysis. The identified metabolites accounted for ≈80% of total radioactivity in plasma.

The following metabolites were identified in plasma: Peak II (M1), Peak III (M2), Peak IV (M3), Peak V (M4), Peak VI (M6), Peak VII (M7), Peak VIII (M8), Peak IX (M9), Peak IX (M10), and Peak X (M13, parent compound). Of these compounds, only M10 was not detected in urine. M10 was identified as CP-88,059 sulfoxide.

The parent compound accounted for ≈7-12% of total plasma radioactivity, depending on the radiolabel. The major plasma metabolite was M8, which accounted for 32-34% of plasma radioactivity. M7 was a major plasma metabolite in females (i.e., ≥10% of total plasma radioactivity). M9 + M10 accounted for 14-20% of total plasma radioactivity.

The major metabolic pathway was oxidation at the sulfur atom, resulting in sulfoxide formation. Minor pathways consisted of the following: (1) "...N-dealkylation of the ethyl side chain attached to the piperazinyl nitrogen...", (2) "...oxidation on the benzisothiazole moiety other than sulfur...", and (3) "...hydroxylation on the 3-position and subsequent oxidation of the benzisothiazole moiety" The sponsor indicated that there were no notable sex differences in metabolic profile. However, M7 was notably more abundant in female than in male plasma (≈10 and 2% of total plasma radioactivity, respectively)

The proposed metabolic pathways for ziprasidone in mouse plasma are illustrated in the following sponsor's Figure 14:

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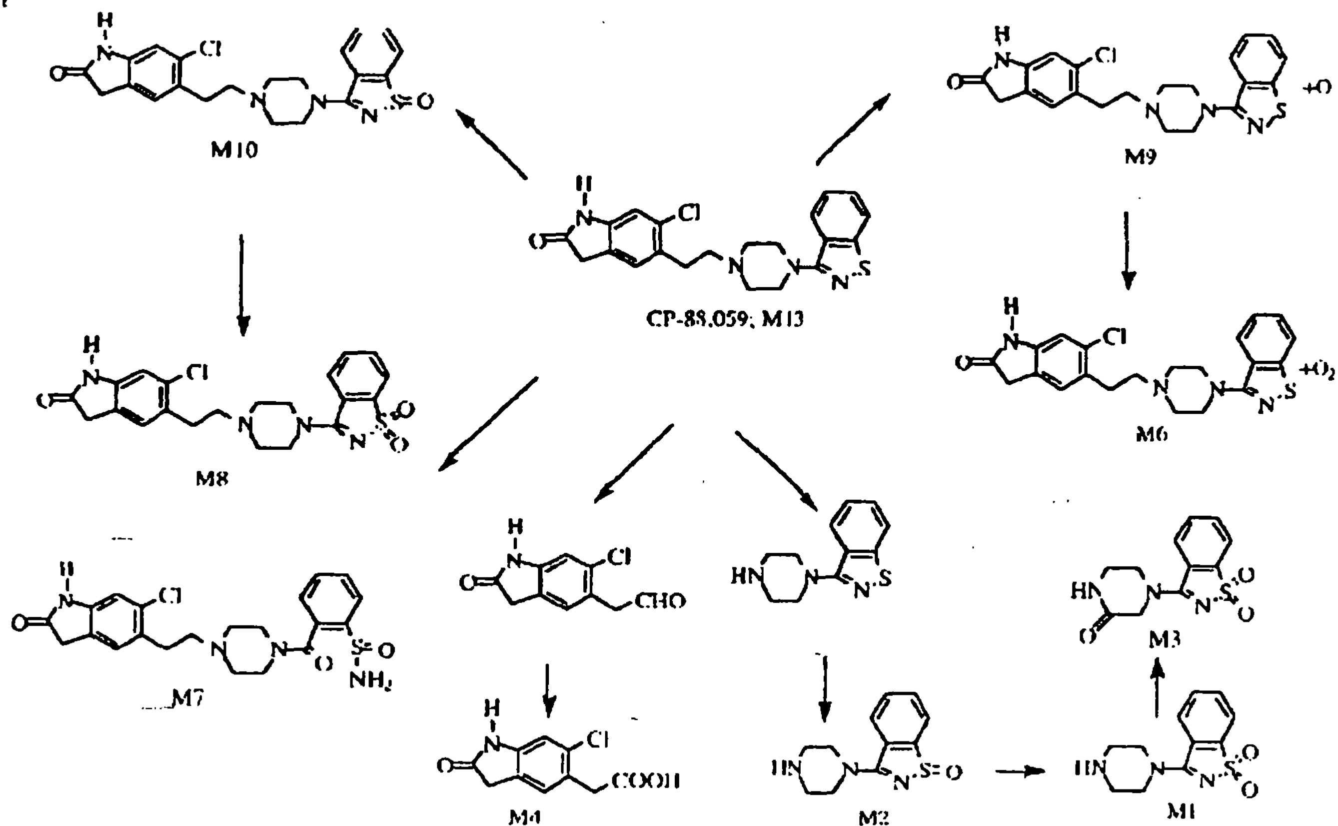

Figure 14. Proposed metabolites of CP-88,059 in mouse plasma

Rat

1. **Pharmacokinetics and oral bioavailability of CP-88,059 in Long Evans rats** (Report No. DM-93-128-2)

Methods: ziprasidone was administered to Long Evans rats as either a single i.v. (1 mg/kg, 5/sex) or oral (25 mg/kg, 3/sex) dose. Blood samples were collected at 0.25, 0.5, 1, 2, 3, and 5 hr after i.v. dosing, and at 0.25, 0.5, 1, 2, 3, 5, 7, 12, and 24 hr after p.o. dosing. For i.v. dosing, the vehicle was glycerol formal, and for p.o. dosing the vehicle was 0.5% methylcellulose.

Ziprasidone was quantitated in plasma using . The LLOQ was µg/mL for p.o. and ng/mL for i.v. dosing.

Results: the PK data are summarized in the following table (data are mean ± S.D.):

DOSE (mg/kg)	ROUTE	SEX	T_{max} (hr)	C_{max} (ng/mL)	AUC* (ng•hr/mL)	Cl (mL/min/kg)	$V_{d}ss$ (L/kg)	$t_{1/2}$ (hr)	F (%)
1	i.v.	M			734 ± 284	25 ± 9	1.4 ± 1.1	0.91	
		F			1215 ± 132	14 ± 2	0.7 ± 0.2	1.1	
25	p.o.	M	0.5 ± 0.0	1753 ± 1346	4920 ± 881				27 ± 5
		F	0.6 ± 0.4	1500 ± 1057	6837 ± 1664				22 ± 6

*$AUC_{(0-\infty)}$ for i.v., $AUC_{(0\text{-last time})}$ for p.o.

The sponsor calculated total body clearance of ziprasidone based on (1) obtained plasma Cl estimates, (2) a blood/plasma ratio of 0.79 for ziprasidone, and (3) <1% of dose appears as unchanged parent in urine. Therefore, total body Cl ≈ 32 (i.e., 25/0.79) and 18 (i.e., 14/0.79) L/min/kg. This is ≈25-50% of hepatic blood flow in this species (estimated to be 68 mL/min/kg, Nies *et al.* Biochem Pharmacol 25:19910-1993, 1976).

2. **Comparison of total radioactivity versus unchanged drug level in plasma of Long Evans rats after oral administration of [^{3}H]- and [^{14}C] labeled CP-88,059** (Study No. DM-93-128-14)

Methods: double-labeled ziprasidone was administered orally (by gavage) to Long Evans rats (14/sex) at a single dose of 10 mg/kg (vehicle: 0.5% methyl cellulose, 1.5 mg/mL). Blood samples were collected from 2/sex at each of the following times: 0.5, 1, 2, 4, 6, 8, and 12 hr postdosing. Radioactivity was quantitated using . Plasma levels of parent compound were quantitated using

Results: the data are summarized in the following table:

SEX	TOTAL RADIOACTIVITY*		CP-88,059		PC AS % TR	
	C_{max} (ng/mL)	AUC (ng•hr/mL)	C_{max} (ng/mL)	AUC (ng•hr/mL)	C_{max}	AUC
M	1932/1897	6554/5935	982	2546	51-52	39-43
F	2459/2375	9561/8877	1435	4429	58-60	46-50

*^{3}H/^{14}C

3. **Excretion of [^{14}C]CP-88,059 in Sprague-Dawley rats** (Study No. DM-93-128-11)

Methods: ^{14}C-ziprasidone was administered to intact and bile-cannulated male Sprague-Dawley rats (4/grp) as a single oral (by gavage) dose of 10 mg/kg. The drug was made up in 0.5% methylcellulose for administration. Of the 4 males cannulated, 1 died at 36-48 hr postdosing (data not included in summary) and 1 was sacrificed because of blood in urine at 96 hr postdosing (data included); therefore, complete data were available for only 2 male rats. Urine, bile, and fecal samples were collected up to 96 hr postdosing in one rat and up to 36 hr postdosing in the other rat. In the remaining 2 cannulated rats, samples were collected up to 168 hr postdosing. In intact rats, urine and fecal samples were collected at 24-hr intervals up to and including 240 hr postdosing. Total radioactivity was quantitated by following sample processing.

Results: the data are summarized in the following table:

GRP	% OF DOSE RADIOACTIVITY			
	URINE	FECES	BILE	TOTAL
intact	18.07	92.53	---	110.6
bile-cannulated*	18.73	48.80	34.64	99.00

* based on data collected up through 96 hr postdosing

4. **Excretion of [^{3}H] and [^{14}C] CP-88,059-1 in the bile cannulated Long Evans rat** (Study No. DM-93-128-12)

Methods: double-labeled ziprasidone was administered to bile-cannulated rats (4-5/sex) as a single oral (gavage) dose of 10 mg/kg. Urine, bile, and fecal samples were collected at 0-6, 6-24, 24-48, 48-72, 72-96, 96-120, 120-144, and 144-168 hr postdosing. One F died at 36-48 hr postdosing, and 1 F lost its bile-duct cannula. Radioactivity was quantitated using following sample preparation.

Results: the data are summarized in the following sponsor's Table 2:

Table 2 Radiolabeled mass balance of CP-88.059 in 7 Long Evan rats administerd a 10 mg/kg PO dose

Percentage of 3H/14C-dose excreted from 0-168 hr post dose

Rat#	Urine	Bile	Feces	Total
1 M	25.06 / 25.18	22.15 / 20.38	52.04 / 50.45	99.25 / 95.05
2 M	29.44 / 28.00	8.54 / 7.71	66.55 / 66.32	104.53 / 102.03
3 M	16.50 / 16.16	18.46 / 16.25	64.95 / 63.08	99.91 / 95.49
4 M	18.82 / 18.23	15.66 / 14.37	70.98 / 65.97	105.46 / 101.57
Average all males	22.46 / 21.89	16.20 / 14.68	63.38 / 62.21	102.29 / 98.54
7 F	19.83 / 18.54	21.70 / 20.24	44.51 / 44.88	86.04 / 83.66
8 F	20.11 / 18.78	16.84 / 15.61	61.05 / 65.74	98.00 / 100.13
10 F	18.85 / 18.57	25.02 / 23.80	43.76 / 44.24	87.63 / 86.61
Average all females	19.60 / 18.63	21.19 / 19.88	49.77 / 51.62	90.55 / 90.13
Overall average	21.03 / 20.26	18.70 / 17.18	56.58 / 56.92	96.42 / 94.34

Approximately 60-70% of dose radioactivity was excreted by 24 hr postdosing. By 24-48 hr postdosing, ≈85 and 94% of dose radioactivity had been excreted in males and females, respectively.

5. **Tissue distribution of CP-88,059 in the Long-Evans and Sprague-Dawley rat** (Study No. DM-94-128-6)

Methods: tissue distribution of double-labeled CP-88,059 was tested in two separate studies.

Exp. 1: CP-88,059 (ziprasidone) was administered (in 0.5% methylcellulose) as a single 5

mg/kg oral (gavage) dose to pigmented rats (Long-Evans, 3/sex/time point). Animals were sacrificed at 2, 24, and 72 hr postdosing. The following tissues were collected at sacrifice: blood, plasma, brain, eyes, heart, lung, liver, kidney, muscle, fat, testis/ovary. In addition, adrenal gland, stomach, small intestine, pigment and nonpigmented skin samples were collected only from males.

Exp. 2: CD-88,059 was administered (in 0.5% methylcellulose) as a single 10 mg/kg oral (gavage) dose to pigmented and nonpigmented male rats (3/strain/time point). Animals were sacrificed at 2, 24, 72, 144, 312, 960 hr, 6 mo, and 1 yr postdosing. The following tissues were collected at sacrifice: eyes, liver, kidney, nonpigmented skin, testis, and pigmented skin (Long-Evans only).

In both studies, tissue radioactivity was determined using The data were expressed as µl-eq of CP 88,059; the LLOQ was µg/g tissue.

Results:

Exp. 1: highest levels of radioactivity were detected at 2 hr postdosing, except in eye. In eye, 24-hr levels were higher than at 24 hr postdosing, particularly in females. Highest levels of radioactivity were detected in liver in both males and females. The next highest levels in females were detected in eye, followed by kidney. In males, levels in stomach and small intestine were lower than liver, followed by eye and kidney which were similar. Tissue:plasma ratios were >1 in all tissue except for brain and muscle (≈1 in pigmented and nonpigmented skin, and in testis). Of all tissues examined, the lowest levels were detected in brain. By 72 hr postdosing, appreciable levels (65-70% of peak) were still evident in eye. Low levels of radioactivity were also detected in all other tissues examined, except for muscle and fat

The tissue $AUC_{(0\text{-}72\ hr)}$ data are summarized in the attached sponsor's Table 2. In terms of AUC, the highest tissue exposure was to eye, with levels in females 60-70% higher than in males. In males, exposure was only slightly higher in eye than in liver. In females, exposure to eye was >2-fold higher than to liver. Brain exposure was similar to plasma AUC.

The data are illustrated in the following sponsor's Fig 1:

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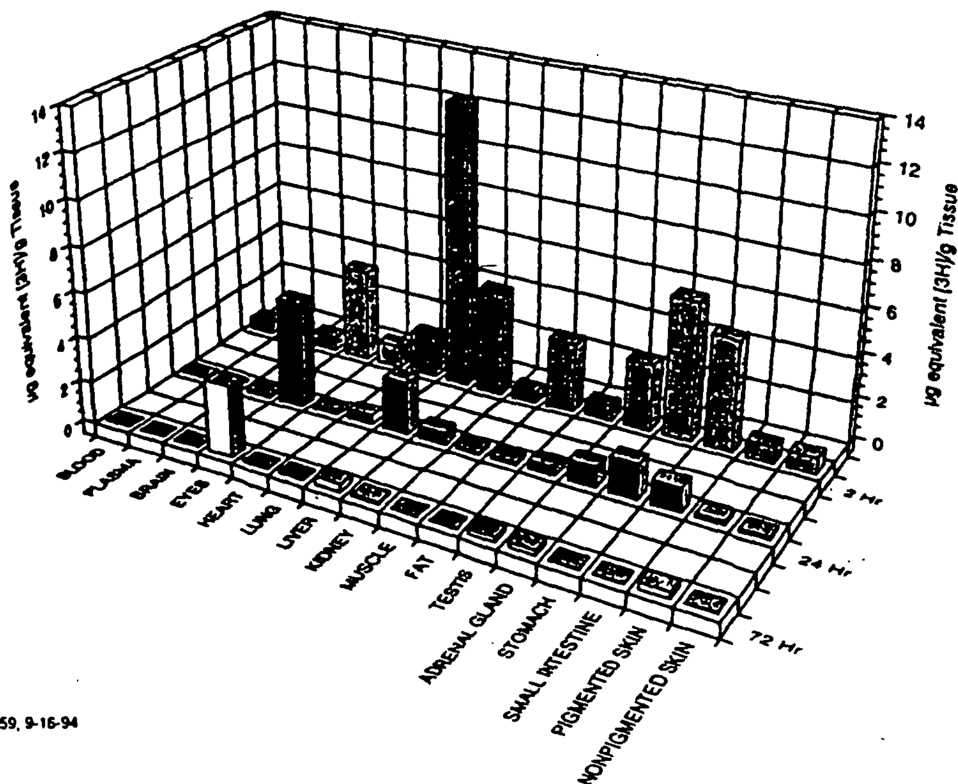

Exp. 2: this experiment assessed prolonged tissue retention of radioactivity in selected tissues in two different strains of rat (pigmented, nonpigmented). Of the 5 tissues examined, only eye (of pigmented strain) and testis had detectable levels of radioactivity at 6 mo to 1-yr; the level in pigmented skin at 6-mo was at the LLOQ. The highest levels of radioactivity in eye (pigmented) were reached by 24-hr postdosing, however, there was a second, lesser peak at 13 days (312 hr). In testis 2- and 24-hr postdosing levels were fairly similar in Long-Evans rats; in Sprague-Dawley rats, peak levels in testis were achieved at 2 hr. Levels of radioactivity in these tissues at 1-yr were 20-26 and 12-10% of peak levels in eye (pigmented) and testis, respectively. In non-pigmented rats, the only tissue with detectable radioactivity at 6-mo to 1-yr was testis; levels at 1-yr were ≈10% of peak levels. Tissue exposure (AUC) data are summarized in the following sponsor's Table 5:

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Table 2

Tissue Exposure (0-72 hr AUC) to Radioactivity and Fraction Remaining at 72 Hours After a Single Oral Dose of [3H/14C]-CP-88,059 (Study #1; 5 mg/kg)

Tissue	0-72 hr AUC (µg equivalent-hr/g)		Fractional Retention (72 hr Conc/Max Conc.)	
	Male 3H/14C	Female 3H/14C	Male 3H/14C	Female 3H/14C
Blood	9/7	13/11	0.036/<0.030	<0.030/<0.030
Plasma	11/9	17/15	<0.030/<0.030	<0.030/<0.030
Brain	13/11	21/16	0.080/0.081	0.083/0.086
Eyes	295/259	474/445	0.705/0.694	0.552/0.526
Heart	22/17	30/31	0.041/0.041	0.077/0.059
Lung	40/30	56/57	0.038/0.040	0.042/0.040
Liver	269/221	191/195	0.027/0.027	0.072/0.029
Kidney	78/66	73/76	0.022/0.017	0.035/0.022
Muscle	12/8	13/13	0.037/0.054	0.050/0.038
Fat	44/41	23/21	0.009/0.010	0.006/0.022
Testes/Ovaries	29/22	47/45	0.267/0.271	0.005/0.065
Adrenal Gland	76/62	N.D.	0.067/0.057	N.D.
Stomach	132/117	N.D.	0.005/0.005	N.D.
Small Intestine	100/84	N.D.	0.008/0.007	N.D.
Pigmented Skin	22/17	N.D.	0.373/0.358	N.D.
Nonpigmented Skin	15/12	N.D.	0.067/0.051	N.D.

N.D. - not determined

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Table 5
Tissue Exposure [AUC (0-∞)] to Radioactivity and Fraction Remaining
After a Single Oral Dose of [3H/14C]-CP-88,059 (Study #2: 10 mg/kg)
In Male Long Evans (LE) and Sprague Dawley (CD) Rats

Tissue	AUC (0-∞) (µg equivalent-hr/g)		FRACTIONAL RETENTION (Day [illegible] Conc/Max Conc.)		FRACTIONAL RETENTION (6-month Conc./Max Conc.)		FRACTIONAL RETENTION (1 Year Conc/Max Conc.)	
	LE 3H/14C	CD 3H/14C	LE 3H/14C	CD 3H/14C	LE 3H/14C	CD 3H/14C	LE 3H/14C	CD 3H/14C
Eyes	33724/31764	201/193	0.515/0.600	0.058/0.045	0.213/0.304	0.080/0.1[illegible]4	0.213/0.261	0.00/0.[illegible]
Testis	2066/1865	2342/2195	0.275/0.318	0.279/0.279	0.138/0.187	0.116/0.128	0.113/0.[illegible]21	0.090/0[illegible]
Nonpigmented skin	81.5/47.5	76.7/69.8	0.090/0.026	0.031/0.034	0.090/0.025	0.050/0.034	0.00/0.00	0.00/0.[illegible]
Pigmented skin	562/471	-/-	0.089/0.101	-/-	0.030/0.043	-/-	0.00/0.00	-/-
Liver	506/404	851/808	0.001/0.001	0.001/0.001	0.001/0.001	0.001/0.001	0.00/0.00	0.00/0.[illegible]
Kidney	220/105	363/314	0.008/0.008	0.006/0.006	0.006/0.005	0.006/0.008	0.00/0.00	0.03/0.[illegible]

Highest tissue exposure (based on AUC) was in eye, followed by testis; exposure in pigmented skin and liver were lower and similar.

6. **Whole-body autoradioluminography of Long Evans and Sprague Dawley male rats after single oral administration of [14C]-CP-88,059-1** (Study No. DM-94-128-7)

Methods: ^{14}C-ziprasidone (in 0.5% methylcellulose) was administered to two strains of rat (pigmented, non-pigmented, n = 2/strain) as a single oral (gavage) dose of 10 mg/kg (actual dose: 9.5 mg/kg) in order to determine the distribution of radioactivity into brain, eye, liver (L-E rats), and reproductive tissues. Animals (1/strain/sampling time) were sacrificed at either 24 or 312 hr postdosing. Quantitation of tissue radioactivity was accomplished by , followed by radioactivity quantitation using the

Results: according to the sponsor, the quantitation method was linear over a concentration range of nCi/g with 4 days of exposure (mean CV = 4.2-7.5% at 24 hr postdosing, and 5-8.4% at 312 hr postdosing).

The data are summarized in the following sponsor's Table 4:

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Table 4. Concentration of radioactivity (nCi/g) in tissues of Long Evans and Sprague Dawley rats at 24 and 312 hours after oral administration of a single 10.0 mg/kg dose of [^{14}C]-CP-88,059.

Tissue	24 Hr Long Evans			24 Hr Sprague Dawley			312 Hr Long Evans			312 Hr Sprague Dawley		
	Mean[a]	S.D.	N	Mean	S.D.	N	Mean	S.D.	N	Mean	S.D.	N
Alimentary Contents												
Caecum	20	4	33	9	1	9	<LLOQ[b]			<LLOQ		
Colon	65	16	62	20	6	37	<LLOQ			<LLOQ		
Stomach	<LLOQ			9	2	8	<LLOQ			<LLOQ		
Brain												
Pineal Gland	29	7	5	<LLOQ			<LLOQ			<LLOQ		
Eye												
Aqueous humor	<LLOQ			<LLOQ			<LLOQ			<LLOQ		
Choroid	65	7	9	<LLOQ			89	14	8	<LLOQ		
Ciliary Body	148	33	16	<LLOQ			177	38	11	<LLOQ		
Iris	120	22	16	<LLOQ			180	44	16	<LLOQ		
Lens	<LLOQ			<LLOQ			<LLOQ			<LLOQ		
Uvea	69	34	17	<LLOQ			108	22	12	<LLOQ		
Vitreous Humor	<LLOQ			<LLOQ						<LLOQ		
Skin												
Eyelid	21	5	10	<LLOQ			29	3	4	<LLOQ		
Non-Pigmented	<LLOQ			<LLOQ			<LLOQ			<LLOQ		
Pigmented	8	1	62	<LLOQ			<LLOQ			<LLOQ		

[a]Mean radioactivity values were calculated by averaging: 1. Tissue concentrations measured at different sectioning levels; and/or 2. Tissue concentrations measured from replicate cryosections obtained from the same sectioning level.
[b]Lower limit of quantitation (LLOQ) was 5.9 nCi/g. Although not listed, all of the other commonly analyzed tissues for a whole-body autoradioluminography study did not contain drug-related radioactivity (i.e. <LLOQ) for Long Evans and Sprague Dawley male rats at 24 and 312 hr post dose.

[The "n's" given in the table are somewhat confusing, and were not clarified in the text.] At 24-hr postdosing, radioactivity was detected in GI tract in both strains, but in brain (pineal gland), eye, and in pigmented skin and eyelid of Long-Evans rat only. At 312 hr postdosing, radioactivity was not detected in the GI tract or brain (pineal gland) for either strain or in pigmented skin, but was still detected in eye and eyelid of the pigmented strain. Radioactivity in the cecum and colon was higher in Long-Evans than in Sprague-Dawley rats at 24 hr postdosing. [No radioactivity was detected in liver (Long-Evans), brain (except pineal; brain regions examined not specified), or reproductive organs (organs examined not specified).] In eye, the highest levels of radioactivity (in Long-Evans rat) were detected in the ciliary body, with slightly lower levels in the iris. The choroid and uvea contained lower but detectable levels of radioactivity; no radioactivity was detected in aqueous or vitreous humor. The same pattern was noted at 312 hr postdosing, except that the absolute levels of radioactivity (expressed as nCi/g) were higher than at 24 hr in all eye regions with detectable radioactivity. In skin (of Long-Evans rat), higher levels of radioactivity were detected in eyelid than in pigmented skin (it was not clear whether or not the eyelid was pigmented) and levels were similar at 24 and 312 hr postdosing.

7. **Identification of metabolites in plasma of Long-Evans rats after oral administration of [^{3}H]- and [^{14}C]-labeled CP-88,059-1** (Study No. DM-94-128-15)

Methods: radiolabeled-labeled ziprasidone (mixture of [^{3}H] and [^{14}C]-labeled) of was administered orally (by gavage) to Long-Evans rats as a single 10 mg/kg dose. Blood samples were collected at 0, 0.5, 1, 2, 4, 6, 8, and 12 hr postdosing from 2/sex/time point. An additional 4/sex were dosed at 10 mg/kg and sacrificed at 1 and 4 hr postdosing. The plasma samples from blood collected at 1, 2, and 4 hr postdosing were pooled across sex for identification of metabolites. Drug-related substances were separated and identified using

Results: 85-91% of total radioactivity was recovered and identified plasma metabolites accounted for 70-84%% of total radioactivity. Peaks were identified as follows:

Peak II (M1): BITP sulfone
Peak III (M2): BITP sulfoxide
Peak IV (M3): BITP sulfone-lactam
Peak V (M4): 6-chloro-5-(2-carboxyethyl)-oxindole
Peak VII (M5): mixture of 3 metabolites. One metabolite was identified as BITP

Peak VII (M5a): 6-chloro-5-(2-hydroxyethyl)-oxindole
Peak VII (M6): intact molecule with two oxidation sites at the benzisothiazole moiety
Peak IX (M9): mixture of two metabolites. M9 was identified as the intact parent compound with one oxidation site at the benzisothiazole moiety
Peak IX (M10): CP-88,059 sulfoxide
Peak XI (M13): parent compound

Therefore, 10 metabolites and the parent compound were identified in plasma. The sponsor noted that there were no major sex differences in terms of metabolic profile.

The proposed metabolic pathways in rat are summarized in the following sponsor's Figure 9. In both males and females, the parent compound accounted for the majority of drug-related material in plasma (33-44 and 27-34%, respectively). Other major peaks were mixtures of 2-3 metabolites (Peaks VII and IX).

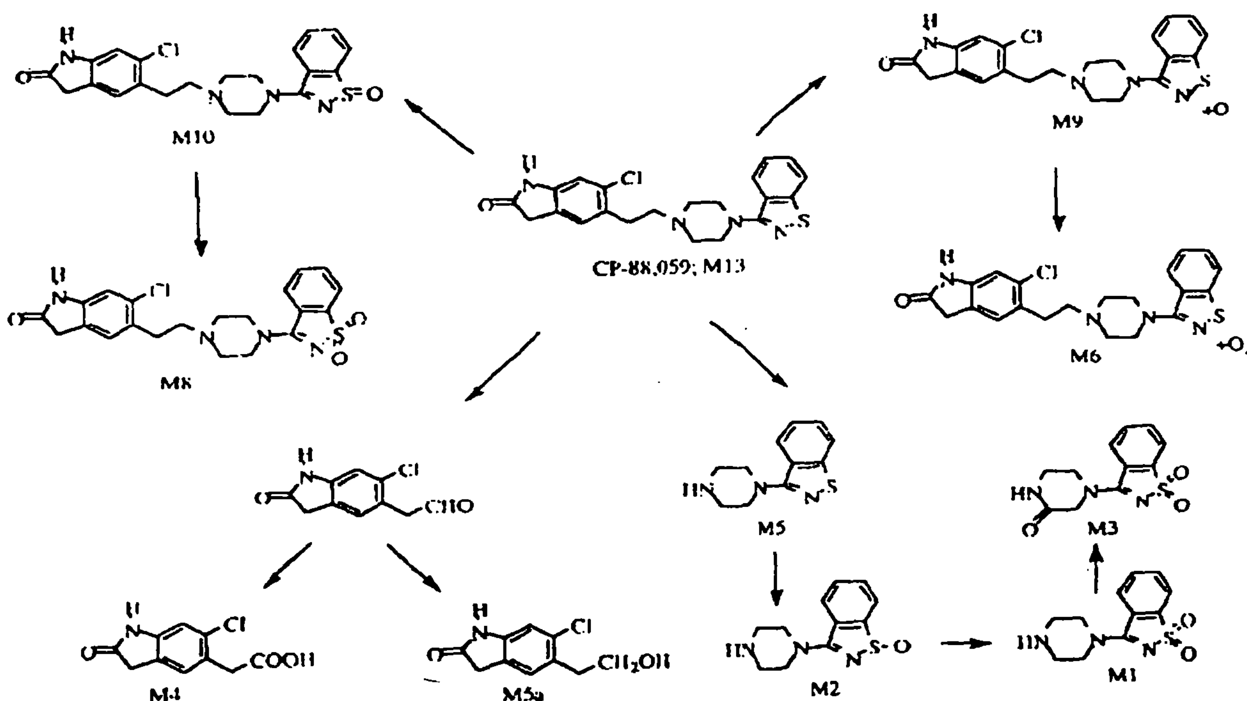

Figure 9. Proposed metabolites of CP-88,059 in rat plasma

8. **Identification of metabolites in urine, bile and feces of Long Evans rats after oral administration of $[^{3}H]$-$[^{14}C]$-labeled CP-88,059-1** (Study #DM-93-128-13).

Methods: a mixture of radiolabeled CP-88,059-1 (unlabeled drug, lot #20480-40-1) was administered (in 0.5% methyl cellulose) to bile duct-cannulated Long-Evans rats (5/sex) at a dose of 10 mg/kg. Urine, fecal, and bile samples were collected (0-24 hr postdosing),