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APPROVAL PACKAGE FOR:

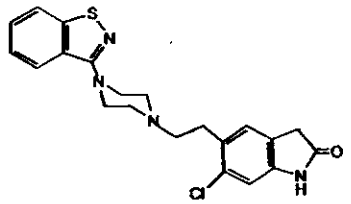
APPLICATION NUMBER

20-919

Pharmacology Review(s)

REVIEW AND EVALUATION OF PHARMACOLOGY/TOXICOLOGY DATA

Reviewer Name: Lois M. Freed, Ph.D.
Division Name: Neuropharmacological Drug Products
HFD#120
Review Completion Date: 6/21/2002
Review number: 3
NDA number: **20-919**
Serial number/date/type of submission: N-(BZ)
Information to sponsor: Y
Sponsor (or agent): Pfizer Inc.
Eastern Point Road
Groton, CT 06340
Drug: ziprasidone mesylate
Code Name: CP-88,059-27
Generic Name: n/a
Trade Name: Zeldox IM™
Chemical Name: 5-[2-[41,2-benzisothiazol-3-yl)-1-piperazinyl]ethyl]-6-chloro-1,3-dihydro-2H-indol-2-one methanesulfonate trihydrate
Molecular Formula/ Molecular Weight: 563.0 (salt)
Structure:



Relevant INDs/NDAs/DMFs: IND#49,045 (ziprasidone i.m.), IND #34,629 (ziprasidone p.o.), NDA 20-285 (ziprasidone p.o.)
Drug Class: D_2, $5HT_2$ receptor antagonist
Indication: agitation
Clinical formulation: 20 mg/mL, formulated for intramuscular injection; each mL of solution contains 20 mg ziprasidone, 4.7 mg methanesulfonic acid, 294 mg sulphobutylether beta-cyclodextrin sodium (SBECD)
Route of administration: i.m.
Studies reviewed within this submission: none. The current submission is a complete response to the Division's Approvable Letter, dated 3/6/01.
Studies not reviewed within this submission: none.

Sponsor's Resubmission: Complete Response to Approvable Letter, dated March 6, 2001.

Nonclinical issues

FDA Comment:

"We remind you that an assessment of reproductive toxicity of IM ziprasidone [to include dosing during all stages of development] needs to be conducted as a Phase 4 commitment (cf. Agency's action letter, December 17, 1998). Please refer to the ICH document, Detection of Toxicity to Reproduction for Medicinal Products [ICH-S5A, Sept 1994], for guidance."

Sponsor's response:

The sponsor provided a "pharmacokinetic based rationale" as to why i.m. reproductive toxicology studies are not necessary. The sponsor made the following points:

(a) "The oral reproductive toxicology studies have adequately defined the safety profile of ziprasidone at exposures higher than those achieved by IM administration to rats.

(b) "...the rat one month IM study was conducted at a dose of 4 mg/kg, the highest attainable IM dose in the rat due to volume considerations..."

(c) "The highest oral dose used in the fertility and teratology studies in rats was 160 mg/kg, which resulted in a Cmax of 3.4µg/ml. This is approximately ten times the Cmax following the human IM dose of 20 ml [sic]."

(d) "The IM dose of 4 mg/kg in the rat one month study resulted in a Cmax approximately 9 times the Cmax observed in humans following the IM administration of 20 mg."

The sponsor provided the following summary table:

Species	Route	Dose	Cmax(µg/ml)
Rat (1)	IM	4 mg/kg	2.8
Rat (2)	Oral	160 mg/kg	3.4
Human (3)	IM	20 mg	3.1

1. 1-month intramuscular study in rats (#00-720-44) (Cmax on day 21)
2. Rat teratology study (#91094-95)
3. Single dose intramuscular clinical study (#033)

The sponsor concluded that "We believe the oral reproduction studies support the IM use of ziprasidone and that additional reproduction studies by the IM route would not provide any additional safety data, because the IM route would not result in an increase in exposure over that in the oral study."

Reviewer comment: the original request for i.m. reproductive toxicology studies was based on the following: (a) a lack of any i.m. reproductive toxicology studies on ziprasidone, either alone or with the excipient, sulphobutylether beta-cyclodextrin sodium [SBECD], (b) the possibility that SBECD may affect the distribution of ziprasidone in the dam and/or fetus, and (c) a concern that SBECD may affect the reproductive toxicity potential of ziprasidone, particularly considering that ziprasidone and SBECD, each alone produces adverse effects on reproduction. The recommendation, as originally submitted to the sponsor [Agency's Non-Approvable Letter, December 17, 1998], did not state the basis for the recommendation.

The sponsor provided data demonstrating that the C_{max} achieved in the 1-mo i.m. toxicity study was similar to that administered at an oral dose of 160 mg/kg [the HD used in the oral fertility and teratology studies in rat]. The sponsor stated that this C_{max} [i.e., ≈3 μg/mL] is '...approximately ten times the Cmax following the human dose of 20 ml [sic]"; however, according to the data in the sponsor's summary table, the C_{max} in humans is similar to that obtained in rats at 4 mg/kg i.m. and 160 mg/kg p.o. [It is possible that the human C_{max} value given in the table is a typographical error.] It should be noted that the dose administered in the 1-mo i.m. toxicity study was 2 mg/day [only one dose level was tested], and was not corrected for increases in body wt. Therefore, the dose on a mg/kg basis decreased over the dosing period. The sponsor did not provide an interspecies comparison of total drug exposure [i.e., AUC].

The sponsor did not address the need to assess the potential reproductive toxicity of the combination of ziprasidone and SBECD. [This is understandable since, as previously noted, the basis for the recommendation was not provided to the sponsor.] Therefore, the information provided by the sponsor does not provide sufficient justification to change the original recommendation for a complete reproductive toxicity assessment of ziprasidone i.m. The stud(ies) should be conducted using a HD sufficient to produce some maternal toxicity. If the HD is limited due to problems with formulation, consideration should be given to alternative dosing regimens, e.g., b.i.d. dosing. As originally recommended, the stud(ies) may be conducted Phase 4.

FDA Comment:

"Please submit final toxicokinetic data for the 1-month i.m. toxicity studies in rat and dog. The reports submitted for both of these studies stated that, "Due to a lack of long term stability data at the time of submission of this report, final reported serum concentrations may change slightly."

Sponsor's response: the sponsor indicated that stability data were collected at 3 concentrations of ziprasidone [50, 500, and 1000 ng/mL] in rat and dog serum. Rat and dog serum samples were stored frozen [temperature not specified] for 88 and 95 days, respectively. The sponsor stated that "Analysis of these samples by the validated HPLC-UV method yielded concentration values that were within [redacted] % of the initially determined concentration values." No data were provided to document this statement.

Since issuance and submission of the final study reports, errors in calculation of the TK data made by the contract laboratory [redacted] were identified. Specifically, the "...contractor...used an incorrect potency calculation in weighing

ziprasidone for the standard curve." Therefore, the TK data were recalculated using the correct factor. The sponsor stated that the error did not "...impact the original interpretation of the study" [i.e., the 1-mo rat and dog toxicity studies].

The sponsor provided the corrected TK data in the following summary tables:

Exposure to CP-88,059 Following Daily Intramuscular Dosing
During 1 Month Study in Sprague Dawley Rats

DSE Study #: 00-720-44

DM Study #: DM2001-88059-001

Day	Sex	Serum Concentrations of CP-88,059 (ng/mL)				
		0 hr	0.5 hr	2 hr	4 hr	8 hr
7	M	-	3310 ± 1500	458 ± 113	85.1 ± 28.7	-
	F	-	3780 ± 1340	524 ± 264	174 ± 51	34.4 ± 20.0
	M+F	-	3550 ± 1360	491 ± 195	130 ± 61	-
21	M	-	2200 ± 1220	291 ± 103	46.4 ± 43.4	-
	F	19.7 ± 19.3	3430 ± 1080	394 ± 108	232 ± 76	66.3 ± 47.8
	M+F	-	2820 ± 1260	342 ± 113	139 ± 114	-

A mean was not calculated if 50% or more of the data were <LLOQ.

Exposure to CP-88,059 Following Daily Intramuscular Dosing
During 1 Month Study in Sprague Dawley Rats

DSE Study #: 00-720-44

DM Study #: DM2001-88059-001

Day	Sex	Cmax (ng/mL)	Tmax (hr)	AUC (0-8hr) (ng•hr/mL)
7	F	3780	0.5	5290
	M	3310	0.5	-
	M+F	3550	0.5	-
21	F	3430	0.5	4950
	M	2200	0.5	-
	M+F	2820	0.5	-

In the original report, $AUC_{(0\text{-last})}$ was 3300 and 3600 ng•hr/mL for M and M+F, respectively, on Day 7 and 2100 and 2800 ng•hr/mL for M and M+F, respectively, on Day 21.

Exposure to CP-88,059 Following Daily Intramuscular Dosing
During 1 Month Study in Beagle Dogs

DSE Study #: 00-720-45

DM Study #: DM2001-88059-003

Day	Sex	Serum Concentrations of CP-88,059 (ng/mL)					
		0 hr	0.5 hr	2 hr	4 hr	8 hr	24 hr
7	M	-	598 ± 96	156 ± 10	78.8 ± 8.1	-	-
	F	-	273 ± 122	108 ± 38	48.2 ± 17.2	-	-
	M+F	-	435 ± 204	132 ± 36	63.5 ± 20.6	-	-
21	M	-	326 ± 190	185 ± 10	69.7 ± 5.8	-	-
	F	-	442 ± 44	110 ± 12	58.3 ± 5.9	-	-
	M+F	-	384 ± 138	148 ± 42	64.0 ± 8.2	-	-

A mean was not calculated if 50% or more of the data were <LLOQ.

Exposure to CP-88,059 Following Daily Intramuscular Dosing
During 1 Month Study in Beagle Dogs

DSE Study #: 00-720-45

DM Study #: DM2001-88059-003

Day	Sex	Cmax (ng/mL)	Tmax (hr)	AUC (0-4 hr) (ng-hr/mL)
7	M	598 ± 96	0.5 ± 0.0	950 ± 85
	F	273 ± 122	0.5 ± 0.0	510 ± 190
	M+F	435 ± 204	0.5 ± 0.0	730 ± 275
21	M	343 ± 165	1.0 ± 0.9	719 ± 176
	F	442 ± 44	0.5 ± 0.0	693 ± 60
	M+F	392 ± 121	0.8 ± 0.6	706 ± 118

For rat and dog, the new C_{max} and AUC values were ≈30% higher than the original values.

Reviewer comments: the sponsor has adequately addressed the FDA's request for updated TK data for the 1-mo i.m. toxicity studies in rat and dog.

RECOMMENDATION

The sponsor should be informed that the pharmacokinetic data submitted do not provide a justification for eliminating the recommendation for an assessment of the reproductive toxicity of IM ziprasidone. This assessment should be conducted [Phase 4] using ziprasidone in combination with sulphobutylether beta-cyclodextrin. The sponsor should be referred to the ICH document, Detection of Toxicity to Reproduction for Medicinal Products [ICH-S5A, Sept 1994] for guidance.

The following information was relayed to the sponsor on 6/19/02:

The pharmacokinetic data submitted do not provide a justification for eliminating our recommendation for an assessment of the reproductive toxicity of IM ziprasidone [to include dosing during all stages of development]. The recommendation was made based on a concern that SBECD may affect the reproductive toxicity potential of ziprasidone, particularly considering that ziprasidone and SBECD, each alone produces adverse effects on reproduction.

Therefore, an assessment of the reproductive toxicity of IM ziprasidone should be conducted Phase 4 using ziprasidone in combination with SBECD. If formulation problems are potentially dose-limiting, you should consider alternative dosing regimens, e.g., b.i.d. dosing. Please refer to the ICH document, Detection of Toxicity to Reproduction for Medicinal Products [ICH-S5A, Sept 1994] for guidance.

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/s/

Lois Freed
6/21/02 10:09:38 AM
PHARMACOLOGIST

Barry Rosloff
6/25/02 04:29:33 PM
PHARMACOLOGIST

5/28/02

Office of Clinical Pharmacology and Biopharmceutics Review

NDA:	**20-919**
Volume:	**1 – 7 volumes**
Compound:	**Zeldox IM (Ziprasidone mesylate)**
Submission Date:	**21 Dec 2001**
Sponsor:	**Pfizer**
Reviewer:	**Joga Gobburu**

Background

The current amendment of the above NDA constitutes a complete response to the Neuropharmacological Drug Product Division's approvable letter dated March 6, 2001. The primary concern cited in the FDA's action letter pertained to the lack of data assessing the effects of ziprasidone IM on the QTc interval. Specifically the letter said:

" ... Therefore, it will be critical for you to submit an adequate assessment of the of the effects of IM ziprasidone on the QTc interval at Tmax after a second 20 mg IM dose. ... In this regard, it will be important for you [sponsor] to address the question of patients who may achieve Cmax plasma levels considerably greater than the mean. Even if we were to conclude that the Cmaxs seen after the maximum IM dose were approximately equal to maximum plasma levels achieved with oral ziprasidone, we would need to be reassured that the highest plasma levels achieved after maximum IM dosing are not only no greater than those achieved after maximum oral dosing, but that there are not an increased number of patients who might achieve these levels after IM compared to oral dosing."

The sponsor conducted study A1281063 in patients with schizophrenia and schizoaffective disorder following administration of 2 injections of ziprasidone or haloperidol given four hours apart (20 mg (first dose) -> 30 mg (second dose) ziprasidone; 7.5 mg (first dose) -> 10 (second dose) mg haloperidol) under highly controlled experimental conditions. A 30 mg ziprasidone dose was examined to assess QTc effects at higher mean concentrations than those associated with the recommended 10 to 20 mg dose range. The approvable letter deals with ziprasidone and only data collected upon administration of ziprasidone IM is considered for review.

The aim of the current review is to (1) quantitate the relationship, if any, between exposure and QTc and (2) simulate scenarios that allow appreciation of the maximal net effect on QTc with the recommended dosing.

Methods

Data

Single blind study A1281063 was designed to characterize the pharmacokinetics, and the effects of multiple IM doses of ziprasidone and haloperidol on the QTc interval at the observed Cmax. Subjects meeting the screening criteria were tapered to the lowest possible dose of their current antipsychotic over approximately 7 days (period 1, days –10 to –4). A drug washout period of 4 days (period 2, day –3 to 0) was followed by a one-day treatment period (period 3, day 1). On the last day of the period 2 (day 0), patients were randomized in a 1:1 ratio to either ziprasidone IM or haloperidol. About 26 plasma concentrations of Ziprasidone (0, 0.25, 0.5, 0.75, 1, 1.25, 1.5, 1.75, 2, 2.5, 3, 4, 4.25, 4.5, 4.75,

5, 5.25, 5.5, 5.75, 6, 6.5, 7, 8, 9, 12, 16, 24, 36, 48, 72 hr) or haloperidol, and ECG measurements (0, 0.25, 0.5, 0.75, 1, 1.25, 1.5, 1.75, 2, 2.5, 3, 4, 4.25, 4.5, 4.75, 5, 5.25, 5.5, 5.75, 6, 6.5, 7, 8, 9, 12, 16 hr) were collected throughout periods 2 and 3. Table 1 shows the demographic characteristics of the patients.

Table 1. Summary of demographic variables of patients in study A1281063.

		Ziprasidone		Haloperidol	
		Males	Females	Males	Females
Subjects		25	6	21	6
AGE, yr	Mean	44.2	41.8	43.6	43.5
	Range	25 - 59	29 - 50	29 - 72	21 - 57
Race					
	White	12	1	11	1
	Black	8	5	7	3
	Asian	1	0	0	1
	Other	4	0	3	1
Weight, kg	Mean	84.8	80.4	89.3	74.4

Analytical Method

Ziprasidone was analysed by [ ] and detected by [ ] The range of the assay was — to — ug/L. As reviewed originally by OCPB, the analytical methodology is acceptable.

Models

Pharmacokinetics

Pharmacokinetic data were manually entered based on the hard copy listings of the concentration-time data (Section 13, Table 1.1). 713 plasma concentrations from 29 subjects were utilized to develop a pharmacokinetic model. Two subjects (50480015 and 50480025) had unusually high concentrations, as high as — ug/L (at 4.75 hr for 50480015) and — ug/L (at 1 hr for 50480025), in comparison to the mean (not including the 2 patients) concentrations of 155 ug/L and 263 ug/L at those particular time points. The reason for this unusually high concentrations was not identified and hence the subjects were deleted from further analyses.

A simple one – compartment model with first-order absorption (ka model) was used to describe time-concentration data, as shown in equation 1:

$$C_{ij} = \frac{Dose \cdot k_{a,i} \cdot F}{V_i \cdot (k_{a,i} - \frac{CL_i}{V_i})} \cdot (e^{-\frac{CL_i}{V_i} \cdot t} - e^{-k_{a,i} \cdot t_{ij}}) \quad (1)$$

In equation 1, C_{ij} is the j^{th} concentration interval of the i^{th} patient, CL_i is the systemic clearance, V_i is the volume of the distribution, $k_{a,i}$ is the rate constant for the first-order absorption, and F is the absolute bioavailability. The parameter F was not estimated due to lack of data from an IV dose. The inter-individual variability of all the PK parameters was described using a log-normal distribution. The residual error was described using a combined additive (variance of σ^2_{SDCP}) and proportional error model (variance of σ^2_{CVCP}).

Pharmacodynamics (QT)

The concentrations predicted using equation 1 were employed in driving the PD model. Both population and individual QT correction methods were evaluated. In total, 4 correction methods were employed: (1) BZT: Bazett's correction that assumes $\beta = 0.5$, (2) FRD: Fredericia's correction that assumes $\beta = 0.33$, (3) NPP: Naïve pooled power model that estimates one population β specific for the current data set and (4) NMP: nonlinear mixed effects model that estimates individual β. Equation 2 describes the relationship between RR interval and QT.

$$QT_{ij} = \alpha_{ij} \cdot RR_{ij}^{\beta_i} \quad (2)$$

In equation 2, QT_{ij} is the j^{th} QT interval of the i^{th} patient, similarly α_{ij} is the corrected QT and RR is the RR interval, and β_i is the exponent coefficient of the i^{th} patient. For the population QT correction methods β would be a constant for all patients. For example, according to Bazett's correction $\beta = 0.5$. The drug effect is added to the above equation assuming a linear relationship between concentration and QTc prolongation, as shown in equation 3.

$$QT_{ij} = \alpha_{ij} \cdot RR_{ij}^{\beta_i} + C_{ij} \cdot Slope_i \quad (2)$$

The presence of a time delay between the concentration and effect profiles was tested using a link model approach. The inter-individual variability of all the PD parameters was described using a log-normal distribution, except for $Slope_i$ which was assumed to follow a normal distribution. The residual error was described using an additive (variance of σ^2_{SDQT}).

Model Selection

The PK and PD models were selected mostly based on visual inspection of the fittings and log-likelihood ratio test. Since rich data are available first-order

conditional estimation (FOCE) methods were used. For the PK model, FOCE with interaction was used.

Simulation

Concentration and QT data were simulated, in 2900 patients, using the final PK and PD model and their parameter distributions. The maximal QTc change in each patient was determined and the various quantiles of this statistic were calculated.

Software

SAS (ver 8.2) was used for all data formatting and statistical procedures. NONMEM (ver. 5, level 1.1) with a Digital Compaq Fortran compiler (ver. 6.1) was used for modeling and simulation.

Results and Discussion

Pharmacokinetics

Figure 1 shows observed and model predicted (posthoc) profiles in 6 patients. The concentration-time profiles of ziprasidone IM could be adequately described using a one-compartment model, as shown in equation 1.

Figure 1. Observed and predicted (individual posthoc) concentration-time profiles in a set of 6 patients.

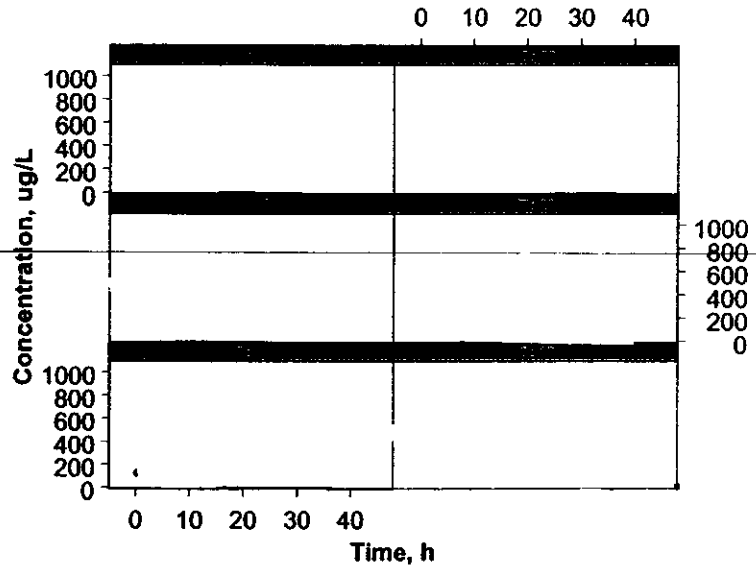
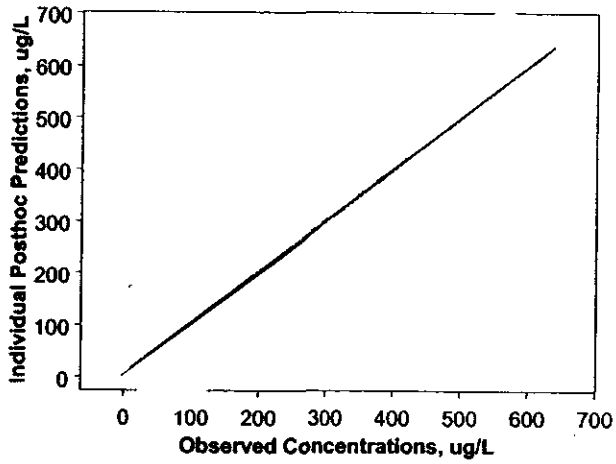


Table 2 shows the final PK parameter estimates and their standard errors.

Parameter	*Mean (%SE)*	*BSV,%CV (%SE)*
CL/F, L/h	27.1 (6)	32 (35)
V, L	100 (8)	40 (25)
ka, 1/hr	3.3 (17)	90 (31)
CVCP (%SE)	22 (13)	
SDCP,ug/L (%SE)	4.5 (31)	

Note: BSV = between-subject variability; CVCP and SDCP are the residual variability model parameters.

Figure 2. Observed and predicted (individual posthoc) concentrations. The predictions are in reasonably close agreement with the observed, except for a few high concentrations.



Since the primary aim of the analysis is to quantitatively assess the concentration dependent QTc prolongation, the good predictions using a one-compartment model were deemed adequate.

Pharmacodynamics

Sponsor's Results

The sponsor provided the following summary table.

QTc Change, Categorical Increases, and Pharmacokinetic Parameters for Ziprasidone and Haloperidol

		Injection 1		Injection 2	
		Ziprasidone	Haloperidol	Ziprasidone	Haloperidol
QTc Change*	N =	25	24	25	24
Mean (95% CI)		4.6 (0.4, 8.9)	6.0 (1.4, 10.5)	12.8 (6.7, 18.8)	14.7 (10.2, 19.2)
Incidence ≥500msec		0	0	0	0
Incidence ≥30msec		12	13	18	14
Incidence ≥60msec		0	0	1	0
Pharmacokinetics	N=	23	22	23	22
Mean Cmax (%CV)		182 (33)	12.6 (43)	319 (41)	17.9 (32)
Mean Tmax (%CV)		1.2 (58)	0.6 (33)	1.1 (45)	1.0 (80)

* Change from Baseline QTc defined as the average of the 3 values surrounding Tmax for each injection; Baseline correction, $QTc = QT/RR^{0.33}$

Reviewer's Results

First the results from the correction methods will be discussed followed by the concentration-effect relationships. Table 3 shows the objective function value for all the 4 different correction methods. NMP has one extra parameter than the other models. Clearly NMP is superior to all methods based on the objective function value. The p-value for rejecting NPP over NMP is < 0.0001 (difference of 31 for one additional degree of freedom).

Table 3. Objective function values obtained for the different QT correction methods.

Correction Method	*Objective function*
Bazett (BZT)	9886
Fredericia (FRD)	9122
Naïve Pooled (NPP)	9044
NONMEM (NMP)	9013

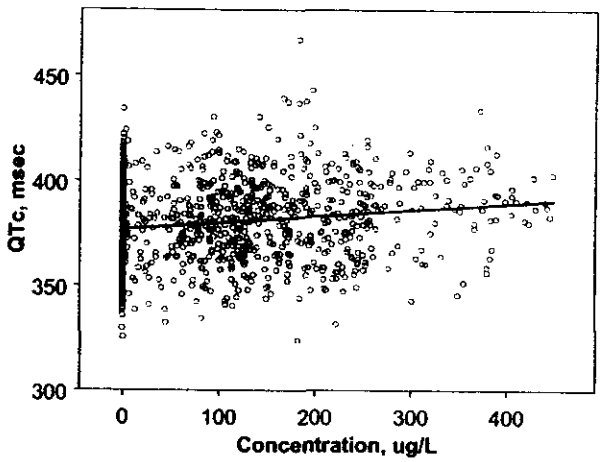
Hence, NMP correction method was selected for further analyses. Inclusion of the slope parameter between concentration-QTc relationship decreased the objective function value by 148 (two additional parameters, mean and BSV of SLOPE), implying a strong significance level. A delay between the time courses of PK and PD was found to be not significant. The final baseline and PD parameter estimates for QTc prolongation are presented in Table 4.

Table 4. Final baseline and PD parameter estimates.

Parameter	*Mean (%SE)*	*BSV,%CV (%SE)*
α, msec	376 (1)	4 (24)
β	0.285 (4)	16 (39)
Slope, msec/ug/L	0.035 (23)	107 (38)
SDCP,ug/L (%SE)	10 (8)	

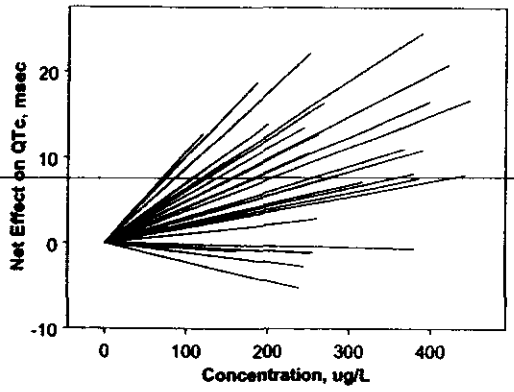
Figure 3 shows the concentration and QTc data with a best fitted linear trend line. Evidently, the data shows a considerable amount of scatter, which is also evident from Table 3.

Figure 3. Concentration versus QTc plot with a best fitted linear trend line.



The between-subject variability for the slope parameter is about 100%. Further observing the distribution of net effects across the concentration range, in Figure 4, depicts that some subjects could potentially have as high as ~25 msec increase in QTc, while some might have almost no change.

Figure 4. Distribution of concentration-net effect across the patient population studied.



The current study design included 20 and 30 mg dosing. The real scenario includes administration of 20 mg doses (highest recommended dose). Simulations were conducted under the design where patients receive 20 mg two time, 4 hr apart. The simulated data were subjected to standard univariate analysis. Table 5 shows the different quantiles of the maximal effect.

Table 5. Quantiles of maximal effect for each patient, over a total of 2900 patients, determined for the simulated data. Patients received 20 mg at 0 hr and another 20 mg at 4 hr.

Quantile	*Maximal Effect, msec*
100%	44
90%	18
75%	12
Mean	8
50%	7
25%	2
0%	-4

Conclusions

1. The primary conclusion from the current analyses is that the QTc prolongation of ziprasidone is concentration dependent. The between subject variability of relationship is considerably high (100%). Although the mean (or median) QTc prolongation is about 8 msec, the variability is considerable, as shown in Table 5. It is possible that there are about 10% of the patients who might have QTc prolongation between 18 and 44 msec.
2. Table 5 presents the various quantiles of net maximal QTc effect in 2900 simulated patients who received two ziprasidone doses of 20 mg q4h. Simulations are needed for at least 2 reasons here: (1) the sponsor studied a higher second dose while the label recommended dosing is 20 mg q4h and (2) the study includes only 31 patients thereby the probability of the worst case scenarios may not be estimated as reliably as would be possible via simulations. The medical reviewer should consider the risk of having the presented QTc prolongation for the perceived benefit that the patient might derive from using ziprasidone.
3. The approvable letter raised another issue for the sponsor to address regarding the distribution of Cmax values after IM injection compared to that after oral. The sponsor reported a mean (CV) Cmax value of 182 (33) ug/L after the first dose (20 mg) and 319 (41) ug/L after the second dose (30 mg). Based on the previous OCPB review, the mean (CV) of Cmax values after 80 mg bid (oral) was 202 (35%) ug/L. Based on the simulations conducted in the present review, the mean (CV) of Cmax values after 20 mg q4h (IM) was 208 (33%) ug/L.
4. Separate univariate analyses to determine the mean change in QTc at Tmax and its variability was conducted. The mean (CV) change in QTc (=QTc at Tmax – Baseline) of haloperidol is 15.5 (65%) msec and that of ziprasidone was 13 (104%) msec. The inter-patient variability of haloperidol (65%) is only slightly less than that of ziprasidone (104%).

5. Another important point is the applicability of the findings of the current review to the oral ziprasidone. In general, the fundamental properties of the drug such as the relationship between given concentration and effect will be independent of the mode of administration. Specifically in the case of ziprasidone, the finding about the high variability in the slope parameter from the IM data will be applicable to the oral case as well. This should be an important consideration in the risk/benefit assessment by the medical team.
6. Two subjects (50480015 and 50480025) were dropped due to unusually high ziprasidone concentrations. These subjects did not have correspondingly high QT intervals. The reviewer conducted a separate analysis including the 2 subjects, but without the 2 high concentrations under doubt. The analysis with all the 31 subjects (without the 2 high concentrations) resulted in very similar PK and PD model parameter estimates.
7. The weakness of the anlaysis is that the metabolites were not tested for relationship with QTc prolongation. Discerning the individual effects of parent and metabolites might not be feasible in the absence of separate administrations of these moieties.

Labeling Recommendations

The following are the sponsor proposed labeling changes that are relevant to the Clinical pharmacology of ziprasidone:

1. INTRAMUSCULAR PHARMACOKINETICS; Metabolism and Elimination: "Although the metabolism and elimination of IM ziprasidone have not been systematically evaluated, the intramuscular route of administration would not be expected to alter the metabolic pathways."
2. WARNINGS; QT prolongation and risk of sudden death: "A study [ ] QT/QTc prolonging effect of intramuscular ziprasidone with intramuscular haloperidol was conducted in patient volunteers. In the trial, ECGs were obtained at the time of maximum plasma concentration following two injections of ziprasidone (20 mg then 30 mg) or haloperidol (7.5 mg then 10 mg) given four hours apart. Note that a 30 mg dose of ziprasidone is 50% higher than the recommended therapeutic dose. The mean change in QTc from baseline was calculated for each drug, using a sample based correction that removes the effect of heart rate on the QT interval. The mean increase in QTc from baseline for ziprasidone was 4.6 msec following the first injection and 12.8 msec following the second injection. The mean increase in QTc from baseline [ ] for haloperidol was 6.0 following the first injection and 14.7 following the second injection. In this study, no patients had a QTc interval exceeding 500 msec.

The labeling language proposed by the sponsor under the Metabolism and Elimination section is acceptable to the Office of Clinical Pharmacology and Biopharmaceutics. The description of the trial, as proposed by the sponsor under WARNINGS section needs to be changed as shown below. The sponsor

did not *per se* conduct the trial to compare, formally, the effects of these two drugs. Also, the units (msec) of QTc prolongation for haloperidol might be helpful.

2. WARNINGS; QT prolongation and risk of sudden death: "A study evaluating the QT/QTc prolonging effect of intramuscular ziprasidone, with intramuscular haloperidol as —— control, was conducted in patient volunteers. In the trial, ECGs were obtained at the time of maximum plasma concentration following two injections of ziprasidone (20 mg then 30 mg) or haloperidol (7.5 mg then 10 mg) given four hours apart. Note that a 30 mg dose of ziprasidone is 50% higher than the recommended therapeutic dose. The mean change in QTc from baseline was calculated for each drug, using a sample based correction that removes the effect of heart rate on the QT interval. The mean increase in QTc from baseline for ziprasidone was 4.6 msec following the first injection and 12.8 msec following the second injection. The mean increase in QTc from baseline [ ] for haloperidol was 6.0 msec following the first injection and 14.7 msec following the second injection. In this study, no patients had a QTc interval exceeding 500 msec.

OCPB Recommendations

Overall, the Office of Clinical Pharmacology and Biopharmaceutics finds that the sponsor has adequately addressed the issue of QTc prolongation, as raised in the approvable letter dated March 6, 2001.

/S/ /S/

Joga Gobburu
Team Leader
Pharmacometrics

Ramana Uppoor
Team Leader
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RD/FT initialed by Ramana Uppoor Ph.D._____

Cc list: NDA 19-865, HFD 110, HFD 860 (Mehta, Gobburu, Marroum, Uppoorr), CDER Document room

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Jogarao Gobburu
5/28/02 10:50:18 AM
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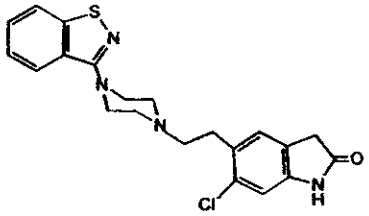
Ramana S. Uppoor
5/28/02 11:55:23 AM
BIOPHARMACEUTICS

REVIEW AND EVALUATION OF PHARMACOLOGY/TOXICOLOGY DATA

Reviewer Name: Lois M. Freed, Ph.D.
Division Name: Neuropharmacological Drug Products
HFD#120
Review Completion Date: 3/2/2001
Review number: 2
NDA number: **20-919**
Serial number/date/type of submission: N-(AP)
Information to sponsor: Y
Sponsor (or agent): Pfizer Inc.
Eastern Point Road
Groton, CT 06340
Drug: ziprasidone mesylate
Code Name: CP-88,059-27
Generic Name: n/a
Trade Name: Zeldox IM™
Chemical Name: 5-[2-[41,2-benzisothiazol-3-yl)-1-piperazinyl]ethyl]-6-chloro-1,3-dihydro-2H-indol-2-one methanesulfonate trihydrate
CAS Registry Number:
Molecular Formula/ Molecular Weight: 563.0 (salt)
Structure:



Relevant INDs/NDAs/DMFs: IND#49,045 (ziprasidone i.m.), IND #34,629 (ziprasidone p.o.), NDA 20-285 (ziprasidone p.o.)
Drug Class: D_2, $5HT_2$ receptor antagonist
Indication: agitation
Clinical formulation: 20 mg/mL, formulated for intramuscular injection; each mL of solution contains 20 mg ziprasidone, 4.7 mg methanesulfonic acid, 294 mg sulphobutylether beta-cyclodextrin sodium (SBECD)
Route of administration: i.m.
Studies reviewed within this submission:
1-mo i.m. toxicity (with *in vivo* micronucleus assay), rat
1-mo i.m. toxicity, dog
Studies not reviewed within this submission: none
Background: the current submission is a complete response to the nonclinical deficiencies described in the Division's non-approvable letters, dated 12/17/98 and 4/20/00. The Division's recommendations were as follows:

> "One month toxicology studies in a rodent and a nonrodent species should be conducted prior to approval. For these studies ziprasidone mesylate in the beta-cyclodextrin sulphobutyl ether formulation should be administered by the intramuscular route, and plasma measurements should be included. We also recommend that an assessment of the effects on micronucleus formation be incorporated into the rodent toxicology study."

TOXICOLOGY

1. Study Title: **One month intramuscular study in Sprague-Dawley rats** [Study No: 00-720-44, Vol #A5.1, and page #: not specified, Conducting laboratory and location: Pfizer Central Research, Groton, CT (except for TK analyses, performed at [redacted] date of study initiation: 5/22/00, report date: 8/00, GLP, QA: Y]

Methods

Dosing

species/strain: Sprague-Dawley rat, [redacted]

#/sex/group or time point: 10/sex/grp

age: 50-52 days

weight: 212.4-253.8 gm for males, 156.8-209.0 gm for females

diet/housing: *ad lib*/individual

satellite groups used for toxicokinetics or recovery: no. However, an additional 5/sex were used for testing of the positive control in the *in vivo* micronucleus portion of the study.

dosage groups in administered units: 0 (sterile water), 0 (SBECD), 2 mg/day (active moiety). **Since only an absolute amount of drug was administered, the dose on a "mg/kg" basis decreased over the 28-day dosing period.**

route, form, volume, and infusion rate: i.m., serile solution, 0.1 mL. The site of injection was rotated daily among 4 injection sites (right/left leg, upper/lower section of thigh).

Drug, lot#, and % purity: ziprasidone mesylate (CP-88,059-27), bulk lot no. CSM01003, [redacted] % CP-88,059 [17.0% methanesulfonic acid, 9.1% water, 0.03% tetrahydrofuran]

Formulation/vehicle: i.m. lyophile, reconstituted with 1.2 mL sterile water for injection; each mL of reconstituted solution contained 20 mg of ziprasidone, 4.7 mg methanesulfonic acid, 294 mg sulphobutylether beta-cyclodextrin sodium. Reconstituted solution stated to be stable for ≤24 hrs at 15-30° C; no data provided. [Solutions were prepared daily.] Concentration of drug solution stated have been ±10% of intended on Days 1 and 28; no data provided. SBECD and DT grps received ≈30 mg/day of SBECD [i.e., SBECD: 0.1 mL/day of 300 mg/mL solution of SBECD; DT: 0.1 mL/day of 1 mL solution containing 294 mg of SBECD].

Observations and times

Clinical signs: animals were observed twice daily.

Body weights: recorded weekly during the dosing period.

Food consumption: recorded weekly during the dosing period.

Ophthalmoscopy: examinations performed prior to start of dosing and on Day 24 (males) or 23 (females). Mydriasis was induced by application of 1% tropicamide for examination.

ECG: no.

Hematology: blood samples were collected (retroorbital sinus puncture) on Day 29 (all animals) for analysis of the following parameters: rbc ct, hgb, hct, platelet ct, MCV, MCH, MCHC, reticulocyte ct, wbc (ct, differential), fibrinogen.

Clinical chemistry: blood samples were collected (retroorbital sinus puncture) on Day 29 (all animals) for analysis of the following parameters: ALT, GGT, total bilirubin, albumin, globulin, glucose, creatinine, K, Ca, AST, 5'-nucleotidase, total protein, cholesterol, BUN, Na, Cl. [Alkaline phosphatase was not measured.]

Urinalysis: urine samples were collected over a 5-7 hr period on Day 24 from all animals for analysis of the following parameters: volume, specific gravity, pH, protein, blood, glucose, urobilinogen, bilirubin, ketones, color.

Gross pathology: a complete necropsy was conducted on all animals following sacrifice on Day 29.

Organ weights: the following organs were weighed: liver, kidneys, heart, brain, adrenals, and

testes. Paired organs were weighed together.

Histopathology: the following tissues were examined microscopically: kidney, urinary bladder, liver (left, right lateral lobes), thymus, spleen, mesenteric lymph node, esophagus, stomach, duodenum, jejunum, ileum, cecum, colon, pituitary gland, salivary gland, pancreas, thyroid gland, testes (both), prostate, seminal vesicle, ovaries, uterus, vagina, trachea, lung (both diaphragmatic lobes), heart, peripheral nerve, brain (cerebrum, cerebellum, pons), spinal cord (cervical), Harderian gland, eyes, skin and adnexa (including mammary gland), bone/bone marrow (sternum), skeletal muscle/injection site, adrenal gland, parathyroid gland, epididymides, gross lesions. Tissues (except for eye and Harderian gland which were fixed in 3% glutaraldehyde/Sorenson's buffer) were preserved in 10% neutral buffered formalin, processed, and stained with H & E for analysis.

Toxicokinetics: blood samples were collected via the retroorbital sinus from 5/sex/grp (drug-treated) prior to start of dosing and on Days 7 and 21 at 0, 0.5, 2, 4, and 8 hrs postdosing. In control animals, blood samples were collected only at 0 and 0.5 hrs postdosing on Days 7 and 21. CP-88,059 was quantitated in serum using HPLC with uv detection. **[note: in the sponsor's protocol, it was stated that "Due to a lack of long term stability data at the time of submission of this report, final reported serum concentrations may change slightly."** Blood samples collected for TK analysis were stored at -20° C "until analysis".

Micronucleus: 5/sex/grp were evaluated for induction of micronuclei. The positive control, Mitomycin C, was tested in an additional 5/sex. Mitomycin C was administered daily at 1.25 mg/kg/day i.p. for 3 consecutive days. Positive control animals were observed as follows: clinical signs (twice daily), body wt (first day of dosing); however, these data were not included in the report. Femoral bone marrow samples were collected from these animals at sacrifice on Day 29 and from the positive control animals "on the day after the completion of their third dose". Bone marrow samples were collected, processed, and examined as detailed in a separate study report (reviewed in the GENOTOXICITY section of this review).

Results

Mortality: there was one unscheduled death: 1 sterile-water CM (found dead on Day 8; cause of death was not determined).

Clinical signs: reduced spontaneous motor activity, prostration, and partially closed eyes were observed in all drug-treated (DT) animals throughout the dosing period. "Unable to rise" was reported in 1/10 DTM only on following the first dose. [It is not clear what distinguished "unable to rise" from "prostration".] Clinical signs were observed within 15 min of dosing; however, behavior was said to normalize by 4-6 hrs postdosing. There was no evidence of local irritation. No clinical signs were observed in C animals.

Body weights: mean body wt was reduced in DTM from Day 8 on (maximum effect on Day 28: 13% reduction compared to SBECD controls). Mean body wt gain was reduced by 47 (Day 8) to 34 (Day 28)% in DTM (compared to SBECD controls). Neither parameter was affected in DTF or in SBECD controls (compared to sterile water Cs).

Food consumption: mean food consumption was only slightly affected in DTM (i.e., 10-12% reduced compared to SBECD controls); differences were statistically significant only on Day 22. Food consumption was not affected in DTF or in SBECD controls.

Ophthalmoscopy: no data or individual observations were provided. According to the sponsor, findings on Day 23/24 were secondary to trauma induced during blood sampling procedures. However, only 5/sex/grp experienced such blood collection prior to ophthalmology examination. The sponsor should have summarized the findings.

Hematology: there were no statistically significant findings in males; however, the following were noted: (1) slight increase in eosinophil ct in SBECD-M and DTM (11 and 14%, compared to sterile water CM), (2) an increase in large unstained cells in SBECD-M and

DTM (39 and 28%, compared to sterile water CM), (3) a small increase in % reticulocytes and fibrinogen in DTM (24 and 11%, respectively, compared to SBECD-M). In females, increases were noted in wbc ct in both SBECD and DT grps (21 and 33%, respectively, compared to water CF), due to increases in lymphocyte and monocyte cts; with the later two parameters, the increases were greater in DTF (10-50%) than in SBECD-F [lymphocyte ct: 23 and 36% in SBECD-F and DTF, respectively; monocyte ct: 13 and 70% in SBECD-F and DTF, respectively]. % reticulocyte ct was increased in DTF (37% compared to SBECD-F); SBECD-F were not affected. Fibrinogen was increased in DTF (14% compared to SBECD-F, 21% compared to water CF); fibrinogen was only slightly increased (6%) compared to water CF. The sponsor considered the wbc ct (and differential) and fibrinogen findings secondary to local irritation. The increased % reticulocyte ct was not considered drug-related by the sponsor due to the lack of significant effects on rbc parameters.

Clinical chemistry: in males, there were no drug-related changes in mean values. ALT, AST, and 5'-nucleotidase were elevated (46, 120, and 70%, respectively, compared to high water CM value) in 1/10 DTM (#23). AST was also elevated (68% above high water CM value) in 1/10 SBECD-M (#16); 5'-nucleotidase was also slightly elevated in this male (46%) and in 1 other SBECD-M (#15, 25%). In females, there were no clear drug-related findings. A small decrease was noted in albumin (4-6%) in DTF, although the sponsor noted that values were within normal range (i.e., the range of healthy animals). Glucose was slightly reduced in DTM and DTF (7-9% compared to SBECD Cs).

Urinalysis: urinalysis data were not summarized. According to the sponsor, there were no drug-related effects. Examination of individual data indicated an increase in the presence of urobilinogen in SBECD-F and DTF compared to water Cs.

Organ Weights: only selected organs were weighed. In males, relative kidney wt was increased in both SBECD-M and DTM compared to water CM [7-6%]; absolute kidney wt was increased in SBECD-M compared to water CM (10%), but was decreased in DTM compared to SBECD-M (14%). In females, absolute and relative adrenal wt was increased in DTF (9-14%) compared to water CF and SBECD-F.

Gross pathology: macroscopic findings were not summarized. According to the sponsor's report, pale or brown discoloration was detected in leg muscles in 4/10 DTM, 9/10 DTF, and 1/10 SBECD-F. This finding was considered drug-related by the sponsor. All other macroscopic findings (not described) were considered unrelated to drug or SBECD.

Histopathology: selected data are summarized in the following table:

TISSUE	FINDING	MALES			FEMALES		
		WC	SBECD	DT	WC	SBECD	DT
urinary bladder	mineralization	0/10	0/10	0/10	0/10	0/10	1/10
liver	necrosis	0/10	0/10	1/10	0/10	0/10	0/10
injection site	foreign body reaction	0/10	0/10	10/10	0/10	0/10	10/10

The foreign body reaction was characterized by the sponsor as follows: "...foreign body giant cells surrounding acicular material (interpreted to be residual administered drug) in connective tissue and/or skeletal muscle, accompanied by varying numbers of mixed inflammatory cells. Foreign body reaction was sparse and isolated (slight), confluent within connective tissue septae (mild) or expanded connective tissue septae slightly, occasionally impinging on skeletal muscle without causing compression of surrounding tissues (moderate)." The local reaction was more severe in females.

The one instance of liver necrosis was described as focal and slight, and was not observed in the DTM in which elevations of AST, ALT, and 5'-nucleotidase were observed. The one instance of urinary bladder mineralization was described as focal and as involving the serosa; no severity was noted.

Toxicokinetics: ziprasidone was rapidly absorbed following i.m. injection. The data are summarized in the following table:

DAY	MALES			FEMALES		
	T_{max} (hr)	C_{max} (ng/mL)	$AUC_{(0\text{-}last)}$ (ng•hr/mL)	T_{max} (hr)	C_{max} (ng/mL)	$AUC_{(0\text{-}last)}$ (ng•hr/mL)
7	0.5	2600 ± 1200	3300	0.5	2900 ± 1000	4100
21	0.5	1700 ± 1000	2100	0.5	2700 ± 800	3900

The differences in exposure between males and females and between Days 7 and 21 are probably due to differences in body wt between sexes and decreases in "mg/kg" dose with growth, and not differences in kinetics. In females, ziprasidone was still detectable (mean: 30-50 ng/mL) at the last measurement time (i.e., 8 hr postdosing).

2. Study Title: **One month intramuscular study in Beagle dogs** (Study No: 00-720-45, Vol #A5.1, page #: not specified, Conducting laboratory and location: Pfizer Central Research, Groton, CT, except for TK analysis: [ ], date of study initiation: 5/19/00report date: 8/00, GLP, QA: Y)

Methods

Dosing

species/strain: Beagle dog [ ]

#/sex/group or time point: 3/sex/grp

age: 5-12 mo.

weight: 6.3-9.3 kg for males, 5.9-7.4 kg for females.

diet/housing: once daily feeding/individual

satellite groups used for toxicokinetics or recovery: no.

dosage groups in administered units: 0 (sterile water), 0 (SBECD, 10 mg/day (active moiety).

route, form, volume, and infusion rate: i.m., solution, 0.5 mL. Injections were rotated among 4 separate sights (left or right leg, upper or lower thigh).

Drug, lot#, and % purity: CP-88.059-27, bulk lot no. CM01003, active moiety/purity: —%.

Formulation/vehicle: solution for sterile injection/17.0% methanesulfonic acid, 9.1% water, 0.03% tetrahydrofuran. Each mL of reconstituted solution contained the following: 20 mg ziprasidone, 4.7 mg methanesulfonic acid, 294 mg sulfobutyl ether beta-cyclodextrin sodium. Stability and documented drug concentrations as per i.m. study in rat (Toxicology, #1). No data were provided.

SBECD and DT grps received ≈150 mg/day of SBECD [i.e., 0.5 mL of a ≈300 mg/mL solution].

Observations and times

Clinical signs: animals were observed twice per day.

Physical: respiratory rate and rectal temperature were recorded once prior to start of dosing, and on Days 9 and 22 (all animals) at 0 and 1 hr postdosing.

Body weights: recorded prior to start of dosing and weekly during the dosing period.

Food consumption: intake was estimated [<25%, 25%, 50%, 75%, 100%, or spill] daily during the dosing period.

Ophthalmoscopy: examinations conducted prior to start of dosing and on Day 23/24. Mydriasis was induced using 1% tropicamide.

ECG: ECG (Leads I, II, aVR, aVL, aVF, CV6LL, V5, and V10), indirect SAP, and heart rate were recorded for all dogs prior to start of dosing, and on Days 9 and 22, at 0 and ≈1 hr postdosing.

Hematology: blood samples were collected (from all animals) once prior to start of dosing and on Days 15 and 28 of dosing for analysis of the following parameters: rbc ct, hgb, hct, platelet ct, MCV, MCH, MCHC, reticulocyte ct, wbc (ct, differential), PT, APTT.

Clinical chemistry: blood samples were collected (from all animals) once prior to start of dosing and on Days 15 and 28 of dosing for analysis of the following parameters: ALT, alkaline phosphatase, total bilirubin, bile acids, albumin, BUN, creatinine, Na, globulin, AST, GGT, K, total protein, cholesterol, glucose, Ca, Cl.

Urinalysis: urine samples were collected over a 5-7 hr period [except in 2-3 animals per collection, urine was collected by cytocentesis] once prior to start of dosing, and on Days 15 and 28 for analysis of the following parameters: volume, specific gravity, pH, protein, blood, glucose, urobilinogen, bilirubin, ketones, color, microscopic analysis of sediment (only if appearance was abnormal or if blood was detected). Ratings of at least 2+ on selected qualitative tests (protein, bilirubin, glucose) were confirmed using quantitative assays.

Gross pathology: a complete necropsy was performed on all animals.

Organ weights: weights of the following were recorded: kidneys, liver, testes, adrenals, brain, pituitary, and heart.

Histopathology: the following tissues were examined microscopically: kidney, urinary bladder, aorta, liver (left, right lateral lobes), thymus, spleen, mesenteric lymph node, esophagus, stomach, duodenum, jejunum, ileum, cecum, colon, pituitary gland, salivary gland, adrenal gland, parathyroid, epididymides, ovaries, uterus, cervix, trachea, lung (both diaphragmatic lobes), heart, peripheral nerve, brain (cerebrum, cerebellum, pons), spinal cord (cervical), eyes, skin and adnexa, mammary gland, bone/bone marrow (sternum), skeletal muscle (including injection site), gallbladder, pancreas, thyroid gland, testes (left, right), prostate, gross lesions. All tissue (except for eyes which were fixed in 3% glutaraldehyde in Sorenson's buffer) were preserved in 10% neutral buffered formalin, processed, and stained with H & E for analysis.

Toxicokinetics: blood samples were collected via jugular venipuncture in DT animals on Days 7 and 21 of dosing at 0, 0.5, 2, 4, 8, and 24 hrs postdosing. In C grps, samples were collected only at 0 and 0.5 hr postdosing. Blood samples were stored at -60° C until analyzed. CP-88,059 was quantitated in serum using HPLC with uv detection (range of quantitation: [ ] ng/mL). **The report noted that "Due to a lack of long term stability data at the time of submission of this report, final reported serum concentrations may change slightly."**

Results

Mortality: there were no unscheduled deaths.

Clinical signs: the only drug-related finding was a decrease in spontaneous motor activity, which was observed in all DT animals throughout the dosing period. According to the sponsor, this effect was observed within 15 min of dosing; behavior normalized by 2-4 hrs postdosing. No local reactions were detected.

Physical: respiratory rate was reduced at 1 hr postdosing on Days 9 and 22 of dosing in DTM and DTF. In DTM, the decrease was 43-45% compared to predosing values. In females, the decrease was 44-48% compared to predosing values; however, predosing values in SBECD and DT grps were higher (80-60%) than in WC grps. Rectal temperature was reduced in DT grps at 1 hr postdosing on Days 9 and 22 of dosing in both males (1.7-1.6° C) and females (1.1-1.0° C).

Body weights: no drug-related effects were noted.

Food consumption: no drug-related effects were noted, according to the sponsor. [Data were qualitative, and were not summarized.]

Ophthalmoscopy: according to the sponsor, no drug-related effects were noted; observations were not given.

ECG: blood pressure was reduced in DTM on Days 9 and 22 (25-33%, comparing predosing values). A similar trend was noted in DTF, however, the post-pre differences were smaller (20-11%). There were no apparent drug-related effects on ECG parameters. Although not statistically significant, heart rate tended to be higher in DT animals. In males, the mean increases (compared to pre-dose values) were 13 and 22 bpm on Days 9 and 22, respectively. However, only 2/3 DTM were affected; in 1/3 DTM, heart rate decreased. In the 2 affected DTM, mean increases were 29 and 39 bpm on Days 9 and 22, respectively. In DTF, the mean increases were 28 and 40 bpm on Days 9 and 22, respectively.

Hematology: in males, wbc ct was elevated on Day 28 (10%) in the DT grp. Neutrophil (10-30%) and monocyte (34-75%) cts were elevated on both sampling days in DTM. Neutrophils and monocytes were elevated, but to a lesser extent (12-20%) in SBECD-M. Hgb and hct were transiently elevated in SBECD-M (10-11%, Day 15). In females, wbc ct, lymphocyte and monocyte cts were elevated on Day 15 in the DT grp (22-67%). Platelet ct was slightly elevated in DTF (36-21%) during the dosing period, and % reticulocyte was increased in SBECD-F on Day 15 (100%), and in DTF in both sampling days (100-130%). The sponsor didn't consider any of these findings drug-related.

Clinical chemistry: the only finding of note was a small increase in total protein in DTF (13-10%) at both sampling times.

Urinalysis: urinalysis data were not summarized. The sponsor considered there to be no drug-related effects. An examination of individual data did not reveal any clear drug-related changes.

Organ Weights: there were no drug-related effects on wts of selected organs.

Gross pathology: according to the sponsor, the only finding of note was "a focal are of pale muscle" at one injection site in 1/3 DTM.

Histopathology: the only drug-related finding was a foreign body reaction at the injection site. This reaction was noted only in DT animals (3/3 M, 3/3 F).

Toxicokinetics: the data are summarized in the following table:

DAY	MALES			FEMALES		
	T_{max} (hr)	C_{max} (ng/mL)	$AUC_{(0\text{-last})}$ (ng•hr/mL)	T_{max} (hr)	C_{max} (ng/mL)	$AUC_{(0\text{-last})}$ (ng•hr/mL)
7	0.5 ± 0.0	460 ± 80	730 ± 60	0.5 ± 0.0	210 ± 100	390 ± 150
21	1.0 ± 0.9	270 ± 130	560 ± 140	0.5 ± 0.0	340 ± 30	540 ± 50

CP-88,059 was not detectable in serum by 8 hrs postdosing in either males or females. In males, serum levels decreased from Day 7 to Day 21, whereas in females the opposite was noted. This resulted in an apparent stable mean exposure from Day 7 to Day 21.

GENOTOXICITY

Study Title: **One month rat micronucleus assay** [Study No: 00-720-46, Vol #A5.1, and page #: not specified, Conducting laboratory and location: Pfizer Central Research, Groton, CT (except for TK analyses, performed at C J date of study initiation: 5/22/00, report date: 8/00, GLP, QA: Y]

Methods: this study was conducted as part of the 1-mo i.m. toxicity study in rat (Study no. 00-720-44). CP-88,059-27 (lot no. CSM01003) was administered to Sprague-Dawley rats (10/sex/grp) at a dose of 2 mg/day i.m. Both sterile water and vehicle (SBECD) controls were included (10/sex/grp). In addition, Mitomycin C (1.25 mg/kg i.p.) was administered to 5/sex/grp for 3 consecutive days as a positive control. Main-study animals were sacrificed on Day 29 (20-24 hrs after the last dose); at sacrifice, samples of femoral bone marrow were collected from 5/sex/grp. Positive control animals were sacrificed 20-24 hrs after the 3rd dose, and bone marrow samples were collected. "At least" 2 bone marrow smears were prepared from each animal. "A minimum" of 2000 PCEs from each animal were scored for micronuclei. The ratio (PCE:NCE) was determined by examination of 1000 rbcs as an index of cytotoxicity. The criterion for a positive response was a "statistically significant [$p<0.05$], dose-related and reproducible increase" in micronuclei compared to vehicle control.

Results: clinical signs in the DT animals consisted of decreased activity, prostration, and ptosis. There was no evidence of cytotoxicity or increase in micronucleated PCEs in the DT animals. In contrast, Mitomycin C produced a decrease in the PCE:NCE ratio (indicative of cytotoxicity) and an ≈10-fold increase in micronuclei.

OVERALL SUMMARY AND EVALUATION

The sponsor conducted 1-mo i.m. toxicity studies in Sprague-Dawley rat and Beagle dog to assess the toxicity of ziprasidone i.m. formulated in sulphobutylether beta-cyclodextrin. The rat study included an assessment in micronucleus formation in bone marrow. In both studies, only one dose level of ziprasidone was tested.

Toxicology

Rat: ziprasidone (or placebo) was administered i.m. to Sprague-Dawley rats (10/sex/grp) at 0 (sterile water), 0 (SBECD), and 2 mg/day for 1 month. Since an absolute amount of drug was administered per day, the mg/kg dose decreased over the dosing period. Based on mean body wt data on Days 1 and 28, the dose decreased from 8.5 mg/kg on Day 1 to 6 mg/kg on Day 28 in males, and from 11 mg/kg on Day 1 to 8.6 mg/kg on Day 28 in females. Ziprasidone was formulated in SBECD, the excipient intended for the clinical formulation; each daily dose contained 294 mg of SBECD. Routine observations were monitored; however, only selected organs were weighed [similar to the procedure used for the oral toxicity studies] and alkaline phosphatase and coagulation parameters were not quantitated.

There were no drug-related deaths. Clinical signs, consisting of reduced spontaneous motor activity, prostration, and partially closed eyes, were observed in all drug-treated (DT) animals. Behavior normalized by 4-6 hrs postdosing. [Individual observations were not provided; therefore, the severity or duration of clinical signs in individual animals could not be determined.] No local irritation was evident. Mean body wt was reduced in DTM (13% compared to SBECD controls), but was unaffected in DTF. Food consumption was only slightly affected in DTM. The sponsor reported no drug-related effects on ophthalmology parameters; however, findings were noted in the 5/sex/grp used for blood sampling (retroorbital puncture). [A listing of findings was not provided.] Hematology findings consisted of increases in reticulocyte ct and fibrinogen in DTM and DTF, increases in eosinophil ct and large unstained cells in DTM and SBECD-M, and increases in wbc ct (due to increases in lymphocyte and monocyte cts) in DTF and SBECD-F [the increases in lymphocytes and monocytes were greater in DTF compared to SBECD-F]. There were no clear drug-related findings on clinical chemistry parameters. ALT, AST, and 5'-nucleotidase were elevated in 1/10 DTM; however, there were no histopathological correlates in the affected male. There were no clear drug-related effects on urinalysis parameters. There was an increase in urobilinogen in DTF and SBECD-F; however, in the absence of other liver findings, this effect is probably incidental. Small increases in kidney and adrenal wts in SBECD and DT grps were without histopathological correlates. The primary gross finding was pale or brown discoloration in leg muscle in DTM and DTF [1/10 SBECD-F was also affected]. The primary microscopic finding was foreign body reaction. This finding was detected in all DT animals, although it was stated to be more severe in females. The foreign body reaction was characterized by presumed drug-related material surrounded by giant cells and mixed inflammatory cells. The increases in wbc ct and fibrinogen probably were secondary to the local effects. Plasma levels of ziprasidone were higher in females than in males. In general, this is consistent with the higher dose administered to females (on a mg/kg basis); however, dose-corrected AUC on Day 21 tended to be higher in females than in males.

Dog: ziprasidone (or placebo) was administered i.m. to Beagle dogs (3/sex/grp) at 0 (sterile water), 0 (SBECD), and 10 mg/day for 1 month. Unlike in rat, the mean dose on a mg/kg basis remained fairly stable over the dosing period since there was little or no change in mean body wt over the dosing period. The mean dose on a mg/kg basis was, however, slightly higher in females (≈1.5 mg/kg) than in males (≈1.25 mg/kg) due to the higher body wt in males. Ziprasidone was formulated in SBECD; the daily dose

contained ≈150 mg/day of SBECD. Routine observations were monitored; however, only selected organs were weighed [similar to the procedure used for the oral toxicity studies].

There were no unscheduled deaths. The only clinical sign was a decrease in spontaneous motor activity, which was observed in all DT animals for up to 2-4 hrs postdosing. [The severity of the effect was not noted.] Upon physical examination, respiratory rate and rectal temperature were noted to be reduced in DTM and DTF. There were no drug-related effects on body wt, food consumption, or ophthalmology. ECG parameters were not affected; however, blood pressure was significantly reduced in DTM (a similar trend was noted in DTF) and heart rate tended to be increased in DT animals (the effects were not statistically significant). Wbc ct was increased in DT animals. The increase was due to increases in neutrophil and monocyte cts in DTM and to increases in lymphocyte and monocyte cts in DTF. Reticulocytes were transiently increased in SBECD-F, but increased throughout the dosing period in DTF. [No changes in rbc parameters were noted in DTF.] The sponsor didn't consider any of these findings drug-related. The only clinical chemistry finding of note was a small increase in total protein in DTF. Urinalysis parameters were not affected and there were no drug-related changes in organ wts. The only gross lesion was a focal area of pale muscle noted in 1/3 DTM. As in rats, the only microscopic finding considered drug-related was foreign body reaction, which was detected only in and in all DT animals.

Conclusion: neither the i.m. toxicity study in rat or in dog was a standard toxicity study since ziprasidone was tested at only one dose level and the dose was not adjusted for body wt. In rats, the dose used was associated with some potentially dose-limiting systemic toxicity [i.e., clinical signs, decreased body wt gain (males)], although it is possible that higher doses could have been used in females. The dose used in the i.m. dog study was low (10 mg/day or 1.25-1.5 mg/kg/day). No dose-limiting toxicity was observed. Higher doses were used in a 2-wk i.v. toxicity study (HD = 8 mg/kg/day) in dog. In that study, severe CNS signs were observed at the HD, but no drug-related deaths occurred. In the 1-mo i.m. studies, the primary drug-related effects in rat and dog were clinical signs (consistent with sedation) and injection site reaction. In neither species was local irritation obvious by visual inspection. Microscopic analysis indicated the presence of drug and an inflammatory reaction at the injection site, involving surrounding connective tissue and/or skeletal muscle in both rat and dog. At each injection site, rat and dog received 0.1 and 0.5 times the volume of injectate compared to the human; the formulation was the same as that to be used clinically.

The i.m. studies alone would not support approval due to their limited design. However, the data from these studies indicate that additional or more severe drug-related effects were not observed when ziprasidone was administered i.m. compared to those observed in the oral and i.v. toxicity studies, with the exception of the local reaction produced by i.m. dosing. [If higher doses had been used in the i.m. dog study, a better comparison of the p.o., i.v., and i.m. routes could have been made; at the dose used, little toxicity would have been expected based on the results of the previous p.o. and i.v. studies.]

One concern regarding the use of the oral nonclinical database in support of the i.m. route was the possibility that plasma exposure might be higher in humans using the i.m. route. Plasma data in humans indicate that exposure to parent compound were fairly similar via the p.o. and i.m. route. comparing the maximum doses via each route [i.e., 20 mg/dose, 40 mg/day for i.m., 100 mg b.i.d., 200 mg/day for p.o.] The human data are summarized in the following sponsor's tables:

i.m.

(Study 128-038)

Mean ± Coefficients of Variation (%CV) of Pharmacokinetic Parameters (n = 6)

Parameter	Ziprasidone 5 mg IM		10 mg IM		20 mg IM	
AUC_{0-t} (ng•hr/ml)[a, b]	222	± 23	460	± 12	841	± 29
$AUC_{0-\infty}$ (ng•hr/ml)[a]	229	± 23	463	± 12	846	± 29
C_{max} (ng/ml)[a]	76	± 7	156	± 14	244	± 37
T_{max} (hr)	0.50	± 38	0.65	± 37	0.71	± 52
K_{el} (hr^{-1})	0.289	± 22	0.309	± 7	0.233	± 20
$T_{1/2}$ (hr)[c]	2.40	--	2.24	--	2.97	--

[a] geometric mean
[b] t=time of the last pharmacokinetic blood sample with quantifiable concentrations of ziprasidone.
[c] mean $T_{1/2}$ = ln (2)/mean K_{el}

(Study No. 128-046)

Mean (Coefficients of Variation - %CV) of Pharmacokinetic Parameters (n=6)

Parameter	Ziprasidone 20 mg/day Group 1		40 mg/day Group 2		80 mg/day Group 3	
	Day 1	Day 3	Day 1	Day 3	Day 1	Day 3
AUC_{0-24} (ng•hr/ml)[a]	648(25)	590 (27)	1363 (26)	1116 (23)	1560 (22)	1504 (30)
Accumulation Ratio[a]	--	0.91 (17)	--	0.82 (9)	--	0.96 (17)
K_{el} (hr^{-1})	0.152 (14)	0.085 (19)	0.176 (16)	0.067 (26)	0.180 (9)	--[c]
$T_{1/2}$ (hr)[b]	4.6	8.1	3.9	10.4	3.8	--[c]

[a] geometric mean
[b] $T_{1/2}$ = 0.693/mean K_{el}
[c] Not determined due to an insufficient time interval over which to estimate K_{el}.

p.o.

(Study No 128-043)

Pharmacokinetic Results:

Mean + Coefficients of Variation (CV%) of Pharmacokinetic Parameters on the Third Day of Each Dosing Regimen

	Ziprasidone 20 mg BID		40 mg BID		80 mg BID	
	Mean	CV%	Mean	CV%	Mean	CV%
AUC_{0-12}[a] (ng•hr/ml)	462	36	768	35	1551	34
C_{max}[a] (ng/ml)	66	51	116	30	202	35
T_{max} (hr)	7.6	22	6.8	40	6.7	45

[a] = Geometric means

[Although data were not available at 100 mg b.i.d., exposure was demonstrated to increase linearly from 20 to 80 mg b.i.d.]

At the maximum human dose of 40 mg/day i.m., the mean plasma C_{max} and AUC did not exceed the levels presumed to be achieved at the maximum human oral dose of 100 mg b.i.d. (200 mg/day). Therefore, the oral nonclinical database may be used to support the use of ziprasidone i.m., thus mitigating the lack of full i.m. toxicity data. Taken together, the p.o., i.v. and i.m. toxicity data in rat and dog are adequate to support the use of ziprasidone i.m. in humans.

Genotoxicity

An *in vivo* micronucleus assay was conducted as part of the 1-mo i.m. toxicity study in rat. Ziprasidone was negative in this assay. As noted previously, the dose in that study produced drug-related effects, however, it was not clear that an MTD was achieved, at least in females. According to the OECD guidelines, it is recommended that 3 dose levels be used in the *in vivo* micronucleus assay, unless the drug produces minimal toxicity at high doses. In that case, a limit dose of 1000 mg/kg/day may be used when the duration of treatment is >14 days, i.e., 3 dose levels are not necessary. If a drug is not minimally toxic, the high dose used should either be associated with "...signs of toxicity such that higher dose levels, based on the same dosing regimen, would be expected to produce lethality..." (quoted from the OECD guidelines, 2/97 draft) or evidence of bone marrow toxicity (i.e., a decrease in the PCE-to-total erythrocyte ratio). The study conducted on ziprasidone tested only one dose level, and it was not clear that the dose used was adequate, particularly in females. Even in males, the only potentially dose-limiting effect other than clinical signs, i.e., a reduction in body wt gain, would not have been sufficient to limit the dose in an acute test and is not a serious toxicity in and of itself. No acute i.m. or i.v. toxicity data were available for rat (the oral and i.p. LD_{50} in rats was >2000 mg/kg). There were no i.m. or i.v. data available for doses higher than 8 mg/kg. Therefore, there are inadequate data to determine that higher i.m. doses could not have been used.

Although the dose used for assessment of the potential for ziprasidone to induce micronuclei *in vivo* was not clearly an MTD, a repeat i.m. study is not recommended. The *in vivo* micronucleus assays (using oral dosing) submitted to the NDA for the oral capsules (NDA 20-825, GEODON™) were inadequate due to deficiencies in methodology. The need for a repeat oral *in vivo* micronucleus assay (to be performed as a Phase 4 commitment) has been conveyed to the sponsor. Plasma exposure to ziprasidone at an oral MTD is expected to be sufficient to provide a safety margin for the i.m. route as well. [Although, it is possible that greater bone marrow exposure could be achieved with i.m. dosing; the tissue distribution data provided in NDA 20-825 were not useful since distribution of radioactivity, ziprasidone, or metabolites into bone marrow was not assessed.] The negative results in the i.m. *in vivo* micronucleus data (collected using adequate methodology, except for dose) and the lack of carcinogenic potential (except for neoplasms in rat associated with serum prolactin elevation) in the mouse and rat carcinogenicity studies somewhat mitigate the concern for genotoxic potential. However, the fact that ziprasidone was positive in the other genotoxicity studies (Ames, *in vitro* gene mutation assay in mammalian cells, *in vitro* chromosomal aberration in mammalian cells) and that the *in vivo* micronucleus assay is part of the standard battery of genotoxicity assays routine supports the need for additional testing (as noted and previously conveyed to the sponsor).

[From a pharmacology/toxicology standpoint, there are no labeling issues at this time.]

RECOMMENDATIONS

From a pharmacology/toxicology standpoint, the data are adequate to support approval of the NDA for ziprasidone i.m. (GEODON™ IM).

If the NDA is approvable, the sponsor should be reminded that an assessment of reproductive toxicity of ziprasidone i.m. [to include dosing during all stages of development] needs to be conducted as a Phase 4 commitment (cf. Agency's action letter, 12/17/98). The sponsor should be referred to the ICH document, Detection of Toxicity to Reproduction for Medicinal Products [ICH-S5A, Sept 1994], for guidance.

Additionally, the following information should be relayed to the sponsor:

> Please submit final toxicokinetic data for the 1-month i.m. toxicity studies in rat and dog. The reports submitted for both of these studies stated that, "Due to a lack of long term stability data at the time of submission of this report, final reported serum concentrations may change slightly."

/s/

Lois Freed
3/6/01 04:06:49 PM
PHARMACOLOGIST

Barry Rosloff
3/6/01 05:10:50 PM
PHARMACOLOGIST

OFFICE OF CLINICAL PHARMACOLOGY AND BIOPHARMACEUTICS REVIEW

Date of Document: 3/10/2000

NDA:	20-919
Name of Drug:	Zeldox IM (Ziprasidone Mesylate Trihydrate (CP-88, 059-27) 20mg/ml, Upon Reconstitution with 1.2 mL of Water For Injection
Indication of Drug:	Acute Control of Agitation
Type of Document:	Correspondence
Sponsor:	Pfizer, Groton, CT
Reviewer:	Hong Zhao, Ph.D

Introduction

This submission is the sponsor's response to FDA Comments dated December 17, 1998. This report is to review the sponsor's response to two Comments made in OCPB's review dated May 16, 1998 that were incorporated into the above mentioned FDA Comments.

OCPB's Previous Comments

1. Cyclodextrin is excreted by filtration and no studies were conducted in renal failure after IM administration. However, in the sponsor" proposed labeling, it is indicated that caution should be taken when administering Zeldox IM in patients with renal impairment.

2. The fate of cyclodextrin has not been investigated in this NDA. It is not known what happens to cyclodextrin at the muscular site after multiple injection.

Sponsor's Response

To Comment 1 - The cyclodextrin vehicle, sulfobutylether beta-cyclodextrin (SBECD), was originally developed [ ] an intravenous [ ] and was subsequently used to formulate ziprasidone for intramuscular administration. The maximum exposure to SBECD after ziprasidone IM (20mg four times a day for 3 days) for an individual weighing 50 kg would be 5.9 mg/kg/dose and 23.5 mg/kg/day.

Much higher doses of SBECD are required for solubilization of the [ ] as compared to intramuscular ziprasidone. As part of the [ ] development program, SBECD doses of up to 200 mg/kg in single dose and multiple dose have been administered to humans. Because of the renal tubule epithelial vacuolation observed in animal toxicology studies, sensitive urinary markers of renal integrity or function were measured in the four clinical studies.

According to the sponsor there were no indications of altered renal function or integrity in humans following SBECD doses of up to 200 mg/kg which suggests that intramuscular ziprasidone would not represent a significant hazard if administered to patients with mild to moderate renal impairment. Cautionary labeling was proposed simply in recognition of the preclinical finding of renal tubular cell vacuolation and the exclusively renal route of elimination for SBECD.

To Comment 2 –SBECD is a hydrophilic compound that is highly ionized at physiologic pH. The chemical characteristics of SBECD predict minimal distribution into tissue following IM administration and also suggest a similar pharmacokinetic profile following intravenous or intramuscular administration. The sponsor claims the fate of single and multiple dose administration of SBECD following intravenous administration has been well characterized in preclinical and clinical trials - elimination half life was 1.6 hours in man; 91% of the dose recovered from rats' urine and feces after 72 hours of dosing; and no systemic accumulation after multiple doses in humans). Furthermore, the clinical safety of SBECD when employed as a vehicle for the intramuscular administration of ziprasidone has been established. Therefore, further exploration of the fate of SBECD following IM administration does not appear warranted.

Comment 1
The sponsor's response to the OCPB's previous two Comments appears to be adequate.

Please convey this Comment to the Clinical Division.

Hong Zhao, Ph.D. /S/

RD/FT Initialed by Raman Baweja, Ph.D. /S/

cc: NDA 20-919 (Zeldox IM), HFD-120, HFD-860 (Zhao, Baweja, Mehta), Central Documents Room (CDR-Biopharm)

/s/

Hong Zhao
3/7/01 04:48:39 PM
BIOPHARMACEUTICS

Raman Baweja
3/7/01 04:56:56 PM
BIOPHARMACEUTICS

Addendum to Pharmacology/Toxicology Team Leader Memo of 12/4/98 to NDA 20-919

A recommendation which Dr. Freed made in her review concerned the possible presence of methanesulfonic acid in the drug product. She notes that, according to the proposed labeling the final injectable solution will include ziprasidone, methanesulfonic acid, and β-cyclodextrin sulphobutyl ether sodium. Because methanesulfonic acid was not stated to be present in any of the definitive toxicity studies submitted, she requested that, if it is to be added to the final drug formulation, it should be present in repeat toxicology studies and that a complete genotoxicity battery should be performed on the compound. I have been informed by our chemistry consultants that methanesulfonic acid will not be in the final formulation, which is described as containing, per vial, — mg. Ziprasidone mesylate trihydrate, — mg. Sulphobutyl ether β-cyclodextrin and water. The problem appears to be a semantic one, which arises because the sponsor's description of the product in their proposed labeling is misleading. This part of labeling needs to be re-written at the time of approval of this formulation.

/S/ 12/9/98

Glenna G. Fitzgerald, Ph.D.

NDA 20-919
c.c. Div File\
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MEMORANDUM

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

DATE: December 4, 1998

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

TO: NDA 20-919
Zeldox IM™ for injection, ziprasidone mesylate
Pfizer, Inc.
20 mg/ml.

RELATED NDA: 20-825, Zeldox capsules, Pfizer, Inc.

SUBJECT: Team Leader Recommendations for Pharmacology and Toxicology

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Zeldox IM is intended for use in acute agitation in psychotic patients. The recommended initial dose is 10 to 20 mg, with subsequent doses of 10 mg every 2 hours or 20 mg every 4 hours, not to exceed a total dose of 80 mg and not to exceed administration for more than 3 days, at which time oral drug is resumed.

The application is not approvable for either chemistry or clinical disciplines. Dr. Freed has reviewed the toxicology package and has also concluded that the NDA is not approvable for several reasons (see page 69 of her review).

This formulation of ziprasidone contains β-cyclodextrin sulphobutyl ether sodium (SBECD) to enhance solubility, an excipient that has not been used in any drug products marketed in the United States. Toxicology studies for ziprasidone submitted to support this NDA consist of the following: acute i.m. studies of ziprasidone tartrate and mesylate in rabbits, a single dose PK study of the two salts in dogs, and 2-week i.v. bolus studies of ziprasidone tartrate (not mesylate) in SBECD in rats and dogs. The following studies were submitted for the new excipient, SBECD, alone: acute i.m. and i.v. studies in mice and rats, 1-month and 6-month i.v. toxicology studies in rats and dogs, a complete reproductive toxicity battery by the i.v. route, and a genetic toxicology battery (Ames test, *in vitro* gene mutation in CHO cells, *in vitro* chromosomal aberration in human lymphocytes, and *in vivo* micronucleus in mice). SBECD alone was associated with kidney and liver toxicity and hematological effects, foamy macrophages and cellular vacuolation in several tissues; it caused embryolethality and early resorptions in rats and

rabbits, decreased pup survival and increased stillbirths in rats; it was not genotoxic

The primary deficiency in the package appears to be the lack of toxicology studies with the combination of ziprasidone and SPECD which are of the appropriate duration and which use the appropriate route to adequately assess the toxicological potential. As outlined in the ICH M3 document, 1-month studies in rodent and nonrodent should be conducted for an NDA for this category of drug, to be used for up to two weeks. Although there are 2-week studies of ziprasidone in SPECD, albeit with the tartrate rather than the mesylate salt, they are by the i.v. route, and PK or plasma data which would allow us to make any comparison of routes is lacking. It also should be remembered that the i.m. regimen in patients could be repeated at some later time if indicated, making 2-week studies, if the i.v. route could be justified, even more inadequate. Given the overall lack of an appropriate toxicological evaluation of drug in vehicle + route + duration to support this indication, I believe that 1-month studies in rodent and nonrodent should be a requirement for approval. These studies should be conducted with the mesylate salt of ziprasidone in SPECD using the i.m. route, and should incorporate appropriate PK parameters. Since the *in vivo* micronucleus assay for SPECD did not conform to OECD guidelines and was negative, an assessment of effects on micronuclei could be incorporated into the rat toxicology study. Although the reproductive toxicology of ziprasidone in SPECD was not examined, pregnancy labeling for oral ziprasidone will already indicate that it is teratogenic in rabbits, causes a decrease in survival in rat pups, and is associated with developmental delays and neurobehavioral functional impairment. These effects were seen at doses close to or lower than clinical doses. Therefore, it would not be required to conduct a reprotox battery prior to approval. However, when and if the IM formulation is approvable, a Phase 4 commitment should be made to conduct a reproductive and developmental toxicity study, using ziprasidone mesylate in SPECD and administered by the i.m. route, to determine if additional and/or synergistic effects are seen with the combination in order to provide more accurate labeling. Plasma level data should be obtained in that study.

Recommendations:

Not approvable. The letter to the sponsor should indicate the following:

One month toxicology studies in a rodent and a nonrodent species should be conducted prior to approval. For these studies ziprasidone mesylate in the beta-cyclodextrin sulphobutyl ether formulation should be administered by the intramuscular route, and plasma measurements should be included. We also recommend that an assessment of the effects on micronucleus formation be incorporated into the rodent toxicology study.

Although not necessary for approval, a reproductive and developmental toxicity study, also with ziprasidone mesylate in the beta-cyclodextrin sulphobutyl ether formulation administered by the intramuscular route, and which incorporates plasma level measurements, should be planned as a Phase 4 commitment.

/S/

Glenna G. Fitzgerald, Ph.D

NDA 20-919
c.c. Div. File
HFD-120\Laughren\Katz\Freed\Hardeman\Fitzgerald

REVIEW AND EVALUATION OF PHARMACOLOGY AND TOXICOLOGY DATA
Review of NDA #20-919

Date: October 1, 1998

Reviewer: Lois M. Freed, Ph.D.

Stamp Date: 12/17/97

PDUFA Date: 12/17/98

Received: 12/22/97

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

Drug: ziprasidone mesylate (i.m.)

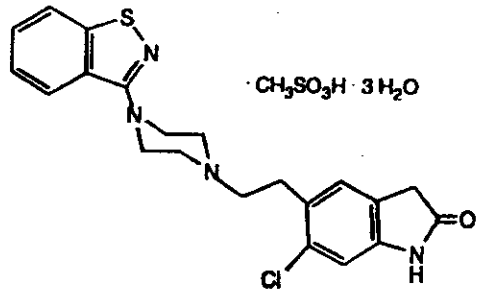
Code Name: CP-88,059

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

Indication: acute agitation in psychotic patients

Structure:

ziprasidone



Molecular weight: 563.09 (mesylate trihydrate salt)

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

Drug Formulation: 20 mg/mL , formulated for intramuscular injection; each mL of solution contains 20 mg ziprasidone, 4.7 mg methanesulfonic acid, 294 mg sulphobutylether beta-cyclodextrin sodium (SBECD)

Excipient structure:

SBECD (ß-cyclodextrin sulphobutyl ether sodium)

Related IND/NDA: IND #49,045 (ziprasidone i.m.), IND #34,629 (ziprasidone p.o.), NDA 20-825 (ziprasidone p.o.)

Reports:

Review and Evaluation of Pharmacology and Toxicology Data (orig rev) (Lois M. Freed, Ph.D., 11/27/95)
Pharmacology/Toxicology Review (Brian Ault, Ph.D.)
Pharmacology/Toxicology Memorandum to IND 49.045 (Lois M. Freed, Ph.D., 2/21/96)
Pharmacology/Toxicology Memorandum to IND 49,045 (Lois M. Freed, Ph.D., 8/13/97)
Pharmacology/Toxicology Memorandum to IND 49,045 (Lois M. Freed, Ph.D., 12/5/97)

Studies Previously Reviewed:

ADME

PK of CP-88,059 in dogs at 1.5 mg/kg i.v. or i.m. (Report DM-95-128-30)
Protein binding of CP-88,059 to human albumin and n 1-acid glycoprotein and the interaction of CP-88,059 with warfarin and propranolol (N-026).

Toxicology (Vol 1.2)

ziprasidone studies:

acute i.m. study in albino rabbits (Protocol 94-720-31)
acute i.m. study in albino rabbits (Protocol 95-720-35)
2-wk i.v. study in Beagle dogs (Protocol 95-720-32)
2-wk i.v. study in Sprague-Dawley rats (Protocol 95-720-33)

SBECD studies:

Intramuscular irritation (Protocol 95-720-35)
acute i.v. in rats and mice (Protocols 93085, 93086)

14-day i.v. range-finding in rats (Protocol 93033)
14-day i.v. range finding in dogs (Protocol 93034)
1-mo i.v. + 1-mo reversibility in rats (Protocol 93082)
1-mo i.v. + 1-mo reversibility in dogs (Protocol 93081)
1-mo i.v. study in dogs (Protocol 95-1033-06)
1-mo i.v. study in rats (Protocol 95-1033-07)

Genotoxicity
Ames test
in vitro chromosomal aberration assay in human lymphocyte
in vitro gene mutation in CHO cells (HGPRT)
in vivo micronucleus assay in mice

Studies Reviewed This Submission:
PK/ADME (Vol 1.11, 1.21)
Toxicology
ziprasidone studies:
exploratory pharmacokinetic and acute toleration study in albino rabbits (Protocol 92-720-24) (Vol 1.11)

SBECD studies:
1-mo i.v. + 2 and 5 mo reversibility in rats (Protocol 95107)
1-mo i.v. + 2 and 5 mo reversibility in dogs (Protocol 95106)
6-mo i.v. in rats (Protocol 60069)
6-mo i.v. in dogs (Protocol 60068)
dermal irritation in rabbit (Protocol 60052)
eye irritation in rabbit (Protocol 60053)
dermal irritation in guinea pig (Protocol 60037)
investigation of relationship between occurrence of renal tubular vacuolation produced by CP-217,861-02 and urinary protein concentrations and urinary enzyme excretion in the rat (Protocol 96092)
effects of CP-217,861-02 and hydroxy-propyl-beta-cyclodextrin on the weight, morphology, and biochemistry of the rat pancreas (Protocol 95069)
in vitro assessment of an interference with the quantitative test for urinary proteins (Protocol 97085)
Reproductive studies (SBECD)
fertility and early embryonic development in rats (Protocol 95128)
maternal range-finding study in rats (Protocol 95125)
maternal range-finding study in rabbits (Protocol 95126)
teratology study in rats (Protocol 95109)
teratology study in rabbits (Protocol 95127)
prenatal and postnatal development in rats (Protocol 95129)
Genotoxicity (SBECD) (Vol 1.20)
Ames test (591-941502)
Ames test (94-1033-01)
Mammalian nutation assays (94-1033-03)
in vitro cytogenetics assays (94-1033-02)
in vivo mouse micronucleus assays (94-1033-04)

Studies Submitted But Not Reviewed

SBECD

[1

TABLE OF CONTENTS

PHARMACOLOGY

The pharmacology data for ziprasidone was cross-referenced from the NDA (20-825) for the oral formulation. The pharmacological effects of the vehicle, SBECD, on a number of physiological systems was tested in more recent studies: cardiovascular studies in anesthetized dog and cat, nictitating membrane resting tension in anesthetized cat, gastrocnemius muscle resting tension in anesthetized cat, respiratory function in anesthetized rat, renal function in conscious rat. These have previously been reviewed by Brian Ault, Ph.D. According to Dr. Ault, SBECD exhibited no 5HT receptor binding affinity and had "...little effect on cardiovascular parameters, autonomic transmission or neuromuscular transmission". An analysis of mean data for the renal function study indicated, however, that SBECD (500 mg/kg i.v., single dose) did increase total excretion of Cl (54%), but had no effect on that of Na or K. The sponsor questioned the biological relevance of this finding.

PK/ADME

Ziprasidone

For ziprasidone, the sponsor cross-referenced the PK/ADME data from the NDA (20-825) for the oral formulation. Additional PK/ADME studies have been conducted under the IND for ziprasidone i.m. These consist of the following: (1) PK of CP-88,059 in dogs (1.5 mg/kg i.v., i.m.; Report DM-95-128-30 or DM-95-128-28) and (2) PK of CP-88,059 in dogs (2.5 mg i.m.): comparison of two different ratios of ziprasidone:SBECD. The first of these two studies has been previously reviewed (P/T Review, L.M.Freed, Ph.D., 11/27/95) and is summarized here. The second study is reviewed below.

The sponsor stated that "Metabolic profiling has not been conducted following IM administration..."

Pharmacokinetics of CP-88,059 in Dogs Administered a 1.5 mg/kg Intravenous of Intramuscular Dose of the Tartrate and Mesylate Salts of CP-88,059 Formulated as a Solution in SBECD (Report No. DM-95-128-28).

In this study, CP-88,059 was administered to 8 male dogs at acute doses of 1.5 mg/kg i.v. and i.m. (formulated as a 20 mg/mL solution of the tartrate salt in 50% ß-cyclodextrin; formulated lot no. 32056-55) and at a dose of 1.5 mg/kg i.m. (formulated as a 36 mg/mL solution of the mesylate salt in 40% ß-cyclodextrin; lot no. 31177-85-1H). Following acute doses, blood samples were collected from 0.083 to 48 hr postdosing for quantitation of serum CP-88,059 levels using HPLC (LOQ = [illegible] ng/mL). No mention was made of the time interval between doses.

The mean absolute i.m. bioavailability of CP-88,059 was determined to be 96-103%. PK parameters were similar after i.m. dosing with the tartrate and mesylate salts. Mean values (tartrate and mesylate, respectively) were as follows: C_{max} = 672 ± 205 and 794 ± 207 ng/mL, T_{max} = 0.34 ± 0.13 and 0.32 ±0.28 hr, $AUC_{0\text{-}8\ hr}$ = 1551 ± 435 and 1424 ± 273 ng•hr/mL, F = 103 ± 20 and 96 ± 17%.

Pharmacokinetics of a 2.5 mg IM dose of Ziprasidone in Dogs: Bioequivalence of a 2.5 mg A/ml Solution to a 20 mg A/ml Solution (Report No. DM96-128-35).

Methods: The purpose of this study was the compare the T_{max} and bioavailability of two different ratios of ziprasidone to SBECD. Ziprasidone was administered i.m. to 4 female Beagle dogs in two different formulations, 2.5 mgA/mL and 20 mgA/mL (vehicle: 30% SBECD); the dose was 0.27 mg/kg for both formulations. Each dog received both formulations. Blood samples were collected at 0.083, 0.25, 0.5, 1, 2, 4, 6, 8, and 24 hr postdosing. Ziprasidone was quantitated in serum by HPLC; the LLOQ was — ng/mL.

Results: for both formulations, serum levels were <LLOQ by 4-6 hr postdosing. The PK parameters are summarized in the following table:

FORMULATION	T_{max} (hr)	C_{max} (ng/mL)	$AUC_{(0\text{-}\infty)}$ (ng•hr/mL)	$T_{1/2}$ (hr)
2.5 mgA/mL	0.25 ± 0.0	126 ± 68	188 ± 87	1.0 ± 0.2
20 mgA/mL	0.12 ± 0.08	170 ± 52	226 ± 79	1.1 ± 0.4

SBECD

For the vehicle, SBECD, eight studies were conducted (DM1-8). Of these, DM1, DM2, and DM 3 have been previously reviewed (Brian Ault, Ph.D.) and will only be summarized here. DM 4-8 are reviewed below.

Mouse

DM5: The disposition and pharmacokinetics of [^{14}C]-CP-217,861-02 in male mice following single intravenous administration at a nominal dose level of 600 mg/kg (Vol 1.21, pg [ ]

Methods: [^{14}C]-SBECD was administered i.v. to male CD-1 mice at a dose of 600 mg/kg. Urine and fecal samples (3 mice) were collected for up to 120 hr postdosing. Blood samples (3 mice/time point) were collected for up to 6 hr postdosing. Sample radioactivity was quantitated using LSC. Identification of drug-related compound(s) in urine (24-hr collection) was accomplished using HPLC/MS.

Results: the PK and elimination data are summarized in the table below, except for AUC value which was 486 µg-equiv•hr/mL.

Rat

1. **DM1: The disposition and pharmacokinetics of [^{14}C]-CP-217,861-02 in rats following single intravenous administration at a nominal dose level of 600 mg/kg** (Vol 1.21, pg 4974).

In this study, [^{14}C]-SBECD (labeled in the sulfobutyl side chain) was administered i.v. to Sprague-Dawley rats (2/sex) at a dose of 600 mg/kg. Blood, urine, and fecal samples were collected for assessment of PK parameters and elimination patterns. The data are summarized in the table below.

2. **DM7: Excretion and plasma pharmacokinetics of [^{14}C]-CP-217,861-02 in Sprague-Dawley rats administered an intramuscular dose** (Vol 1.21, pg 4984, Pfizer Central Research, Sandwich, Kent, UK).

Methods: [^{14}C]-SBECD (30%) was administered i.m. to male Sprague-Dawley rats (n = 5) at a dose of 160 mg/kg. Urine and fecal samples were collected for up to 72 hr postdosing. Blood samples were collected for up to 7 hr postdosing. Sample radioactivity was quantitated using LSC.

Results: the data are summarized in the table below, except for C_{max} and AUC data were 142-230 µg-equiv/mL and 206-254 µg-equiv•hr/mL, respectively.

3. **DM3: Tissue distribution of radioactivity in male and female rats after a single intravenous administration of [^{14}C]-CP-217,861-02 at a dose level of 600 mg/kg** (Vol 1.21, pg 5029).

In this study, [^{14}C]-SBECD was administered i.v. to Lister-hooded rats (4 M, 2 F; 1 animal/time point) at a dose of 600 mg/kg. Males were sacrificed at 0.1, 1, 24, and 96 hr postdosing , while females were sacrificed at 24 and 96 hr postdosing.

Radioactivity was concentrated in renal cortex and medulla (highest peak levels); high levels

were also detected in lung, skin (dermis, and sebaceous gland). The $t_{1/2}$ for radioactivity was longer in kidney cortex than in medulla. In kidney cortex, 40, 26, and 11% of peak levels were detected at 1, 24, and 96 hr postdosing, respectively, while in kidney medulla, tissue levels at these sampling times were only 14, 2, and <1%, respectively. Brain levels were low, with radioactivity being detected only at 0.1 hr postdosing; by 1 hr postdosing, brain levels were <LLOQ.

Dog

DM2: The disposition and pharmacokinetics of [^{14}C]-CP-217,861-02 in male and female Beagle dogs following single intravenous administration at a nominal dose level of 240 mg/kg (Vol 1.21, pg 4991).

In this study, [^{14}C]-SBECD (labeled in the sulfobutyl side chain) was administered i.v. to Beagle dogs (2/sex) at a dose of 240 mg/kg. Blood, urine, and fecal samples were collected for assessment of PK parameters and elimination patterns. The data are summarized in the table below.

Human

DM4: The excretion and pharmacokinetics of [^{14}C]-CP-217,861-02 in male volunteers following single and multiple intravenous administration at nominal dose levels of 100 and 50 mg/kg, respectively (Vol 1.21, pg [ ]

Methods: [^{14}C]-SBECD was administered, according to the methods section, to 9 healthy male volunteers at doses of 100 mg/kg b.i.d. (Day 1), 50 mg/kg b.i.d. (Days 1-9), and 50 mg/kg (Day 10). However, since all subjects received all doses, it is unclear how 100 and 50 mg/kg could both be administered on Day 1. According to the tables, a single 100 mg/kg i.v. dose of [^{14}C]-SBECD was administered on Day 1, or at least samples were collected after a single dose. The second collection period was on Day 10, after a single 50 mg/kg dose. At least during Days 2-9, subjects received [^{14}C]-SBECD at 50 mg/kg b.i.d. Blood, urine, and fecal samples were collected on Days 1 and 10 for analysis of sample radioactivity (LSC). Urine samples were additionally analyzed by HPLC/MS for identification of radiolabeled drug-related compound(s).

Results: the elimination data are summarized in the table below. [The sponsor noted that recovery of urinary radioactivity was low in 2 subjects (50-60%); therefore, calculations were made with and excluding data from these subjects.] HPLC/MS analysis of urine (Day 1) indicated a single peak, corresponding to the administered compound. Radioactivity was also noted to be distributed into plasma, with no detectable distribution into rbc.

Rabbit

DM5: the excretion and pharmacokinetics of [^{14}C]-CP-217,861-02 in female rabbits following single intravenous administration at a nominal dose level of 600 mg/kg (Vol 1.21, pg [ ]

Methods: [^{14}C]-SBECD was administered to female New Zealand White rabbits at a dose of 600 mg/kg i.v. Urine and fecal samples (3 rabbits) were collected for up to 120 hr postdosing, and blood samples (3 rabbits) were collected for up to 6 hr postdosing. Sample radioactivity was quantitated using LSC. Identification of drug-related compound(s) in urine was conducted using HPLC/MS on 24-hr urine collections.

Results: the PK and elimination data are summarized in the table below. Radioactivity in blood was distributed almost entirely into plasma. HPLC/MS analysis of urine samples

documented the existence of a single peak, corresponding to [^{14}C]-SBECD.

Interspecies comparison

summary table:

SPECIES	DOSE (mg/kg)	PK PARAMETERS			% OF DOSE RADIOACTIVITY	
		$t_{1/2}$ (hr)	Cl (mL/min/kg	V_d (L/kg)	URINE	FECES
CD-1 mouse	600 i.v.	0.1, 0.6	20	0.9	83.9-90.7	0.5-7.2
S-D rat	600 i.v.	0.3	9.8	0.3	84	3.5
	160 i.m.	0.68			70-93	2-13
Beagle dog	240 i.v.	1.1	4.7	0.4	93	0.2
NZW rabbit	600 i.v.				96.6-108.4	0.07-0.23
human	100 i.v., acute				85.67 ± 20.16	<LLOQ
	50 i.v., multiple				91.01 7 ± 11.79	<LLOQ

DM8: Mass spectrometric characterization of CP-217-861 in mouse, rat, rabbit, dog and human urine (Vol 1.21, pg 4940, Pfizer Central Research, Sandwich, Kent, UK).

Methods report for HPLC/MS analyses performed on urine samples (spiked and samples from PK studies), which were injected directly into the MS. The report noted that SBECD can exist in a number of protonated/deprotonated, or n-substituted forms (Na, H, or CH_2-(CH_2)-SO_3Na).

SBECD was "..essentially unretained..." on the column, and did not form complexes with other ions, e.g., K^+ or NH_4^+. Comparison of the distribution of sulphobutyl substituents detected (1-10) between spiked samples and samples from animals dosed with [^{14}C]-SBECD indicated that SBECD is excreted unchanged in the urine.

Ziprasidone

1. **An exploratory pharmacokinetic and acute toleration study in albino rabbits** (Study No. 92-720-24, Pfizer Central Research, USA, study dates: 10/92, report date: 12/92, Vol 1.11).

The report indicated that this study was conducted using ziprasidone formulated in Encapsin HPB (i.e., hydroxypropyl-ß-cyclodextrin). As reported in the literature, drug substances may be markedly affected by the type of cyclodextrin used for solubilization. Therefore, the results are not relevant to the current NDA since the drug formulation intended for marketing contains SBECD.

SBECD

1. **1-month intravenous toxicity study in Sprague-Dawley rats-with 2 and 5 month reversibility.** (Study No. 95107, Pfizer Central Research, study dates: 10/95-4/96, report date: 12/96, GLP, Vol 1.14-1.15).

Methods: SBECD (batch no. TS-94-242X; vehicle: saline) was administered to Sprague-Dawley rats (10/sex/grp) at doses of 0, 300, 1000, and 3000 mg/kg i.v. for 1-mo. Additional grps (5/sex/grp/time) were dosed for 28 days at 0, 1000, and 3000 mg/kg, and maintained without dosing for 2 and 5 mo after the end of the dosing period to study reversibility of effects. Observations consisted of the following: clinical signs, food/water consumption, ophthalmology (C, HD reversibility grps), hematology, clinical chemistry, urinalysis, terminal studies [gross pathology, organ wts (only adrenal, brain, heart, kidney, liver, spleen), histopathology, EM analysis of kidney, liver, lungs from "randomly selected" C and HD animals]. In recovery animals, only the following organs were examined microscopically: cervical node, heart, kidneys, liver, lungs, mesenteric node, ovaries, pituitary, spleen, testes, urinary bladder, and uterus.

Results: there was one unscheduled death (HDF 802, Day 5), which was considered accidental by the sponsor. No drug-related clinical signs were observed, and no clear drug-related effects on body wt, food consumption, ophthalmology,

Drug-related changes were noted on a number of hematology parameters [measured 24 hr after the last dose, and at 2 and 5 mo in recovery (R) animals]. The sponsor summarized the findings (Day 29) in main study animals (not clearly specified in the report) in the following table (taken directly from sponsor's report):

Variations in haematologic parameters in high-dose animals compared to controls

	Treatment groups		Reversibility groups	
	Males	Females	Males	Females
Red Blood Cell Count	-10% **	-14% **	-8% **	-13% **
Haemoglobin	-6% **	-9% **	-5% *	-10% **
Haematocrit	-6% **	-7% **	-6% **	-8% **
Mean corpuscular volume	+5% *	+8% **	+2%	+6% **
Mean corpuscular Haemoglobin	+4% *	+6% **	+2%	+4% *
White blood cell count	+29% *	+39% **	+15%	+19%
Neutrophils	+106% **	+56% **	+89% **	+104% **
Platelets	+10%	+17% *	+15% **	+24% **

*, ** = statistically significant at p=0.05 and p=0.01, respectively.

In addition to the findings listed in the table, the following were noted: (1) increases in large unstained cells in HDM and HDF (28 and 80%, respectively, and (2) increases in lymphocytes, monocytes, and basophils, particularly in females [effects were observed in LDF and HDF, except for an increase in basophils, which was noted only in HDF].

Similar effects were observed on Day 30 in recovery grps; however, total wbc cts were somewhat less affected: 16 and 19% in HDM-R and HDF-R, respectively.

All effects appeared to be reversible, with no consistent significant changes in the affected parameters at either 2 or 5 mo.

Drug-related changes were also noted on a number of clinical chemistry parameters. These are summarized in the following table (*p<0.05, **p<0.01:

PARAMETER	MAIN STUDY				RECOVERY			
	MALES		FEMALES		MALES		FEMALES	
	MD	HD	MD	HD	MD	HD	MD	HD
K	--	--	--	--	--	--	-9%*	-10%*
Cl	--	-2%*	--	-3%*	--	-2%**	--	-3%**
Phosphate	--	+6%*	--	+9%	--	+13%**	--	+13%*
urea	--	+28%**	--	+18%*	--	+21%*	--	+37%**
creatinine	--	--	--	--	--	+18%*	--	+13%*
cholesterol	--	+23%*	+28%**	+16%	--	+23%**	+29%*	+31%**
total bilirubin	--	--	--	--	--	--	-12%	-30%*
TG	--	-44%**	--	--	--	-50%**	--	--
AST	--	+170%**	--	+94%**	--	+150%**	--	+50%*
ALT	--	+340%**	--	+79%*	--	+200%**	--	--

As noted in the table, effects were observed primarily at the HD, and total bilirubin and K were affected only in the recovery animals [for recovery animals, parameters were measured only in those animals being sacrificed].

In recovery animals, increases in urea, AST, and ALT (33, 94, and 130%, respectively) were still evident in HDM on Day 85. The effect on urea was similar in magnitude to that observed at the end of the dosing period; AST and ALT were less affected. On Day 180, alkaline phosphatase and ALT were increased at the HD (120-150%, respectively). An examination of the individual data for males indicated that bilirubin, AST, and ALT were elevated in 1 MDM-R (#260: 110, 188, and 240%, respectively) and 1 HDM-R (#357: 240, 130, and 470%, respectively); in addition, alkaline phosphatase was increased (560%) in the HDM-R. The sponsor discussed effects only in the HDM-R, and concluded they were "unlikely" to be due to treatment.

On urinalysis parameters, drug-related effects were noted on urinary pH and hgb (males only). These data are summarized in the following sponsor's tables:

Mean pH values at the end of the treatment period

Dose (mg/kg)	Treatment groups		Reversibility groups	
	Males	Females	Males	Females
Control	7.35	6.55	7.40	6.80
300	7.25	6.65	-	-
1000	6.80 *	5.90 *	7.05	6.80
3000	6.30 **	5.60 **	6.55 **	6.15 **

*, ** = statistically significant at p=0.05 and p=0.01, respectively.

Summary of incidence of "grade 2 or 3" qualitative test for haemoglobin in males

Dose (mg/kg)	Treatment period: Treatment group	Treatment period: Reversibility group	Reversibility period: After 2 months	Reversibility period: After 5 months
Control	2/10	0/10	0/5	0/5
300	2/10	"	"	"
1000	7/10	1/10	2/5	1/5
3000	9/10	8/10	0/5	1/5

It should be noted that the effect on pH was greater during the recovery period than during dosing in females [Day 29-30: 7-10, 14-10% at MD and HD, respectively; Day 85: 5 and 20% at MD and HD, respectively; Day 180: 14 and 20% at MD and HD, respectively]. In males, urinary pH was still lower at the MD and HD on Day 85 [9 and 12%, respectively, compared to 5 and 12%, respectively, on Day 30], but not on Day 180. An increased incidence/severity of hgb in urine was observed only during the dosing period.

Drug-related changes in organ/tissue wts were summarized in the following sponsor's table:

Variations in absolute and relative organ weights in high-dose animals

	Kidneys		Liver		Spleen	
	Absolute	Relative	Absolute	Relative	Absolute	Relative
Treatment period						
Males	+22% *	+22% **	+21% *	+21% **	+18% *	+19% *
Females	+22% **	+25% **	+37% **	+41% **	+25% **	+28% **
Two months after treatment period						
Males	+18%	+12%	+21% *	+16% **	+11%	+6%
Females	+24% **	+21% **	+19% *	+16% *	+9%	+6%
Five months after treatment period						
Males	+14%	+12%	+1%	-1%	+8%	+6%
Females	+15% *	+15%	+2%	+1%	+32% *	+32% *

*, ** = statistically significant at p=0.05 and p=0.01, respectively.

In addition to these findings, kidney wt was significantly increased in MDF (main study; 12-15%, absolute and relative). In recovery animals, kidney wt was increased (≈10%) in MDF at Day 85 (absolute and relative wt) and Day 180 (relative wt only); these differences did not achieve statistical significance.

[Markedly elevated organ wts were not noted in the 2 males (MD-R, HD-R) in which LFTs were particularly elevated.]

Macroscopic findings were not summarized in tabular form. According to the sponsor, macroscopic findings consisted of "... pale discoloration of the kidneys in 7/19 high-dose and 2/20 mid-dose rats, an enlarged kidney in 1/10 high-dose males and pale discoloration of the lungs in 1/10 high-dose males." None was observed in recovery animals.

Selected microscopic findings are summarized in the attached tables. No microscopic findings were noted in MDM-R #260. In HDM-R #357, liver findings consisted of the following: mononuclear cell infiltration, pericholangial inflammation, necrosis (focal), and hepatocyte vacuolation (minimal);also noted: "Occasional vacuolated hepatocytes undergo single cell necrosis. Kupffer's cells have a large eosinophilic or slightly vacuolated cytoplasm and a peripherally located nucleus (hypertrophic macrophages)."

The sponsor considered tubular vacuolation and vacuolation of the transitional epithelium to be drug-related effects. Upon EM analysis, it was noted that increases in the severity of tubular vacuolation was accompanied by extension of the effect from the cortex to the medulla. The vacuoles were either empty or contained "...a faintly granular eosinophilic PAS-positive material". With increase in severity, tubular cells appeared "...markedly swollen and obliterated the lumen of the tubules..." No necrosis was detected. Vacuolation of the transitional epithelium was noted to involve the "...superficial layer of the epithelium".

The increase in pyelonephritis, described as a common finding in rats, particularly in females, was considered to be a drug-related increase in a spontaneous finding. The sponsor noted that such a high incidence of this finding "..has never been observed previously..."

Vacuolation of hepatocytes was characterized by the sponsor as marked, diffuse, and staining positive with PAS. Vacuolated (foamy) macrophages in liver, lung, and testis also stained positive with PAS. In heart, foamy macrophages were detected in cardiac valves; in the pituitary gland, foamy macrophages were detected in the vestigial lumen.

The pituitary gland adenoma was considered incidental because of the low incidence (1 animal) and the lack of preneoplastic signs (e.g., hyperplasia) in other animals.

Upon EM analysis of selected tissues, the following were noted: (1) the vacuolated epithelial cells detected in kidney were located in "...proximal tubules and Henle's loop..." and were determined to be of lysosomal origin. It was also noted that "...occasional epithelial cells from the proximal tubules presented cytoplasmic area of dissolution of organelles, dilation of mitochondrial cristae and a decreased number of apical microvilli". (2) in liver, vacuoles, detected in hepatocytes, were of lysosomal origin, and occasionally were noted to be large enough to displace the nucleus. It was noted that "Occasional cells had mitochondria with dilated cristae and irregularly-shaped nucleus with peripheral condensation of the chromatin. Kupffer and sinusoidal cells were found to contain "...slightly dilated lysosomal profiles...". (3) in lung, vacuolated macrophages, containing "...numerous small lysosomal vacuoles filled with dense lamellar profiles...", were detected in the alveolar lumen and fibroblasts detected in the interstitium.

Appears This Way
On Original

TISSUE	FINDING	MALES				FEMALES			
		C	LD	MD	HD	C	LD	MD	HD
MAIN STUDY									
kidney	vacuolation, tubular								
	minimal	0/10	10/10	0/10	0/10	0/10	6/10	0/10	1/10
	mild	0/10	0/10	4/10	0/10	0/10	2/10	7/10	0/10
	moderate	0/10	0/10	6/10	3/10	0/10	0/10	2/10	7/10
	marked	0/10	0/10	0/10	7/10	0/10	0/10	0/10	2/10
	pyelonephritis	0/10	0/10	0/10	0/10	0/10	3/10	4/10	8/10
	vacuolation, trans. epith	0/10	0/10	6/10	9/10	0/10	0/10	7/10	6/10
liver	hypertrophic macrophages	0/10	0/10	2/10	10/10	0/10	0/10	3/10	10/10
	fibrosis	0/10	0/10	0/10	1/10	0/10	0/10	0/10	0/10
	infarct	0/10	0/10	0/10	1/10	0/10	0/10	0/10	0/10
	vacuolation, hepatocyte								
	mild	0/10	0/10	0/10	0/10	0/10	0/10	0/10	1/10
	marked	0/10	0/10	0/10	10/10	0/10	0/10	0/10	9/10
heart	foamy macrophages	0/10	0/10	0/10	3/10	0/10	0/10	0/10	4/10
cervical node	foamy macrophages	0/10	0/10	9/10	10/10	0/10	0/10	9/10	10/10
pituitary gland	foamy macrophages	0/10	1/10	3/10	6/10	0/10	0/10	0/10	2/10
testes	foamy macrophages	0/10	10/10	10/10	10/10				
ovaries	foamy macrophages					0/10	0/10	0/10	9/10
uterus	foamy macrophages					0/10	0/10	0/10	9/10
spleen	foamy macrophages	0/10	0/10	1/10	7/10	0/10	0/10	0/10	4/10
mesent. lymph node	foamy macrophages	0/10	0/10	8/10	10/10	0/10	0/10	6/10	9/10
urinary bladder	vacuolation, epith. cell	0/10	4/10	6/10	10/10	0/10	2/10	7/10	7/10
lungs	foam cell foci								
	minimal	2/10	5/10	6/10	0/10	2/10	6/10	8/10	1/10
	mild	0/10	0/10	3/10	0/10	0/10	0/10	2/10	5/10
	moderate	0/10	0/10	1/10	2/10	0/10	0/10	0/10	4/10
	marked	0/10	0/10	0/10	8/10	0/10	0/10	0/10	0/10
injection site	hemorrhage	1/10	4/10	4/10	7/10	3/10	6/10	4/10	4/10

DAY	TISSUE	FINDING	MALES			FEMALES		
			C	MD	HD	C	MD	HD
85	kidney	vacuolation, tubular						
		minimal	0/5	1/5	0/5	0/5	0/5	0/5
		mild	0/5	2/5	2/5	0/5	4/5	0/5
		moderate	0/5	1/5	2/5	0/5	1/5	3/5
		marked	0/5	0/5	0/5	0/5	0/5	2/5
		pyelonephritis	0/5	0/5	0/5	1/5	3/5	2/5
		vacuolation, trans. epith	0/5	3/5	4/5	0/5	4/5	4/5
	liver	hypertrophic macrophages	0/5	0/5	5/5	0/5	0/5	4/5
		vacuolation, hepatocyte	0/5	0/5	5/5	0/5	0/5	5/5
	heart	hyperplasia, mesothelial	0/5	0/5	0/5	0/5	0/5	1/5
	cervical node	foamy macrophages	0/5	0/5	2/5	0/5	0/5	0/5
	pituitary	foamy macrophages	0/5	0/5	1/5	0/5	0/5	0/5
	testes	foamy macrophages	0/5	5/5	5/5			
	mesenteric node	foamy macrophages	0/5	0/5	4/5	0/5	0/5	2/5
	urinary bladder	vacuolation, epith. cell	0/5	2/5	4/5	0/5	3/5	3/5
	lungs	foam cell foci	0/5	0/5	1/5	0/5	0/5	1/5
180	kidney	hydronephrosis	0/5	0/5	1/5	0/5	0/5	0/5
		hyaline droplets	0/5	1/5	2/5	1/5	0/5	0/5
		glomerulonephritis						
		minimal	1/5	2/5	1/5	0/5	0/5	0/5
		mild	1/5	0/5	2/5	0/5	0/5	0/5
		moderate	0/5	0/5	1/5	0/5	0/5	0/5
		hyperplasia, trans. cell	0/5	0/5	0/5	1/5	0/5	2/5
		vacuolation, tubular						
		minimal	0/5	4/5	1/5	0/5	4/5	1/5
		mild	0/5	0/5	3/5	0/5	1/5	2/5
		moderate	0/5	0/5	1/5	0/5	0/5	1/5
		pyelonephritis	0/5	0/5	0/5	0/5	0/5	2/5
		vacuolation, trans. epith.	0/5	2/5	3/5	0/5	4/5	4/5
	liver	hypertrophic macrophages	0/5	0/5	2/5	0/5	0/5	3/5
		leukocytosis, sinusoidal	0/5	0/5	1/5	0/5	0/5	0/5
		inflammation, pericholangial	0/5	0/5	1/5	0/5	0/5	0/5
		necrosis	0/5	0/5	1/5	0/5	0/5	0/5
		vacuolation, hepatocyte	0/5	0/5	5/5	0/5	0/5	4/5
	heart	infiltration, mononuclear cell	1/5	4/5	4/5	0/5	2/5	1/5
	pituitary	B-adenoma, incidental	0/5	0/5	1/5	0/5	0/5	0/5
	testes	foamy macrophages	0/5	5/5	5/5			

DAY	TISSUE	FINDING	MALES			FEMALES		
			C	MD	HD	C	MD	HD
180 (con't)	urinary bladder	infiltration, mononuclear cell	0/5	0/5	0/5	0/5	1/5	1/5
		vacuolation, epith. cell	0/5	3/5	4/5	0/5	4/5	3/5
	lungs	foam cell foci, minimal	1/5	0/5	4/5	1/5	0/5	4/5
		pigmentation	0/5	0/5	1/5	0/5	0/5	2/5

2. **1-month intravenous toxicity study in Beagle dogs-with 2 and 5 months reversibility** (Study No. 95106, Pfizer Central Research, study dates: 2/96-8/96, report date: 3/28/97, GLP, Vol 1.16)

Methods: CP-217,861-02 (batch no. 3043-089X) was administered to Beagle dogs (3/sex/grp) at doses of 0, 300, 750, and 1500 mg/kg i.v. (vehicle: saline) for 28 days. Additional dogs received doses of 0, 750, and 1500 and then followed for an additional 2 (1/sex/grp) or 5 (1/sex/grp) mo. to assess reversibility of findings. [The sponsor provided homogeneity data on drug formulations; analysis was by HPLC. Homogeneity was confirmed on 1 formulation/dose on Day 29.] Observations consisted of the following: clinical signs, body weight, food consumption ("semi-quantitative estimate"), ophthalmology (all C, HD, HD-R animals; baseline and on Day 21), cardiovascular parameters [ECG, hr, SAP; baseline and Wk 3 (0, i.e., 24-hr after previous dose, and 2 hr postdosing; in all grps], hematology (baseline, Day 14 and 29, 2 and 5 mo), clinical chemistry (16.5 hr overnight collection; baseline, Day 14 and 29, 2 and 5 mo), urinalysis (baseline, Day 28, 2 and 5 mo), terminal studies [gross pathology (Day 29, 86, 181), organ wts (adrenal gland, brain, heart, kidneys, liver, ovaries, spleen, testis), histopathology (battery in main study animals; only cervical node, kidneys, liver, mesenteric node, and urinary bladder in recovery animals], EM [liver, kidney in all main study and recovery animals].

[According to the sponsor, the i.v. route was selected since this is a proposed route for humans. The HD was selected on the basis of solubility (limit 300 mg/mL) and dosing volume (maximum acceptable: 5 mL/kg).] It should be noted that 1/sex/grp for recovery grps is too small to adequate assess reversibility, particular of sex-specific findings.

Results: there were no unscheduled deaths or drug-related clinical signs observed. There were no clear drug-related effects on the following: body weight or food consumption (qualitative assessment only), cardiovascular parameters (in 1 C, 1 MD, and 1 HD animal, heart rate either remained unchanged or increased and QT interval increased), ophthalmology, hematology, clinical chemistry, urinalysis.

No significant differences among grps were noted on organ wts; however, the following were of note: (1) decrease in absolute and relative (A-R) adrenal wt in HDM (main study; 12-19%), (2) increase in liver wt in HDM (main study; 33-23%, A-R), (3) decrease in spleen wt in MDM and HDM (main study; 14-16 and 19-26%, respectively, A-R), (4) dose-related increase in ovary wt (A-R) in main study animals (40-38, 100, and 120-100% at the LD, MD, and HD, respectively). The number of animals per grp were too small to assess effects in recovery animals.

According to the sponsor, there were no gross pathology findings; these data were not provided in summary form. Selected histopathology findings are summarized in the following tables.

TISSUE	FINDING	MALES				FEMALES			
		C	LD	MD	HD	C	LD	MD	HD
MAIN STUDY									
liver	vacuolation, hepatocytes								
	minimal	0/3	1/3	2/3	1/3	0/3	2/3	1/3	2/3
	mild	0/3	0/3	1/3	1/3	0/3	0/3	2/3	1/3
	moderate	0/3	0/3	0/3	1/3	0/3	0/3	0/3	0/3
	hypertrophic macrophages								
	minimal	0/3	0/3	3/3	2/3	0/3	1/3	3/3	1/3
	mild	0/3	0/3	0/3	1/3	0/3	0/3	0/3	2/3
kidney	vacuolation, tubular								
	minimal	0/3	2/3	2/3	0/3	0/3	3/3	2/3	0/3
	mild	0/3	1/3	1/3	0/3	0/3	0/3	1/3	1/3
	moderate	0/3	0/3	0/3	3/3	0/3	0/3	0/3	2/3
	vacuolation, trans epithel.								
	minimal	0/3	1/3	2/3	2/3	0/3	1/3	3/3	3/3
cervical node	foamy macrophages								
	minimal	0/3	1/3	0/3	1/3	0/3	0/3	1/3	0/3
	mild	0/3	0/3	2/3	0/3	0/3	0/3	2/3	3/3
	moderate	0/3	1/3	0/3	2/3	0/3	0/3	0/3	0/3
lungs	bronchopneumonia								
	moderate	0/3	0/3	0/3	1/3	0/3	0/3	0/3	1/3
adrenal	vacuolation, zona fasc.								
	mild	0/3	0/3	0/3	0/3	0/3	0/3	0/3	1/3
mesenteric node	foamy macrophages								
	minimal	0/3	1/3	2/3	1/3	0/3	1/3	3/3	1/3
	mild	0/3	0/3	0/3	1/3	0/3	0/3	0/3	1/3
	moderate	0/3	0/3	0/3	0/3	0/3	0/3	0/3	1/3
urinary bladder	vacuolation, epithelial								
	minimal	0/3	1/3	3/3	2/3	0/3	3/3	2/3	0/3
	mild	0/3	0/3	0/3	1/3	0/3	0/3	0/3	3/3
	moderate	0/3	0/3	0/3	0/3	0/3	0/3	1/3	0/3

TISSUE	FINDING	MALES			FEMALES		
		C	MD	HD	C	MD	HD
RECOVERY ANIMALS (ALL, DAY 86, 181)							
liver	vacuolation, hepatocytes minimal mild	 0/2 0/2	 0/2 0/2	 1/2 1/2	 0/2 0/2	 2/0 0/2	 1/2 1/2
	hypertrophic macrophages minimal	 0/2	 0/2	 2/2	 0/2	 0/2	 2/2
kidney	vacuolation, tubular minimal mild	 0/2 0/2	 1/2 0/2	 2/2 0/2	 0/2 0/2	 1/2 0/2	 1/2 1/2
cervical node	foamy macrophages minimal mild	 0/2 0/2	 1/2 0/2	 1/2 0/2	 0/2 0/2	 0/2 1/2	 0/2 1/2
mesenteric node	foamy macrophages minimal	 0/2	 1/2	 0/2	 0/2	 2/2	 2/2
urinary bladder	vacuolation, epithelial mild	 0/2	 2/2	 2/2	 0/2	 2/2	 2/2

Upon EM analysis of main-study C and HD animals, renal and liver findings were additionally characterized as follows:

Kidney: vacuolated cells were detected in the proximal tubules and Henle's loop. Vacuoles appeared to be membrane-bound and of lysosomal origin. Compression of the nucleus was noted with large vacuoles.

Liver: vacuoles (membrane-bound, lysosomal origin) were observed in hepatocytes; the large vacuoles were noted to cause compression of the nucleus. "...numerous slightly dilated lysosomal profiles..." were observed in Kupffer and sinusoidal cells; the affected lysosomes contained both granular material and lamellar bodies.

3. **6-month intravenous toxicity study in Sprague-Dawley rats** (Study No. 94-28-21, Pfizer Pharmaceuticals Inc, Japan, study dates: 8/22/94-2/28/95, report date: 1/96, GLP/Japan, Vol 1.17-1.18)

Animals: Sprague-Dawley rats [ ]
initial body wts: 168*9-200.2 gm in males, 133.5-155.4 gm in females
initial age: ≈6 wks
diet/housing: housed individually, food and water *ad lib.*
n = 20/sex/grp

Drug: CP-217,861-2 (lot no. 3669-018)
vehicle: 0.9% NaCl
storage/stability: drug substance stored at rm temperature. Stability of drug formulation (300 mg/mL) said to be stable for 1 yr at rm temperature (no data were provided to support this statement).
purity: "activity" was reported to be [ ]
route: i.v.; stated by sponsor to be an intended clinical route.
doses: 0, 200, 320, and 600 mg/kg

dosing volume: 1.82 mL/kg
duration: 190-191 consecutive days

Observations

Clinical signs: all animals were observed twice daily.

Body weight/food consumption: body weight and food consumption were recorded weekly during the dosing period.

Ophthalmology: examinations were conducted on all animals prior to the start of dosing, on Days 95-96, and on Days 177-178. Mydriasis was induced prior to evaluation with slit lamp and hand-held camera.

Hematology: blood samples were collected (cervical vein bleed) on Days 65-67, 120-122, and 184-186 for analysis of the following parameters: rbc, hgb, hct, wbc (total count and differential), platelet ct, reticulocyte ct, MCV, MCH, MCHC.

Clinical chemistry: blood samples were collected (cervical vein bleed) on Days 65-67, 120-122, and 184-186 for analysis of the following parameters: Na, K, Cl, BUN, creatinine, alkaline phosphatase, glucose, TG, ALT, AST, total protein, albumin, globulin, A/G, total bilirubin, total cholesterol.

Urinalysis: urine samples were collected over a 15-hr period prior to each blood collection for analysis of the following parameters: volume, specific gravity, pH, color, clarity, protein, occult blood, glucose, urobilinogen, bilirubin, ketones, microscopic analysis of sediment.

Terminal studies

Gross pathology: all surviving animals were sacrificed within 24 hrs of dosing (Days 191-192) and necropsies were performed.

Organ/tissue wts: weights of the following organs/tissues were recorded: kidney (combined), liver, lung, heart, spleen, adrenals (combined), pancreas, brain, pituitary, testes (combined), ovaries/oviduct (combined).

Histopathology: the following tissues were examined microscopically only in C and HD animals: kidneys, urinary bladder, liver, salivary gland, pancreas, trachea, lung, heart, testes, epididymides, prostate, seminal vesicle, cerebrum, cerebellum, spinal cord (cervical, thoracic), sciatic nerve, aorta (thoracic), lymph node (mesenteric, submandibular), spleen, thymus, esophagus, stomach, small intestine (duodenum, jejunum, ileum), cecum, colon, pituitary, adrenal, thyroid, eye, Harderian gland, skin with mammary gland (thoracic), skeletal muscle (femoral), bone and bone marrow (femoral, sternum, vertebral column), injection site, ovaries/oviduct, uterus, vagina, gross lesions.

In LD and MD animals, only kidneys, lung, liver, urinary bladder, and gross lesions were examined microscopically.

All tissues were preserved in 10% formalin, except for testes, seminal vesicle, prostate, epididymides, ovaries, uterus, vagina, and eyes which were preserved in Bouin's fluid. Tissues were stained with H & E for examination; additional sections of kidney, lung, and liver were stained with PAS for saccharides, and liver sections from selected C and HD animals were stained with Sudan II for lipid.

EM: EM analysis was performed on sections of left renal cortex and lung from selected C and HD animals (3/sex/grp).

Results

Mortality: there were no unscheduled deaths.

Clinical signs: according to the sponsor, there were no drug-related clinical signs (no summary table was provided).

Body weight: there were no clear drug-related effects on body weight; however, body weight tended to be lower (4%) in HDM (compared to CM) from Day 57 on.

Food consumption: there were no drug-related effects.

Ophthalmology: according to the sponsor, there were no drug-related effects (no listings were provided).

Hematology: the summary table and individual line listings provided by the sponsor were almost unreadable.

In males, wbc ct was slightly elevated at the MD and HD (2 and 11%), due to relative high values in 1 MDM and 2 HDM; neutrophil cts were elevated in MDM and HDM (45 and 50%, respectively) on Day 184. This latter effect was not due to responses in a few individual animals.

In females, decreases were noted in rbc parameters on Day 120 and 184. At the MD, rbc ct, hgb, and hct were reduced (4-5%) on Day 120 only; at the HD, these same parameters were reduced (4-7%) on Days 120 and 184. These effects were not considered toxicologically relevant by the sponsor.

Clinical chemistry: in males, the following were noted: (1) increase in ALT in HDM on Day 120 and 184 (47 and 70%, respectively); ≈2-fold increases were observed in HDM #132 and 137, (2) 45% increase in AST in HDM on Day 184; increases in individual values were <2-fold; AST values in HDM #132 and 137 were elevated. (3) decrease in glucose at all doses (LD: 8-12% at all time points; MD: 11% at Day 184, HD: 7-8% at all time points), (4) increase in BUN at all doses (LD: 7-10%, Day 65 and 184, and 13-10% at the MD and HD on Day 184).

In females, the following were noted: (1) a transitory decrease in TG (20-25% at all doses on Day 65; 15% at HD on Day 120) and (2) an increase in creatinine (13%) at the HD on Day 184 (no marked increases in individual animals).

Urinalysis: the primary drug-related effect was a decrease in pH in males and females at all doses and all sampling times. At all doses, the magnitude of the effect increased with duration of dosing (males: 5-10, 6-14, and 8-15% at LD, MD, and HD, respectively; females: 6-13, 5-11, and 13-15% at LD, MD, and HD, respectively).

Terminal studies

Gross pathology: the only apparent drug-related finding was discoloration of the kidney (not otherwise specified) at all doses in males (0/20 C, 14/20 LD, 18/20 MD, and 19/20 HD) and females (0/20 C, 4/20 LD, 9/20 MD, and 20/20

HD).

Organ/tissue wts: increases in kidney wt (absolute and relative) were noted in MDM, (8-10%) and HDM (20-22%) and at all doses in females (10-8, 12-15, and 30-29% at LD, MD, and HD, respectively); relative, but not absolute, kidney wt was increased (6%) in LDM. Spleen wt (A-R) was increased at all doses in females only (9-7, 10-13, and 17% at LD, MD, and HD, respectively). Relative, but not absolute, liver wt was increased (7%) in HDF.

Histopathology: selected microscopic findings are summarized in the following table:

TISSUE	FINDING	MALES				FEMALES			
		C	LD	MD	HD	C	LD	MD	HD
kidney	vacuolation, tubular								
	grade 1*	0/20	2/20	0/20	0/20	0/20	1/20	0/20	0/20
	grade 2	0/20	16/20	1/20	2/20	0/20	16/20	6/20	0/20
	grade 3	0/20	2/20	19/20	17/20	0/20	2/20	14/20	14/20
	grade 4	0/20	0/20	0/20	1/20	0/20	0/20	0/20	6/20
	vacuolation, trans. epithel.	0/20	16/20	20/20	20/20	0/20	19/20	20/20	18/20
lung	foam cell foci	2/20	9/20	6/20	17/20	3/20	4/20	4/20	11/20
	granuloma	4/20	5/20	6/20	12/20	8/20	8/20	9/20	7/20
urinary bladder	vacuolation, epithel.	0/20	18/20	18/20	19/20	0/20	20/20	19/20	17/20
liver	hypertrophy, macrophages	0/20	3/20	6/20	18/20	0/20	0/20	0/20	3/20
	light staining hepatocytes	0/20	7/20	12/20	8/20	0/20	0/20	4/20	3/20
	infiltration, mononuclear cells	2/20	3/20	3/20	1/20	4/20	4/20	4/20	10/20

*grade 1: <25% cortical tubules affected; grade 2: 25-50% cortical tubules affected; grade 3: 50-75% cortical tubules affected; grade 4: >75% cortical tubules affected.

Findings were further characterized by the sponsor as follows:

kidney: tubular vacuolation involved an increase in the "...number and size of cytoplasmic vacuoles within the epithelial cells..." Larger vacuoles were detected at grades 3 and 4. At grades 1 and 2, vacuolated cells were restricted to the renal cortex, whereas at the higher grades, affected cells were noted in the medulla as well. Vacuoles were found to contain "...a small amount of granular or punctiform, eosinophilic, PAS-positive material, but no acicular pseudocrystals".

lung: "...foam cell foci (alveolar histiocytosis)..." were noted to be grade 1.

liver: hypertrophic macrophages were mostly detected in portal areas. Contents were PAS-positive.

The light staining hepatocytes was not considered drug-related by the sponsor, but rather a possible artifact. The staining was characterized as eosinophilic and faint, with no pattern of distribution or dose-related increase in incidence or severity.

lung: the granulomas were not considered to be drug-related, but a result of "...foreign materials introduced by venipuncture."

4. **6-month intravenous toxicity study in Beagle dogs** (Study No. 94-28-22, Pfizer Pharmaceuticals, Inc., Japan, study dates: 11/14/94-5/26/95, report date: 1/96, GLP, Vol 1.18)

Animals: Beagle dogs [ ]
initial body wts: 8.36-11.02 kg in males, 7.88-10.42 kg in females
initial age: ≈9 mo
housing/diet: animals were housed individually; food and water were available *ad lib.*
n = 4/sex/grp

Drug: CP-217,861-2 (lot no. 3669-018, RPP-94-CDSBE-BA#-2)
purity: stated to be [ ]
vehicle: 0.9% NaCl
stability: drug formulation was stated to be stable at rm temperature for 1 yr; no data were provided to document stability.
doses: 0, 150, 300, and 600 mg/kg
dosing volume: 1.82 mL/kg
route: i.v.
duration: dosing was daily for 190-193 days.

Observations

Clinical signs: all animals were observed twice daily.

Body weight/food consumption: body weight and food consumption was recorded weekly.

Ophthalmology: all animals were examined at baseline, on Days 92 or 99, and 177-180. Mydriasis was induced for portions of the examination.

Cardiovascular: ECG, SAP, and DAP were obtained in all animals prior to the start of dosing, on Day 100-103, and Day 184-187 at 24 hr after the prior dose and 2 hr postdosing. ECG and hr measurements were taken in conscious dogs using Lead II.

Hematology: blood samples were collected from all surviving animals prior to the start of dosing, on Days 66, 121, and 183 for analysis of the following parameters: rbc, hgb, hct, wbc (total, differential), platelet ct, reticulocyte ct, MCV, MCHC, MCH, PT, APTT.

Clinical chemistry: blood samples were collected from all surviving animals prior to the start of dosing, on Days 66, 121, and 183 for analysis of the following parameters: Na, K, Cl, BUN, creatinine, alkaline phosphatase, glucose, TG, ALT, AST, total protein, albumin, total bilirubin, total cholesterol, globulin, A/G.

Urinalysis: urine samples (15-hr) were collected prior to each blood sample collection for assessment of the following parameters: volume, specific gravity, pH, color, clarity, protein, occult blood, glucose, urobilinogen, bilirubin, ketones, microscopic analysis of sediment.

Terminal studies

Gross pathology: a complete necropsy was performed on all animals.

Organ/tissue wts: wts of the following organs/tissues were recorded: kidneys (combined), liver/gallbladder, lung, heart, spleen, adrenals (combined), pancreas, brain, pituitary, testes (combined), ovaries (combined).

Histopathology: the following tissues were microscopically examined in all animals: kidney, urinary bladder, liver, gallbladder, salivary gland (submandibular gland), pancreas, trachea, lung, heart, aorta (thoracic), spleen, thymus, lymph node (mesenteric, submandibular), esophagus, stomach, small intestine (duodenum, jejunum, ileum), cecum, colon, pituitary, thyroid/parathyroid, testes, epididymides, prostate, ovaries, uterus, vagina, cerebrum, cerebellum, spinal cord (cervical, thoracic), sciatic nerve, eye, adrenal, skin with mammary gland (thoracic), skeletal muscle (femoral), bone/bone marrow (femoral, sternum), injection site (cephalic vein with skin), gross lesions.

For evaluation, tissues were preserved in 10% buffered neutral formalin, except for testis, prostate, epididymides, ovaries, uterus, vagina, and eyes which were preserved in Bouin's fluid, and stained with H & E.

Additional evaluations were as follows: (1) analysis of kidney, lung, and liver sections using PAS for saccharides, (3) liver sections from 3 HDF and 1 CF were stained with Sudan III for lipids.

EM: sections of left renal cortex and lung from all C and HD animals were examined by EM.

Results

Mortality: there were no unscheduled deaths.

Clinical signs: according to the sponsor, there were no drug-related clinical signs (no individual line listings or summary table were provided).

Body weight: there were no clear drug-related effects, although body weight was slightly lower (6%) at the end of the study in HDM as compared to CM; this difference was not statistically significant.

Food consumption: food consumption data were not discussed by the sponsor, nor was a summary table provided; data were supplied in the form of individual line listings. There did not appear to be any drug-related effects.

Ophthalmology: according to the sponsor, there were no drug-related effects. No summary or individual data were provided.

ECG/bp: there were no clear drug-related effects on ECG. sinus arrhythmia and/or sinus arrest were reported in 1 MDM (postdosing, Day 184), 1 HDM (prior to dosing on Days 101 and 185), 1 CF (postdosing on Day 186), 1 LDF (pre- and postdosing, Day 186), 1 MDF (pre- and postdosing, Day 186).

There were no apparent effects on blood pressure, heart rate, or respiratory rate.

Hematology: there were no apparent drug-related effects.

Clinical chemistry: the only drug-related effect was an increase in ALT in HDF on Day 183. The mean value for ALT in HDF was 2.2 fold higher compared to both baseline and CF values on Day 183. An examination of the individual data indicated high values in 2/4 HDF (#31 and 32; 3.3 and 2.4 fold increases over baseline, respectively). There were no similar increases in HDM.

Urinalysis: there were no apparent drug-related effects.

Terminal studies

Gross pathology: the only apparent drug-related effect was discoloration of the kidneys, noted only in 4/4 HDM and 2/4 HDF.

Organ/tissue wts: there were no significant drug-related effects. Pancreas weight (absolute and relative) was dose-dependently reduced in females (7-13, 17-22, 23-30% at LD, MD, and HD, respectively); however, none of the differences were statistically significant.

Histopathology: selected findings are summarized in the following table:

TISSUE	FINDING	MALES				FEMALES			
		C	LD	MD	HD	C	LD	MD	HD
kidney	vacuolation, tubular								
	grade 1*	0/4	4/4	1/4	1/4	0/4	4/4	3/4	1/4
	grade 2	0/4	0/4	3/4	2/4	0/4	0/4	1/4	1/4
	grade 3	0/4	0/4	0/4	1/4	0/4	0/4	0/4	2/4
	vacuolation, trans. epithel.								
	grade 1**	0/4	3/4	3/4	2/4	0/4	4/4	4/4	4/4
	grade 2	0/4	0/4	0/4	2/4	0/4	0/4	0/4	0/4
liver	infiltration, monon. cells.	2/4	4/4	4/4	3/4	1/4	2/4	4/4	4/4
	hypertrophy, macrophages								
	grade 1***	0/4	1/4	0/4	3/4	0/4	1/4	0/4	2/4
	vacuolation, hepatocellular	0/4	0/4	0/4	0/4	0/4	0/4	0/4	2/4
urinary bladder	vacuolation, epithelial								
	grade 1**	0/4	3/4	3/4	2/4	0/4	3/4	3/4	3/4
	grade 2	0/4	0/4	0/4	1/4	0/4	0/4	0/4	1/4

*grade 1, 2, and 3 refer to "<25%, 25-50%, 50-75, and >75% of affected cortical tubules affected, respectively.

**grade 1 = cytoplasmic microvacuolation; grade 2: cytoplasmic macrovacuolation resulting in cell enlargement.

***grade 1 = scattered, primarily in portal area

In HDF #31 and 32, both exhibiting marked increases in ALT on Day 184, liver findings consisted of mononuclear cell infiltration, hypertrophic macrophages, and hepatocellular vacuolation; however, effects were "slight" in both females.

Findings were additional characterized by the sponsor as follows:

kidney: effects were noted primarily in the proximal tubule; cytoplasmic vacuoles were unstained.

liver: cytoplasm of hypertrophic cells appeared foamy "...and often contained an accumulation of pigment and amorphous material, some of which was PAS positive". Vacuoles were cytoplasmic, and some "...contained eosinophilic hyaline bodies which were PAS positive and Sudan III negative".

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1. **Investigation of the relationship between the occurrence of renal tubular vacuolation produced by CP-217,861-02 and urinary protein concentrations and urinary enzyme excretion in the rat** (Study No. 96092, Pfizer Centre de Recherche, France, study dates: 11/12/96-12/10/96, report date: 10/97, GLP, Vol 1.20).

Animals: Sprague-Dawley rats (Pfizer Central Research, Sandwich, U.K.)
initial body weight: 227 gm in males, 203 gm in females
initial age: ≈7 wks
diet/housing: housed under "standard conditions" and fed *ad lib.*
n = 10/sex/grp

Drug: CP-217,861-02 (batch no. 3043-176)
vehicle: saline
purity/stability: no data on purity or stability. Expiration date for drub substance was given as "December 1996"; study was conducted on 11-12/96. Batch was manufactured on 12/95.
drug formulation homogeneity/stability: homogeneity was documented twice during the dosing period. No stability data were provided.
doses: 0, 300, and 3000 mg/kg
dosing volume: 10 mL/kg
route: i.v.
duration: 28 days

Observations

Clinical signs: animals were observed, but how often was not specified.

Body weight: body wts were taken on Days 1, 7, 14, 21, and 28, but were not reported.

Clinical chemistry: blood samples were collected from all rats on Day 29 to assess the following parameters: Na, K, Cl, Ca, P, alkaline phosphatase, ALT, AST, lipids (cholesterol, TG), glucose, urea, creatinine, total bilirubin, total protein, and albumin.

Urinalysis: urine samples were collected from all animals on Days 1, 7, 14, and 28 for 24 hrs postdosing for analysis of the following parameters: volume, density, pH, qualitative observations (NOS), total protein, creatinine, enzymes (LDH, N-acetyl-ß-D-glucosaminidase (NAG), gamma-glutamyl transferase (GGT), ß-galactosidase, alanine aminopeptidase (AAP).

Terminal studies

Gross pathology: a complete necropsy was performed on all animals.

Organ/tissue wts: only kidney was weighed.

Histopathology: on kidney was microscopically examined. Sections were fixed in 10% formalin and stained with H & E for analysis.

Results

Mortality: there were no unscheduled deaths.

Clinical signs: the only clinical sign reported by the sponsor was red discoloration of the urine; the incidences were as follows:

Incidence of red discolouration of urine

Group	Day 1	Day 7	Day 14	Day 28
Control				
300 mg/kg	2/20	2/20	2/20	3/20
3000 mg/kg	4/20	11/20	11/20	7/20

Clinical chemistry: the following were noted: (1) small increases in urea (19%), creatinine (17%), and cholesterol (26%) in HDM, and in urea (34%) and cholesterol (19%) in HDF. (2) increases in AST and ALT in HDM (3.1 and 4.7 fold, respectively) and HDF (65 and 82%, respectively).

Examination of individual data indicated >2-fold elevations in AST and/or ALT in 10/10 HDM (AST: range = 138-389; ALT: 61-341 IU/L, in affected animals), and in AST in 3/10 HDF (108-156 IU/L).

Urinalysis: effects were observed on a several parameters. The data are summarized in the following sponsor's tables/figs:

Changes in mean urinary volumes[a] and pH[b] 0-3 hours after treatment in high-dose groups

Day in study	Volume		pH	
	Males	Females	Males	Females
1	76 **	92 **	- 1.86 **	-1.35 **
7	66 **	117 **	- 1.29 **	-1.55 **
14	78 **	154 **	- 1.27 **	-1.17 **
28	79 **	113 **	- 1.51 **	-1.82 **

a : percent change from control values

b : pH units difference from control values

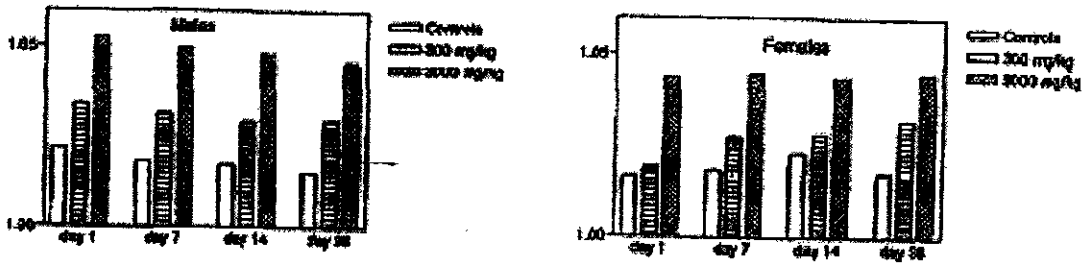
**: statistically significant at p = 0.01

The sponsor noted that 24-hr urinary volume was fairly similar among grps (sponsor's table follows):

Ranges of daily urinary volumes (ml) in controls and high-dose groups during the whole study period.

Dose (mg/kg)	Males	Females
Controls	22-26	13-15
3000	22-24	14-18

Increase in mean urinary densities 0-3 hours after treatment in both treated groups



Specific gravity and pH were fairly similar among grps during the rest of the collection periods. Specific gravity (i.e., density) was also increased consistently and significantly during 0-3 hr postdosing at the LD; however, the effect was slight (≈1%). Urinary volume and pH were not notably or consistently affected at the LD.

As noted in the following sponsor's tables, urinary protein excretion was elevated, both within 3-hr postdosing and over the 24-hr postdosing period.

Increases in median urinary protein excretion 0-3 hours after treatment
(x fold from median control values)

Dose (mg/kg)	300		3000	
Day in study	Males	Females	Males	Females
1	1	3 ***	7 ***	19 ***
7	1	2 ***	6 ***	22 ***
14	2 **	3 **	6 ***	18 ***
28	2 **	3 **	8 ***	24 ***

, *: statistically significant at p = 0.01 and 0.001 respectively

Daily increases in median urinary protein excretion
(x fold from median control values)

Dose (mg/kg)	300		3000	
Day in study	Males	Females	Males	Females
1	1	2 **	2 *	4 ***
7	1	2	2 **	4 ***
14	1	1 *	2	3 **
28	1	1 *	2 **	3 ***

*, **, ***: statistically significant at p=0.05, 0.01 and 0.001 respectively

Of the urinary enzymes assayed, only LDH and NAG showed consistent changes. Both of these latter enzymes were increased in males and females. Differences are summarized in the following table; data are expressed in

percent increase in HD grps compared to C grps (-- = no change):

PARAMETER	SEX	DAY	SAMPLING TIME (hrs postdosing)				
			0-3	3-6	6-9	9-24	0-24
LDH	M	1	44	170	50	--	55
		7	210	290	170	73	150
		14	330	310	110	100	190
		28	190	230	110	190	170
	F	1	300	--	160	--	120
		7	290	29	--	110	100
		14	160	80	220	32	120
		28	310	170	100	53	70
NAG	M	1	150	68	--	--	67
		7	230	22	230	--	63
		14	220	--	--	--	56
		28	540	120	60	89	68
	F	1	400	--	--	--	52
		7	840	--	--	72	62
		14	350	--	--	--	63
		28	710	--	--	68	56

In females, LDH was also elevated (based on 24-hr total collection) at the LD, primarily on Day 1 (150%), as was NAG (29-50% on all days).

Terminal studies

Gross pathology: no summary table was provided. According to the sponsor, "Pale discoloration of the renal cortex..." was observed in 10/10 HDM and 7/10 HDF.

Organ/tissue wts: absolute and relative kidney wt was increased in HDM and HDF (13-20%).

Histopathology: microscopic findings in kidney are summarized in the following table. Vacuolation of the transitional epithelium was characterized as "...minimal to mild..."

FINDING	MALES			FEMALES		
	C	LD	HD	C	LD	HD
vacuolation, tubular						
grade 1	0/10	8/10	0/10	0/10	3/10	0/10
grade 2	0/10	2/10	0/10	0/10	7/10	0/10
grade 3	0/10	0/10	8/10	0/10	0/10	8/10
grade 4	0/10	0/10	2/10	0/10	0/10	2/10
vacuolation, trans. epithel.	0/10	0/10	8/10	0/10	1/10	8/10

2. **Effects of the administration of CP-217,861-02 and hydroxypropyl-beta-cyclodextrin on the weight, morphology and biochemistry of the rat pancreas** (Study No. 95069, Pfizer Centre de Recherche, France, study dates: 7/13/95-8/11/95, report date: 3/96, GLP except for biochemical analyses, Vol 1.21).

Methods: CP-217,861-2 (lot no. RPP-94-CDSBE-BA 2) was administered at doses of 0, 500, and 5000 mg/kg to Sprague-Dawley rats (10/sex/grp) for 1 mo. For comparison, hydroxypropyl-beta-cyclodextrin (HPBCD) was administered at the same doses, also for 1 mo. Dosing route was oral as a drug-diet admixture. Observations consisted of the following: mortality, body wt/food intake, clinical chemistry (CCK, end of study after 17 hr fast), terminal studies [gross pathology, organ wt, and histopathology of pancreas only, determination of protein content and trypsin activity in pancreatic tissue].

Results: there were no unscheduled deaths during the study. According to the sponsor, body weight was not affected in females or in LDM. In HDM, body weight was 7-6% lower than in CM with both CP-217,861-2 and HPBCD. Food intake was not affected. [Achieved doses were calculated to be up to 18-21% lower than the intended doses.]

There was no drug-related effect on plasma CCK levels in either CP-217,861-2 or HPBCD-treated animals. Trypsin activity was slightly higher in treated animals (10-56%), although the differences were not statistically significant or dose-related.

Pancreatic wt was elevated in HDM, LDF, and HDF treated with CP-217,861-2 and in females (both doses) treated with HPBCD. Effects at the LD were small with both compounds (5-8%). There were no clear drug-related microscopic findings (in pancreas) with either compound.

3. **In vitro assessment of an interference with the quantitative test for urinary proteins** (Study No. 97085, Pfizer Centre de Recherche, study dates: not specified, report date: 10/8/97, GLP, Vol 1.21).

Methods: CP-217,861-02 (lot no. 4687-059) was added to urine samples collected from untreated Sprague-Dawley rats (5/sex) in order to assess interference with urinary protein analysis. Urinary protein was quantitated using the Coomassie Brilliant Blue method. Urine samples were spiked with CP-217,861-02 at concentrations of 0.6 to 75 mg/mL

Results: at concentrations of ≥18.8 mg/mL in males and ≥4.7 mg/mL in females, CP-217,861-02 produced artifactual increases in urinary protein. Increases in urinary "protein" ranged from 15 to 169% in males (18.8-75.0 mg/mL) and from 27 to 600% (4.7-75.0 mg/mL) in females.

4. **Skin irritation to the rabbit** [Study No. PFZ 593/940371/SE, [illegible] study date: 5/94, report date: 7/15/95, GLP except for drug characterization (no data), Vol 1.20].

Methods: CP-217,816-02 (lot no. 3005-117) was applied to the shaved skin of New Zealand White rabbits (n = 3) at a dose of 0.5 gm in a 25 mm x 25 mm gauze pad. The pad was applied at one site per animal and covered with [ ] adhesive dressing for 4 hrs. Observations included the following: clinical signs, dermal response (dermal irritation: 0-4 for erythema/eschar formation and for edema). Dermal responses were judged =60 min following removal of the pad, and for an additional 4 days.

Results: no drug-related clinical signs were evident. No irritation (erythema/eschar/edema) was observed in 1/3 animals and a score of "1" for both erythema/eschar and edema was obtained in 1/3 animals only at the first sampling time. In the third animal, both erythema/eschar formation and edema were detected on Day 1 (scores of 2 and 1, respectively) and on Day 2 (scores of 1 and 1); no symptoms were detected on the remaining two days of observation. [erythema/eschar formation: 1 = "very slight erythema (barely perceptible)", 2 = "well-defined erythema"; edema: 1 = "very slight oedema (barely perceptible)", 2 = "slight oedema (edges of area well-defined by definite raising"]

5. **Eye irritation to the rabbit** [Study No. PFZ 594/940440/SE, [ ] tudy date: 5/94, report date: 7/15/95, GLP except for drug characterization (no data), Vol 1.20].

Methods: this study was conducted in New Zealand White rabbit (n = 3). CP-217,816-02 (lot no. 3005-117) was applied to one eye at a dose of 82 mg as a fine powder. After application, the eye was held shut for one sec. Animals were observed for clinical signs and ocular responses. Ocular responses were scored for corneal (0-4), iris (0-2), conjunctivae (0-3), and chemosis (lids/nictating membranes) (0-4) at 1, 24, 48, and 72 hr and 4 and 7 days postapplication.

Results: no irritation was detected in cornea or iris of any of the animals. Minimal redness (score of 1) and chemosis (score of 1) of the conjunctiva were evident at one hr postapplication in all 3 animals. By 24 hr postapplication, only minimal redness of the conjunctiva was observed in these animals.

6. **Skin sensitization in the guinea pig** [Study No. PFZ 631/951054/SS, [ ] study date: report date: 8/30/95, GLP except for drug characterization (no data), Vol 1.20].

Methods: CP-217,816-02 (lot no. 3005-117) was tested in the guinea pig (Dunkin/Hartley) maximization test for allergic contact dermatitis. In a preliminary study, "...concentrations of the test substance that would produce irritation suitable for the induction phase of the main study and...a maximum non-irritant concentration ..." were identified. Based on the results of this study, 7.5 and 30% (w/v in distilled water) were selected as the maximum non-irritating and the highest concentration producing irritation but no other adverse effects, respectively.

The Induction phase consisted of the following. Animals (n = 20) received injections of Freund's complete adjuvant, CP-217,861 (30%), or CP-217,816 (30%) in Freund's complete adjuvant to clipped skin. One week later, the same area of skin was shaved and a 20 mm x 40 mm patch saturated with =0.4 mL of 30% drug solution was applied. The patch was held in place with plastic adhesive tape and [ ] and left for 48 hrs. Control animals (n = 10) received the same procedure only without drug.

The Challenge phase consisted of the following: Both drug-treated and control animals received topical application (20 x 20 mm patch) of 3.75 and 7.5% CP-217,861 to a posterior site and the flank, respectively. Patches remained in place for 24 hrs secured as during the induction phase.

Observations consisted of the following: daily assessment of clinical signs, body wt, and dermal responses [0-4 scales for erythema/eschar formation and edema formation; diameter of skin response]. Dermal responses were evaluated at 24, 48, and 72 hr after removal of patches.

Results: no clinical signs or body wt effects were reported. During the induction phase, necrosis was noted with injection of Freund's adjuvant, either with or without drug. With drug in water, slight irritation was reported. With topical application, moderate erythema was observed at all three application sites (i.e., Freund's adjuvant, 30% drug in water, and 30% drug in Freund's adjuvant) in drug-related animals, but not in controls.

During the challenge phase, minimal skin reactions (localized dermal reaction, dryness and/or sloughing of epidermis) were observed in 4/10 controls. In treated animals, positive reactions were obtained in 18/20 animals, and positive sites were concentration-dependent (17/20 at 7.5% and 13/20 at 3.75%). In 2 treated animals, necrotic patches were observed at the application site. Other signs included erythema (scores of 1-2), edema (scores of 1-2), localized dermal reaction, and dryness/sloughing of the epidermis.
should be labeled as possibly causing "... sensitization by skin contact...".

REPRODUCTION

1. Reproductive study 1: fertility and early embryonic development in Sprague-Dawley rats (Study no. 96-28-41, Pfizer Pharmaceuticals Inc., Japan, study dates: 6/30/96-10/4/96, report date: 8/97, Japanese GLP, Vol 1.18).

Animals: Sprague-Dawley rat [illegible]
initial age: 9 wks
initial body wt: 309-343 gm for males, 191-218 gm for females
diet/housing: 1/sex/cage during mating, individually thereafter. Food and water were provided *ad lib.*
n = 20/sex/grp

Drug: CP-217,861-02 (lot no. 3669-018)
vehicle: 0.9% saline
purity/stability: "activity" was reported as — stability of drug substance for 2 yrs at rm temperature was noted. No data were provided for either of these statements.
doses: 0, 100, 400, and 1500 mg/kg
route: i.v.
dosing volume: 0.49 mL/100 gm
duration: males were dosed from 28 days prior to mating, throughout the mating period, and until sacrifice (total of 63-66 days of dosing). Females were dosed from 14 days prior to mating, throughout the mating period, and through Day 7 of gestation.

Observations

Clinical signs: animals were observed daily.

Mating/estrus: the following parameters were recorded: length of estrus cycles, incidence of estrus, copulation and pregnancy indices.

Body weight: body weight was recorded daily in females, and every 3 days during the dosing period in males.

Food consumption: food consumption was recorded daily (except during mating) in females and at 3-day intervals in males.

Terminal studies

Gross pathology/litter parameters: necropsies were performed on males and females. In females, the uterus was examined and the no. of corpora lutea and implantation sites were recorded, as well as the no. of resorptions and live fetuses.

Histopathology: testis (all grps), prostate (C, HD) and seminal vesicles (C, HD only) were examined microscopically in males. Also, sperm count and motility were evaluated on epididymal samples. Tissues were not examined microscopically in females.

Results

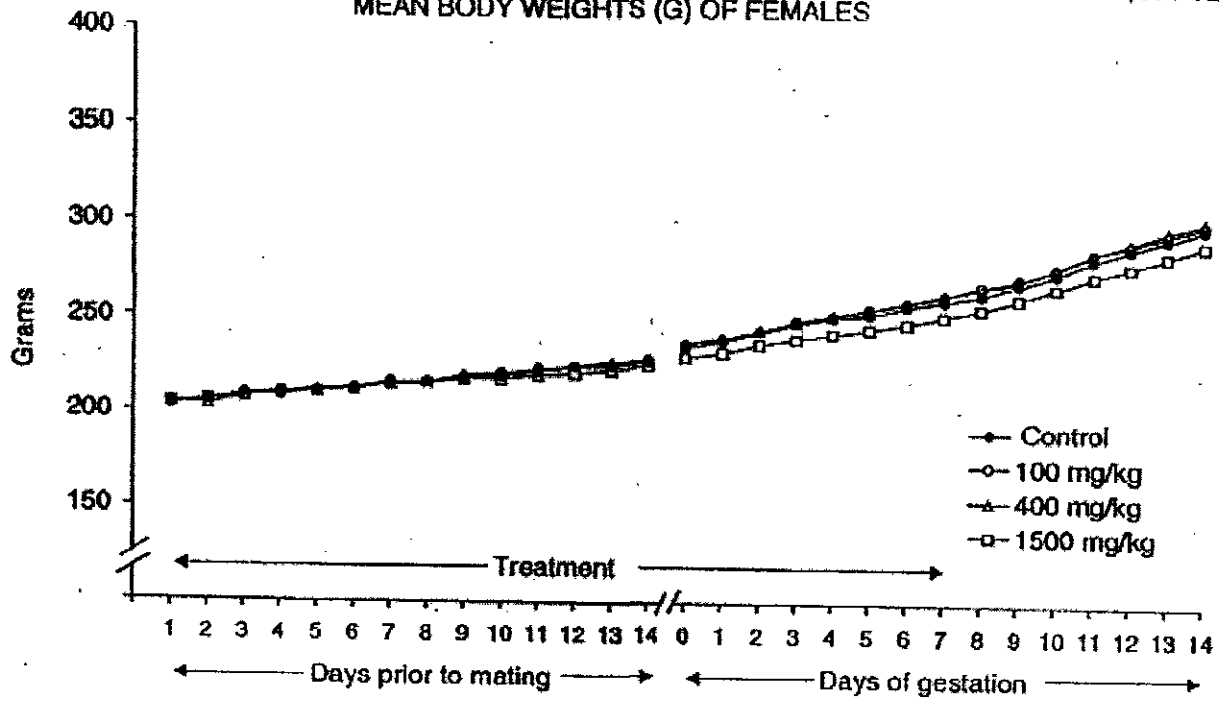
Mortality: there were no unscheduled deaths.

Clinical signs: there were no clear drug-related clinical signs. Reddish-brown stained fur was detected in 1 HDM (Day 31 of dosing on).

Body weight: body weight was not affected in males. In females, body weight was similar among grps during the mating period, but reduced at the HD (3-4%) during gestation (sponsor's Fig).

FIGURE 2

96-28-41
CP-217,861-02



Body weight gain in females was reduced by 7% during the first 8 days of gestation.

Food consumption: in males, food intake was reduced (6-13%) slightly, but significantly, at the HD throughout the dosing period, and from Day 16 of dosing on at the MD (3-8%).

In females, food intake was reduced sporadically during the end of the mating period, and during gestation.

Reproductive parameters (mating, fertility): there were no apparent drug-related effects on estrus cycle (incidence/duration), cohabitation period, pregnancy rate, or mating index.

The HDF (#539) mated with the HDM (#79) in which microscopic findings were detected was not pregnant.

Terminal studies

Litter parameters: there were no drug-related effects on the no. of corpora

lutea, implantation sites, or live fetuses. Although not statistically significant, there was an increase in resorptions at the HD (70%, cf. sponsor's Table 8 below). An examination of the individual data indicated that 11/19 C and 12/19 HD litters had at least one resorption; therefore, the increase in resorptions at the HD was due primarily to a higher no. of resorptions in a few HD litters.

TABLE 8

96-28-41
CP-217,861-02

REPRODUCTIVE DATA ON GESTATION DAY 14

	Control	100 mg/kg	400 mg/kg	1500 mg/kg
No. of females mated	20	20	20	20
No. of females copulated, (%)	20 (100.0)	19 (95.0)	20 (100.0)	20 (100.0)
No. of females pregnant, (%)	19 (95.0)	18 (94.7)	19 (95.0)	19 (95.0)
No. of corpora lutea				
Total	315	304	319	323
Mean ± S.D.	16.6 ± 1.9	16.9 ± 2.2	16.8 ± 1.7	17.0 ± 2.2
No. of implantation sites				
Total	281	260	301	280
Mean ± S.D.	14.8 ± 3.6	14.4 ± 4.4	15.8 ± 1.7	14.7 ± 3.8
No. of resorptions, (%)				
Mean ± S.D.	6.8 ± 10.2	8.3 ± 7.9	7.5 ± 7.6	11.6 ± 17.1
No. of live fetuses				
Total	264	237	279	250
Mean ± S.D.	13.9 ± 3.8	13.2 ± 4.1	14.7 ± 2.1	13.2 ± 4.4

Gross pathology: bilateral pale discoloration of the kidney was detected in 16/20 HDM. One HDM (#79) had "...dilated renal pelvis, dilated ureter and atrophy of the prostate and seminal vesicles.

No drug-related findings were apparent at necropsy in females.

Sperm analysis: there were no apparent drug-related effects on sperm count or motility. Both parameters, however, were reduced in 1 HDM (#74): 53% motility (C range: 69-89%), 547.55 x 10^6 sperm/gm epididymis (C range:
It should be noted that this was not the HDM in which microscopic findings were detected. The HDF (#534) mated with HDM #74 only 2 implantation sites and only 2 live fetuses.

Histopathology: the primary drug-related finding was foamy interstitial macrophages (slight) in testes in all dose grps: 0/20 CM, 18/20 LDM, 20/20 MDM, and 20/20 HDM. In 1 HDM, delayed release of spermatid (retained step 19 spermatids in Stage IX-XI seminiferous tubules) and slight, diffuse atrophy of the prostate and seminal vesicles were also observed (as noted above).

2. Maternal range finding study in Sprague-Dawley rats (Study no. 95-28-51, Pfizer Pharmaceuticals Inc., Japan, study dates: 5/16/95-6/14/95, report date: 11/8/96, GLP, Vol 1.18).

Methods: CP-217,861-02 (lot no. 3669-018) was administered to Sprague-Dawley rats (7/grp) at doses of 0, 300, 1000, and 3000 mg/kg i.v. from Day 6 through Day 17 of gestation. Observations consisted of the following: clinical signs, body weight, food consumption, terminal studies [no. of corpora lutea and implantation sites, placental wt, gross pathology of dams, histopathology on lung and kidney in dams], fetal examination [no. of live and dead fetuses, sex, body wt, external malformations].

Results: there were no unscheduled deaths in dams. Body weight gain was reduced (24%) in HDF only during the first wk (i.e., Days 6-12). Food consumption was lower in LDF and HDF; however, the sponsor noted that these differences were evident prior to the start of dosing and, therefore, were not drug-related effects. No drug-related effects were observed at necropsy. One CF and 1 MDF were not pregnant.

There were no apparent drug-related effects on any of the reproductive or fetal parameters.

Microscopic findings in dams are summarized in the following table:

TISSUE	FINDING	CF	LDF	MDF	HDF
kidney	vacuolation, tubular				
	grade 1*	0/7	4/7	1/7	0/7
	grade 2	0/7	0/7	5/7	0/7
	grade 3	0/7	0/7	0/7	7/7
	grade 4	0/7	0/7	0/7	0/7
lung	foam cell foci				
	grade 1**	1/7	5/7	2/7	0/7
	grade 2	0/7	0/7	3/7	2/7
	grade 3	0/7	0/7	1/7	5/7

*grades: 1 = <25%, 2 = 25-50%, 3 = 50-75%, and 4 = >75% of affected cortical tubules.

**grades: 1 = <20, 2 = <50, and 3 = ≥50 pulmonary foam cell foci.

3. Teratology study (reproductive study III) in Sprague-Dawley rats (Study no. 95-28-52, Pfizer Pharmaceuticals Inc., Japan, study dates: 9/20/95-10/23/95, report date: 2/25/97, Japanese GLP, Vol 1.19).

Animals: Sprague-Dawley rat [ ]
initial body wt: 202-249 gm
initial age: 9 wks
diet/housing: animals were housed individually except during the mating period; food and water were provided *ad lib.*
n = 20/grp

Drug: CP-217,861-02 (lot no. 3669-018)
vehicle: 0.9% saline
purity/stability: "activity" was given as — stability of i.v. injection solution was given as 2 yrs. No data were provided to document these statements.
doses: 0, 100, 600, and 3000 mg/kg
route: i.v.
dosing volume: 0.98 mL/100 gm body wt
duration: Days 6 through 17 of gestation

Observations

Dams

Clinical signs: animals were observed daily.

Body weight/food consumption: measured daily.

Terminal studies

Gross pathology: females were sacrificed on Day 21 of gestation. Uterine contents were examined. In those females not visibly pregnant, the uterus was stained for examination of possible implantation sites. The no. of corpora lutea and implantation sites, and placental weight were recorded.

Histopathology: not performed in dams.

Fetuses

Litter parameters: no. of live and dead fetuses, sex, body wt.

Examinations

External: all fetuses were examined for buccal and external malformations.

Visceral: even-numbered fetuses (i.e., ≈1/2 of each litter) were examined for visceral malformation using the Wilson technique.

Skeletal: odd-numbered fetuses were stained with Alizarin red S and alcian blue for analysis of skeletal findings.

Results

Dams

Mortality: there were no unscheduled deaths.

Clinical signs: according to the sponsor, there were no drug-related clinical signs.

Body weight: the only drug-related effect on body weight was a decrease in body weight gain (20%) in HDF during the first wk of dosing. Mean body weight in HDF on Days 12 and 17 were 98-97% of the mean CF values.

Food consumption: overall food consumption (Days 6-20 of gestation) was reduced by 7% in HDF. Food consumption was reduced significantly in HDF only during the dosing period (7-9%).

Terminal studies

Gross pathology: there were no drug-related macroscopic findings, according to the sponsor. Three CF, 1 MDF, and 1 HDF were found not to be pregnant.

Fetuses

Litter parameters: the data were summarized in the following sponsor's table:

TABLE 3

95-28-52
CP-217,661-02

REPRODUCTIVE AND FETAL DATA

	Control	100 mg/kg	600 mg/kg	3000 mg/kg
No. of pregnant females	17	20	19	19
No. of females died	0	0	0	0
No. of corpora lutea				
Total	267	319	304	300
Mean ± S.D.	15.7 ± 1.9	16.0 ± 1.9	16.0 ± 1.2	15.8 ± 1.4
No. of implantation sites				
Total	240	270	283	283
Mean ± S.D.	14.1 ± 3.9	13.5 ± 3.5	14.9 ± 1.2	13.8 ± 2.7
Placental weight, mg				
Mean ± S.D.	418.6 ± 54.8	422.5 ± 60.1	421.8 ± 34.9	442.0 ± 40.3
Embryomortality, %				
Mean ± S.D.	1.5 ± 2.7	8.4 ± 20.0	4.5 ± 7.6	7.2 ± 10.9
No.of early resorptions	3	16	13	14
late resorptions	0	1	0	0
macerated fetuses	1	0	0	2
dead fetuses	0	0	0	0
No. of F1 live fetuses				
Total (Males/Females)	236 (121/115)	253 (126/127)	270 (138/132)	247 (118/129)
Mean ± S.D.	13.9 ± 3.8	12.7 ± 4.1	14.2 ± 1.6	13.0 ± 3.1
Fetal body weight, g				
Males Mean ± S.D.	5.06 ± 0.31	5.13 ± 0.32	5.19 ± 0.21	5.09 ± 0.42
Females Mean ± S.D.	4.81 ± 0.27	4.84 ± 0.31	4.89 ± 0.27	4.89 ± 0.31
No. of F1 fetuses with external malformation	1[a]	0	1[b]	1[c]

a) Rhinocephaly
b) Agnathia
c) Kinky tail

Of note were the following: (1) increases in embryomortality at all doses (not dose-related) and (2) increases in early resorptions at all doses. The no. of litters in which at least 1 early resorption was detected was as follows: 3/17 CF, 6/20 LDF, 8/19 MDF, and 9/19 HDF. The increase in the embryomortality rate [i.e., (dead/macerated + resorptions)/total no. of implantation sites] reflected primarily these resorption; no dead fetuses were observed in any of the grps.

Examinations

External: there were no dose-related increases in external malformations. Malformations were detected in 3 fetuses, as noted in the above table.

Visceral: visceral findings were summarized in the following sponsor's table:

TABLE 4

95-28-52
CP-217,861-02

SKELETAL AND VISCERAL EXAMINATIONS OF FETUSES

	Control	100 mg/kg	600 mg/kg	3000 mg/kg
No. of dams	17	20	19	19
Skeletal examination				
No. of fetuses examined	123	130	140	128
Variations				
No. of fetuses with				
normal 13 pairs	121 (98.3)	125 (96.4)	136 (97.3)	122 (93.7)
cervical rib	0	1 (0.7)	2 (1.4)	2 (3.3)
extra 14th rib(s)	2 (1.7)	4 (2.9)	2 (1.3)	4 (3.0)
split of vertebral arch	0	0	1 (0.7)	0
Malformations	1 (0.6)	0	0	1 (0.6)
agenesis of the 2nd sternebra	1	0	0	0
multiple malformation[a]	0	0	0	1
Visceral examination				
No. of fetuses examined	113	123	130	119
No. of fetuses with anomalies	6 (5.6)	4 (2.7)	6 (5.0)	6 (4.9)
cleft palate and anophthalmia	0	0	1	0
dilatation of the renal pelvis	2	0	2	3
dilatation of the renal pelvis and hydroureter	1	0	0	0
dilatation of the ureter	1	0	0	0
thymic remnant in the neck	2	3	3	3
thymic remnant in the neck and dilatation of the ureter	0	1	0	0

Figures in parentheses represent mean percent of variations or anomalies by litter.
a) Fusion of the cervical vertebrae, sternebrae, rib and caudal vertebrae and agenesis of the thoracic vertebrae and rib.

It should be noted that visceral malformations, e.g., cleft palate and anophthalmia, are listed under "anomalies". Neither the number of affected fetuses or litters (5/17 C, 3/20 LD, 5/19 MD, and 5/19 HD litters) was dose-related.

Skeletal: skeletal findings were summarized in the above sponsor's Table 4 and in the following sponsor's table (Table 5). Of note was an increase in the no. of fetuses/litters with cervical rib (variation) in dosed grps and the occurrence of multiple malformations in 1 HD fetus.

It should be noted that individual fetal data were not provided. The "individual" line listing provided observations per dam, not observations for individual fetuses. These data should be requested from the sponsor.

TABLE 5

95-28-52
CP-217,861-02

SKELETAL EXAMINATION OF FETUSES
DEGREE OF OSSIFICATION

		Control	100 mg/kg	600 mg/kg	3000 mg/kg
No. of dams		17	20	19	19
No. of fetuses examined		123	130	140	128
No. of fetuses with rudimentary skull bones		0	0	0	0
5th sternebra, (%)[a]		8 (6.3)	14 (9.8)	9 (6.6)	8 (5.9)
No. of fetuses with ossified calcaneus		0	0	0	0
No. of ossified caudal vertebral centra		6.8 ± 0.5[b]	6.7 ± 0.6	6.6 ± 0.5	6.6 ± 0.7
(forepaw)					
metacarpal bones,	right	4.0 ± 0.0	4.0 ± 0.0	4.0 ± 0.0	4.0 ± 0.0
	left	4.0 ± 0.0	4.0 ± 0.0	4.0 ± 0.0	4.0 ± 0.0
first phalanges,	right	2.1 ± 0.5	2.2 ± 0.4	1.8 ± 0.3	2.0 ± 0.5
	left	1.9 ± 0.6	2.2 ± 0.5	1.7 ± 0.4	1.8 ± 0.5
middle phalanges,	right	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
	left	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
(hindpaw)					
metatarsal bones,	right	4.9 ± 0.2	4.9 ± 0.2	4.8 ± 0.2	4.8 ± 0.3
	left	4.9 ± 0.3	5.0 ± 0.1	4.8 ± 0.2	4.9 ± 0.2
first phalanges,	right	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.1	0.0 ± 0.0
	left	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.1	0.0 ± 0.0
middle phalanges,	right	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
	left	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0

a) Figures in parentheses represent mean litter percent.
b) Each value represents the mean and standard deviation.

Based on the sponsor's summary, there was no apparent drug-related effects on skeletal ossification.

4. Maternal range finding study in Japanese White rabbits (Study no. 95-28-72, Pfizer Pharmaceuticals Inc., Japan, study dates: 5/17/95-6/29/95, report date: 11/96, Japanese GLP, Vol 1.19).

Methods: CP-217,861-02 (lot no. 3669-018) was administered to Japanese White rabbits (7/grp) at doses of 0, 250, 600, and 1500 mg/kg i.v. from Day 6 through Day 18 of gestation. Observations consisted of the following: clinical signs, body weight, food consumption, terminal studies [no. of corpora lutea and implantation sites, placental wt, gross pathology of dams, histopathology on lungs and kidney of dams], fetal examinations [no. of live and dead fetuses, sex, body wt, buccal and external malformations].

Results: there were no unscheduled deaths. One LDF was sacrificed on Day 21 of gestations with evidence of hindlimb paralysis (from Day 18 of gestation on). Body weight gain was not affected by treatment. Food consumption was lower at all doses throughout most of the dosing period; however, differences were statistically significant at all doses only during the postdosing period. Also, reductions were not dose-related.

No drug-related findings were evident at necropsy, either in survivors or in the LDF sacrificed moribund. Four females were not pregnant (1 CF, 2 MDF, 1 HDF). There were also no drug-related microscopic findings in the dams, except for a grade 1 tubular vacuolation of the kidney in 1/7 HDF.

The reproductive data are summarized in the following sponsor's table. Of note are the increases in early resorptions in MDF and HDF, and the 1 HD fetus with an external malformation (omphalocele, i.e., herniation of the umbilical cord).

TABLE 3

95-28-72
CP-217,661-02

REPRODUCTIVE AND FETAL DATA

	Control	250 mg/kg	600 mg/kg	1500 mg/kg
No. of pregnant females	6	6	5	6
No. of females aborted	0	0	0	0
No. of females died	0	0	0	0
No. of corpora lutea				
Total	70	70	54	60
Mean ± S.D.	11.7 ± 2.5	11.7 ± 1.5	10.8 ± 1.3	11.3 ± 2.3
No. of implantation sites				
Total	35	59	49	55
Mean ± S.D.	5.8 ± 4.3	9.8 ± 1.5	9.8 ± 2.7	9.2 ± 1.7
Placental weight, g				
Mean ± S.D.	5.93±1.47	4.88±0.41	4.86±0.80	5.19±0.66
Total No. of dead fetuses,(%)[a]	4 (11.4)	3 (5.1)	4 (8.2)	3 (5.5)
early resorptions	1	0	4	3
late resorptions	0	0	0	0
macerated fetuses	3	3	0	0
dead fetuses	0	0	0	0
No. of F1 live fetuses				
Total	31	56	45	52
Mean ± S.D.	5.2 ± 3.7	9.3 ± 1.5*	9.0 ± 2.1	8.7 ± 2.0
Fetal body weight, g				
Mean ± S.D.	48.34±9.82	37.06±4.71*	40.17±2.82	42.86±3.63
No. of F1 fetuses with external malformation	0	0	0	1[b]

Values marked with asterisks differ significantly from the control value; * P<0.05, ** P<0.01, *** P<0.001.
a) (No. of dead fetuses/No. of implantation sites) X 100
b) Omphalocele

5. Reproductive study III, teratology in Japanese White rabbits (Study no. 96/28/73, Pfizer Pharmaceuticals Inc., Japan, study dates: 5/22/96-6/24/96, report date: 7/97, Japanese GLP, Vol 1.19).

Animals: Japanese White rabbit (Ł)
initial age: 17 wks
initial body wt: 3.11-3.90 kg
diet/housing: females were housed individually; food and water were available

ad lib.
n = 20/grp

Drug: CP-217,861-02 (lot no. 3669-018)
vehicle: 0.9% saline
purity/stability: "activity" was given as —— stability of drug substance stated to be 2 yrs at rm temperature. Data were not provided in the report to support these statements.
doses: 0, 100, 400, 1500 mg/kg
route: i.v.
dosing volume: 4.87 mL/kg
duration: Day 6 through Day 18 of gestation

Observations

Dams

Clinical signs: animals were observed daily.

Body weight/food consumption: body wt and food intake were measured daily.

Terminal studies

Gross pathology: dams were sacrificed on Day 29 of presumed gestation. Uterine contents were examined and no. of corpora lutea, implantation sites, placental wt, and gross findings were recorded.

Histopathology: not performed.

Fetuses

Litter parameters: no. of live and dead fetuses, body wt.

Examinations: all fetuses were examined for buccal and external malformations, sex and visceral malformations/anomalies/variations. Visceral findings were examined using the Stuckhardt and Poppe (1984) fresh dissection method. Skeletal findings (examined in all fetuses) were conducted using Alizarin red S and alcian blue stains.

Results

Dams

Mortality: there were 2 unscheduled deaths: 1 CF and 1 HDF. Both animals were sacrificed following evidence of hindlimb paralysis. Paralysis, according to the sponsor, was "due to accidents".

Clinical signs: absorptions were evident in 1 MDF (Day 26) and 1 HDF (Day 22). The sponsor noted that food consumption was decreased or absent in these animals from the "...middle of pregnancy..." and "..continued until the animals aborted".

No drug-related clinical signs were evident.

Body weight: there were no drug-related effects.

Food consumption: there were no drug-related effects during the dosing period. From Day 24 of gestation on, food intake tended to be lower in MDF 29-45%) and HDF (11-48%).

Terminal studies

Gross pathology: according to the sponsor, there were no drug-related findings. Six females were not pregnant: 2 CF, 1 LDF, 2 HDF.

Fetuses

Litter parameters: the data were summarized in the following sponsor's table:

Appears This Way
On Original

Appears This Way
On Original

TABLE 3

96-28-73
CP-217,861-02

REPRODUCTIVE AND FETAL DATA

	Control	100 mg/kg	400 mg/kg	1500 mg/kg
No. of pregnant females	17	19	20	16
No. of females aborted	0	0	1	1
No. of females died	0	0	0	0
No. of corpora lutea				
Total	208	230	223	174
Mean ± S.D.	12.2 ± 2.4	12.1 ± 2.0	11.7 ± 1.9	11.8 ± 1.7
No. of implantation sites				
Total	139	165	170	142
Mean ± S.D.	8.2 ± 3.1	8.7 ± 3.6	9.0 ± 2.0	9.5 ± 2.0
Placental weight, g				
Mean ± S.D.	5.35 ± 1.09	5.50 ± 1.46	4.91 ± 1.03	4.95 ± 0.93
Embryonortality, %				
Mean ± S.D.	12.3 ± 15.3	9.9 ± 13.6	7.7 ± 8.6	5.4 ± 9.4
No. of early resorptions	6	4	7	0
late resorptions	1	1	1	0
macerated fetuses	7	7	5	8
dead fetuses	1	1	1	0
No. of F1 live fetuses				
Total (Males/Females)	124 (72/ 52)	152 (88/ 64)	156 (77/ 79)	134 (67/ 67)
Mean ± S.D.	7.3 ± 3.1	8.0 ± 3.6	8.2 ± 1.9	8.9 ± 2.1
Fetal body weight, g				
Males Mean ± S.D.	44.46 ± 9.78	41.24 ± 7.40	39.52 ± 7.76	38.40 ± 4.78
Females Mean ± S.D.	39.16 ± 9.27	41.03 ± 8.24	38.25 ± 7.14	38.52 ± 5.24
No. of F1 fetuses with				
external malformation	0	1[a)]	1[b)]	1[c]

a) Omphalocele
b) Oligodactyly
c) Spina bifida

There were no apparent drug-related effects. [The number of dams at the HD was slightly lower than the recommended 16-20/grp.] External malformations were noted in 1 fetus in each of the dose grps.

Examinations: as noted above, no drug-related external malformations were evident. The visceral and skeletal findings were summarized in the following sponsor's tables:

TABLE 4

96-28-73
CP-217,861-02

SKELETAL AND VISCERAL EXAMINATIONS OF FETUSES

	Control		100 mg/kg		400 mg/kg		1500 mg/kg	
No. of does	17		19		19		15	
Skeletal examination								
No. of fetuses examined	124		152		158		134	
No. of fetuses with								
Variations								
normal 12 pairs, (%)	91	(74.9)	85	(58.5)	92	(59.9)	85	(63.0)
cervical rib, (%)	1	(1.0)	3	(1.4)	0		1	(0.8)
extra 13th rib(s), (%)	30	(22.5)	64	(40.1)	64	(40.1)	48	(36.2)
no. of pairs	22		44		46		36	
no. of unilateral	8		20		18		12	
accessory sternebrae, (%)	4	(8.4)	6	(4.3)	2	(1.3)	3	(1.8)
asymmetry of sternebrae, (%)	1	(0.5)	0		0		0	
split of sternebrae, (%)	1	(0.5)	0		0		0	
13 thoracic vertebrae, (%)	4	(4.4)	12	(7.3)	19	(10.3)	18	(15.2)
8 lumbar vertebrae, (%)	0		1	(0.6)	4	(2.5)	8	(6.3)
lumbarization, (%)	0		5	(4.0)	3	(2.3)	2	(2.3)
Malformations (Total), (%)	4	(2.8)	1	(0.9)	0		3	(2.3)
Caudal hemivertebra	1		0		0		2	
Fused caudal vertebrae	0		1		0		0	
Thoracic hemivertebra	1		0		0		0	
Lumbar vertebral arch rudimentary and lumbar hemivertebra	0		0		0		1	
Nodulated ribs	2		0		0		0	
Visceral examination								
No. of fetuses examined	124		152		156		134	
No. of fetuses with								
Malformations (Total), (%)	1	(0.6)	0		1	(0.5)	1	(0.6)
Circumcorneal hemorrhage	1		0		0		0	
Hydrocephaly	0		0		0		1	
Microphthalmia	0		0		1		0	

Figures in parentheses represent mean percent of variations or malformations by litter.

Visceral malformations were detected in 3 fetuses: 1 C, 1 MD, and 1 HD. Visceral anomalies/variations were either not observed or were not reported.

Skeletal malformations were detected in 8 fetuses: 4 C, 1 LD, 3 HD. None appeared to be drug-related. The following skeletal findings were noted: (1) an increase in no. of extra 13th rib (pairs/unilateral). Neither the fetal or litter incidences were dose-related (fetal: 100-125, 100-125, and 63-50% increase at the LD, MD, and HD, respectively; litter: 59-47, 74-68, 63-47, 67-53% at LD, MD, and HD, respectively). (2) the litter incidences of 13 thoracic and 8 lumbar vertebrae were increased in a dose-related manner, (3) lumbarization was observed only in treated fetuses; however, neither the fetal or mean litter incidences were dose-related.

TABLE 5

96-28-73
CP-217,061-02

SKELETAL EXAMINATION OF FETUSES
DEGREE OF OSSIFICATION

		Control	100 mg/kg	400 mg/kg	1500 mg/kg
No. of does		17	19	19	15
No. of fetuses examined		124	152	156	134
No. of fetuses with rudimentary					
skull bones		0	0	0	0
5th sternebra, (%)[a]		54 (37.3)	57 (37.7)	67 (45.1)	71 (51.2)
No. of ossified					
caudal vertebral centra		15.1 ± 0.6[b]	15.1 ± 0.4	15.1 ± 0.4	15.0 ± 0.4
(forepaw)					
metacarpal bones,	right	5.0 ± 0.1	5.0 ± 0.1	5.0 ± 0.1	5.0 ± 0.0
	left	5.0 ± 0.1	5.0 ± 0.1	5.0 ± 0.1	5.0 ± 0.0
first phalanges,	right	5.0 ± 0.0	5.0 ± 0.0	5.0 ± 0.0	5.0 ± 0.0
	left	5.0 ± 0.0	5.0 ± 0.0	5.0 ± 0.0	5.0 ± 0.0
middle phalanges,	right	4.0 ± 0.0	4.0 ± 0.0	4.0 ± 0.0	4.0 ± 0.0
	left	4.0 ± 0.0	4.0 ± 0.0	4.0 ± 0.0	4.0 ± 0.0
(hindpaw)					
metatarsal bones,	right	4.0 ± 0.0	4.0 ± 0.0	4.0 ± 0.0	4.0 ± 0.0
	left	4.0 ± 0.0	4.0 ± 0.0	4.0 ± 0.0	4.0 ± 0.0
first phalanges,	right	4.0 ± 0.0	4.0 ± 0.0	4.0 ± 0.0	4.0 ± 0.0
	left	4.0 ± 0.0	4.0 ± 0.0	4.0 ± 0.0	4.0 ± 0.0
middle phalanges,	right	4.0 ± 0.0	4.0 ± 0.0	4.0 ± 0.0	4.0 ± 0.0
	left	4.0 ± 0.0	4.0 ± 0.0	4.0 ± 0.0	4.0 ± 0.0

a) Figures in parentheses represent mean litter percent.
b) Each value represents the mean and standard deviation.

Regarding the degree of ossification data, there were dose-related increases in the fetal and litter incidence of rudimentary 5th sternebra.

[Litter incidence was calculated by the sponsor as follows: no. of affected fetuses/total no. of fetuses for each litter, divided by the total number of litters. It is not the no. of litters with at least one affected fetus. It should also be noted that individual data were provided for dams, but not for fetuses. The sponsor should be asked to provide these data.]

6. Reproduction study II: prenatal and postnatal development in Sprague-Dawley rats (Study no. 96-28-61, Pfizer Pharmaceuticals Inc., Japan, study dates: 9/3/96-2/12/97, report date: 10/97, Japanese GLP, Vol 1.19).

Animals: Sprague-Dawley rat
initial age: 9 wks
initial body wt: 167-227 gm

diet/housing: animals were housed individually, food and water were provided *ad lib.*
n = 23/grp

Drug: CP-217,816-02 (lot no. 3669-018)
vehicle: 0.9% saline
purity/stability: "activity" given as — drug formulation reported to be stable for 2 yrs at room temperature. No data were provided to document these statements.
doses: 0, 100, 600, 3000 mg/kg
dosing volume: 0.98 mL/100 gm
route: i.v.
duration: daily from Day 6 of gestation to Day 21 of lactation. All dams allowed to deliver.

Observations

Dams

Clinical signs: dams were observed daily from Day 19 on. Litters born during the day were designated "Day 0"; litters born overnight were designated "Day 1".

Body weight: dams were weighed daily during gestation and lactation.

Food consumption: food intake was recorded daily during gestation.

Reproductive parameters: the following parameters were recorded: gestation length, time/duration of parturition, dystocia, no. of viable litters, lactation performance (" qualitative estimation by observation of teats and stomachs of neonates").

Terminal studies

Gross pathology: dams were sacrificed on Days 21-23 of lactation and necropsied (no. of implantation determined). Females determined not to be pregnant were sacrificed on presumed Day 24 of gestation and uteri were examined for signs of pregnancy/abnormalities.

F_1

Mortality: litters were checked at birth for no. of live/dead pups and sex. Pup survival was recorded on Days 4, 21, an 56 postpartum.

Clinical signs: pups were observed daily.

Body weight: body wts were recorded on Days 1, 34, 7, 10, 14, and 21 of lactation and weekly thereafter.

Postnatal development: external examination was conducted on Day 1. The following developmental tests were conducted: surface righting (Day 1 on), appearance of incisors (Day 7 on), eye opening (Day 10 on), air righting (Day 14 on), visual cliff avoidance (Day 18), vaginal opening (Day 28 on), auditory function (Day 30), preputial separation (Day 35 on), motor activity, i.e., total distance traveled in 20 min (2 pup/sex/litter) on Days 22-24, FOB (2/sex/litter on Days 24-26, Cincinnati Water Maze (1/sex/litter) on Days 55-63, Gemini Avoidance System

(passive; 1/sex/litter) on Days 55-61.

Reproductive performance: in the F_1 females selected, the following were recorded: copulation and pregnancy indices, body weight during gestation and throughout lactation (Day 21 postpartum), food consumption during gestation, gestation and parturition length, no. of viable litters, lactation performance.

Terminal studies

Gross pathology: pups were sacrificed on Day 4 postpartum (litters culled to 4/sex), Day 21 postpartum (all pups except those selected for assessment of reproductive parameters), and Day 21 of lactation for those F_1 females selected for assessment of reproductive performance (all were allowed to deliver) and necropsied. The no. of implantation sites were determined in mated F_1 females at sacrifice.

F_2

Mortality: litters were checked daily for survival. Survival rates were recorded on Day 4 and Day 21 postpartum.

Clinical signs: pups were observed daily.

Body weight: body weights were recorded on Days 1, 4, 7, 10, 14, and 21 postpartum.

Reproductive parameters: litters were examined for viable pups at birth, sex of pups.

Terminal studies

Gross pathology: F_2 pups were sacrificed on Day 4 postpartum (litters culled to 4/sex/litter) and Day 21-23 (all remaining pups) and necropsies were performed.

Results

Dams

Mortality: there were no unscheduled deaths.

Clinical signs: no drug-related effects were reported.

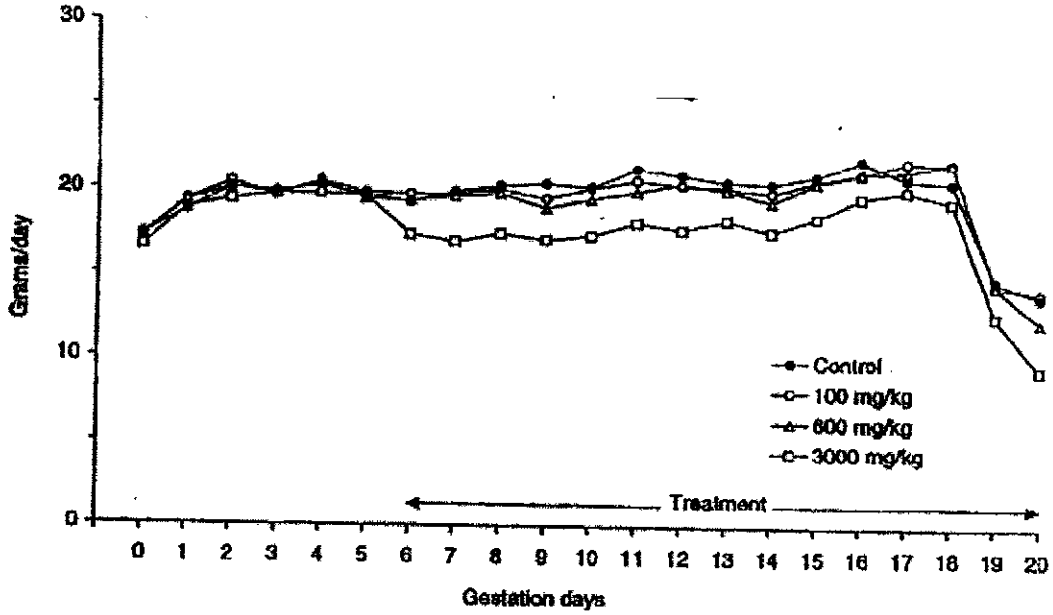
Body weight: body weight was slightly (3-4%), but significantly lower in HDF during gestation compared to CF. Men body wt gain was reduced during Days 6-13 of gestation (24%), but increased during the lactation period in HDF. No body weight effects were evident during lactation.

Food consumption: food consumption was reduced during most of the gestational period in HDF (5-31%) (sponsor's table follows).

FIGURE 3

96-28-61
CP-217,851-02

MEAN GESTATIONAL FOOD CONSUMPTION (G/DAY) OF F0 RATS



Reproductive parameters: the data were summarized in the following sponsor's table.

TABLE 6

96-28-61
CP-217,861-02

REPRODUCTIVE DATA OF LITTERING F0 RATS AND F1 PUP VIABILITY

		Control	100 mg/kg	600 mg/kg	3000 mg/kg
No. of pregnant females		20	21	21 [a]	22
No. of females with viable litters		20	21	20	21
Length of gestation, days	Mean ± S.D.	21.4 ± 0.6	21.4 ± 0.6	21.5 ± 0.6	21.7 ± 0.7
No. of implantation sites	Mean ± S.D.	15.1 ± 3.4	14.4 ± 3.4	14.1 ± 4.7	14.1 ± 4.6
No. of resorptions	Mean ± S.D.	1.7 ± 1.7	1.1 ± 1.5	1.4 ± 1.5	1.2 ± 1.6
No. of stillbirths	Mean ± S.D.	0.1 ± 0.3	0.3 ± 0.7	0.4 ± 0.9	1.4 ± 2.3 *
No. of F1 pups					
At birth No. of litters		20	21	21	22
Total	Mean ± S.D.	13.9 ± 3.0	13.0 ± 3.2	12.3 ± 4.4	11.5 ± 4.6
Male	Mean ± S.D.	6.2 ± 2.6	6.3 ± 2.1	5.8 ± 2.6	6.1 ± 3.2
Female	Mean ± S.D.	7.1 ± 3.1	6.7 ± 2.8	6.5 ± 2.4	5.4 ± 2.9
Day 4 No. of litters		20	21	20	21
Total	Mean ± S.D.	13.1 ± 2.8	11.9 ± 3.9	11.1 ± 4.4	9.7 ± 6.0 **
Day 21 No. of litters		17	19	15	16
Total	Mean ± S.D.	8.0 ± 0.0	7.9 ± 0.3	7.9 ± 0.4	7.9 ± 0.3
Day 56 No. of litters		17	19	15	16
Total	Mean ± S.D.	4.0 ± 0.0	4.0 ± 0.0	4.0 ± 0.0	4.0 ± 0.0
No. of F1 pups with					
External malformations		0	0	0	0
Visceral malformations		1 [b]	1 [b]	3 (2 [b], 1 [c])	1 [b]

* $P < 0.05$, ** $P < 0.01$

a) Data from one dam(# 633) sacrificed on GD 26 because of no sign of parturition, was excluded only for calculations of mean length of gestation.
b) Diaphragmatic hernia
c) Hydronephrosis and hydroureter

Eight females were not pregnant: 3 C, 2 LD, 2 MD, and 1 HD. The mean number of stillbirths was increased (14-fold) in HDF. Upon examination of the individual data, the number of stillbirths and the number of affected litters (i.e., litter with at least one stillbirth) were as follows [expressed as # stillbirths(# litters)]: 2(2), 6(4), 9(5), and 3(11) in CF, LDF, MDF, and HDF, respectively. Other parameters were not significantly affected, although no. of live pups at birth was slightly reduced as a result of the increase in stillbirths.

Terminal studies

Gross pathology: pale discoloration of the kidney (bilateral) was detected in 12/23 HDF.

F_1

Mortality: the no. of litters was reduced on Day 4 in HDF (26%) (cf. sponsor's Table 6 above). The sponsor did not express the data in terms of pup survival. An examination of the individual data indicated that the no. of pup deaths (total) and the number of affected litters (i.e., litter with at least one pup death by Day 4) were as follows [expressed as total pup deaths (affected litters)]: 4(3), 23(11), 37(13), and 49(11) in CF, LDF, MDF, and HDF, respectively.

Clinical signs: no drug-related clinical signs or external malformations were evident.

Body weight: pup body wt was significantly reduced throughout the lactation at the HD in males (7-10%) and females (5-9%). Body wt also tended to be lower in MD pups (1-8%), but the differences were not statistically significant.

Body wt was not significantly affected after weaning; however, body wt tended to remain slightly lower (≤5%) in HD pups (compared to C pups).

Postnatal development: the sponsor set criteria for inclusion of litters in the assessment of postnatal development, i.e., litters had to have at least 2 pups/sex or a total of 7 pups. Therefore, 3 C, 2 LD, 5 MD, and 5 HD litters were not included in the evaluation of drug-related effects on postnatal development.

There were no statistically significant drug-related effects on developmental parameters/paradigms. The following, however, are of note: (1) the time to step through in the passive avoidance paradigm was shorter in MD (10-18%) and HD (19-22%) male and female pups, (2) the number of errors made on the Cincinnati Maze tended to be higher in HDF (≤33%, 19% in mean score).

Reproductive parameters: body wt was reduced (≈5%) in MDF and HDF (compared to CF) throughout gestation and lactation; the effect was statistically significant only at the HD. These differences probably reflect, at least in part, prior effects on body weight since body wt gain during gestation was not affected; however, body wt gain during the first wk of lactation tended to be lower in MDF and HDF (14-18%, respectively).

Food intake during gestation was not significantly affected; however, it was slightly reduced (≈7%) during Days 13-20 of gestation in MDF and HDF.

The reproductive data were summarized in the following sponsor's table (Table 27). There were no apparent drug-related findings.

TABLE 27

96-28-61
CP-217,861-02

REPRODUCTIVE DATA OF LITTERING F1 RATS AND F2 PUP VIABILITY

		Control	100 mg/kg	600 mg/kg	3000 mg/kg
No. of females mated		17	19	15	16
No. of females with successful copulation (%)		16 (94.1)	18 (94.7)	14 (93.3)	15 (93.8)
No. of pregnant females (%)		16 (100.0)	18 (100.0)	14 (100.0)	15 (100.0)
No. of females with viable litters		16	18	14	15
Length of gestation, days	Mean ± S.D.	21.4 ± 0.5	21.5 ± 0.5	21.4 ± 0.5	21.3 ± 0.6
No. of implantation sites	Mean ± S.D.	14.8 ± 2.6	14.2 ± 3.7	14.5 ± 2.9	14.9 ± 2.7
No. of resorptions	Mean ± S.D.	1.4 ± 1.3	1.7 ± 1.6	1.5 ± 1.9	1.7 ± 1.7
No. stillbirths	Mean ± S.D.	0.6 ± 2.5	0.2 ± 0.7	0.8 ± 2.4	0.7 ± 1.7
No. of F2 pups					
At birth No. of litters		16	18	14	15
Total	Mean ± S.D.	12.6 ± 3.2	12.3 ± 3.9	12.2 ± 3.8	12.4 ± 3.5
Male	Mean ± S.D.	6.1 ± 2.3	5.7 ± 2.8	6.1 ± 2.4	6.4 ± 2.5
Female	Mean ± S.D.	6.5 ± 1.8	6.6 ± 2.4	6.1 ± 2.4	6.0 ± 2.5
Day 4 No. of litters		14	18	14	15
Total	Mean ± S.D.	11.5 ± 4.1	11.4 ± 5.0	9.9 ± 4.9	9.9 ± 5.0
Day 21 No. of litters		14	16	12	14
Total	Mean ± S.D.	7.1 ± 2.2	7.8 ± 0.7	7.0 ± 2.5	6.8 ± 2.7
No. of F2 pups with					
External malformations		0	0	0	0
Visceral malformations		0	0	0	0

Terminal studies

Gross pathology: no drug-related gross pathology findings were reported in F_1 animals sacrificed on Day 4 of lactation, after weaning, after assessment of development, or mating.

F_2

Mortality/survival: (cf sponsor's Table 27 above). There were no apparent drug-related effects on survival.

Clinical signs: no drug-related effects were detected.

Body weight: body weight was significantly lower in MDM, HDM, and HDF on Days 14 and 21 of lactation. In males, the effect was not dose-related. The sponsor did not attribute these to drug.

Terminal studies

Gross pathology: no drug-related effects were detected.

GENOTOXICITY

1. **Bacterial mutation assay** (Study no. PFZ 591/941502, [redacted], report date: 1/25/95, study dates: 5/94, GLP, Vol 1.19)

Methods: the mutagenic potential of CP-217,861-02 (SBECD) (batch no. 3005-117) was tested using the following *S. typhimurium* tester strains: TA 1535, TA 1537, TA 1538, TA 98, and TA 100. [This battery lacked the recommended strain, *E. coli* WP2 uvrA, *E. coli* WP2 uvrA pKM101, or *S. typhimurium* TA 102.] Testing was conducted in the absence and presence of metabolic activation (Aroclor 1254-induced S9, male Sprague-Dawley rat). Positive controls were as follows: -S9: N-ethyl-N'-nitro'N'nitrosoguanidine (TA 1535, TA 100), 9-aminoacridine (TA 1537), 2-nitrofluorene (TA 1538, TA 98); +S9: 2-aminoanthracene. [The activity of S9 was tested with carcinogens, 7, 12-dimethylbenzathracene and 2-aminoanthracene.]

SBECD was tested at concentrations of 0 (solvent: water), 50, 150, 500, 1500, and 5000 µg/plate (±S9). There were three replicates per concentration, and 2 separate experiments were conducted. Plates were incubated at 37° C for 3 days.

Criteria for a positive response were as follows: a increase in revertants of ≥2-fold, with evidence of dose-relationship, and reproducible in 2 separate experiments.

Results: there were no increases in revertants with any tester strain, either with or without S9. Positive controls produced increases in revertants consistent with positive responses. The concentration of 9-aminoacridine (TA 1537) used was too high, producing such an increase in revertants that they could not be scored in either experiment; therefore, the sensitivity of the TA 1537 assay was not documented.

2. **Microbial reverse mutation assays** (Study no. 94-1033-01, Pfizer Central Research, Groton, Conn., report date: 7/15/94, study dates: 3/94, GLP, Vol 1.20).

Methods: the mutagenic potential of SBECD (lot no. 3005-117) was tested using *S. typhimurium* tester strains, TA 1535, TA 1537, TA 98, TA 100, and *E. coli* WP2 uvrA pKM101. [Tester strains were tested to verify characteristic genetic markers.] Testing was conducted in the absence and presence of metabolic activation (Aroclor-induced S9, rat strain not specified) at concentrations of 0 (water), 0.01, 0.05, 0.2, 1, and 5 mg/plate. There were 3 replicates per concentration, and incubation was at 37° C for 48-72 hr. Positive controls were as follows: -S9: sodium nitrite (TA 1535), 9-aminoacridine (TA 1537), 2-nitrofluorene (TA 98), nitrofurantoin (TA 100), and N-ethyl-N'-nitro-nitrosoguanidine (*E. coli*); +S9: 2-anthramine for all tester strains. [Activity of the S9 fraction did not seem be have been verified using a compound other than 2-anthramine, as recommended in the OECD guidelines.]

Criteria for a positive response were as follows: "..a dose-related, reproducible, three-fold increase in the average number of revertant colonies per plate compared to...negative control..."

Results: there were no increases in revertants with any tester strain, either with or without metabolic activation. [All plates could not be evaluated for TA 1535 due to plate contamination (0.2, 1.0 mg/plate, -S9) or toxicity (1.0 mg/plate, +S9).]

3. **Mammalian mutation assays** (Study no. 94-1033-03, Pfizer Central Research, Groton, Conn., report date: 8/23/94, study dates: 3-5/94, GLP, Vol 1.20).

Methods: the mutagenic potential of SBECD (lot no. 3005-117) was tested in CHO cells at the HPRT locus in the absence and presence of metabolic activation (Aroclor 1254-induced Sprague-Dawley rat liver S9). SBECD was tested in duplicate (at all but LC, -S9) at

concentrations of 2-5 mg/plate (6 concentrations). Incubation with test or vehicle (water) was for 5 hrs. Ethyl methanesulfonate (-S9) and 3-methylcholanthrene (+S9) were used as positive controls. Criteria for a positive response were as follows: (1) dose-response, (2) statistically significant increase in MF, with at least one concentration producing an average MF of >20 or at least 2 consecutive concentrations producing statistically significant increases in MF, (3) an increase in MF outside the range of historical control, (4) reproducibility of finding.

Results: no cytotoxicity was evident at any concentration, with or without S9. There were also no increases in MF, either with or without S9.

4. **In vitro cytogenetic assays** (Study no. 94-1033-02, Pfizer Central Research, Groton, Conn., report date: 7/26/94, study dates: 3-4/94, GLP, Vol 1.20).

Methods: the clastogenic potential of SBECD (lot no. 3005-117) was tested *in vitro* in human lymphocytes, with and without metabolic activation (Aroclor 1254-induced male Sprague-Dawley rat liver S9). SBECD was tested in duplicate at 3 concentrations (3200, 4000, and 5000 µg/mL). Cells were treated for 24-hr (-S9) or 3 hr (+S9). Mitomycin-C (-S9) and cyclophosphamide (+S9) were used as positive controls. 100 metaphases were examined per culture (i.e., 200/concentration).

The criteria for a positive response were as follows: "...a statistically significant, reproducible and dose-related increase in the number of abnormal cells compared to the concurrent ...controls."

Results: there were no increases in structurally or numerically aberrant cells, either with or without S9.

5. **Mouse micronucleus assays** (Study no. 94-1033-04, Pfizer Central Research, Groton, Conn., report date: 11/2/94, study dates: 4-5-94, GLP, Vol 1.20).

Methods: the clastogenic activity of SBECD (lot no. 3005-117) was tested in CD-1 mice (5/sex/grp) at doses of 750, 1500, and 3000 mg/kg i.v., administered for 3 consecutive days. Mitomycin C (5/sex) was administered at a dose of 0.5 mg/kg/day i.p. for 3 days as a positive control. Bone marrow was sampled ≈24 hr after the last dose. 1000 PCEs/animal were scored for presence of micronuclei. [This is only one-half of the number, i.e., 2000 PCEs/animal, recommended by the OECD guidelines.] The proportion of immature to mature erythrocytes was determined on 200 cells/slide (i.e., 400 cells/animal).

Results: there were no drug-related deaths or "...substantial adverse clinical signs..." (not otherwise specified by the sponsor) in treated animals. There were also no differences in PCE/NCE ratio, indicating a lack of bone marrow toxicity. Body weight was not affected in SBECD-treated animals.

There were no increases in micronucleated PCEs at any dose of SBECD. In contrast, Mitomycin C produced marked increases in micronuclei.

SUMMARY AND EVALUATION

Pharmacology

CP-217,816-02 (SBECD) was tested in *in vitro* receptor studies and in a number of safety pharmacology studies (i.e., cardiovascular, respiratory, renal). These studies were previously reviewed by Dr. Brian Ault. In the *in vitro* receptor studies, SBECD "...demonstrated a lack of $5HT_2$ activity...", according to Dr. Ault's review. Also, Dr. Ault noted that "little effect" was observed in the safety pharmacology studies in Beagle dogs, cats, and/or rats at doses up to 250 mg/kg.

The pharmacology of ziprasidone was addressed by referencing the NDA for oral ziprasidone (NDA 20-825).

Conclusion: SBECD appeared to exhibit no pharmacological activity in the limited number studies of mechanism of action and safety pharmacology conducted.

PK/ADME

Ziprasidone: the sponsor cross-referenced the PK/ADME data from the NDA (20-825) for the oral formulation. Two additional studies were conducted under the IND for ziprasidone i.m.; both of these studies were conducted in dog.

In one study, the PK of the tartrate and mesylate salts of ziprasidone (in SBECD, 50%) were compared after i.v. and i.m. dosing in 8 Beagle dogs. Absolute i.m. bioavailability was high (96-103%) with both salts. There were no marked differences in PK between the two salts.

In the second study, two different formulations of ziprasidone i.m. (2.5 and 20 mgA/mL; SBECD, vehicle) were tested. There were no notable differences in PK parameters between the two formulations.

The sponsor noted that no metabolic profile data were obtained for ziprasidone using the i.m. route. Therefore, the possibility exists that different metabolites may be encountered with the i.m. as compared to the oral route. Concern is somewhat mitigated by the fact that ziprasidone, given orally, is extensively metabolized via a number of pathways.

SEBCD: eight studies were conducted (in mouse, rat, dog, rabbit, human) to assess the PK/ADME of SEBCD. Of these, 3 were previously reviewed (Brian Ault, Ph.D.).

Selected PK parameters and balance data are summarized in the following table:

SPECIES	DOSE (mg/kg)	PK PARAMETERS			% OF DOSE RADIOACTIVITY	
		$t_{1/2}$ (hr)	Cl (mL/min/kg	V_d (L/kg)	URINE	FECES
CD-1 mouse	600 i.v.	0.1, 0.6	20	0.9	83.9-90.7	0.5-7.2
S-D rat	600 i.v.	0.3	9.8	0.3	84	3.5
	160 i.m.	0.68	[redacted]	[redacted]	70-93	2-13
Beagle dog	240 i.v.	1.1	4.7	0.4	93	0.2
NZW rabbit	600 i.v.	0.5	5.2	0.2	96.6-108.4	0.07-0.23
human	100 i.v., acute	[redacted]	[redacted]	[redacted]	85.67 ± 20.16	<LLOQ
	50 i.v., multiple	1.6	1.8	0.2	91.01 7 ± 11.79	<LLOQ

The data on i.m. dosing were minimal. In rat, ^{14}C-SBECD was eliminated rapidly and was primarily excreted in urine in all species tested, particularly in humans. Also, the V_d in animals was consistent with limited tissue distribution.

Tissue distribution was assessed in Lister-hooded rats following a single i.v. dose (600 mg/kg) of ^{14}C-SBECD. Radioactivity was concentrated in renal cortex and medulla. Radioactivity levels in other tissues were lower than plasma levels, although levels of radioactivity in lung and skin (dermis) were only slightly lower. Tissue retention was longest in renal cortex, with 26% of peak levels still detectable at 24 hr postdosing. [In plasma, levels of radioactivity were <LLOQ by 24 hr postdosing.] Brain levels were low, and fell below the LLOQ by 1 hr postdosing.

HPLC/MS analysis of urine samples from mouse, rat, rabbit, dog, and human indicated that SBECD was excreted unchanged.

Conclusion: there was minimal study of the PK/ADME of ziprasidone in the novel excipient, SBECD. A comparison of i.v. and i.m. dosing was limited and conducted only in Beagle dog and only with acute dosing. Absolute i.m. bioavailability was nearly complete (96-103%). However, comparisons of other PK parameters (T_{max}, $t_{1/2}$) between the two routes and compared to the oral route were not assessed. As noted, no comparison of routes was conducted in rat. [A PK/toleration study was conducted in rabbit. However, since the drug formation contained hydroxypropyl-ß-cyclodextrin instead of SBECD, the results were not relevant.]

A comparison of the tartrate and mesylate salts of ziprasidone (in SBECD) was assessed (in dogs) because the only toxicity studies conducted on ziprasidone in SBECD were conducted using the tartrate salt, whereas it is the mesylate salt that is intended for marketing. There were no marked differences in PK between the two salts in the acute dosing study in dogs; however, these data were minimal (i.e., single dose level, acute dosing).

The metabolism of ziprasidone (with or without SBECD) was not assessed following i.m. dosing, either in animals or in humans. As pointed out by the sponsor, ziprasidone is extensively metabolized via several pathways when administered orally. However, there is the potential for different metabolites to be formed via the i.m. route and without data it is not possible to rule out this possibility.

Toxicology

Acute i.m. (2 in albino rabbits) and 2-wk i.v. (Beagle dog, Sprague-Dawley rat) toxicity studies were conducted using ziprasidone in SBECD. The following toxicity studies were conducted on SBECD alone: i.m. irritation, acute i.v. (rat, mouse), 14-day i.v. range-finding (rat, dog), 1-mo + 1-mo reversibility i.v. (rat, dog), 1-mo i.v. (rat, dog), 1-mo + 2 and 5 mo reversibility i.v. (rat, dog, and 6-mo i.v. (rat, dog) toxicity studies.

For the current NDA, the 2-wk i.v. toxicity studies are probably the most relevant. However, the subchronic toxicity studies with the excipient alone are also important since SBECD is a new chemical entity. The longer term (i.e., 6-mo) studies are not as relevant considering the short-term human used proposed (≤3 days).

It should be noted that no subchronic or chronic i.m. toxicity studies were conducted, either with ziprasidone in SBECD or with SBECD alone.

Ziprasidone

Acute: the acute studies conducted were previously reviewed (L.M. Freed, Ph.D., P/T Review, 11/27/95). The summary from that review follows:

"Acute i.m. studies: The tartrate and mesylate salts of CP-88,059 were tested in separate acute i.m. irritation studies. The tartrate salt was tested at 6.8 mg/kg, given as two separate injections (0.1 and 1 mL of a 20 mg/mL solution). The mesylate salt was tested at 10 mg/kg (0.1 and 1.0 mL of a 36 mg/mL solution). Placebo (vehicle) and saline injections were also administered. Each animal received 4 injections into 4 separate sites (sacrospinalis muscle, bilateral) in one of the following combinations: (1) CP-88,059 + placebo, (2) CP-88,059 + saline, (3) saline + placebo. Animals (5/time point) were sacrificed at either 3 or 7 days postdosing.

With the tartrate salt, physical discomfort was evident following the 1.0 mL injection of CP-88,059; other injections were not associated with signs of physical discomfort. In animals sacrificed 3 days postdosing, the following observations were made with the tartrate and mesylate salts. The placebo was associated with redness and induration, with these signs being more notable with the 1.0 mL dose. Saline injections were associated primarily with redness (more consistently noted at 1.0 mL). With the tartrate salt, induration and redness were evident at both 0.1 and 1.0 mL, but the findings were more severe at the higher dose. With the mesylate salt, induration was noted consistently at 0.1 mL; at the higher dose, local changes (induration and redness) was noted only at one site. The microscopic findings consisted of evidence of necrosis and fibroplasia. These were observed with saline and placebo sites as well as with CP-88,059. The findings were minimal with saline, and were evident almost entirely with the 1.0 mL dose. With placebo, changes were also primarily noted at 1.0 mL, and were somewhat more severe than with saline. With CP-88,059, necrosis and inflammation occurred at both doses (i.e., 0.1 and 1.0 mL), but the severity of the findings was greater at 1.0 mL and greater than with 1.0 mL doses of either saline or placebo.

In animals sacrificed 7 days postdosing, placebo effects at the injection site were minimal and tended to be less than noted in animals sacrificed after 3 days. No changes were observed with saline. The effects of CP-88,059 differed slightly between the tartrate and mesylate studies, due, in all probability, to interassay variation. In the tartrate study, the effects of CP-88,059 were less severe than in animals sacrificed after 3 days, whereas, in the mesylate study, the effects of CP-88,059 were somewhat greater. Additional findings upon microscopic examination were fibroplasia and regeneration at the injection site. These were seen minimally with placebo. With CP-88,059, necrosis, inflammation, fibroplasia, and regeneration were evident at both doses, and tended to be more severe, but only slightly, at 1.0 mL as compared to 0.1 mL.

The data indicate that both the vehicle and CP-88,059 induce irritation and tissue injury at the injection site. There was also evidence of tissue repair in animals analyzed 7 days postdosing."

Subchronic: the 2-wk i.v. toxicity studies in Sprague-Dawley rat and Beagle dog were previously reviewed (L.M. Freed, Ph.D., P/T Review, 11/27/95). [It should be noted that the tartrate salt was used in both studies; it is the mesylate salt that is to be marketed. The summary from that review follows:

"**Dog**. A 2-wk i.v. bolus toxicity study was conducted in Beagle dogs at doses of 0, 1, 2, and 4 mg/kg b.i.d. (i.e., 2, 4, and 8 mg/kg). SBECD was used as the vehicle, the doses being 20, 40, and 80 mg/kg b.i.d. (i.e., 40, 80, and 160 mg/kg).

There were no unscheduled deaths. Clinical signs were summarized by the sponsor (i.e., no data were provided) as including "...tremors, face pressing, pawing, decreased activity, recumbency, elevated posterior, splayed limbs, hunched posture, ataxia, circling, cage biting, cage licking, muscle fasciculation, limb lifting, and ptosis" at all doses. Other signs noted at higher doses included aggressiveness, urination, and recumbent posture. Behavior normalized within 24 hr of dosing. Heart rate (↑)and rectal temperature (↓) were affected at all doses. There were no clear drug-related effects on body weight, food consumption, hematology, clinical chemistry, or urinalysis parameters. There was a non-dose-related increase in large unstained cells in DTF. Serum levels of CP-88,059 were quantitated on Day 8. Unfortunately, AUC measurements were based only on plasma samples taken from 0.25 to 6 hr after the first daily dose (i.e., 2 hr after the second daily dose); therefore, estimates of daily exposure cannot be made. AUC increased in a greater-than dose-proportionate manner at the HD. The $t_{1/2}$ was not estimated, but visually appeared to be <2 hrs.

There were no gross pathology findings, according to the sponsor. Absolute and relative liver weights were elevated in MDM and HDM (15-29%). The primary microscopic findings involved kidney (tubular vacuolation; primarily Grade 1, <20% of cortical tubules affected) and skin (hemorrhage, inflammation). Both findings were attributed by the sponsor to the vehicle. This is reasonable considering that (1) the incidence of kidney findings were similar in C and HD animals (these animals received the same dose of vehicle), and (2) injection site changes were noted in all animals. Since a saline control was not included, the effects of injection alone could not be determined.

Rat. A 2-wk i.v. bolus toxicity study was conducted in Sprague-Dawley at doses of 0, 2, 4, and 8 mg/kg. SBECD was used as the vehicle (1 mL/kg; 400 mg/kg).

There were no unscheduled deaths. Clinical signs data were not provided, but according to the sponsor, decreased activity and/or sedation were noted at all doses. In addition, urogenital staining were observed in MD and HD animals. No drug-related effects were noted on body weight, food consumption, or on urinalysis parameters. On hematology parameters, the only finding was a decrease (21%) in platelets in HDM; blood coagulation indices were not evaluated. On clinical chemistry parameters, there were no drug-related findings in males and only minimal changes in several parameters (↑ TG, ↓ Cl, ↓ SDH, ↓ total protein, ↓ albumin) in females. Serum levels of CP-88,059 were quantitated on Day 7 of dosing. Unfortunately, AUC was determined using serum samples collected only from 0.25 to 4 hr postdosing. Therefore, an accurate assessment of daily exposure could not be made. The AUC increased in a greater-than dose-proportionate manner at the MD and HD.

According to the sponsor, there were no gross pathology findings. Absolute and relative liver weights tended to be reduced in HDF, but the effect was minimal. The primary drug-related microscopic findings were detected in kidney (chronic progressive nephropathy, vacuolation of affected tubules) and at the injection site (inflammation, hemorrhage, mineralization;

examined only in C and HD grps). The sponsor attributed microscopic findings in kidney to the vehicle. This would seem reasonable considering the similarity in the incidence of kidney findings in males in C and HD grps; however, the incidence in females (at all doses lower than in males) was slightly higher in dosed groups (particularly MD and HD). The incidence of inflammation at the injection site was slightly higher in HD grps, as was hemorrhage in males. However, hemorrhage in females and mineralization in both males and females were noted to a greater extent in control (vs HD) animals.

Summary: the results of the acute i.m. irritation studies indicated that both ziprasidone mesylate and SBECD produced local injection site effects. Tissue regeneration was observed primarily at sites at which ziprasidone was applied. In general, recovery was almost complete at SBECD sites by 7 days postinjection, but at ziprasidone sites, necrosis, inflammation,and fibroplasia (as well as regeneration) were still evident.

Doses in the 2-wk i.v. studies in both species appeared to be limited by drug-related clinical signs. This was most evident in dogs. The decreased activity and sedation observed in rats were presumed dose-limiting; however, the degree of sedation did not affect food intake or body wt. Kidney and injection site were target organs in both studies. Microscopic findings consisted of tubular vacuolation (rat, dog) and chronic progressive nephropathy (rat) in kidney and inflammation, hemorrhage, and/or mineralization at the injection site. The sponsor considered both effects to be due to the excipient. This is not an unreasonable conclusion considering the similar effects in C (SBECD-treated) and HD grps, with two exceptions. The incidence of renal tubular vacuolation was slightly higher in dosed grps in female rats, and local injection effects were slightly greater at the HD.

No NOEL was established in either species. In rats, clinical signs were observed at all doses, and microscopic changes in kidney and injection site were observed in all grps. In dogs, drug-related clinical signs and heart rate effects were observed at all doses, and microscopic findings (kidney, injection site) were detected in all grps.

The doses of ziprasidone administered were 0.2-1 and 0.8-3.2 times the maximum proposed human dose on a mg/m^2 basis in rats and dogs, respectively. For the excipient, doses were 0.3-1.3 and 1-4.4 times the maximum proposed human dose on a mg/m^2 basis in rat and dog, respectively.

Previous i.v. studies (IND 34,629)

Two-wk i.v. studies in Sprague-Dawley rat and Beagle dog have previously been submitted. However, apparently due to solubility problems, the doses (0, 0.05, 0.1, 0.2 mg/kg) were inadequate to characterize i.v. toxicity in either species."

SBECD

[SBECD is referred to as "drug" in the following discussion.]

Acute: the acute i.m. (irritation) and i.v. (bolus) studies were previously reviewed (Brian Ault, Ph.D., P/T Review). The following summary statement was taken directly from Dr. Ault's review:

> "SBECD was administered in 0.1 or 1 ml saline (40%w/v) = 40, 400 mg IM in 4 animals in sacrospinalis muscle. After 3 or 7 days only 1 ml produced mild tissue damage (redness). At 7 days tissue repair was ongoing with macrophage infiltration."

The following summary was based on information provided in Dr. Ault's review:

Acute i.v. (bolus) toxicity was tested at a dose of 2000 mg/kg in CD-1 mouse and Sprague-Dawley rat (5/sex). Only limited observations were made: clinical signs, body weight, gross pathology; data were provided only for body wt. No adverse effects were observed in either species at the single dose tested.

Subchronic: the following studies were previously reviewed by Dr. Ault: 14-day i.v. range finding studies in rat (0, 160, 240, 600, 1500 mg/kg) and dog (0, 160, 240, 750 mg/kg), 1-mo + 1-mo reversibility i.v. studies in rat (160, 240, 320 mg/kg) and dog (100, 200, 300 mg/kg), and 1-mo i.v. studies in rat (40, 80, 160 mg/kg) and dog (30, 60, 120 mg/kg). [All studies involved bolus injection.]

The following summary was taken directly from Dr. Ault's review:

> "Although the clinical route of administration is IM, all toxicology studies have been performed IV, and no bridging studies are available.
>
> Clinical signs, hematology and urinalysis were unremarkable up to high dose levels [1500 mg/kg (rat) and 750 mg/kg (dog) 14 day studies]...
>
> The primary toxicology finding was of a dose-dependent vacuolation of renal proximal tubule epithelial cells, and to some degree urinary bladder epithelial cells, as well as development of pulmonary foamy macrophages at high doses. The proximal tubule epithelial cell vacuolation is probably lysosomal in origin, representing the retention of parent drug substance. Distribution studies of labelled SBECD were consistent with prolonged retention within proximal tubular cells. There was a NOAEL for 30 mg/kg in 1 month dog studies, but no no-effect level was determined for rats (minimum dose 40 mg/kg). However, at 40 mg/kg only a few regenerating epithelial cells (due to chronic nephropathy) were vacuolated. The human maximal dose may therefore be close to the threshold for vacuolation in subchronic rat studies. 1 month treatment with 1 month washout suggested that the vacuoles induced by high drug doses (300-320 mg/kg in rat and dog) may reverse, but not necessarily resolve.
>
> In these studies no abnormal hematology or urinalysis was observed, although this may be expected to occur at high doses..."

Two studies (i.e., 1-mo + 2, 5 mo reversibility i.v. studies in rat and dog) were reviewed in this NDA review.

Rat. In Sprague-Dawley rat, SBECD was administered i.v. (bolus) at doses of 0, 300, 1000, and 3000 mg/kg for 1 mo. The main study was conducted in 10/sex/grp; additional C, MD, and HD animals (5/sex/grp/sampling time) were followed for 2 and 5 mo after the end of the 1-mo dosing period to assess potential reversibility of findings. Observations included only limited organ/tissue wt data, limited microscopic analysis in recovery animals (i.e., cervical lymph node, heart, kidneys, liver, lungs, mesenteric node, ovaries, pituitary, spleen, testes, urinary bladder, uterus), and EM analysis of kidney, liver, and lungs from "randomly selected C and HD main-study animals.

There was only one unscheduled death (HDF, Day 5), which was not considered drug-related by the sponsor. No drug-related clinical signs, or effects on body wt, food consumption, or ophthalmology were noted. Red blood cell and wbc parameters were affected at the HD. Anemia was evidenced in both males and females by decreases in rbc ct, hgb, and hct; MCV and MCH were both slightly increased, consistent with a macrocytic anemia. Wbc ct, neutrophil ct, platelets, and large unstained cells were increased in males and females. [These effects were not observed after either 2 or 5 mo. of recovery.] Drug-related effects were also observed on a number of clinical chemistry parameters. Increases in P_i and urea, as well as slight decreases

in Cl were noted in HDM and HDF, suggesting effects on renal function. These effects were also observed in HD recovery animals, as well as an increase in creatinine (not observed in main study animals). Changes in serum lipids (increased cholesterol, decreased TG (in HDM only), and increases in LFTs were suggestive of hepatic involvement, primarily at the HD. [These effects were also observed in recovery animals, as well as a decrease in total bilirubin in MDF and HDF (not observed in main study animals.] In main study animals, the only effect observed at the MD was an increase in cholesterol in females only. For most affected parameters (but not LFTs), the magnitude of the effect in recovery animals was similar or larger than in main study animals.

On urinalysis parameters, drug-related effects were observed on pH and hgb (males only). Urinary pH was statistically reduced in MD and HD males and females. Urinary hgb was increased in MDM and HDM. Urinary pH was still reduced at 2 and 5 mo postdosing in females, but only at the 2 mo recovery period in males. Interestingly, hgb was not notably affected at the MD in the recovery animals (at the end of the dosing period); however, at the HD, recovery males were as affected as main-study males. Urinary hgb was detectable only in 1-2/5 MD and 1/5 HD animals after 2-5 mo of recovery.

A complete battery of organs was not measured. Of the limited organs weighed, drug-related changes were observed in kidney, liver, and spleen. Weights of all three organs were increased in HDM and HDF. In recovery animals, the effect on spleen in HDF was greater than in main study HDF, whereas that in kidney (males, females) and spleen (in males) had lessened substantially by 5 mos. Liver was not affected in recovery animals by 5 mos.

Microscopic changes were noted in a number of organs. Foamy macrophages (or foam cells) were detected in heart, lymph nodes, pituitary gland, testes, ovaries, uterus, spleen, urinary bladder, and lung. Deposition of SBECD-related material was suggested by the PAS-positive material detected in several of these organs/tissues. Vacuoles detected in renal tubules/epithelium and liver were also shown to contain PAS-positive material. For most organs/tissues, microscopic changes were evident primarily at the MD and HD; however, in testes, kidney, urinary bladder, and/or lungs, the LD was also affected. Therefore, no NOEL was established in this study. Foamy macrophages were detected in cardiac valves and in the vestigial lumen of the pituitary gland. Pyelonephritis was detected in kidney only in females, but at all doses; the sponsor considered this an exacerbation of a spontaneous finding. Hemorrhage at the injection site was dose-related in males, but not in females.

In recovery animals, microscopic findings were evident at both doses (i.e., MD, HD) at 2 and 5 mos.

Selected tissues were examined using EM. In liver and kidney, vacuoles were determined to be of lysosomal origin. In kidney and liver, cellular disruption, i.e., dilation of mitochondrial cristae, dissolution of organelles, peripheral condensation of nuclear chromatin) was noted in a few or "occasional" cells.

No attempt was made to quantitate systemic exposure during this study.

Dog. In Beagle dog, SBECD was administered at doses of 0, 300, 750, and 1500 mg/kg i.v. for 28 days (n = 3/sex/grp). Additional dogs (1/sex/grp/time point) were dosed at the MD and HD for 28 days and then followed for either 2 or 5 mos after the end of dosing. Observations included on limited organ/tissue wt data and complete microscopic analysis of tissues only in main-study animals. In recovery animals, only lymph nodes, kidneys, liver, and urinary bladder were examined microscopically. Sections of liver and kidney were examined by EM in all main-study and recovery animals.

There were no unscheduled deaths or drug-related clinical signs, according to the sponsor.

There were also no apparent drug-related effects on the following: body weight or food consumption (qualitative assessment only), cardiovascular parameters (in 1 C, 1 MD, and 1 HD animal, heart rate either remained unchanged or increased and QT interval increased), ophthalmology, hematology, clinical chemistry, urinalysis, or gross pathology.

Although there were no statistically significant effects on organ/tissue wts, decreases in adrenal (HDM) and spleen wt (MDM, HDM), and increases in liver (HDM) and ovary (all doses) wt were notable. The no. of recovery animals was too small to assess effects in these animals.

Foamy macrophages were detected in lymph nodes in at all doses in males and females. Vacuolation was noted in liver (hepatocytes), kidney (tubular, transitional epithelium), and urinary bladder (epithelium). Hypertrophic macrophages were detected in liver primarily at the MD and HD. In recovery animals (considered together due to the small "n"), foamy macrophages or vacuolation was still evident (both doses) in previously affected tissues; however, the effect tended to be milder.

Due to the microscopic effects observed at the LD, no NOEL was established in this study. No attempt was made to quantitate systemic exposure.

Chronic: 6-mo i.v. toxicity studies were conducted in Sprague-Dawley rat and Beagle dog. No data were submitted to document stability of the excipient in either study report.

Rat. SBECD was administered at doses of 0, 200, 320, and 600 mg/kg i.v. for 190-191 days (n = 20/sex/grp). Observations consisted of the following: clinical signs, body wt, food consumption, ophthalmology, hematology, clinical chemistry, urinalysis, terminal studies [gross pathology, organ/tissue wts (limited battery), histopathology (C, HD, except that kidneys, lung, liver, urinary bladder, and gross lesions were also examined at the LD and MD), EM (renal cortex, lung from 3/sex for C, HD)].

No unscheduled deaths or drug-related clinical signs were reported. There were also no notable effects on body wt, food consumption, or ophthalmology. Hematology findings were as follows: (1) slight elevation of wbc ct in MDM and HDM, due to relatively high values in a few animals, (2) elevated neutrophil cts in MDM and HDM, (3) decreases in rbc parameters in MDF and HDF (not considered toxicologically relevant by the sponsor). On clinical chemistry parameters, the primary findings were increases in ALT and AST in HDM; however, the increases were <2-fold, except for ALT in 2 HDM. Small increases were noted in BUN (males, all doses) and creatinine (HDF) at the end of the dosing period.

Urinary pH was decreased in males and females at all doses and at all sampling times. This effect increased with duration of dosing at all doses.

The only gross finding was discoloration of the kidneys in males and females at all doses. Kidney wt was elevated in all but LDM. Spleen wt was increased at all doses, but only in females.

As in previous studies, the primary drug-related microscopic findings were related to deposition of excipient-related material in a number of tissues. In this study, kidney, lung, urinary bladder, and liver were affected. Foam cells, vacuolation, and/or hypertrophic macrophages were detected at all doses. Mononuclear cell infiltration was increased only in HDF, and the incidence of lung granulomas were increased in HDM. Cytoplasmic vacuoles within tubular epithelial cells and hypertrophic macrophages in liver were found to stain positive for PAS.

Dog. SBECD was administered to Beagles (n = 4/sex/grp) at doses of 0, 150, 300, and 600 mg/kg i.v. for 190-193 days. Documentation of drug stability or purity was not provided.

Observations consisted of the following: clinical signs, body wt, food consumption, ophthalmology, cardiovascular, hematology, clinical chemistry, urinalysis, and terminal studies [gross pathology, organ/tissue wts (kidneys, liver/gallbladder, lung, heart, spleen, adrenals, pancreas, brain, pituitary, testes, ovaries), histopathology, EM (renal cortex, lung from C, HD animals)]. No attempt was made to quantitate or characterize systemic exposure.

There were no unscheduled deaths or drug-related effects on clinical signs, body wt, food consumption, ophthalmology, ECG/bp, hematology, or urinalysis. The only drug-related change in clinical chemistry was an increase (>2-fold) in ALT in HDF at the final sampling time.

At necropsy, discoloration of the kidneys in HD animals was the only drug-related finding. No significant changes were noted in organ wt, although pancreas wt was dose-dependently reduced in females. Microscopic findings were limited to kidney, liver, and urinary bladder. Cellular vacuolation was observed in all three organs/tissues. In kidney and urinary bladder, effects were observed at all doses, whereas in liver, hepatocellular vacuolation was detected only in HDF and hypertrophic macrophages were detected in HDM and HDF. In two HDF in which ALT was markedly elevated, microscopic findings in liver consisted of mononuclear cell infiltration, hypertrophic macrophages, and hepatocellular vacuolation. Vacuolation of the kidney was noted tubules and transitional epithelium; cytoplasmic vacuoles were unstained.

Special toxicity: special toxicology studies were conducted in order to further investigate the renal (in Sprague-Dawley rat) and irritating (rabbit, guinea pig) effects of SBECD. The effects of SBECD (0, 300, and 3000 mg/kg i.v.) on various renal indices were tested in a 1-mo study. Observations consisted of the following: clinical signs, body wt, clinical chemistry, urinalysis, and microscopic examination of kidney. The results were consistent with renal toxicity. Red discoloration of the urine, increases in serum urea and creatinine, and increases in urinary volume and protein excretion were observed at both doses. Urinary pH was decreased at both doses. Increases in ALT and AST were suggestive of liver toxicity at the HD. Activities of 2 (i.e., LDH, NAG) of the 5 urinary enzymes assayed were increased. For the most part, urinary effects were obtained in the early sample collection (0-3 hr). Kidney wt was increased in both males and females at the HD, whereas tubular and transitional epithelial vacuolation was detected at both doses (although the severity was greater at the HD). Interesting, increases in urinary protein were not reported in the general toxicity studies, even though doses as high as 3000 mg/kg were used; hemoglobinuria was reported in males in one of the 1-mo studies.

To further investigate the apparent increase in urinary protein, a study was conducted in which urine samples from untreated Sprague-Dawley rats were spiked with SBECD in order to assess possible assay interference. At concentrations of ≥18.8 mg/mL in male and ≥4.7 mg/mL in female urine, SBECD produced artifactual increases in urinary protein measurements (up to 169 and 600% in males and females, respectively).

Additional special toxicology studies consisted of the following: (1) effect of SBECD administered orally on the pancreas in Sprague-Dawley rats, (2) skin irritation in rabbit, (3) eye irritation in rabbit, and (4) skin sensitization in guinea pig. At doses of 500 and 5000 mg/kg p.o. for 1 mo, SBECD had no effect on plasma CCK levels, but did produce increases in pancreatic wt in HDM and in females at both doses. No microscopic changes in pancreas were noted; however, hydroxypropyl-ß-CD (tested at the same doses for comparison), previously shown to produce preneoplastic and neoplastic changes in pancreas, also produced no microscopic changes. Therefore, the study may not have been long enough to adequately assess the tumorigenic potential of SBECD.

In the irritation studies, minimal signs of irritation were observed; however, the results of the sensitization study indicated that SBECD has the potential to produced sensitization.

Summary: SBECD was irritating when injected i.m., as observed in the i.m. studies with ziprasidone + SBECD.

In subchronic and chronic i.v. studies in rat and dog, SBECD produced toxicities similar to those previously reported for other cyclodextrins. In rat, no NOEL was established due to microscopic findings detected at all doses (40-3000 mg/kg i.v.) in all studies. In the 1-mo and 1-mo + 1-mo reversibility studies (dose range: 40-320 mg/kg i.v.), microscopic findings were noted in kidney (vacuolation of renal proximal tubule epithelial cells), urinary bladder (vacuolation of epithelial cells), and lung (foamy macrophages, but there was no evidence of altered renal function or hematological effects. In the 1-mo + 2 and 5 mo recovery study, higher doses were used. At these higher doses (i.e., 1000 and 3000 mg/kg; LD = 300 mg/kg i.v.), effects were noted on clinical pathology parameters, suggesting hematological effects and effects on kidney and liver function, primarily at the HD. Kidney, liver, and spleen wt were also increased at the HD. In addition, microscopic findings consistent with SBECD deposition (i.e., foamy macrophages, foamy cells, vacuolation; PAS-positive stains) were detected in a wide variety of organs/tissues: heart, lymph nodes, pituitary gland, testes, ovaries, uterus, spleen, urinary bladder, lung, and kidney. Pyelonephritis was detected at all doses in females; the sponsor considered this an exacerbation of a spontaneous lesion. Although there was some recovery evident at 2 and 5 mo postdosing, recovery was not complete and SBECD-related effects were still evident at 5 mo in MD and HD animals. In the chronic study, clinical pathology parameters were affected, suggesting possible functional effects, spleen wt was increased in females at all doses (the decreases in rbc parameters were observed only in MD and HD females), and microscopic changes consistent with deposition of SBECD-related material were detected in kidney, lung, urinary bladder, and liver. [Increases in ALT and AST were observed, however, only in 2 HDM were the increases >2-fold.]

In the dog, a NOAEL of 30 mg/kg i.v. was established in one of the 1-mo studies. At higher doses in the subchronic studies, clinical pathology and ECG parameters were not affected. However, microscopic changes consistent with deposition of SBECD-related material were observed at all doses. Affected tissues consisted of lymph nodes, liver, kidney, and urinary bladder. Some degree of reversibility (but not resolution) of SBECD-related effects was noted in the 1-mo + 1-mo recovery study. In the 1-mo + 2 and 5 mo recovery study, the number of animals per grp was too small (i.e., 1/sex) to make definitive comments about recovery; however, in the few animals studied, microscopic findings, although milder, were still evident. In the 6-mo study, the primary SBECD-related effects were increases in ALT in HDF and microscopic findings (e.g., cellular vacuolation, hypertrophic macrophages) in kidney, liver, and urinary bladder.

Conclusions

The i.v. toxicity studies conducted in rat and dog demonstrated toxicities consistent with those previously reported for other cyclodextrin derivatives (e.g., cytoplasmic vacuolation, foamy cells). No-effect levels were either not established (rat) or were similar to the maximum proposed human dose (MPHD, i.e., NOAEL in dog was 1.5 times the MPHD). Effects suggestive of hemolysis (i.e., decreased rbc parameters, increased spleen wt) or functional organ changes (i.e., changes in clinical pathology parameters) were minimal and/or tended to be observed only at the highest doses. Some recovery from SBECD-related effects was noted in subchronic studies, however, complete reversibility was not. At the highest dose tested in rats (i.e., 3000 mg/kg i.v.), cellular disruption (i.e., dissolution of organelles, dilation of mitochondrial cristae in renal proximal tubules, altered shape of nuclei with condensation of chromatin in hepatocytes) was detected (similar changes were not observed at 600 mg/kg i.v.; the 1000 mg/kg dose was not evaluated by EM).

For an NCE intended for clinical use for ≤3 days, with a maximum of 8 doses per day (i.e., 8 x 10 mg, as described in the sponsor's proposed labeling), general requirements for general toxicity studies for an NDA would be 1-mo studies in two species (1 rodent, 1 nonrodent),

using the clinical route (or other appropriate route with justification). For ziprasidone mesylate i.m., only 2-wk studies were conducted (rat, dog). These studies did not use the clinical route (i.e., i.m.), the clinical drug substance (i.e., the mesylate salt), or the clinical drug formulation (preclinical drug formulation did not contain methanesulfonic acid). [Note: according to the sponsor's proposed labeling, the clinical formulation (reconstituted) contains "...20 mg of ziprasidone and 4.7 mg of methanesulfonic acid solubilized by 294 mg of sulphobutylether beta-cyclodextrin sodium..." However, according to the chemistry information submitted, methanesulfonic acid is not listed as an ingredient in the final clinical formulation. The sponsor should be asked to clarify this apparent discrepancy.]

The use of 2-wk toxicity studies was justified by the sponsor by cross-referencing the NDA database for the oral formulation. For the oral studies to be useful in supporting the safety of the i.m. formulation, it would need to be demonstrated that plasma levels of ziprasidone achieved in humans via the oral route do not exceed those obtained with the i.m. route. If they do, it would be important to determine whether or not the plasma levels of ziprasidone achieved in animals in the definitive oral toxicity studies adequately covered the plasma levels expected to be achieved in humans at the highest recommended i.m. dose.

Comparing plasma levels across species and between routes (in humans) was difficult because comparable data were not necessarily available. According to summary tables provided by the sponsor, the following values were obtained in humans (healthy volunteers or special populations; variation expressed as %CV) in multiple-dose studies:

At 80 mg b.i.d. (p.o.)
- T_{max} = 7 ± 45 hr
- C_{max} = 202 ± 35 ng/mL
- $AUC_{(0\text{-}12\ hr)}$ = 1551 ± 34 ng•hr/mL

At 20 mg q.i.d. (i.m.)
- T_{max} = not given
- C_{max} = not given
- $AUC_{(0\text{-}24\ hr)}$ = 1556-1591 ng•hr/mL

At 20 mg (single dose, i.m.)
- T_{max} = 0.7 ± 52 hr
- C_{max} = 258 ± 34 ng/mL
- $AUC_{(0\text{-}\infty)}$ = 846 ± 29 ng•hr/mL

Note that the AUCs are not based on the same sampling intervals, and that the AUC for the 20 mg q.i.d. i.m. dosing regimen is not proportionately higher than the AUC for the single 20 mg i.m. dose. This is, only in part, due to the fact that the sampling intervals are different between these AUC estimates. This apparent lack of dose-proportionality is an issue that has been raised by the clin pharm/biopharm reviewer (cf. Clinical Pharmacology and Biopharmaceutics Review, NDA-20-919, Sayed Al-Habet, Ph.D., HFD-860). Unfortunately, with the lack of PK/metabolism data following i.m. dosing, it is not possible to determine the reason(s) for the non-linearity; deposition at the injection site or increased metabolism with repeat dosing are both possible reasons for the non-linearity. [Absolute i.m. bioavailability was reported as ≈100% in humans and dogs; however, these estimates were made with single doses and at relatively low dose levels, i.e., 5 mg and 1.5 mg/kg in human and dog, respectively.]

Another observation (regarding human exposure) is the lower plasma levels on Day 3 as compared to those obtained on Day 1. Considering the $t_{1/2}$ of ziprasidone in human plasma with i.m. dosing (i.e., 4-5 hrs), it would seem reasonable that multiple daily dosing (e.g., 20 mg

q.i.d.) would result in substantially higher peak levels (i.e., C_{max}) than obtained with a single 20 mg dose. However, the exact opposite was obtained. The reason(s) for this apparent discrepancy is not clear.

The available data would suggest that there is a possibility that, in humans, i.m. dosing would produce higher systemic exposure than that achieved after the highest proposed oral dose. There is also some evidence in humans of drug deposition at the injection site (i.e., T_{max} = 0.7 hr after i.m. dosing). The potential for systemic exposure to be higher in humans with the i.m. route warrants the comparison of systemic exposure achieved in the preclinical animal species with that predicted in humans via the i.m. route in order to determine whether or not toxicity was assessed at sufficiently high plasma levels. Unfortunately, it not possible to make comparisons between systemic exposure in humans and that in the preclinical species used for toxicity testing (i.e., rat, dog) with any certainty due to the lack of data. In addition to the potential problems with the human data, only C_{max} data for ziprasidone were collected in the subchronic and chronic oral toxicity studies in rat; both C_{max} and AUC were assessed in dog. Peak plasma levels of ziprasidone were 9-10 µg/mL in the rat 1- and 6-mo oral studies, suggesting that total daily exposure was higher than anticipated in humans via the i.m. route. In dog, however, plasma levels were ≤2-fold higher than the predicted human levels. Therefore, the systemic exposure obtained in dog may not have been sufficiently high to cover that anticipated in humans with i.m. dosing. Systemic exposure was markedly higher in the 2-wk i.v. toxicity study in dogs (i.e., 2235 ± 479 ng/mL for C_{max} and 4009 ± 1198 ng•hr/mL for $AUC_{(0.25\text{-}6\ hr)}$ (HD, b.i.d. dosing; note the limited sampling interval for AUC). Therefore, higher systemic exposure to the parent compound was achievable in dog using parenteral dosing.

Other issues that needs to be addressed when considering the adequacy of the 2-wk i.v. toxicity studies is the route used, i.e., i.v. instead of i.m. dosing, are as follows:

(1) a primary difference between the two routes, from a toxicity standpoint, is the lack of evaluation of local drug effects with i.v. dosing. The acute i.m. irritation studies in rabbit demonstrated local irritation and tissue damage by drug and excipient (SBECD). Considering that multiple injections may be administered to humans, and the possibility of drug deposition at the injection site, the possibility of microscopic changes in muscle following multiple dosing needs to be evaluated.

(2) the tartrate rather than the mesylate salt was used in the 2-wk i.v. studies. To address the differences in salts, the sponsor conducted an acute PK study in Beagle dogs at a single dose level, and demonstrated that the PK at that dose was fairly similar with the two salts. No such comparison was conducted in rat. Therefore, the PK of the mesylate salt was compared with that of the tartrate salt in only one species, and the toxicity of the mesylate salt was not tested in either rodent or non-rodent.

The possibility of different toxicities with the mesylate salt rests, in part, on the potential for formation of methanesulfonic acid or an ester derivative *in vivo* following dissociation of the salt. Esters of methanesulfonic acid (e.g., methyl, ethyl ester) are known direct-acting genotoxic compounds. Whether or not the acid is also potentially genotoxic or if esters form *in vivo* are not known. This concern is heightened by the possibility that methanesulfonic acid itself is present in the final clinical formulation, as discussed above. [This compound was not listed as being present in any of the drug formulations used for toxicity testing.] Concern for the salt alone is ameliorated by the fact that numerous mesylate salts are currently approved for marketing.

(3) the possibility of qualitatively different metabolites being formed following i.m. injection is probably remote, but has not been ruled out.

Several issues cannot be resolved based on the data provided:

(1) PK bridging studies comparing the i.m. and i.v. routes were not conducted in rat and only minimal data were provided for dog.

(2) the i.v. toxicity studies provided no assessment of the local effects (including histopathology) of repeated i.m. dosing

(3) none of the toxicity studies (as far as this reviewer is aware) was conducted using methanesulfonic acid in the drug formulation.

(4) the human data suggest the possibility that systemic exposure to ziprasidone may be higher with i.m. than achieved with oral dosing. [As discussed, the human data are somewhat contradictory, but provide some suggestion of nonlinearity.] Although the exposure data are difficult to compare among species, it would appear that plasma levels of ziprasidone achieved in dogs in the oral toxicity studies were only slightly higher than those achieved in humans at the maximum recommended human dose. The toxicokinetic data collected in the 2-wk i.v. toxicity study in dogs indicate that higher plasma levels of ziprasidone may be achieved using i.v. (and my inference, i.m.) dosing.

Therefore, it is recommended that the sponsor conduct 1-mo i.m. toxicity studies in rodent and non-rodent (i.e., rat and dog), using the clinical formulation and the i.m. route. One additional issue is the lack of documentation of the stability of SBECD under conditions used in the toxicity studies. These data should be requested.

Reproduction

The following reproduction studies were conducted on SBECD (referred to as "drug") alone: fertility and early embryonic development in Sprague-Dawley rats, embryofetal development in Sprague-Dawley rats, embryofetal development in Japanese White rabbits, prenatal and postnatal develop in Sprague-Dawley rats.

Fertility and early embryonic development: SBECD was administered i.v. at doses of 0, 100, 400, and 1500 mg/kg (n = 20/sex/grp). Males were treated from 28 days prior to mating, throughout the mating period, and until sacrifice (total of 63-66 days of dosing). Females were dosed from 14 days prior to mating, throughout the mating period, and through Day 7 of gestation. Observations consisted of clinical signs, body wt, food consumption, mating performance, litter parameters, gross pathology, and limited histopathology in males [i.e., testes, prostate (C, HD only), seminal vesicles (C, HD only)], analysis of sperm (motility, count). [No microscopic examination of tissues was performed in females.]

There were no unscheduled deaths or drug-related clinical signs. Body wt was slightly reduced in HDF (3-4%) during gestation (up to Day 14). Body wt was not affected in males; however, food intake was reduced slightly, but significantly, (6-13%) at the HD. In females, food intake was sporadically reduced during the end of the mating period and during gestation. There were no clear drug-related effects on estrus cycle (incidence or duration), cohabitation period, pregnancy rate, mating index, or litter parameters. However, there was an increase (70%), although not statistically significant, in resorptions at the HD. An examination of the individual data indicated that the increase was due to multiple resorptions in a few HD litters.

At necropsy, bilateral pale discoloration of the kidney was detected in the majority of HDM. One HDM had dilated renal pelvis and ureter, and atrophy of the prostate and seminal vesicles. There were no apparent drug-related effects on sperm motility or count, although both parameters were reduced in one HDM. [The female mated with the affected male had

only 2 implantation sites and 2 live fetuses. This HDM was not the same animal in which macroscopic findings were detected.]

The primary macroscopic finding was foamy interstitial macrophages (degree was "slight") in testes in all dose grps; all MD and HD males were affected. Microscopic findings in one HDM (i.e., slight, diffuse atrophy of the prostate and seminal vesicles, retained spermatid) confirmed the findings at necropsy

Embryofetal development: in **rat**, SBECD was administered at doses of 0, 100, 600, and 3000 mg/kg i.v. from Day 6 through Day 17 of gestation (n = 20/grp). [Dose selection was based on the results of a preliminary study in Sprague-Dawley rats at doses of 300-3000 mg/kg i.v. In that study, minimal effects were observed; however, body wt gain was reduced by 24% during the first wk of dosing.] In the main study, observations consisted of clinical signs, body wt, food consumption, gross pathology (dams sacrificed at Day 21 of gestation), litter parameters, and fetal examinations. All fetuses were examined for external and buccal malformations; skeletal (Alizarin red S, alcian blue) and visceral (Wilson's technique) findings were each examined in 1/2 of the fetuses.

There were no unscheduled deaths and no drug-related clinical signs were evident in the dams. Body wt gain was reduced (20%) in HDF during the first wk of dosing; however, mean body wt at the end of the dosing period were similar among grps. Food consumption was reduced slightly (7%) in HDF, consistent with body wt changes. No gross pathology findings were noted; 3 CF, 1 MDF, and 1 HDF were found to be not pregnant. Embryomortality and the no. of early resorptions were increased at all doses. The increases in early resorptions, but not embryomortality, were dose-related (in terms of affected litters).

There were no drug-related increases in fetal findings (i.e., malformations, skeletal ossification); however, individual fetal data were not provided. [The sponsor has been asked to provided the missing data.]

In **rabbit**, SBECD was administered at doses of 0, 100, 400, and 1500 mg/kg i.v. from Day 6 through Day 18 of gestation (n = 20/grp). [Doses were selected on the basis of results obtained in a preliminary study in Japanese White rabbits at doses of 250-1500 mg/kg i.v. In that study, there were no effects on survival, clinical signs, body wt, or food consumption. There was, however, an increase in early resorptions in MDF and HDF; omphalocele was detected in 1 HD fetus.] In the main study, observations consisted of clinical signs, body wt, food consumption, gross pathology, litter parameters, and fetal examinations [external, skeletal (Alizarin red S, alcian blue), visceral (Stuckhardt & Poppe fresh dissection) in all fetuses].

There were two unscheduled deaths (1 CF, 1 HDF) due to hindlimb paralysis (considered "accidental" by the sponsor). Abortion of entire litter occurred in 1 MDF and 1 HDF. No drug-related clinical signs, or effects on body wt, food consumption, gross pathology, or litter parameters were observed in dams. Six females were not pregnant: 1 CF, 1 LDF, 2 HDF. The total number of dams per grp was 17, 19, 20, and 16 in C, LD, MD, and HD grps, respectively.

Upon fetal examination, there were no drug-related malformations or visceral findings. Skeletal findings consisted of the following: (1) a dose-related increase in the litter incidence of 13 thoracic and 8 lumbar vertebrae, (2) lumbarization was observed only in fetuses from treated dams; differences were not dose-related, (3) dose-related increases in fetal and litter incidence of rudimentary 5th sternebra.

Prenatal and postnatal development: SBECD was administered to Sprague-Dawley rats at doses of 0, 100, 600, and 3000 mg/kg i.v. from Day 6 to Day 21 of lactation. All dams were allowed to deliver. Observations in the F_0 generation consisted of the following: clinical

signs, body wts, reproductive (litter) parameters, gross pathology (on Day 21-23 of lactation). Observations in the F_1 generation were as follows: pup survival, clinical signs, body wt, postnatal development (physical, developmental), reproductive performance (selected animals), gross pathology (Day 4 of lactation for culled pups, Day 21 postpartum for remainder not selected for assessment of reproductive performance, Day 21 of lactation for animals selected for reproductive assessment). [For assessment of postnatal development, the sponsor set a criteria of ≥2 ups/sex or a total of 7 pups per litter for that litter to be included in the evaluation; therefore, 3 C, 2 LD, 5 MD, and 5 HD litters were excluded.] Observations in the F_2 generation were as follows: survival, clinical signs, body wt, litter parameters, gross pathology (Day 4 postpartum in culled pups, Day 21 postpartum for remaining pups.

In the F_0 generation, there were no unscheduled deaths or drug-related clinical signs. Body wt was slightly (3-4%), but significantly, reduced in HDF (compared to CF) during gestation. Reductions in food consumption in HDF were consistent with those in body wt gain. The primary effect was an increase in stillbirths in HDF, although the number of live pups was reduced slightly at the HD as a result of the stillbirths. At necropsy, pale discoloration of the kidney was detected in HDF.

In the F_1 generation (not directly dosed), pup survival was reduced on Day 4 in all dosed grps, both in terms of affected pups and litters. No drug-related clinical signs or external malformations were evident. Body wt was reduced throughout lactation at the HD (compared to C pups). There were no clear drug-related effects on postnatal development measures, although the following were of note: (1) time to step through was reduced in the passive avoidance paradigm in MD and HD pups and (2) the number of errors in the Cincinnati Maze tended to be higher in HDF. Neither of these differences was statistically significant.

In females selected for assessment of reproductive potential, there were no marked effects on body wt during gestation or lactation; however, body weight was reduced (≈5%) in MDF and HDF. Food intake was similarly affected (≈7%). There were no apparent drug-related effects on litter parameters in the F_1 generation and no findings at necropsy.

In the F_2 generation, there were no apparent differences in survival, clinical signs, or gross pathology. Body wt was significantly lower in MDM, HDM, and HDF during lactation; the differences were not dose-related in males. The sponsor did not consider these body wt effect to be drug-related.

Summary: all the reproductive toxicity studies were conducted with the excipient alone, i.e., the reproductive toxicity of ziprasidone mesylate or of ziprasidone mesylate in SBECD was not assessed. In addition, the reproductive studies were conducted using i.v. dosing instead of the intended clinical route (i.e., i.m.).

SBECD had no apparent effects on mating or fertility in male and female rats at doses up to 1500 mg/kg i.v.. In embryofetal toxicity studies in rat and rabbit, no drug-related malformations were observed. However, embryolethality and early resorptions were increased in both species. In rat, only the litter incidence of early resorptions was increased in a dose-related manner (all doses). An increase in resorptions was also observed in the mating and fertility study in rats, at the HD (1500 mg/kg). In rabbit, the increase in early resorptions was minimal and observed only in the dose-range finding study (at 600 and 1500 mg/kg i.v.). There were increases in certain skeletal variations in both species [cervical rib in rats, dose-related; 13 thoracic (all doses) and 8 lumbar (MD, HD) vertebrae in rabbits]. Skeletal ossification was affected in rabbits, as evidence by a dose-related increase in rudimentary 5th sternebra at the MD and HD (400 and 1500 mg/kg i.v.).

In the perinatal-postnatal development study in Sprague-Dawley rats, dams were dosed from

Day 6 of gestation to Day 21 of lactation. In terms of pup survival, there were increases in stillbirths at all doses (statistically significant only at the HD = 3000 mg/kg i.v.) and decreases in pup survival on Day 4 at all doses (both no. of pups and affected litters, i.e., litters with at least one pup death). Maternal effects were observed at the HD [i.e., small, but significant decrease in body wt compared to CF (3-4%), decrease in food consumption, pale discoloration of the kidney); body wt effects were observed during gestation only. The primary finding in offspring was reduced body wt (compared to Cs). In F_1 pups, body wt was reduced throughout lactation in MD and HD grps (significant at HD only); body wt in the HD grp tended to remain slightly lower during lactation, although there were no statistically significant differences. In animals selected for assessment of reproductive performance, body wt was slightly, but significantly reduced (compared to Cs) in MD and HD grps throughout gestation and lactation; food intake was not notably affected. No SBECD-related effects on physical or behavioral development. [It must be noted, however, that the sponsor did not evaluate these parameters in 15 litters (3C, 2 LD, 5 MD, and 5 HD) because they did not meet the criteria of having at least 2 pups/sex or a total of 7 pups. In the F_2 generation, body wt was significantly lower in MD and HD pups during lactation. In males, this finding was not dose-related, and the sponsor did not consider it drug-related.

Conclusions: as previously noted, only the reproductive effects of SBECD were tested (i.e., those of ziprasidone in SBECD were not) and the intended clinical route (i.m.) was not used; all studies were conducted using i.v. dosing.

Mating and fertility were not adversely affected in either males or female rats. However, foamy interstitial macrophages were detected in testes at all doses, a finding also observed in a subchronic toxicity study at doses of 300-3000 mg/kg i.v. [In that study, ovaries were also affected (3000 mg/kg i.v.).] Although testis effects were not consistently observed (i.e., the testis was not affected in the 26-wk study at doses up to 600 mg/kg i.v.], these finding do suggest that SBECD might have adverse effects on fertility, depending upon a variety of factors (e.g., dose, duration, susceptibility).

SBECD did not induce malformations in either rat or rabbit at the doses tested (up to 1500 mg/kg i.v.). SBECD did, however, exhibit embryofetal toxicity as demonstrated by increases in embryolethality and early resorptions in rats at all doses in the embryofetal toxicity study and at the HD (i.e., 1500 mg/kg i.v.) in the mating and fertility study. There were increases in skeletal variations rats and rabbits at all doses, and delayed ossification in rabbits at doses of 400-1500 mg/kg i.v. In the peri- and postnatal study in rats, SBECD exhibited no clear adverse effects on physical or behavioral development. It should be noted, however, that certain litters were not represented in the developmental assessment because they did not meet the sponsor's criteria for inclusion. Stillbirths were increased at all doses (significantly at the HD, i.e., 3000 mg/kg i.v.), and pup survival to Day 4 postpartum was reduced at all doses. Adverse effects on pups was further demonstrated by long-term effects on pup body wt at doses of 600-3000 mg/kg i.v. Body wt was also lower at these doses in the F_2 generation, although the sponsor did not consider this SBECD-related.

The primary problem with the battery of tests performed is that they were conducted only using SBECD. While they may be adequate assessments of reproductive toxicity for SBECD, the question of how this novel excipient may affect the reproductive toxicity potential of ziprasidone. Therefore, it is recommended that the sponsor conduct additional studies to assess the effects of ziprasidone together with the excipient on all stages of reproduction. This evaluation is considered particularly important since both ziprasidone and SBECD alone have been shown to produce reproductive toxicity.

One additional issue is the lack of individual fetal data in the embryofetal toxicity studies. These data have been requested from the sponsor.

Genotoxicity

The genotoxic potential of SBECD was tested in the following assays: Ames test (2 studies), *in vitro* gene mutation in CHO cells, *in vitro* chromosomal aberration in human lymphocytes, *in vivo* micronucleus in mice.

The results of all assays performed were negative. The design and conduct of the studies were adequate, except for the *in vivo* mouse micronucleus assay. In the latter assay, only one-half of the OECD-recommended number of PCEs per animal was examined for presence of micronuclei. Regarding the *in vivo* study, a separate (tissue distribution) study in rats indicated that SBECD did distribute to bone marrow, with peak levels ≈20% of peak plasma levels, after i.v. dosing.

Conclusions: SBECD had no demonstrated genotoxic potential in the battery of assays performed. All assays were adequate except for the *in vivo* mouse micronucleus assay. In this latter assay, less than the recommended number of cells were examined for micronuclei (cf. OECD guidelines).

The drug substance, ziprasidone mesylate, was not tested for genotoxic potential. [The genotoxicity data in the oral database were generated using ziprasidone HCl.] In addition, methanesulfonic acid, potentially present in the clinical formulation, was not tested for genotoxic potential.

Repeating the full genotoxicity battery using ziprasidone mesylate is not recommended because of the numerous mesylate salts currently on the market. However, considering the possibility that dissociation of the salt *in vivo* may result in formation of potentially genotoxic substance(s) (e.g., methanesulfonic acid or esters), it is recommended that in the repeat *in vivo* micronucleus assay the mesylate salt be used. It is also recommended that the genotoxic potential of methanesulfonic acid be tested, particularly if it is present in the final clinical formulation.

RECOMMENDATIONS

It is this reviewer's understanding that this NDA is not considered approvable on the basis of clinical and chemistry deficiencies. From a pharmacology/toxicology standpoint, there are also deficiencies that would preclude a recommendation for approval.

The following information should be relayed to the sponsor:

1. one-month i.m. toxicity studies should be conducted in rodent and nonrodent using the clinical drug formulation. These studies are needed for the following reasons: (1) no bridging pharmacokinetic data were provided, comparing the i.v. and i.m. routes, in rat and minimal data were provided in dog, (2) higher plasma levels of ziprasidone may be achieved in dog using the i.m. as compared to the oral route. Assessment of the safety of higher exposures in dog is recommended since human exposure to ziprasidone with i.m. dosing may exceed that obtained with oral dosing, and the plasma levels of ziprasidone achieved in the oral toxicity studies in dog were only slightly higher than those achieved following oral dosing in humans, (3) local effects (including histopathology) of multiple i.m. dosing were not assessed in either rat or dog, and (4) according to the proposed labeling, the final injectate is to include ziprasidone, methanesulfonic acid, and ß-cyclodextrin sulphobutyl ether sodium. Methanesulfonic acid was not stated to be present in any of the definitive toxicity studies submitted.

2. ziprasidone and the novel excipient, ß-cyclodextrin sulphobutyl ether sodium (SBECD), were each tested alone in a battery of reproductive toxicity studies. Considering, however, that (1) SBECD may influence the distribution of ziprasidone in the dam and/or fetus and that (2) both ziprasidone and SBECD produce adverse effects on reproduction, the potential adverse effects of ziprasidone in SBECD on all stages of reproduction need to be evaluated. If methanesulfonic acid is to be added to the final drug formulation, it should also be present in the drug formulation used in the repeat reproductive studies.

3. the *in vivo* micronucleus assay should be repeated using ziprasidone mesylate and the recommended number of metaphases (cf. OECD guidelines for genotoxicity) for evaluation of micronuclei. In addition, if methanesulfonic acid is to be added to the final drug formulation, a complete genotoxicity battery should be performed on the compound.

4. please provide data documenting the stability of SBECD under the conditions of the general (i.e., subchronic/chronic) and reproductive toxicity studies.

11/20/98

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cc
NDA orig (20-919)
div file
HFD-120
/G.Fitzgerald/L.M.Freed/S.Hardeman

/S/
Sept 2
memo