CENTER FOR DRUG EVALUATION AND RESEARCH

APPLICATION NUMBER:

21-346

PHARMACOLOGY REVIEW

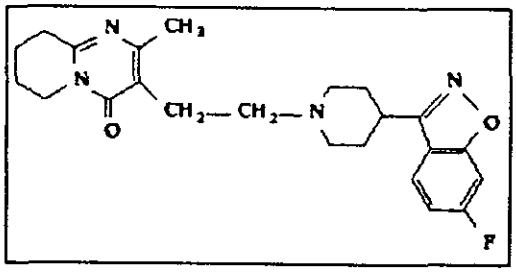
PHARMACOLOGY/TOXICOLOGY COVER SHEET

NDA number: **21-346**
Review number: 2
Sequence number/date/type of submission: 4/28/2003
Information to sponsor: Yes (X) No ( )
Sponsor and/or agent: Johnson & Johnson Pharmaceutical Research & Development, LLC
1125 Trenton-Harbourton Rd
Titusville, NJ 08560-0200
Manufacturer for drug substance: sponsor

Reviewer name: Lois M. Freed, Ph.D.
Division name: Neuropharmacological Drug Products
HFD #: 120
Review completion date: 10/29/03

Drug:

Trade name: RISPERDAL CONSTA™
Generic name (list alphabetically): risperidone i.m. depot
Code name: n/s
Chemical name: 3-[2-[4-(6-fluoro-1,2-benzisoxazol-3-yl)-1-piperidinyl]ethyl]-6,7,8,9-tetrahydro-2-methyl-4*H*-pyrido[1,2-a]pyrimidin-4-one
CAS registry number: n/s
Mole file number: n/s
Molecular formula/molecular weight: 410.49
Structure:



Relevant INDs/NDAs/DMFs: ——— (drug substance), I52982 (RISPERDAL i.m. depot, Janssen), N20-272 (RISPERDAL tablets, Janssen)
Drug class: D_2, $5HT_2$ antagonist
Indication: schizophrenia
Clinical formulation: solution for injection [25, 37.5, 50 mg]; excipient: 75:25 polylactide-co-glycolide (PLG); 381 mg risperidone per gm microspheres.
Route of administration: i.m. depot injection
Proposed use: n/a
Introduction and drug history: NDA was originally submitted on 8/31/2001. A Not-Approvable letter was issued on 6/28/2002 due to the following: (a) the tumor profile observed in the 2-yr i.m. depot carcinogenicity study in rat, (b) the lack of an i.m. embryofetal development study in rat, considering the finding of osteodystrophy in the 1-yr chronic toxicity and the 2-yr carcinogenicity studies in rat, (c) the lack of adequate data on qualification of an impurity ——— unique to the i.m. depot formulation. The current submission is the sponsor's complete response to the Division's Not-Approvable letter.
Studies reviewed within this submission:
Expert Review (Gordon C. Hard, BVSc, Ph.D., DSc, Section 1.2)

Cell Proliferation assessment (Section 1.3)
Substrain Differences assessment (Section 1.4)
Overall Discussion and Conclusions (Section 1.6)

Studies not reviewed within this submission: none

Executive Summary

1. Recommendations

   A. Recommendation on Approvability

      1. The renal tubular and adrenomedullary tumors observed in the 2-yr i.m. depot carcinogenicity study in rat cannot be dismissed as irrelevant to humans, and must be included in labeling.

      2. The sponsor is in the process of conducting an i.m. depot embryofetal development study. It is this reviewer's recommendation that this study be completed and reviewed prior to approval.

      3. It is recommended that the sponsor be asked to commit to further investigating the osteodystrophy observed in the 1-year i.m. depot toxicity and the 2-year i.m. depot carcinogenicity studies in rat. Additional studies to be conducted Phase 4 should address the exact nature of the bone lesion(s) and possible mechanism(s) underlying this finding.

      4. It is recommended that the sponsor conduct Phase 4 an *in vitro* chromosomal aberration assay in mammalian cells or an *in vitro* mouse lymphoma assay (with colony sizing) to assess the genotoxic potential of the [redacted] impurity [redacted] The study can either be conducted using a drug batch enriched in [redacted] or directly testing [redacted]

   B. Recommendations on Labeling: proposed revisions (bold, strikeouts) to the sponsor's labeling are indicated below:

3 Page(s) Withheld

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

_____ § 552(b)(5) Deliberative Process

✓ § 552(b)(5) Draft Labeling

2. Summary of Nonclinical Findings

The sponsor submitted the following:

(a) published literature, an Expert Report, and data from special toxicology studies to address the concerns regarding the tumor data from the 2-yr i.m. depot carcinogenicity study.

(b) a description of the plan to address the need for an i.m. depot embryofetal development study in rats.

(c) information to address the need to qualify a — impurity and — degradants.

Although the relevance of the tumor findings to humans is unclear, the data provided by the sponsor did not provide a justification for dismissing the renal tubular or adrenomedullary tumors observed in the 2-yr i.m. depot (but not the oral) carcinogenicity study in rat.

The sponsor's proposal for addressing the reproductive toxicology issue appears adequate.

Regarding the impurities issue, the sponsor lowered the specification for the degradants to below the qualification threshold. The specification for the — impurity is slightly above the qualification threshold ——— however, it is the same specification as for the drug substance for the currently marketed oral dosage form. It was determined that further qualification of the general or reproductive toxicity of the — impurity was not necessary, but that there was a need for further assessment of genotoxic potential (i.e., an *in vitro* chromosomal aberration assay in mammalian cells or an *in vitro* mouse lymphoma assay [with colony sizing]).

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PHARMACOLOGY/TOXICOLOGY REVIEW

I. PHARMACOLOGY

No additional pharmacology studies were submitted.

II. SAFETY PHARMACOLOGY

No additional safety pharmacology studies were submitted.

III. PHARMACOKINETICS/TOXICOKINETICS

No PK/ADME studies were submitted. Additional TK data were reviewed under the SPECIAL TOXICOLOGY STUDIES section.

IV. GENERAL TOXICOLOGY

No acute, subchronic, or chronic general toxicity studies were submitted.

V. GENETIC TOXICOLOGY

No genetic toxicology studies were submitted.

VI. CARCINOGENICITY

The sponsor submitted the following: (a) Expert Report prepared by Dr. Gordon C. Hard (New Zealand), (b) amendment to the final report of the 24-month intermittent repeated dose i.m. carcinogenicity study in Wistar rat (Exp. No. 4729): Section 3.11 Histopathology, non-GLP. (c) amendment to the final report of the 12-month intermittent repeated dose i.m. toxicity study in Wistar rat (Exp. No. 4729A): Histopathology section, non-GLP.

The results of these investigations were summarized in an overall summary provided by the sponsor.

1. Expert Report on Renal Histopathologic Changes in Rat Toxicology Studies with Risperidal Consta (Compound R064766) (Gordon C. Hard, BVSc, PhD, DSc, FRCPath, FRCVS, FAToxSci, Tairua, 2853, New Zealand, October 28, 2002).

Dr. Hard provided an expert evaluation of the kidney findings in Hannover Wistar rats in the following studies: (a) 2-yr carcinogenicity study (Exp No. 4729; males only except for analysis of selected females in which "focal tubule hyperplasia" had been diagnosed), (b) 12-mo interim sacrifice from 2-yr carcinogenicity study (Exp No. 4729A; males only), (c) 6-mo subcutaneous study (Exp No. 4731; males only), (d) 7-wk i.m. mechanistic study (Exp No. 5459; males only). As noted by Dr. Hard, "...the scope of work focused on seeking histologic evidence that might support a mode of action underlying a small incidence of renal tumors at the high-dose in male rats of the 2-year carcinogenicity study."

Dr. Hard examined kidney sections using "conventional brightfield microscopy" and, for selected tissues, "ultraviolet illumination....in order to assess the presence of lysosomes or hyaline droplet accumulation...". Dr. Hard scored the incidence and severity of tubule dilation, pelvic mineralization, chronic progressive nephropathy, and tumors.

The incidence and severity of tubule dilation were summarized in the following table (from Dr. Hard's expert report) (it was noted that criteria used to assess this finding were those described by the Society of Toxicologic Pathology and/or IARC; severity grades were not defined in Dr. Hard's report):

Table 1. Group incidence and severity of tubule dilation in male rats

Study	Dose group (mg/kg)	Rats in group	Rats with severity grade:				
			0	1	2	3	4
2-year carc. I/M	0	50	**33**	15	2	0	0
	0 (veh)	50	**35**	13	2	0	0
	40	50	4	17	**22**	7	0
12-mo. I/M	0	19	**13**	6	0	0	0
	40	19	5	**11**	3	0	0
6-mo. S/C	0	19	**13**	6	0	0	0
	40-160	19	**11**	7	1	0	0

The mode is shown in bold
veh = vehicle control

Dr. Hard noted the following regarding tubule dilation: (a) in males, the severity was greatest at the HD in the 2-yr carcinogenicity study, with the distal tubules being primarily affected. (b) tubule dilation and other changes (i.e., infiltration by neutrophils, distal and proximal tubule basophilia, distal tubule hypertrophy) were also observed in control grps (in males), and in HDF with a similar incidence and severity as in HDM. (c) there appeared to be an increase in severity (or "mild exacerbation") in tubule dilation in the 2-yr study as compared to the 12-mo study; no drug-related increase in tubule dilation was observed in the 6-mo study.

The incidence and severity of pelvic mineralization were summarized in the following table (from Dr. Hard's expert report) (severity grades were not defined in Dr. Hard's report):

Table 2. Group incidence and severity of pelvic mineralization in male rats

Study	Dose Group (mg/kg)	Rats in group	Rats with severity grade:				
			0	1	2	3	4
2-year carc. I/M	0	50	**29**	14	5	2	0
	0 (veh)	50	**24**	17	7	1	1
	40	50	3	13	**18**	14	2
12-mo. I/M	0	19	**15**	3	1	0	0
	40	19	**11**	6	1	1	0
6-mo. S/C	0 (veh)	19	**18**	1	0	0	0
	40-160	19	**12**	4	3	0	0

The mode is shown in bold
veh = vehicle control

Dr Hard noted that the severity of pelvic mineralization in males was greatest at the HD in the 2-yr study. The mineralization was described as occurring "...mainly in the pelvic fornices often in association with simple hyperplasia of the urothelial lining, and sometimes inflammatory debris." Pelvic mineralization was observed as a background finding in control grps (in males) and in HDF (with an incidence and severity similar to that in HDM) in the 2-yr study.

It was Dr. Hard's opinion that there was no evidence of "sustained cytotoxicity" in distal tubules (i.e., "...no evidence of an increase in single cell death or apoptotic change, mitotic activity, simple tubule hyperplasia, or karyomegaly...") in any of the studies, nor evidence of hyaline droplets in male rats. An examination of kidney tissue from 3 animals "that were sacrificed or had died relatively early in the 2-year study" indicated no evidence of hyaline droplets and "no increase in lysosomal autofluorescence". In addition, no "marked linear mineralization in the lumens of Henle limbs in the papilla" of HDM was observed at either the 12-mo or 2-yr sacrifice times.

The following table (copied from Dr. Hard's report) summarizes the incidence of CPN in the three studies:

Table 4. Group incidence and severity of chronic progressive nephropathy (CPN)

Study	Dose group (mg/kg)	Effective no. of rats*	Rats with severity grade:							
			0	1	2	3	4	5	6	7
2-year carc. I/M	0	48	6	**13**	6	11	10	2	0	0
	0 (veh)	50	8	**12**	9	10	8	3	0	0
	40	50	9	11	**13**	7	8	2	0	0
12-mo. I/M	0	19	**16**	2	1	0	0	0	0	0
	40	19	**14**	5	0	0	0	0	0	0
6-mo. S/C	0 (veh)	19	**17**	2	0	0	0	0	0	0
	40-160	19	**16**	3	0	0	0	0	0	0

* Effective no. excludes those rats in which CPN could not be assessed due to autolysis
The mode is shown in bold
veh = vehicle control

(The severity grades were defined by Dr. Hard as follows: 1 = minimal, 2-4 = mild to high-moderate, with disease remaining focal, 5 = early severe, with "...foci...beginning to coalesce into areas", 6 = severe,

with the majority of the kidney being affected, and 7 = end stage, with "virtually no normal parenchyma" remaining.)

As evident from the Table 4, there was no evidence of a drug-related increase in incidence or severity of CPN in the 12-mo interim or 6-mo studies.

Dr. Hard noted that, although the severity was greater in HDM compared to the male control grps, the incidence was higher in the control grps, resulting in a mean severity score of 2.25 for both HD and C males in the 2-yr study. In particular, Dr. Hard stated that "There were no rats affected with grades 6 (severe) or 7 (end-stage) in any group. According, the tumors did not occur in rats with an advanced grade of CPN..." (cf Table 3 below).

Table 3. Summary of tumor characteristics

Animal number	Tumor type	Morphology	Approx. size in mm	Grade of CPN	No. of tumors
HM336	adenoma	basophilic, tubule diff.	2.6 0.7	2	2
HM339	carcinoma	basophilic, tubule diff.	12.5	1	1
HM345	adenoma	basophilic, tubule diff.	1.0	3	1
HM361	adenoma	basophilic, tubule diff.	0.5	3	1
HM366	adenoma	basophilic, tubule diff.	4.4	5	1

Dr. Hard confirmed the presence of renal tubular adenomas in 4 HDM and a renal tubular carcinoma in 1 HDM (a total of 5 affected HDM) the 2-yr study (Table 3). However, he noted that "No solid foci of atypical tubule hyperplasia were observed in the high dose males of any of the study groups examined". Of the females in the 2-yr study, only one female (LD) was found to have evidence of atypical tubular hyperplasia.

Based on his examination of the data, Dr. Hard concluded that "...it seems likely that the tumors encountered with intramuscularly-administered Risperidone may possibly be of spontaneous origin and not treatment-related..." based on the following observations:

(1) risperidone was negative in "short-term tests", indicating a lack of genotoxic effects; therefore, "mechanisms involving direct or indirect (via oxidative stress) DNA damage can be excluded".

(2) renal tubular tumors "...were not accompanied by a background incidence of histologically relevant atypical tubule hyperplasia". "DNA-damaging renal carcinogens produce renal tumors in high incidence and with shortened latency" and are "always [accompanied by] an increased background of atypical tubule hyperplasia.

(3) there was no evidence for the presence of any of the "three known pathways" of "non-genotoxic or epigenetic mechanisms of renal tubule tumor induction by chemicals", i.e., "sustained direct cytotoxicity and compensatory cell regeneration, indirect cytotoxicity/regeneration through an α_{2u}-globulin mode of action, or via exacerbation of the spontaneous disease progress..."

Of particular relevance to the mechanism proposed by the sponsor in the original NDA submission (i.e., exacerbation of chronic nephropathy [CPN]), Dr. Hard stated "...the requirements for ascribing that process [advanced CPN] to Risperidone were not met in these studies. Thus, the compound did not cause

a biologically significant exacerbation of CPN to advanced grades (*severe* or *end-stage*), and the tumors were not associated with high grades of CPN, or with areas of CPN change."

However, Dr. Hard also noted that "[his] conclusion can be enlightened by applying more sensitive techniques, such as immunohistochemical procedures, to determine the presence or absence of sustained cytotoxicity/cell regeneration."

2. Amendment to the final report: Twenty-four months intermittent repeated dose intramuscular carcinogenicity study in the Wistar rat (Exp. No. 4729, R No. R064766, signed 3/17/03, non-GLP).

In this Amendment, the results of an evaluation of renal and parathyroid gland sections from selected CM and HD for evidence of cell proliferation and apoptosis (kidney) and diffuse hypertrophy or hyperplasia (parathyroid). Analyses were conducted (non-GLP) by Johnson & Johnson Pharmaceutical Research and Development (a division of Janssen Pharmaceutica N.V.).

These analyses were conducted in response to Dr. Hard's recommendation that additional studies be conducted to investigate possible mechanisms (e.g., cytotoxicity/regeneration) underlying the renal tumors.

Methods:

renal tissue: additional renal sections were prepared from the original tissue blocks for "the first 18" terminally sacrificed CM and HDM "without a renal corticotubular tumor", and from the 5 HD with renal corticotubular tumor(s) (all terminally sacrificed). Sections were immunostained for the presence of PCNA (Proliferating Cell Nuclear Antigen; the stain used was not specified) or apoptosis (commercially available DNA fragmentation detection kit). The PCNA data were expressed as the "...total number of PCNA-positive renal corticotubular nuclei and the number of PCNA-positive renal corticotubular nuclei per mm^2 of non-tumorous cortical tissue..." The apoptosis data were expressed as the "...total number of apoptosis-positive nuclei of non-tumorous cortical tissue...", and not corrected for total area (due to the "extremely low" counts).

parathyroid tissue: the evaluation was conducted on the original tissue slides from "the first" 20 CM and HDM that were terminally sacrificed. Films [redacted], were made of the sections, "taking care that no focal hyperplasia was present on the picture", and digitalized. "Representative" areas (4 samples per animal, each with a surface area of 0.0049 mm^2) were examined for total number of parathyroid glandular cell nuclei.

Results: the PCNA data were summarized in the following sponsor's tables (individual data could not be found):

non-renal tumor-bearing males:

Table : 24 Months - Mean number of PCNA positive renal corticotubular nuclei per mm^2 of cortex

Dose group (mg/kg)	Mean	SE	Significance p-value 2-tailed	p-value 1-tailed
Control (0)	1.769	0.23		
High (40)	2.427	0.346	0.1946	0.0973

renal tumor-bearing males:

Table : 24 Months extra - Mean number of PCNA positive renal corticotubular nuclei per mm^2 of cortex
Renal routine sections of rats with corticotubular neoplasia

Dose group (mg/kg)	Mean	SE	Significance p-value 2-tailed	p-value 2-tailed
High (40)	5.34	1.5	0.0073 **	0.0307 @

Significance computed by Mann-Whitney U test : versus Control - 24 months: * p<.05 ** p<.01 *** p<.001
versus High - 24 months: @ p<.05 @@ p<.01 @@@ p<.00

The apoptosis data were summarized in the following sponsor's tables:

non-renal tumor-bearing males;

Table : 24 Months - Mean number of APOP positive renal corticotubular nuclei

Dose group (mg/kg)	Mean	SE	Significance p-value 2-tailed
Control (0)	3.1	3.1	
High (40)	3.1	3.7	0.7110

Significance computed by Mann-Whitney U test : * p<.05 ** p<.01 *** p<.001

renal tumor-bearing males:

Table : 24 Months extra - Mean number of APOP positive renal corticotubular nuclei
Renal routine sections of rats with corticotubular neoplasia

Dose group (mg/kg)	Mean	SE	Significance p-value 2-tailed	p-value 2-tailed
High (40)	0.6	0.4	0.0370 *	0.0421 @

Significance computed by Mann-Whitney U test : versus Control - 24 months: * p<.05 ** p<.01 *** p<.001
versus High - 24 months: @ p<.05 @@ p<.01 @@@ p<.001

The following conclusions were reached based on these data:

1. the number of PCNA-positive renal corticotubular nuclei (per mm^2 of non-tumorous cortical tissue) was "marginally increased" in non-renal tumor-bearing HDM compared to CM and significantly increased in renal tumor-bearing HDM compared to non-renal tumor-bearing HDM and CM.

2. no difference was detected in the incidence of apoptosis-positive renal corticotubular nuclei between CM and non-tumor bearing HDM. Although not commented on by the sponsor, the mean number of apoptosis-positive renal corticotubular nuclei was significantly higher in tumor-bearing HDM compared to CM and non-tumor bearing HDM.

The parathyroid data were summarized in the following sponsor's table:

Table 2: mean number of parathyroid glandular cell nuclei per 0.0049 mm^2 per group

Group	one area	median
Control	49	57
High dose	58**	62*
(p-value)	(p=0.0065)	(p=0.0263)

Statistics computed by Mann-Whitney U test (two-tailed)
* $p < .05$
** $p < .01$

Median values ranged from 41 to 65 in CM and from 43 to 74 in HDM. Values exceeding the control range occurred in 8/20 HDM (i.e., 67, 74, 70, 71, 70, 69, 70, 69 in HDM #322, 324, 326, 328, 332, 334, 335, 339, respectively).

It was concluded that there was "...a significant increase in nuclei per surface unit..." in HDM compared to CM, and that "This might indicate that slight diffuse hyperplasia of the parathyroid gland is present in the high dosed male group."

Amendment to the final report: 12-month intermittent repeated dose intramuscular toxicity study in the Wistar rat (Exp. No. 4729A, R. No. R064766, Histopathology section, non-GLP).

In this amendment, the sponsor provided the report for an additional evaluation of renal sections collected at the end of the 12-month interim sacrifice (during the 2-yr carcinogenicity study) toxicity study.

Methods: it would appear that similar methodology was used as described in the previously described amendment to the final report of the 24-month carcinogenicity study regarding examination of renal sections for presence of PCNA and apoptosis. Examinations were conducted on additional sections made from the original tissue blocks for 18 CM (#1-18) and 18 HDM (#301-315 and 317-319), all sacrificed at Wk 52.

Results: the PCNA and apoptosis data were summarized in the following sponsor's tables:

Exp 4729 - PCNA-immunostain- positive renal corticotubular cells

Table : 12 Months - Mean number of PCNA positive renal corticotubular nuclei per mm^2 of cortex

Dose group (mg/kg)	Mean	SE	Significance p-value 2-tailed	p-value 1-tailed
Control (0)	0.744	0.158		
High (40)	2.26	0.308	0.0002 ***	0.0001 ***

Table : 12 Months - Mean number of APOP positive renal corticotubular nuclei

Dose group (mg/kg)	Mean	SE	Significance p-value 2-tailed
Control (0)	1.1	0.6	
High (40)	0.2	0.2	0.1171

Significance computed by Mann-Whitney U test : * p<.05 ** p<.01 *** p<.001

It was concluded that:

1. the number of "...PCNA-positive renal corticotubular nuclei per mm^2 was 3 fold increased in..." HDM.

2. there was no effect of drug on the number of apoptosis-positive renal corticotubular nuclei.

Note: in the sponsor's overall response, the following summary table of renal PCNA data in non-tumor-bearing HDMs was provided:

Table 1: Cell proliferation in renal cortical tissue of non-tumor bearing male Wistar Hannover rats from the IM carcinogenicity study[R1]: mean number of PCNA-positive cell nuclei per mm^2 of cortex[R2]

Time point	Dose (mg kg 2 weeks)	Mean	SE	Statistical significance *p*-value two-tailed	*p*-value one-tailed
12 months	0	0.744	0.158	-	-
12 months	40	2.260	0.308	0.0002***	0.0001***
24 months	0	1.769	0.230	-	-
24 months	40	2.427	0.346	0.1946	0.0973

Statistical significance computed by Mann Whitney U-test for the comparison of dosed versus control groups at 12 months and at 24 months:
* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$

Individual animal data on PCNA were provided for tumor-bearing HDMs (24-mo data) in the following sponsor's table:

Table 2: Cell proliferation in non-tumor renal cortical tissue of tumor bearing male Wistar Hannover rats from the IM carcinogenicity study at 24 months[a]

Animal No.	Cell proliferation: PCNA-positive nuclei (number mm² cortex)
366	10.549
361	4.965
345	6.280
336	2.708
339	2.203
Mean number ± SE	5.34 ± 1.5[b,c]

Statistical significance computed by:

- Mann-Whitney U test (two- tailed): *p< .05, **p< .01, ***p< .001, for comparison versus controls (24 months)
- Mann-Whitney U test (two- tailed), [#]p< .05, [##]p< .01, [###]p< .001, for comparison versus non-tumor bearing high-dose males (24 months)

The sponsor noted the increase in PCNA-positive nuclei in CM at 24-mo, compared to 12-mo, and suggested that this increase "...possibly reflects the increased incidence of age-related non-neoplastic renal cortical tissue changes observed...", as summarized in the following sponsor's table (taken from data presented in Attachment 3 of the Response Integrated Summary):

Table 4: Quantitative and qualitative comparison of spontaneous age-related non-neoplastic renal changes between male Wistar Wiga and male Wistar Hannover rats (see Attachment 3)

	Wiga 1	Wiga 2	Hannover 1	Hannover 2
Basophilic tubuli, (multifocal, plus thickened basement membranes (cortex)	19/20 *2.50*	19/20 *2.25*	18/20 *1.55*	16/20 *1.55*
Basophilic dilated tubuli, diffuse (cortex)	1/20 *0.05*	2/20 *0.10*	13/20 *** *0.65*	13/20 *** *0.65*
Dilated tubules, (multifocal (cortex)	16/20 *1.45*	14/20 *1.35*	8/20* *0.65*	8/20* *0.55*
Hyaline casts	19/20 *1.80*	15/20 *1.35*	16/20 *1.05*	11/20** *0.90*
Glomerulopathy	15/20 *1.70*	14/20 *1.25*	6/20* *0.50*	7/20* *0.65*
Interstitial fibrosis inflammatory infiltrate	17/20 *1.70*	14/20 *1.05*	5/20*** *0.40*	9/20* *0.65*
Minerals (pelvis)	0/20 *0.00*	4/20 *0.20*	9/20** *0.60*	10/20*** *1.25*
Hyperplasia diffuse (transitional epithelium)	0/20 *0.00*	1/20 *0.10*	5/20* *0.30*	8/20** *0.55*
Inflammation pelvis	1/20 *0.05*	1/20 *0.10*	9/20** *0.60*	6/20 *0.40*

Twenty male, terminally sacrificed control rats of four different J&JPRD carcinogenicity studies were re-evaluated by one pathologist for individual age-related non-neoplastic renal changes:

- Wiga 1: results of carcinogenicity study No. 3026 (*in vivo* phase 1993-1995) (incidences and *mean severity grade*)
- Wiga 2: results of carcinogenicity study No. 3097 (*in vivo* phase 1993-1995) (incidences and *mean severity grade*)
- Hannover 1: results of carcinogenicity study No. 4044 (*in vivo* phase 1997-1999) (incidences and *mean severity grade*)
- Hannover 2: results of carcinogenicity study No. 4101 (*in vivo* phase 1996-1998) (incidences and *mean severity grade*)

Statistical significance versus Wiga 1 computed by:

- Fisher Exact test (two-tailed): *p< .05, **p< .01, ***p< .001, for comparison of incidences
- Mann-Whitney U test (two-tailed): #p< .05, ##p< .01, ###p< .001, for comparison of mean severity grade

The sponsor concluded that:

"These data demonstrate that at 12 months there is a clear-cut treatment-related increase in cell proliferation at the 40 mg/kg/2 weeks dose level as compared to controls.. This observation is in accordance with a marked increase in relative kidney weight at 12 months in high-dose male Hannover rats as compared to control and vehicle-dosed animals. This treatment-related increase in relative kidney weight continues up to 24 months of dosing...parallel with sustained high-level cell proliferation.

"As there could be an interval of considerable length between the increased cell proliferation (first observed at 12 months) and the formation of renal tubular tumors (seen at 24 months), the suggestion by Dr. Hard that the tumors might be of a spontaneous nature...becomes questionable. Rather, the data suggest the existence of a continuum of tissue changes including enhanced cell proliferation, increased kidney weights and eventually the development of tumors."

3. RISPERDAL CONSTA™ (NDA 21-346): Response to FDA Action Letter of June 29, 2002.

This response summarized all information/data submitted in response to the FDA action letter. Points made by the sponsor in the Pharmacology/Toxicology portion of the integrated summary response relevant to Carcinogenicity are summarized below:

Response to Request #1 (Request #1 [below] copied from the sponsor's report):

> **1. The tumor profile in the 2-yr intramuscular (IM) depot carcinogenicity study in rat was different than that observed in the 2-yr oral studies in mouse and rat [NDA 20-272, RISPERDAL tablets]. Two tumor types, renal tubular adenomas and adrenomedullary tumors, were observed only with the IM depot formulation. This raises the concern that the IM depot formulation may be more tumorigenic than oral risperidone. You concluded that the renal tubular adenomas and adrenomedullary tumors were related to elevations in serum prolactin. However, the information/data provided did not support this mechanism. For example, there was not convincing evidence of an exacerbation of chronic renal disease in high-dose males, either as a group or in the individual animals with renal tubular adenomas. In addition, the mechanistic studies conducted in rats did not provide adequate data for dismissing the possibility of a unique tumor profile [with the IM depot formulation] on the basis of substrain differences or differential effects of route on serum prolactin. When serum prolactin effects were assessed following oral and IM depot administration, the AUC for serum prolactin was greater following oral dosing. This finding undermines the view that elevated prolactin is primarily responsible for the tumors seen with IM dosing.**
>
> **The data from the genotoxicity studies indicate that risperidone is not genotoxic; therefore, there is a presumed threshold for tumorigenic effects. However, in the IM depot study, there was no safety margin between plasma exposures at the no-effect doses for renal and adrenomedullary tumors and that expected at the maximum recommended clinical dose.**
>
> **These findings would preclude approval of this application in the absence of any demonstration of a clinical advantage of this product. Of course, if you have additional data or information that would support the conclusion that the renal tubular adenomas and adrenomedullary tumors are irrelevant in terms of human risk, such data/information should be submitted for review.**

Regarding the renal tumors:

1. The renal tubular adenomas observed in the i.m. depot carcinogenicity study in Wistar Hannover

rats were noted in the original NDA reports to be a result of "an exacerbation of age-related spontaneous chronic renal disease in Wistar Hannover rats. This increase in chronic renal disease was considered to be related to elevated serum prolactin levels".

2. In response to the FDA action letter, the sponsor has provided additional information, including additional investigations, addressing potential mechanism(s) underlying the renal tumors. These include the following: (a) an Expert Review conducted by Dr. Gordon C. Hard, (b) assessment of cell proliferation and apoptosis in the original tissue from males in the 2-yr rat i.m. depot carcinogenicity study. (c) historical control data regarding substrain differences in spontaneous tumors, (d) an 8-wk mechanistic study in male Wistar Wiga and Wistar Hannover rats to address substrain and route differences, (e) a reanalysis of renal tissue from 4 previously conducted carcinogenicity studies in Wistar Wiga and Wistar Hannover rats (2 studies each), (f) literature references.

•The Expert Review by Dr. Hard, the assessment of cell proliferation and apoptosis, and the 8-wk study are reviewed separately.

•Regarding the historical control data, the sponsor noted that "It is well known that there are differences between strains of rats with regard to spontaneous tumor profiles". The sponsor summarized historical control data for various tumor types in Wistar (Wiga, Hannover), Fischer 344, and Sprague-Dawley rats in the following table:

Table 3: Historical control data on incidences of selected tumor types in male rats of the Wistar Wiga, the Wistar Hannover, the Fischer 344 and the Sprague-Dawley strain

Tumor type	Wistar Hannover*	Wistar Wiga**	Fischer 344	Sprague-Dawley
Mammary gland: fibroadenoma	0 %	0.89 %	2.63 %	0.4 %
Pituitary gland: adenoma	30.86 %	24.09 %	22.32 %	62.2 %
Endocrine pancreas: adenoma	6.91 %	14.22 %	3.16 %	7.5 %
Adrenal gland: pheochromocytoma	3.10 %	12.28 %	25.54 %	19.0 %
Kidney: tubular adenoma	0.24 %	0.44 %	0.36 %	0.7 %

* historical control data mentioned in the study report of the IM carcinogenicity study, based on eight different carcinogenicity studies performed at J&JPRD[P1]

** historical control data mentioned in the study report of the oral carcinogenicity study, based on nine different carcinogenicity studies performed at J&JPRD[P2]

The sponsor provided copies of published literature reporting the spontaneous incidences of tumors in Fischer 344 rats (Haseman JK *et al.* In: *Pathology of the Fischer Rat.* Boorman GA *et al.*, eds., pg 555-564, Academic Press Inc., San Diego, 1990):

	Male Rats						Female Rats					
	Untreated			Corn Oil Gavage			Untreated			Corn Oil Gavage		
	Animals with Tumors[a]	Rate[b] %	Range %	Animals with Tumors[a]	Rate[b] %	Range %	Animals with Tumors[a]	Rate[b] %	Range %	Animals with Tumors[a]	Rate[b] %	Range %
Kidney	(1928)			(1943)			(1977)			(1944)		
Tubular Cell Adenoma	7	0.4	0–4	3	0.2	0–2	2	0.1	0–2	2	0.1	0–2
Tubular Cell Adenocarcinoma	3	0.2	0–2	7	0.4	0–2	2	0.1	0–2	0	0.0	—
Transitional Cell Papilloma	3	0.2	0–2	1	0.1	0–2	0	0.0	—	0	0.0	—
Transitional Cell Carcinoma	2	0.1	0–2	0	0.0	—	0	0.0	—	0	0.0	—
Sarcoma	1	0.1	0–2	1	0.1	0–2	0	0.0	—	0	0.0	—
Nephroblastoma	1	0.1	0–2	1	0.1	0–2	0	0.0	—	0	0.0	—
Lipoma	2	0.1	0–2	2	0.1	0–2	0	0.0	—	1	0.1	0–2
Liposarcoma	0	0.0	—	1	0.1	0–2	0	0.0	—	0	0.0	—
Leiomyosarcoma	0	0.0	—	1	0.1	0–2	0	0.0	—	0	0.0	—

and in Sprague-Dawley rats (table below from McMartin DN *et al. Toxicol Pathol* 20(2):212-225, 1992; numbers in parentheses represent total numbers of animals examined):

TABLE I.—Neoplasms and associated lesions in Sprague-Dawley rats.

Site: Neoplasm or finding

	Males			Females		
		Range %	Cumulative average %		Range %	Cumulative average %
Kidney	(585)			(584)		
Adenoma		0.0–2.9	0.7		0.0–0.0	0.0
Adenocarcinoma		0.0–1.7	0.5		0.0–0.0	0.0
Nephroblastoma		0.0–0.0	0.0		0.0–1.7	0.2
Lipoma		0.0–1.7	0.5		0.0–1.7	0.3
Liposarcoma		0.0–2.9	0.7		0.0–0.0	0.0
Polyp		0.0–1.4	0.2		0.0–0.0	0.0
Nonneoplastic lesions						
Focal tubular hyperplasia		0.0–1.4	0.2		0.0–0.0	0.0

The sponsor acknowledged that "There are many sources of variability in tumor incidence. Historical control data therefore should be used carefully in the evaluation of tumor incidences...Nevertheless, the limited data presented in Table 3 tentatively demonstrate that, although some spontaneous tumor incidences are comparable between strains, spontaneous incidences of other tumor types may be 2- to 8-fold higher in some rat strains compared to others."

Regarding the use of historical control data, the sponsor provided copies of two published articles (Haseman JK *et al. Toxicol Pathol* 12(2):126-135, 1984; Haseman JK. *Reg Toxicol Pharmacol* 21:52-59, 1995). Haseman *et al.* (1984) stated that, "Although the concurrent control group is always the first and most appropriate control group used for decision making..., there are certain instances in which the use of historical control information can aid...in the overall evaluation of tumor incidence data", e.g., rare tumors, tumors demonstrating "a borderline increase relative to concurrent controls". However, Haseman *et al.* (1984) also pointed out that a number of issues must "all be addressed before historical control data can be used in a formal testing framework": (a) differences in tumor nomenclature and diagnostic criteria among studies/laboratories (the authors noted that these "should be identical to insure unambiguous identification" of tumors), (b) differences in pathology protocols, quality assurance and review procedures, "diets, caging regimens, and various environmental parameters", treatment of controls (vehicle, treated/untreated), and other sources of variability (e.g., "calendar year, laboratory, pathologist, supplier)". Similar issues were raised in the Haseman (1995) article.

•The sponsor also noted that the incidence of spontaneous tumors may also vary between substrains of rats, and noted that differences between substrains of Wistar rats have been documented. Walsh & Poteracki (Walsh KM, Poteracki J. *Fund Appl Toxicol* 22:65-72, 1994;

copy provided by the sponsor) reported on the incidence of spontaneous tumors in Wistar rats. The authors presented a compilation of data from 10 carcinogenicity bioassays conducted in Wistar (Crl:(WI)BR, Hla:(WI)BR) rats (from two different suppliers). The incidences of renal adenomas were as follows (total no. animals: 685/sex):

TABLE 3
Spontaneous Neoplasms Occurring in Three or More Control Wistar Rats

System Neoplasm	Male			Female		
	N	%	% Range	*N*	%	% Range
Urinary						
Renal adenoma	3	0.44	0–2.0	1	0.15	0–2.0

In addition, the authors discussed similarities and differences between their data and those previously published for Wistar rats, in both similar and different substrains. The authors also noted differences in their own database between Crl and Hla substrains; however, time and animal sources were confounding factors. There were no references specifically to differences between Wiga and Hannover substrains.

The sponsor noted that the profile of drug-induced tumors "...is highly dependent upon the strain of rats used...", and cited a published article by Munro *et al.* (Munro IC *et al. Reg Toxicol Pharm* 21:60-70, 1995). Regarding intraspecies differences, Munro *et al.* (1995) stated that "Although there is less intraspecies genetic variability compared to the interspecies level, differences in strain [i.e., interstrain] sensitivity to spontaneous and chemically induced tumor development can be remarkable". (Substrain differences did not appear to be specifically addressed.) Interspecies and interstrain differences may also be due to a variety of factors, e.g., differences in PK/ADME (in particular, metabolism). Munro *et al* (1995) also state that "The route by which the chemical is administered also can greatly affect the distribution, metabolism, and sites of metabolism of the chemical within the body (i.e., different target organs may be affected by both the route of exposure and the relationship between administered and tissue-delivered dose)...." and that "It has been known for years that the route of exposure can greatly modulate the carcinogenic response in experimental animals".

The sponsor provided examples of interstrain (not intersubstrain) and sex differences in drug-induced tumor profiles (i.e., increased susceptibility of male Fischer 344 rats [compared to female Fischer 344 and male Sprague-Dawley rats] to hydroquinone-induced renal tubule adenomas, increased susceptibility of Fischer 344 rats [compared to Sprague-Dawley rats] to oxazepam-induced renal tubule neoplasms.

(The sponsor discussed quantitative and qualitative differences between Wistar Wiga and Hannover rats in spontaneous age-related renal changes. These data have been reviewed in detail in the original NDA review. It is the sponsor's opinion that the "...increase in non-neoplastic renal changes induced by RISPERDAL CONSTA™ in Wistar Hannover rats...may have contributed to the differential renal tumor response in IM-dosed Wistar Hannover rats...versus orally dosed Wistar Wiga rats.")

•Attachment 3: A quantitative and qualitative comparison of spontaneous age-related non-neoplastic renal histological changes between control male Wistar Wiga and Wistar Hannover rats (carcinogenicity studies).

In Attachment 3 of the Integrated Summary, the sponsor provided the results of a re-analysis of

original H & E stained renal tissue section of "...the first 20 terminally sacrificed male rats of four different J&JPRD carcinogenicity studies...evaluated histologically by one pathologist for individual age-related non-neoplastic renal changes." Two studies were conducted in Wistar Wiga rats (April 1993-April 1995 and July 1993-August 1995), and two studies were conducted in Wistar Hannover rats (March 1997-March 1999 and December 1996-December 1998).

Tissue findings were graded on scale of 0 to 5, with 0 = absent, 1 = minimal or small quantity of change and 5 = severe or large quantity of change. Findings were summarized in the following sponsor's tables:

Organ or Tissue - Observation	Terminally sacrificed control rats			
	Wiga 1	Wiga 2	Hannover 1	Hannover 2
Kidneys *Number examined:*	*20*	*20*	*20*	*20*
- basophilic tubuli, (multi)focal + thickened basement membranes (cortex)	19	19	18	16
- basophilic dilated tubuli, diffuse (cortex)	1	2	13 ***	13***
- cyst(s) cystic	2	2	2	3
- cystic	0	0	1	0
- dilated pelvis	0	1	2	6 *
- dilated tubules, (multi)focal (cortex)	16	14	8 *	8 *
- dilated tubuli, diffuse (cortex + medulla)	2	1	0	0
- glomerulopathy	15	14	6 *	7 *
- hyaline cast(s)	19	15	16	11 **
- hyalinization (tubuli)	1	1	0	0
- hyperplasia, diffuse (transitional epithelium)	0	1	5 *	8 **
- hyperplasia, focal (transitional epithelium)	0	1	2	1
- hypertrophy, (multi)focal (cortical tubuli)	2	5	0	3
- inflammation (pelvis)	1	1	9 **	6
- inflammation, granulomatous microabscess(es) (cortex)	2	1	3	2
- interstitial fibrosis inflammatory infiltrate	17	14	5 ***	9 *
- minerals (pelvis)	0	4	9 **	10***
- pigmentation cytoplasm tubular epithelium	0	0	1	0
- vacuolization, (multi)focal (cortical tubuli)	0	0	1	0

Significance versus Wiga 1 computed by the Fisher Exact test (two tailed) : * P < .05 ** P < .01 *** P < .001
Statistics are only performed if more than 50 % of the animals of the group are examined

Organ or Tissue - Observation	Terminally sacrificed control rats			
	Wiga 1	Wiga 2	Hannover 1	Hannover 2
Kidneys *Number examined:*	*20*	*20*	*20*	*20*
- basophilic tubuli, (multi)focal + thickened basement membranes (cortex)	2.50 (0.28)	2.25 (0.26)	1.55 ** (0.21)	1.55 * (0.26)
- basophilic dilated tubuli, diffuse (cortex)	0.05 (0.05)	0.10 (0.07)	0.65 *** (0.11)	0.65*** (0.11)
- cyst(s) cystic	0.25 (0.20)	0.10 (0.07)	0.20 (0.14)	0.15 (0.08)
- cystic	0.00 (0.00)	0.00 (0.00)	0.05 (0.05)	0.00 (0.00)
- dilated pelvis	0.00 (0.00)	0.05 (0.05)	0.15 (0.11)	0.65 ** (0.25)
- dilated tubules, (multi)focal (cortex)	1.45 (0.23)	1.35 (0.28)	0.65 * (0.21)	0.55 ** (0.17)
- dilated tubuli, diffuse (cortex + medulla)	0.15 (0.11)	0.05 (0.05)	0.00 (0.00)	0.00 (0.00)
- glomerulopathy	1.70 (0.28)	1.25 (0.29)	0.50 ** (0.21)	0.65 ** (0.22)
- hyaline cast(s)	1.80 (0.20)	1.35 (0.24)	1.05 ** (0.17)	0.90 ** (0.22)
- hyalinization (tubuli)	0.05 (0.05)	0.05 (0.05)	0.00 (0.00)	0.00 (0.00)
- hyperplasia, diffuse (transitional epithelium)	0.00 (0.00)	0.10 (0.10)	0.30 * (0.13)	0.55 ** (0.17)
- hyperplasia, focal (transitional epithelium)	0.00 (0.00)	0.05 (0.05)	0.10 (0.07)	0.10 (0.10)
- hypertrophy, (multi)focal (cortical tubuli)	0.15 (0.11)	0.35 (0.15)	0.00 (0.00)	0.20 (0.12)
- inflammation (pelvis)	0.05 (0.05)	0.10 (0.10)	0.60 ** (0.17)	0.40 * (0.15)
- inflammation, granulomatous microabscess(es) (cortex)	0.15 (0.11)	0.05 (0.05)	0.15 (0.08)	0.10 (0.07)
- interstitial fibrosis inflammatory infiltrate	1.70 (0.25)	1.15 (0.25)	0.40 *** (0.18)	0.65 ** (0.20)
- minerals (pelvis)	0.00 (0.00)	0.20 * (0.09)	0.60 *** (0.18)	1.25*** (0.32)
- pigmentation cytoplasm tubular epithelium	0.00 (0.00)	0.00 (0.00)	0.05 (0.05)	0.00 (0.00)
- vacuolization, (multi)focal (cortical tubuli)	0.00 (0.00)	0.00 (0.00)	0.05 (0.05)	0.00 (0.00)

Significance versus Wiga 1 computed by Mann-Whitney U test (two tailed) : * P < .05 ** P < .01 *** P < .001
Standard Error is shown between brackets
Statistics are only performed if more than 50 % of the animals of the group are examined

The sponsor noted that;

> "Quantitatively, the [spontaneous age-related non-neoplastic renal] changes were, in general, more pronounced in Wistar Wiga versus Wistar Hannover rats, except for pelvic mineralization and related changes (diffuse transitional epithelial hyperplasia and pelvic inflammation) and diffuse basophilic, dilated cortical tubuli. These changes were more pronounced in Wistar Hannover versus Wistar Wiga rats....In the Wistar Hannover Substrain, tubular basophilia and hyaline casts were mostly associated with pelvic mineralization and related changes and with diffuse basophilic, dilate cortical tubuli., which were more pronounced than interstitial fibrosis/inflammation and glomerulopathy. These data demonstrate that especially pelvic mineralization and diffuse basophilic,

dilated cortical tubuli are spontaneous age-related renal changes, which are differently observed with regard to incidence, severity grade and relative importance in male Wistar Hannover rats compared to Wistar Wiga rats."

Regarding the adrenomedullary tumors:

1. The sponsor summarized the adrenomedullary findings from the IM depot carcinogenicity study in rats in the table below (taken from the sponsor's submission):

Table 5: Incidence of adrenomedullary hyperplasia and adrenomedullary tumors in the IM carcinogenicity study with risperidone in Wistar Hannover rats[21]

Observation	Dosage group (mg/kg body weight) 0 (control)	0 (vehicle)	5	40	Statistical significance
Males					
Adrenomedullary hyperplasia, focal	8/50	9/50	9/50	33/50	p 0.001
Adrenomedullary tumor, benign (phaeochromocytoma)	2/50	2/50	2/50	11/50**	p 0.0013
Adrenomedullary tumor, malignant (phaeochromocytoma)	1/50	1/50	1/50	1/50	p 0.4345
Adrenomedullary tumor, benign and or malignant (phaeochromocytoma)	2/50	3/50	3/50	12/50**	p 0.0006
Females					
Adrenomedullary hyperplasia	8/50	5/50	17/50	13/50	p 0.01
Adrenal medullary tumor, benign (phaeochromocytoma)	0/50	1/50	1/50	3/50 *	p 0.0238
Adrenomedullary tumor, malignant (phaeochromocytoma)	0/50	0/50	1/50	0/50	p 0.4738
Adrenomedullary tumor, benign and or malignant (phaeochromocytoma)	0/50	1/50	2/50	3/50**	p 0.0350

* Fisher Exact test (two-tailed)
** age-adjusted analysis (according to Peto), asymptotic *p*-value (one-sided) of Peto's trend statistic (no correction for continuity)

The sponsor indicated that drug-related adrenomedullary tumors were also observed in the oral carcinogenicity study in Wistar Wiga rats. Adrenomedullary findings in the oral study were summarized in the following sponsor's table:

Table 6: Incidence of adrenomedullary hyperplasia and adrenomedullary tumors in the oral carcinogenicity study with risperidone in Wistar Wiga rats[22]

Observation	Dosage group (mg/kg body weight) 0 (control)	0.63	2.5	10	Statistical significance
Males					
Adrenomedullary hyperplasia	11/50	12/50	11/50	19/49	p 0.05
Adrenomedullary tumor, benign (phaeochromocytoma)	3/50	7/50	6/50	6/49	p 0.0687*
Females					
Adrenomedullary hyperplasia	8/50	7/50	5/50	9/50	p 0.05
Adrenal medullary tumor, benign (phaeochromocytoma)	1/50	3/50	1/50	3/50	p = 0.2445*
Adrenomedullary tumor, malignant (phaeochromocytoma)	0/50	1/50	0/50	0/50	p 0.6416*
Adrenomedullary tumor, benign and or malignant (phaeochromocytoma)	1/50	4/50	1/50	3/50	p 0.2966*

* age-adjusted analysis (according to Peto); asymptotic *p*-value (1-sided) of Peto's trend statistic (no correction for continuity)

According to the sponsor, "...this study showed a trend towards a 2-fold increase in the incidence of benign adrenomedullary tumors in dosed males as compared to controls. This trend was of borderline statistical significance (p = 0.0687...). Therefore, it was not retained as being drug-related. No such trend was apparent in females." The sponsor further stated that "These data (Table 5 and 6) demonstrate that there is no essential qualitative difference between the IM and the oral carcinogenicity study with

respect to the induction of adrenomedullary tumors. In both studies, a treatment-related increase in the incidence of adrenomedullary tumors was found in male animals."

The sponsor proposed hyperprolactinemia and hypercalcemia as potential mechanisms underlying the adrenal pheochromocytomas observed with IM risperidone, although the sponsor noted that "No hypercalcemia was observed in the oral rat carcinogenicity study with risperidone." The difference in what the sponsor referred to as "...the quantitative difference in adrenomedullary tumor response between the two carcinogenicity studies..." was postulated to be due to the fact that hypercalcemia and hyperprolactinemia were both observed in the IM study whereas only hypercalcemia was observed in the oral study. i.e., "In the case of the IM study...the prolactin- and calcium-related mechanisms....probably acted in concert." The sponsor noted that both factors result in stimulation of "...chromaffin cell proliferation by neurally derived signals that also regulate catecholamine production and release." The sponsor also argued that adrenomedullary tumors in rats are fundamentally different from human pheochromocytomas. (This issue was discussed in detail in the original NDA review.)

The sponsor concluded that "The increased incidence of adrenomedullary tumors in risperidone-treated rats...is considered to be a rat-specific phenomenon, which is of low relevance to humans...." This conclusion was based on the following:

(a) "...the morphological and functional characteristics of adrenomedullary tumors differ markedly between rats and humans."

(b) prolactin and calcium are involved in the induction of adrenomedullary tumors in rats, but not in humans.

(c) no risperidone-induced adrenomedullary tumors have been observed postmarketing.

2. The sponsor provided copies of a number of published studies addressing the association between hyperprolactinemia and/or hypercalcemia and adrenomedullary tumors in rats:

•Tischler and DeLellis (Tischler AS, DeLellis RA. *J Am Coll Toxicol* 7(1): 23-44, 1988) have reviewed the data on spontaneous and drug-induced proliferative lesions of the adrenal gland in rat. Among the points made by the authors are the following:

(a) there are clear strain differences in the occurrence of spontaneous lesions. The Wistar strain was noted to be particularly vulnerable to spontaneous lesions of the adrenal medulla (as high as 74-86% in one study cited). And within any strain, adrenal proliferative lesions are more frequently observed in males and in older animals.

(b) "...it has been suggested by some authors that prolactin might play a role in the etiology of adrenal medullary proliferative lesions" (no reference was cited). The authors cited a publication reporting that hypophysectomy "might eliminate these lesions in a susceptible rat strain." Although the authors included "Neuroleptics" in a list of drugs associated with adrenomedullary proliferative lesions, no specific neuroleptic was identified, and they stated that "The specific neuroleptics that produce adrenal medullary lesions have not been disclosed..."

(c) numerous agents have been reported to increase adrenomedullary proliferative lesions. The authors noted that the wider diversity of pharmacological activities among these agents would "...strongly suggest that multiple different mechanisms are able to stimulate proliferation of adrenal medullary cells. Perhaps these mechanisms act by a final common path, but this remains to be determined."

(d) the discussion of the relationship between rat adrenomedullary nodules and human pheochromocytomas was previously reviewed (original NDA review).

•Tischler *et al.* (Tischler AS *et al. Arch Histol Cytol* 52(Suppl):209-216, 1989) investigated the potential of reserpine-induced tumors to serve as model for studying mechanisms underlying the development of chromaffin cell proliferation and tumors, and discussed the data on the pharmacological activity of drugs that are associated with adrenomedullary tumors. The following comments were of note:

(a) reserpine (5-20 mg/kg) produced a dose-related increase in chromaffin cell proliferation in Sprague-Dawley and Long-Evans rats following 5 days of daily dosing. The magnitude of the response was similar in the two strains.

(b) spontaneous or drug-induced adrenomedullary proliferative lesions in rats are of interest since, among other factors, "...they resemble the proliferative changes seen in the adrenal glands of patients with Multiple Endocrine Neoplasia syndromes..."

(c) the induction of adrenomedullary proliferative lesions is affected by endogenous and exogenous factors. Among the exogenous factors are the following: (1) "...the excessive intake or absorption of calcium...", (2) drugs inducing neurogenic stimulation of the adrenal gland (e.g., reserpine-induced increases in secretion and synthesis of catecholamines, nicotine-induced stimulation of nicotinic cholinergic receptors in the adrenal gland, (3) drug-induced elevations in serum prolactin, although it was noted that "Any effects of prolactin on chromaffin cell proliferation are likely to be indirect, however, because the normal rat adrenal medulla has no demonstrable prolactin receptors. The apparent lack of prolactin receptors in PC12 pheochromocytoma cells ("...which are representative of spontaneously occurring and drug-induced adrenal medullary tumors in many respects") was also noted.

(d) the relationship between human and rat adrenal medulla proliferative responses. While there are differences between the species (e.g., proliferation during development), the authors noted that "As in rats..., mitoses are almost never observed in random histological sections of adrenals from normal human adults. Interestingly, the proliferative changes seen in the adrenal glands of patients with Multiple Endocrine Neoplasia syndromes...strongly resemble those which occur in rats under diverse circumstances, suggesting that the pharmacologically stimulated rat might provide an experimental model for studying the pathogenesis of those disorders."

•Rosol TJ *et al.* (Rosol TJ *et al. Toxicol Pathol* 29(1):41-48, 2001) described adrenal gland structure and function and mechanisms of chemically induced lesions. The following comments related to the adrenal medulla were of note:

(a) the adrenal medulla is a less common site for chemical-induced toxicity than is the adrenal cortex, although proliferative lesions "occur frequently" either spontaneous or as a result of dietary factors or exposure to chemicals.

(b) there is a "strong genetic component to the pathogenesis of pheochromocytomas in rats", being more common in certain strains (e.g., Wistar, Long-Evans, Sprague-Dawley) than others (e.g., Osborne-Mendel, Charles River, Holtzman) and in males compared to females.

(c) in addition to genetics, a variety of factors are associated with the pathogenesis of pheochromocytomas, e.g., "chronic high levels of growth hormone or prolactin associated with pituitary tumors, dietary factors, and stimulation of the autonomic nervous system". (No references were cited for the prolactin association.)

(d) a number of agents induce pheochromocytomas, including reserpine, Vitamin D, and "slowly absorbed sugars and starches". Reserpine induces adrenal medullary hyperplasia and pheochromocytomas, presumably due to neural stimulation of the adrenal medulla as a result of drug-induced hypotension. In some rat strains (e.g., F344), pheochromocytomas may be associated with "...more severe chronic progressive glomerulopathy...". Hypercalcemia has been postulated to underlie the cellular proliferation and pheochromocytomas associated with Vitamin D and sugars/starches. Although the mechanism(s) by which hypercalcemia may exert this effect is unknown, it is hypothesized that hypercalcemia-induced catecholamine synthesis may be involved.

•Roe and Bar (Roe FJC, Bar A *Human Toxicol* 4:27-52, 1985) reviewed published literature on the relationship between adrenal medullary hyperplasia and endocrine disturbances. The following were of note:

(a) in humans, proliferative lesions of the chromaffin cells of the adrenal medulla are rare (i.e., 0.005%), and "...in general, phaeochromocytomas in humans contain much higher quantities of noradrenaline, and sometimes also of adrenaline, than normal adrenal medullary cells." In the rat, examine of adrenal medullary tissue by H & E does not allow for a distinction between chromaffin-positive and chromaffin-negative cells. Therefore, it is uncertain whether the term, "pheochromocytoma", always refers to proliferation of cells capable of synthesizing catecholamines. Related to this was the distinction between functional and non-functional adrenal medullary tumors, and the suggestion that such tumors may be non-functional in rats. The functionality of these tumors in humans is also controversial. It would appear that, in both humans and rats, pheochromocytomas are generally benign.

(b) in the rat, pheochromocytomas may result from a variety of factors, including ionizing radiation, drug administration (e.g., nicotine, "various neuroleptics"), and endocrine imbalance (e.g., increased growth hormone). In all cases of drug-induced adrenal medullary hyperplasia and neoplasia observed by the authors, "...both kinds of lesion have been chromaffin-negative and the lesions have arisen against a background of high incidences of tumours of endocrine origin at other sites both in treated and control animals." Data from an NIH/NCI study of reserpine were summarized by the authors in the following Table 2 (from the published article):

Table 2 NCI study on reserpine in groups of 50 F344 rats (NIH, 1979)

	Males			*Females*		
Disease	*Control*	*Low dose*	*High dose*	*Control*	*Low dose*	*High dose*
Phaeochromocytoma	3	18**	24**	1	3	4
Pituitary adenoma	17	13	6*	21	27	28
Mammary fibroadenoma				14	18	14

Significance of difference from controls: *$P < 0.01$; **$P < 0.001$

Data on several sugars/sugar alcohols were summarized by the sponsor in the following Table 5 (from the published article):

Table 5 Incidences of adrenal medullary hyperplasia or neoplasia in rats exposed to xylitol, sorbitol or sucrose for 79 or more weeks [Review by A. B Russfield (unpublished work) of data reported by Hunter *et al.*, 1978 a]

% xylitol (X), sorbitol (So) or sucrose (Su) in diet	*0*	*2(X)*	*5(X)*	*10(X)*	*20(X)*	*20(So)*	*20(Su)*
Males							
No. of rats dying or killed at or after 79 weeks for which adequate sections were available	30	32	34	36	35	40	33
% with hyperplasia of one or both adrenal medullae	10	22	24	25	29	38**	6
% with neoplasia of one or both adrenal medullae	17	6	9	8	31	13	6
Females							
No. of rats dying or killed at or after 79 weeks for which adequate sections were available	42	38	37	41	44	46	39
% with hyperplasia of one or both adrenal medullae	5	5	24*	29**	25**	17	3
% with neoplasia of one or both adrenal medullae	2	0	5	5	14	11	8

Significance of difference from controls: * $P < 0.05$; ** $P < 0.01$
Trend statistics for xylitol: hyperplasia, males not significant and females*; neoplasia, males* and females**

(c) in comparing humans and rats, it was noted that in both humans and rats, adrenal medullary tumors may be associated with other endocrine tumors (e.g., thyroid, parathyroid). The authors stated that "...in rats, high incidences of adrenal medullary proliferative lesions are rarely seen in the absence of high incidences of tumours of other endocrine sites. This is true for the 'spontaneously' occurring adrenal lesions seen in untreated control rats..., also for lesions seen in rats exposed to various neuroleptic drugs...". The data for "...one such drug [neuroleptic]..." were summarized in the following Table 9 (from the published article):

Table 9 Percentages of control and treated rats bearing adrenal medullary and certain other endocrine tumours in a 2-year carcinogenicity study on a particular neuroleptic drug in Wistar rats

	Males		*Females*	
	Control	*High dose*	*Control*	*High dose*
Adrenal				
medulla	15	72***	19	24
cortex	7	6	16	4
Mammary	0	13*	52	78***
Pancreas endocrine	3	42***	3	28***
Pituitary	21	67***	73	82
Thyroid				
C-cell	26	8**	15	8
follicular	1	6	10	8

* Significantly higher than controls $P < 0.05$ ** Significantly lower than controls $P < 0.01$
*** Significantly higher than controls $P < 0.001$

(The neuroleptic tested was not identified.)

(d) several of the agents producing adrenal medullar tumors (e.g., lactose, xylitol) increase Ca absorption from the GI tract. The authors suggested that the rat is less able to tolerate excessive levels of absorbed Ca than other species, e.g., mouse, although they relate this possibility to progressive nephropathy: "The rat..., particularly the overfed rat...is highly susceptible to a form of progressive nephropathy which predisposes to parathyroid hyperplasia and metastatic calcification. The existence of these renal changes may make it more difficult for rats that are absorbing excess amounts of calcium from the gut to avoid albeit short but physiologically

significant excursions into hypercalcemia which constitute an immediate cause of the adrenal medullary lesions." They conclude that there is no human equivalent to the "...epizootic adrenal medullary proliferative disease..." observed in rat.

•Lynch *et al* (Lynch BS *et al. Reg Toxicol Pharmacol* 23:256-297, 1996) reviewed the available data on the association between low digestable carbohydrates (mannitol) and adrenal medullary proliferative lesions in one or more strains of rat. In considering the relevance of adrenal medullary tumors in rats to humans, the authors noted that the anatomy of the adrenal medulla in rat and human are generally similar; however, important anatomical differences exist: (a) in rat, there are two distinct populations of chromaffin cells, i.e., epinephrine- and norepinephrine-producing cells. In humans, there is no such distinction. (b) a cell type referred to as "the small granule containing cell" is present in rat, but not in human, adrenal medulla. The function of this cell type is "largely unknown". (c) "...the peptide and protein composition of secretory granules differs between rats and humans". However, the authors did indicate that the significance of these differences is unclear.

Lynch *et al.* (1996) discussed the differences in responsiveness to Ca between rats and other species, although it was noted that "...the channels in human chromaffin cells have not yet been characterized..." Nonetheless, it was suggested that rat is potentially more sensitive to alterations of Ca homeostasis than are other animal species for which information is available. Also, rat adrenal medulla would appear to be more sensitive to mitogenic stimulation (via, e.g., increased catecholamine stimulation) than the human adrenal medulla. According to Lynch *et al* (1996),

> "The most notable difference between rats and humans is the frequency with which spontaneous lesions of the adrenal medulla develop. In the rat, the spontaneous incidence of proliferative lesions of the adrenal medulla, including diffuse and focal hyperplasia as well as pheochromocytomas, is considerably greater and shows much more variability than similar lesions in the normal human population...The spontaneous incidence rate of tumors of the adrenal medulla in rats ranges from 0.5% in the Holtzman rat strain....to 16% in the F344 rat...and to 69% in the Wistar rat..." In contrast, the spontaneous incidence rate in humans has been reported to range from 0.005 to 0.1%..., with most data favoring the lower part of this range....Interestingly, mice, like humans, have chromaffin cells resistant to *in vitro* mitogenic stimuli...Mice also have a low spontaneous incidence of adrenal medullary lesions (about 1%)..."
>
> "A second important species difference is that pheochromocytomas are inducible in rats by many pharmacologically unrelated substances...but have never been reported to be associated with any substance in humans. Induction of pheochromocytomas in rats appears to reflect an exacerbation of their tendency for spontaneous development..."
>
> "A third difference...is that in rats these lesions are generally not hyperfunctional (i.e., do not induce symptoms of hypertension)....In humans, however, pheochromocytomas are often accompanied by symptoms indicative of hypertension (i.e., increased catecholamine output)..."

Lynch *et al* (1996) stated that "...the adrenal medullary proliferative lesions reported in rats administered high dietary concentrations of certain polyols is a species-specific phenomenon, likely of no relevance to humans." This conclusion was based, in part, on the lack of relevance to humans of the mechanism proposed to underlie the sugar alcohol-induced adrenomedullary tumors, i.e., altered Ca homeostasis. Sugar alcohols have been demonstrated to produce hypercalcuria "...and to a lesser extent increased serum calcium concentrations...", an effect thought to be due to increased Ca absorption from the gut. The authors summarized data on xylitol-induced lesions from a study conducted in Wistar-derived animals) in the following Table 4 (taken directly from the published article):

TABLE 4
Effects of Xylitol-Calcium on Incidence of Adrenal Medullary Proliferative Lesions in Rats

Group	Treatment	No. with animals	No. with hyperplasia (%)	No. with neoplasia (%)	Urinary calcium (mg/24 hr) Week 13/14	Week 32/32	Week 53/54
Control	0% Xylitol/0.4% Ca	63	40 (64)	6 (9.5)	0.6	0.6	0.8
1	20% Xylitol/0.4% Ca	56	31 (55)	24 (43)	6.1	11.1	7.0
2	20% Xylitol/0.2% Ca	57	39 (68)	17 (30)	2.7	8.6	7.8
3	20% Xylitol/0.05% Ca	63	55 (87)	7 (11)	0.8	2.9	3.0

The authors noted that, although a decrease in dietary Ca reduced the no. of rats with neoplasia, the total incidence of hyperplasia + neoplasia remained the same regardless of dietary Ca content. Also, "...several human studies conducted with [various sugar alcohols] have shown that several polyols either inhibited calcium absorption or produced no changes in the urinary calcium levels..." The authors cited studies demonstrating that other compounds, e.g., Vitamin D and various NSAIDS, may also produce adrenal medullary cellular proliferation as a result of increasing Ca absorption.

The authors concluded that "Because hypercalciuria (inferred to indicate increased absorption) occurs in a dose-related manner in the rat, but not to any significant extent in humans ingesting up to 100 gm of individual polyols per day...and because altered calcium homeostasis (a phenomenon that predisposes to adrenal medullary tumor development in rats) does not appear to operate in humans ingesting dietary amounts of polyols, the rat adrenal medullary lesions appear, on mechanistic grounds alone, to be irrelevant to humans." This conclusion was based on the following: (a) sugar alcohols and their metabolites are not genotoxic, (b) no effect of polyols on the adrenal medulla in either mice or dogs, (c) the development of adrenal medullary cellular proliferation in the rat occurs only at high doses, (d) no sugar alcohol/polyol-induced toxicity or adrenal medullary tumors have been demonstrated in humans, (e) species differences in structure and function of the adrenal medulla, (f) susceptibility of the adrenal medulla in rat to spontaneous and induced cellular proliferation, (g) strong evidence implicating increased Ca absorption as a mechanism underlying increased adrenal medulla cellular proliferation in the rat, and the lack of such evidence (i.e., an increase in Ca absorption) in humans consuming up to 100 gm per day of polyols.

•Kurokawa *et al.* (Kurokawa Y *et al. JNCI* 74:715-723, 1985) reported the results of a 2-yr carcinogenicity study of retinol acetate conducted in F344 rats. Retinol acetate was administered in the drinking water at doses of 0 (vehicle "beadlets"), 0.125%, and 0.25%. The results of genotoxicity studies indicated that retinol acetate is mutagenic, testing positive in the Ames assay (TA100, but negative with TA98), the *in vitro* chromosomal aberration assay in human fibroblasts, among others. Adrenomedullary tumors were significantly increased in HD males and females. According to the authors, tumors of other organs (i.e., mammary gland in males, thyroid and clitoral glands in females) were decreased. However, according to the data in Table 3, clitoral gland tumors were increased in HDF (see data from authors' Table 3 below).

TABLE 3.—*Tumor incidences in F344 rats given RAC*

Tumor	Incidences,[a] No. (%), in: Males after RAC doses of: 0%	0.125%	0.25%	Females after RAC doses of: 0%	0.125%	0.25%
Preputial (clitoral) gland	1 (2)	5 (10)	5 (10)	1 (2)	1 (2)[b]	7 (14)[b]
Adenoma	1	4	5	1	1	7
Adenocarcinoma	—	1	—	—	—	—

Neoplastic findings in adrenal medulla were summarized in the following table (taken directly from the published article):

TABLE 4.—*Incidences of adrenal medulla neoplastic lesions in F344 rats given RAC*

RAC dose, %[a]	Effective No. of rats	No. (%) of rats bearing:				
		Pheochromocytomas			Hyperplasias	Bilateral lesions
		Benign	Malignant	Both types		
Males						
0 (controls)	49[b]	15(30.6)	3(6.1)	18(36.7)	9(18.4)	13(26.5)
0.125	50	23(46.0)	4(8.0)	27(54.0)	8(16.0)	25(50.0)[c]
0.25	48	28(58.3)[c]	11(22.9)[c]	39(81.3)[d]	7(14.6)	36(75.0)[d]
Females						
0 (controls)	50	3(6.0)	0	3(6.0)	8(16.0)	5(10.0)
0.125	49[b]	10(20.4)[c]	1(2.0)	11(22.4)[c]	16(32.7)	7(14.3)
0.25	48	20(41.7)[d]	0	20(41.7)[d]	15(31.3)	24(50.0)[d]

[a] Percent RAC in drinking water.
[b] 1 ganglioneuroma.
[c] $P<.05$ relative to controls.
[d] $P<.001$ relative to controls.
[e] $P<.01$ relative to controls.

The sponsor noted that the incidence of pheochromocytomas in controls was high ("...almost equal to the highest rates observed in the historical controls."), suggesting that "...the animals used in our bioassay were relatively susceptible to the unknown factors responsible for the development of adrenal medullary tumors." Increased Ca absorption was proposed as a possible mechanism underlying the increase in pheochromocytomas; serum Ca was significantly increased in LDM (10.8 ± 0.6, 11.4 ± 0.9, and 11.0 ± 0.7 mg/dL in CM, LDM, and HDM, respectively) and at both doses in females (10.3 ± 0.7, 10.8 ± 0.6, and 10.9 ± 0.5 mg/dL in CF, LDF, and HDF, respectively). The authors noted that a number of compounds have been shown to increase the incidence of pheochromocytomas, e.g., "...nicotine, thiouracil, growth hormone, and radioation...reserpine and 4-chloro-*m*-phenylenediamine...).

VII. REPRODUCTIVE AND DEVELOPMENTAL TOXICOLOGY

1. RISPERDAL CONSTA™ (NDA 21-346): Response to FDA Action Letter of June 29, 2002.

The sponsor responded to the FDA action letter (6/29/02) in an integrated summary response. The portion of the sponsor's integrated summary relevant to Reproductive and Developmental Toxicology is summarized below:

Response to Request #2 (FDA's Request #2 [below] is copied directly from the sponsor's report):

> **2. No reproductive toxicology studies were conducted using the IM depot formulation of risperidone. The reproductive toxicology studies conducted using oral risperidone were used to support the IM depot formulation. Findings observed in the 1-yr chronic and the 2-yr carcinogenicity studies in rat using the IM depot formulation suggest that the IM depot formulation may have different toxicities than the oral formulations [for which a complete battery of reproduction studies was conducted]. Specifically, the osteodystrophy detected in the 1-yr and 2-yr studies and the additional tumor types observed with the IM depot formulation raise a concern that the oral reproductive toxicity studies may not provide an adequate test of the potential for the risperidone IM depot formulation to produce reproductive toxicity. It is recommended that, at a minimum, you conduct an embryofetal development study in rat using the clinical IM depot formulation. It is further recommended that an oral dose group be included in the study.**

The sponsor indicated that a dose-range finding study is ongoing. The design of the definitive study will be based on the results of the dose-range finding study.

Regarding the osteodystrophy observed in the i.m. depot, but not the oral, nonclinical studies, the sponsor has concluded that the osteodystrophy findings are "...a consequence of the non-neoplastic renal changes, which are most probably a substrain-specific response to risperidone treatment rather than a response specific to the IM route of administration...". The sponsor based this conclusion on the following:

1. Published literature reports an association between osteodystrophy and "...parathyroid hyperplasia and severe non-neoplastic renal changes in male Fischer 344 rats...". The sponsor provided two references relevant to this association.

   (a) Bucher *et al.* (Bucher JR *et al. Toxicol Sci* 42:1-12, 1998) reported the results of toxicity and carcinogenicity studies of Oxazepam (marketed as an anxiolytic under the tradename, Serax) conducted in F344 rats. Oxazepam was administered in the diet at doses of 0, 625, 2500, 5000, and 10,000 ppm (≈25, 100, 225, and 600 mg/kg in males and ≈25, 110, 220, and 615 mg/kg in females).

   In kidney, a "...slightly greater..." incidence ("...at the upper limit of the historical range...") of renal tubule adenomas was detected in males at 100 mg/kg. Based on this, additional sections of kidney were examined (i.e., "...an extended step-section evaluation...") in males only. "Additional rats with renal tubule adenoma and numerous additional occurrences of renal epithelial hyperplasia were identified." The data were summarized in Table 2 (taken directly from the published study):

TABLE 2
Incidences of Neoplasms and Nonneoplastic Lesions of the Kidney of Rats in the 2-Year Feed Study of Oxazepam

	Parts per million				
	0	625	2500	5000	10,000 (Stop-exposure)
Male					
Single sections (standard evaluation)					
Number examined microscopically	50	50	50	50	42
Nephropathy	49[a] (1.9)[b]	44 (2.3)	49 (2.7)**	50 (3.2)**	42 (3.3)**
Renal tubule hyperplasia	0	1 (1.0)	3 (2.3)	1 (2.0)	0
Renal tubule adenoma	1[c]	0	3	1	0
Step sections (extended evaluation)					
Number examined microscopically	50	50	50	50	50
Renal tubule hyperplasia	5	6	9	8	21**
Renal tubule hyperplasia, oncocytic	0	1	2	2	3
Renal tubule adenoma, multiple	0	1	1	1	1
Renal tubule adenoma, all	1	1	4	5	6*
Renal tubule, oncocytoma	0	0	0	1	0
Single sections and step sections (combined)					
Number examined microscopically	50	50	50	50	45
Renal tubule hyperplasia	5	6	12*	9*	21**
Renal tubule hyperplasia, oncocytic	0	1	2	2	3
Renal tubule adenoma, multiple	0	1	2	1	1
Renal tubule adenoma, all	2	1	7*	6	6*
Renal tubule, oncocytoma	0	0	0	1	0
Female					
Number examined microscopically	50	50	50	50	1
Nephropathy	32 (1.1)	43** (1.3)	41** (1.3)	48** (1.7)**	1 (2.0)

[a] Number of animals with lesion.
[b] Average severity of lesions in affected animals: 1, minimal; 2, mild; 3, moderate; 4, marked.
[c] Historical dosed feed study control incidence 0.7% ± 1.5%, range 0 to 6%.
* Significantly different ($p < 0.05$) from the control group by the logistic regression test (incidence) or by the Mann–Whitney U test (severity)
** $p < 0.01$.

As noted in Table 2, in males the severity, but not the incidence, of nephropathy was increased, being characterized as "mild" in control and LD grps and "moderate to marked" in the higher dose grps. (Dosing at the HD was terminated after Wk 26 due to poor condition.) The authors also noted that "Parathyroid gland hyperplasia and fibrous osteodystrophy of the bone (secondary to renal lesions) were increased in exposed males; the incidences of parathyroid gland hyperplasia (0 ppm, 3/39; 625 ppm, 6/41; 2500 ppm, 9/46; 5000 ppm, 16/40) and fibrous osteodystrophy of the bone (0/50, 1/50, 6/50, 8/50) occurred with positive trends." In females, there was an increase in nephropathy at all doses; the severity was significantly increased only at 5000 ppm (220 mg/kg). There was no mention of parathyroid gland hyperplasia or osteodystrophy in females.

The authors ascribed the nephropathy to normal aging, but noted that "...this condition is worsened when the animals are maintained on a relatively high-protein diet, such as the NIH-07 diet used in these studies...". The authors also noted that "The parathyroid gland hyperplasia and fibrous osteodystrophy of bone that occurred in the males are consistent sequelae to severe nephropathy and secondary hyperparathyroidism of chronic renal failure..."

(b) Nyska *et al.* (Nyska A. *et al. Toxicol Pathol* 27(4):456-462, 1999) investigated the potential correlation between "...the severity of chronic progressive glomerulonephropathy (CPN) and the incidence of adrenal pheochromocytoma..." in selected studies conducted in male Fischer 344 rats by NTP. The authors also assessed the effects of CPN on mineral metabolism. The relationship between CPN and parathyroid function was described by the authors as follows:

> "Chronic renal failure is known to be associated with the inability to secrete phosphate, which results in hyperphosphatemia. Also associated with this disease are reduced production of the active metabolite of vitamin D (because of the decreased number of nephrons) and hypocalcemia (secondary to decreased calcium intestinal absorption)....The low serum calcium levels stimulate parathyroid hormone secretion. In severe cases of CPN in rats, which are associated with disturbed calcium/phosphorus homeostasis, there might be chronic stimulation of the chromaffin cells toward proliferation, which may eventually lead to hyperplasia and neoplasia. Thus, the possible association between the severity of CPN, associated changes related to secondary hyperparathyroidism, and adrenal pheochromocytoma were examined in selected studies carried out at the....(NTP)."

In the studies selected for examination, CPN was graded for severity on a scale of 0 to 4 (based on the % of renal tissue involved), with "0" being no involvement and "4" being "marked, >76% involvement". In the inhalation studies, the incidence of grade 4 CPN in controls was 237/900. In the dietary studies, the incidence of grade 4 CPN in controls was 73/894. According to the authors, "The incidences of parathyroid hyperplasia, mineralization of the glandular stomach, and osteodystrophy, which are often associated with severe CPN...were low or variable among the studies (data not shown). These nonneoplastic lesions were not significantly correlated with adrenal pheochromocytoma". However, the authors conclude that "In the present investigation, severe CPN was often associated with parathyroid hyperplasia and fibrous osteodystrophy" and noted that "Secondary hyperparathyroidism associated with chronic renal disease has been described in humans, rats, and dogs....Fibrous osteodystrophy is known to result from increased secretion of parathyroid hormone as a manifestation of primary or secondary hyperparathyroidism...It is considered to be the most significant consequence of increased parathyroid hormone levels because of chronic renal disease and associated uremia..."

The authors further describe the relationship between severe CPN or "Advanced renal failure" as follows:

> "Advanced renal failure may lead to hypercalcemia...Hypercalcemia in the rat (secondary to hyperparathyroidism) is well documented in the literature...Hypercalcemia leads to calcification of normal tissue...This metastatic calcification of soft tissues occurs in rats as relatively late manifestation of CPN...Metastatic calcification occurs in epithelial cells and basement membrane of the gastric glands; connective tissue of the lamina propria and muscularis mucosae in the stomach; muscular layers in the stomach and intestine; epithelial cells and the basement membranes of the renal tubules and Bowman's capsule in the kidney; alveolar walls in the lung, tunica media in the large arteries; and tunica media of the moderate-sized arteries in the kidney, testis, pancreas, stomach, heart, tongue, lulng, spleen, salivary gland, and skeletal muscle..."
>
> In human patients suffering from chronic renal disease associated with hyperparathyroidism, the serum levels of calcium, alkaline phosphatase, and parathyroid hormone and associated soft-tissue calcification were reduced after subtotal parathyroidectomy...Although serum Ca^{+2} analysis was not performed in the studies included in our investigation, the pathologic evidence of metastatic mineralization (i.e., gastric glandular mineralization and bone fibrous

osteodystrophy) is evidence for a state of hypercalcemia. An association between hypercalcemia and increased incidence of pheochromocytomas has been previously reported in rats..."

It was also noted that some drug (e.g., retinol acetate) may produce hypercalcemia by increasing Ca absorption from the gut.

The authors pointed out that "...most NTP studies with marked treatment-related increases in the severity of CPN did not show a corresponding increase in adrenal pheochromocytoma incidence." Although the reason for this is unknown, it was hypothesized that "...one possible explanation is that although the severity of CPN may be increased, the resulting enhanced CPN in certain studies may not be severe enough to cause marked alterations in the calcium homeostasis. Interestingly, each of the...studies with the strongest increase in pheochromocytoma incidence...showed significant increases in both bone fibrous osteodystrophy and severity of CPN. In contrast, many other studies with less striking increases in CPN severity and/or no corresponding increase in fibrous osteodystrophy showed no increase in the incidence of pheochromocytoma..."

2. The sponsor summarized the serum Ca and parathyroid gland findings in the 12-mo IM toxicity (interim sacrifice) and the 2-yr IM carcinogenicity assessment in rat: (a) increased serum Ca in females at 5 and 40 mg/kg and in males at 40 mg/kg at the 12-mo interim sacrifice, (b) increased serum Ca at 40 mg/kg in males and females at the end of the 2-yr study, (c) osteodystrophy was observed at the 12-mo sacrifice (nos), and in both sternum and stifle joint (femur) in males and females at the end of the 2-yr dosing period, (d) no drug-related microscopic changes in parathyroid gland at either the 12-mo or the 2-yr examination periods. The sponsor also noted that "In the oral rat carcinogenicity study, ...no treatment-related changes in renal histology and serum calcium levels, and no signs of osteodystrophy were seen. The parathyroids were not affected either upon routine examination."

3. Parathyroid tissue from "the first 20" terminally sacrificed C and HD male rats in the 2-yr carcinogenicity study were re-analyzed. It was determined that there was a "slight, statistically significant increase in the mean number of glandular cell nuclei per unit area, indicative of the occurrence of slight diffuse hyperplasia." (These data are reviewed separately, cf. CARCINOGENICITY section.) Therefore, it was concluded that "...the response of the parathyroid and the development of osteodystrophy are secondary to disturbance of serum calcium levels caused by the age-related non-neoplastic renal changes at the 40 mg/kg/2 weeks dose level."

VIII. SPECIAL TOXICOLOGY STUDIES

1. Study title: Eight-week mechanistic study in the Wistar Wiga and Wistar Hannover rat (Study no: EDMS-PSDB-2412254, Exp. No. 5626, Conducting laboratory and location: Date of study initiation: 8/27/02, GLP, QA report: Y)

Purpose: to compare serum prolactin effects following administration of risperidone as a drug-diet admixture and an i.m. depot formulation to Wistar Wiga and Wistar Hannover rats, respectively.

Drug, lot #, radiolabel, and % purity: risperidone [batch no. ZR064766PUA401 (diet), ZR06466EIA771 (microspheres)]

Formulation/vehicle: drug-diet admixture, depot formulation (0.1 mL/100 gm body wt). The depot formulation consisted of a suspension of microspheres (batch no. 164-3500AA); the composition of the microspheres was not specified.

Animals:

Wistar Hannover

≈12 wks of age (Day 0), body wt: 265-341 gm (Day 0), 80 males/grp

Wistar Wiga

≈12 wks of age (Day 0), body wt: 315-448 gm (Day 0), 80 males/grp

Animals were housed individually.

Methods:

Doses: 0, 10, 40 mg/kg. Dose grps and frequency of dosing was summarized by the sponsor as follows (directly from submission):

Dosage groups: (mg kg b.w.)	0 (group A: control Wistar Wiga)
	*10 (Group B: Wistar Wiga)
	40 (Group D: Wistar Wiga)
	0 (group E: control Wistar Hannover)
	*10 (Group F: Wistar Hannover)
	40 (Group G: Wistar Hannover)
Frequency of dosing:	*continuous
	on 4 occasions, each occasion spread by 2 weeks

Doses were administered over an 8-wk period. According to the above, HD animals (both Wiga and Hannover) received i.m. doses every 2 wks; LD animals (both Wiga and Hannover) received risperidone via the diet which was available continuously throughout the 8-wk dosing period.

Analysis of the drug-diet admixture documented drug concentration, homogeneity, and stability. According to the sponsor, the drug concentration varied with an acceptable range (80-120%), and was adjusted in order to maintain doses during the study. Achieved doses were provided (e-report; pg 172); weekly mean doses ranged from 9.63 to 11.4 mg/kg in Wiga rats and from 9.14 to 12.1 mg/kg in Hannover rats.

I.M. injections were administered into the left or right m. biceps femoris (hindlimb) on Days 0, 14, 28, and 42. Control groups were not treated.

Observations and times:

Clinical signs: daily.

Body weight: recorded "initially", weekly during the dosing period, and at the end of the study.
Food consumption: recorded at weekly intervals during the dosing period.
Clinical pathology: at autopsy, blood samples were collected from animals (#7, 28, 107, 128, 207, 228, 307, 407, 428, 507, and 528) sacrificed on Day 49 for quantitation of serum creatinine and creatinine clearance, and from all animals sacrificed on Day 56/57 for quantitation of a battery of clinical chemistry parameters (Na, K, Cl, Ca, P_i, total protein, albumin, glucose, cholesterol, TG, BUN, creatinine, total bilirubin, alkaline phosphatase, AST, ALT, GGT).

Urinalysis was conducted on 30 rats/grp on Day 45 or 46; urine samples were collected over a 12-hr period (daytime). Urine parameters evaluated consisted of the following: specific gravity, pH, volume, glucose, ketones, occult blood, proteins, microscopic analysis of sediment, Na, K, Cl, Ca, IPH (phosphate), BUN, creatinine, creatinine clearance (calculated).

For analysis of serum prolactin, 10/grp were sacrificed "starting on hour 0" (i.e., 7:00 a.m.) on Day 49 and 10/grp/time point were sacrificed on Day 56/57 (0 [7:00 a.m.], 4, 8, 12, 16, 20, and 24 hrs).
Gross pathology/organ wt: animals were sacrificed under anesthesia (Nembutal 0.07 mL/100 gm body wt i.p.), and "Samples of mammary gland, kidney (bilateral), seminal vesicles and prostate (dorsal+ventral)" and organs with gross lesions were collected from animals sacrificed on Day 56/57. Tissues were preserved in 10% buffered formalin. It was noted that "The corpses may have been kept in the refrigerator until organ sampling." The duration of refrigeration was not specified. Kidney wt was recorded.
Histopathology: the tissues selected for microscopic evaluation were stained with H & E for examination. Kidney sections were prepared from 18 C and 18 drug-treated Hannover rats, i.e., 10 rats at time point 0 (at 7:00 a.m.) on Day 56 and "the first 8 rats" at the first time point (at 11:00 a.m.) on Day 56. Severity was scored as follows: 0 = absent, 1 = minimal or small quantity, 5 = severe or large quantity (the severity of scores of 2, 3, and 4 was not defined). In the analysis, "When tissues were not scored for a histological change the "0.00" (zero) was used for further statistical analysis".

Additional kidney sections from the same animals were immunostained (nos) for quantitation of PCNA. This procedure and analysis was repeated due to unexpected results (i.e., a significant decrease in PCNA-positive nuclei in drug-treated animals) obtained in the first analysis.
TK: blood samples were collected at the same times as described for quantitation of serum prolactin. Risperidone and 9-OH-risperidone were quantitated in plasma using LC/MS/MS (LOQ = [redacted] ng/mL). The data were presented for risperidone, 9-OH-risperidone, and the "active moiety" (i.e., risperidone + 9-OH-risperidone).

Results:

Mortality: there were no unscheduled deaths.
Clinical signs: drug-related clinical signs consisted of ptosis and decreased general activity, and were observed with dietary and i.m. depot administration in both Wiga and Hannover substrains. The incidences are summarized in the table below. The sponsor noted that both signs were observed to a "slightly" greater extent in Wiga rats (compared to Hannover) and with dietary administration (compared to i.m. depot). The sponsor also noted that, with i.m. depot dosing, ptosis was seen most frequently after the first dose.

SIGN	WIGA			HANNOVER		
	C	DIET (10 mg/kg)	I.M. (40 mg/kg)	C	DIET (10 mg/kg)	I.M. (40 mg/kg)
ptosis	0/80	75/80***	61/80***	0/80	59/80***	41/80***
decreased activity	0/80	76/80***	50/80***	0/80	65/80***	35/80***

*** $p<0.001$

Subcutaneous masses were observed with i.m. depot administration in both Wiga (3/80) and Hannover (2/80) rats. These were considered by the sponsor to reflect "probably residual of injected volume".

body weight: mean body wt was significantly, but not markedly, reduced in all treated grps throughout most of the dosing period. Final mean body wts were decreased by 8 and 4% at 10 (diet) and 40 (i.m. depot) mg/kg (compared to untreated C), respectively, in Wiga rats and by 10 and 5% at 10 (diet) and 40 (i.m. depot) mg/kg (compared to untreated C), respectively, in Hannover rats.

Overall mean body wt gain was reduced by 37 and 16% at 10 (diet) and 40 (i.m. depot) mg/kg, respectively, in Wiga rats and by 56 and 30% at 10 (diet) and 40 (i.m depot) mg/kg, respectively, in Hannover rats.

The sponsor noted that body wt was "slightly more" affected in dietary grps.

food consumption: in the Wiga rat, food consumption was consistently reduced in the 10-mg/kg (diet) grp from Wk 3 on (5-11%); at 40 mg/kg (i.m. depot), food consumption was increased during Wks 4-5 (3-4%).

In the Hannover rat, food consumption was transiently increased at 10 mg/kg (diet) (7-9% during Wks 1-2), but consistently decreased from Wk 3 on (5-13%); at 40 mg/kg (i.m. depot), food consumption was reduced only during the last 2 wks of dosing (4%).

clinical chemistry: serum creatinine was fairly similar among grps at Wk 7, although slightly lower in Hannover rats at 10 mg/kg (diet) (0.48 mg/dL vs 0.56-0.69 mg/dL for other grps, including Cs).

The following findings were noted in all treated grps: (a) decreases in K (Wiga: 8% in dietary and i.m. grps; Hannover: 10 and 6% in dietary and i.m. depot grps, respectively), (b) small, but significant increases (1-2%) in Ca in all treated grps, (c) decreases in glucose (Wiga: 17 and 9% in dietary and i.m. depot grps, respectively; Hannover: 14 and 6% in dietary and i.m. depot grps, respectively), (d) decreases in urea N (Wiga: 9 and 7% in dietary and i.m. depot grps, respectively; Hannover: 11 and 7% in dietary and i.m. depot grps, respectively), (e) decreases in creatinine (6-7%) in all treated grps, (f) decreases in alk phos (Wiga: 12 and 10% in dietary and i.m. depot grps, respectively; Hannover: 18 and 14% in dietary and i.m. depot grps, respectively).

Other notable findings were as follows: (a) an 8% increase in P_i in Wiga rats at 10 mg/kg (dietary), (b) an increase in cholesterol (34%) in Hannover rats at 10 mg/kg (dietary), (c) increases in total bilirubin (17%) in both Wiga and Hannover rats at 10 mg/kg (dietary). ALT and AST were not affected in the 10-mg/kg grps (dietary). ALT was decreased (12%) in Hannover rats at 40 mg/kg (i.m. depot), and AST was decreased in both 40-mg/kg (i.m. depot) grps.

urinalysis: the following findings were observed in all treated grps: (a) small (1-2%) increases in specific gravity, (b) decreases in pH (Wiga: 16 and 10% [1.1 and 0.7 units] in dietary and i.m. depot grps, respectively; Hannover: 19 and 12% [1.3 and 0.8 units] in dietary and i.m. depot grps, respectively), (c) decreases in urinary volume (Wiga: 40-41%; Hannover:

22-24%), (d) increases in ketones (Wiga: 110 and 150% in dietary and i.m. depot grps, respectively; Hannover: 28 (n.s) and 71% in dietary and i.m. depot grps, respectively), (e) increases in Ca (Wiga: 62 and 38% in dietary and i.m. depot grps, respectively; Hannover: 140 and 100% in dietary and i.m. depot grps, respectively), (f) increases in phosphate (Wiga: 66 and 35% in dietary and i.m. depot grps, respectively; Hannover: 140 and 80% in dietary and i.m. depot grps, respectively).

Other notable findings were as follows: (a) an increase in rbcs in Wiga rats at 10 mg/kg (dietary) (0.23 vs 0.00), (b) increases in wbcs in Wiga rats at 10 mg/kg (dietary) (0.5 vs 0.13 for C) and in both Hannover treated grps (0.00, 0.13, and 0.3 for C, 10 mg/kg [dietary], and 40 mg/kg [i.m. depot], respectively), (c) an increase in granular casts in Wiga rats at 40 mg/kg (i.m. depot) (0.17 vs 0.00 for C), (d) an increase in protein in Wiga rats (82 and 150% in dietary and i.m. depot grps, respectively), but a decrease in protein in Hannover rats at 10 mg/kg (dietary) (38%). (e) increases in Na in Hannover rats only (both grps: 48-46%), (f) increases in K in both Hannover treated grps (20 and 12% in dietary and i.m. depot grps, respectively), (g) decreases in Cl in Wiga rats (17 and 24% in dietary and i.m. depot grps, respectively) and increases in Cl in Hannover rats (49 and 20 [n.s.]% in dietary and i.m. depot grps, respectively), (h) increases in urea N only in Hannover rats (5 and 13% in dietary and i.m. depot grps, respectively), (i) decreases in creatinine in both dietary grps (11 and 14% in Wiga and Hannover rats, respectively).

Hormones: serum prolactin was elevated in all treated grps on Day 56-57. The data were summarized in the following sponsor's tables:

WISTAR WIGA

Dosage group	A : Control	B : 10 mg/kg/day	D : 40 mg/kg/2 weeks
Prolactin (ng/ml.h) AUC_{24h}	463	3351	2676
SE	(36)	(147)	(164)

WISTAR HANNOVER

Dosage group	E : Control	F : 10 mg/kg/day	G : 40 mg/kg/2 weeks
Prolactin (ng/ml.h) AUC_{24h}	247	2625	2200
SE	(36)	(147)	(164)

SE : Standard Error

The sponsor conducted additional statistical analysis of the serum prolactin levels. That is, in addition to the "additive model" used to assess differences in absolute prolactin levels, a "multiplicative model (employing the logarithm of prolactin concentrations) has been used to investigate the ratios of prolactin concentrations..." and an analysis comparing single samples collected on Day 49 vs Day 56. It was the sponsor opinion that "By employing the additive and multiplicative models of statistical analysis, the prolactin concentrations were viewed from different angles", that the multiplicative model "evaluates the magnitude of change in treated versus control animals, and therefore, "These models...yield fundamentally different results, which supplement each other."

Using the additive method, it was concluded that serum prolactin levels were higher (a) in the Wiga substrain regardless of route and (b) with oral dosing than with i.m. depot dosing. Using the multiplicative method, it was concluded that (a) the ratios of prolactin levels (IM/CT and OR/CT) were greater in Hannover (OR/CT and IM/CT ≈ 11 and 9-fold, respectively) than in Wiga rats (OR/CT and IM/CT ≈ 7 and 6-fold, respectively). An analysis of single samples collected on Day 49 vs 56 indicated that serum prolactin levels were different between these two sampling times only for Hannover rats. (There was no overall significant effect of Days.)

Gross pathology: gross findings were summarized in the following sponsor's tables:

	MALE WIGA RATS		
	Dosage group (mg/kg)		
Organ or tissue : observation	A:Control not treated	B:Dosed OR/FOOD:10	D:Dosed IM:40
	X/ N	X/ N	X/ N
Eye,adnexae : red	0/70	0/70	1/70
Jaw : malformed incisors	0/70	1/70	0/70
Kidneys : cyst	0/70	2/70	1/70
Kidneys : pale	1/70	0/70	0/70
Kidneys,renal pelvis : dark	1/70	0/70	0/70
Kidneys,renal pelvis : dilated	7/70	6/70	10/70
Mammary gland : stimulation	11/70	25/70*	24/70*
Prostate : stippled, yellow	8/70	69/70***	44/70***
Prostate : swollen	0/70	20/70***	2/70
Skin : alopecia	0/70	0/70	1/70
Subcutis : nodule, yellow	0/70	0/70	1/70
Subcutis : tissue mass, white	0/70	0/70	1/70
Urinary bladder : dilated	1/70	0/70	0/70

MALE HANNOVER RATS			
Organ or tissue : observation	Dosage group (mg/kg) E:Control not treated X/ N	F:Dosed OR/FOOD:10 X/ N	G:Dosed IM:40 X/ N
Kidneys : changed surface, rough	0/70	1/70	0/70
Kidneys : cyst	2/70	1/70	1/70
Kidneys : focus, dark	1/70	0/70	0/70
Kidneys : pale	1/70	2/70	1/70
Kidneys : pale, irregular, +	1/70	1/70	0/70
Kidneys : swollen	0/70	1/70	0/70
Kidneys,renal pelvis (content) : grit	1/70	0/70	1/70
Kidneys,renal pelvis : dilated	4/70	0/70	0/70
Mammary gland : inspissated secretion	0/70	0/70	7/70*
Mammary gland : stimulation	7/70	20/70*	21/70**
Prostate : stippled, yellow	1/70	70/70***	63/70***
Prostate : swollen	0/70	16/70***	1/70
Skin : alopecia	1/70	1/70	0/70
Subcutis : nodule, yellow	0/70	0/70	2/70
Urinary bladder : dilated	0/70	1/70	1/70

Significance computed by Chi-square test (two tailed) : * P < .05 ** P < .01 *** P < .001

+ This observation is already included in the major observation of this tissue

X : Number of positive animals
N : Total number of animals

Organ wt: absolute kidney wt was significantly decreased in Hannover rats (4-5% in both treated grps), consistent with decreases in body wt (relative to C). Body-wt corrected kidney wt was increased in both dietary grps (5-6%).

Histopathology: the incidences of microscopic changes in kidney were summarized in the following sponsor's table (only tissue from Hannover rats was examined for histopathology):

MALES

Organ or Tissue - Observation	Dosage group (mg / kg) Control Hannover	G Hannover:IM:40
Kidneys *Number examined:*	*18*	*18*
- (intra)tubular proteinaceous material (cortex)	10	9
- autolytic changes	0	18
- basophilic tubuli, (multi)focal	4	1
- cyst(s)/cystic	1	0
- dilated pelvis	3	0
- hyaline cast(s)	2	4
- inflammation (pelvis)	3	0
- inflammation, chronic, (multi)focal	0	1
- minerals (pelvis)	2	0

Significance versus Control Hannover computed by the Fisher Exact test (two tailed) : * P < .05 ** P < .01 *** P < .001
Statistics are only performed if more than 50 % of the animals of the group are examined

The sponsor addressed the increase in the incidence of "autolytic changes" in the 40-mg/kg grp by noting that "This was considered related to the design of the study. (Priority was given to blood sampling. Necropsy was performed afterwards.) The time

between the start of necropsy of group E [control] and group G [40 mg/kg] was estimated to be approximately 1 hour."

Cellular proliferation: the PCNA data were summarized in the following sponsor's tables:

Table : Mean number of PCNA positive renal corticotubular nuclei per mm^2 of cortex

Dose group (mg/kg)	Mean	SE	Significance p-value 2-tailed	Significance p-value 1-tailed
Control (0)	2.277	0.241		
High (40)	0.989	0.128	0.0002 ***	0.0001 ***

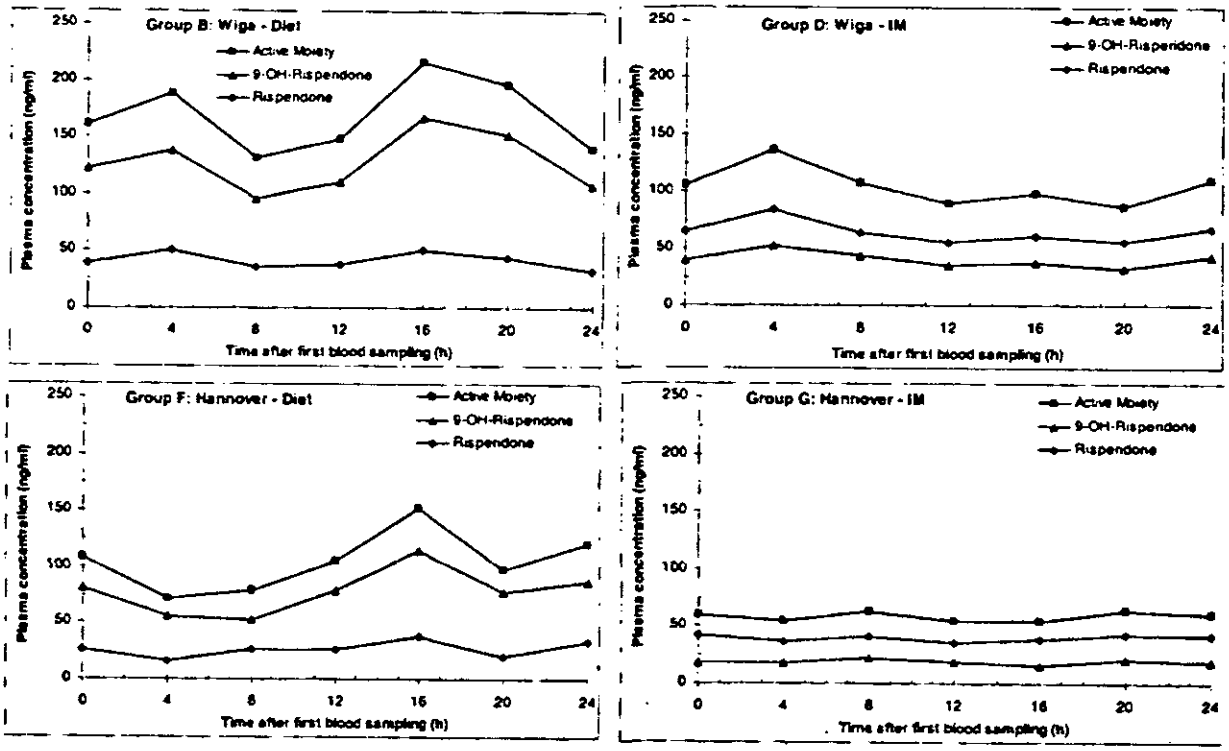
Significance computed by Mann-Whitney U test : * p<.05 ** p<.01 *** p<.001

The sponsor considered the decrease in PCNA-positive nuclei in the HD grp to be an artifact of the increased autolysis observed in this grp, based on the results of Study EDMS-PSDB-2498049.

TK: plasma levels of risperidone, 9-OH-risperidone, and the "active moiety" were summarized in the following sponsor's tables and figures:

Day	Time after first sampling (h)	Group B: Wiga - Diet		
		Risperidone (ng/ml)	9-OH-risperidone (ng/ml)	Active Moiety (ng/ml)
49	0	36.3 ± 11.0	127 ± 34	163 ± 44
56	0	38.5 ± 10.8	122 ± 28	160 ± 38
	4	50.3 ± 27.7	138 ± 52	188 ± 79
	8	35.4 ± 20.2	95.9 ± 33.8	131 ± 54
	12	38.2 ± 19.3	110 ± 45	148 ± 63
	16	50.3 ± 21.6	167 ± 40	217 ± 60
57	20	44.3 ± 13.5	152 ± 30	197 ± 43
	24	32.3 ± 16.8	107 ± 44	140 ± 60
56-57	C_{min} (C_{max}/C_{min}-ratio)	32.3 (1.6)	95.9 (1.7)	131.2 (1.7)
	C_{max}	50.3	167	217
	9-OH-risp./risp.-ratio		3.3 ± 0.9[1]	
	$AUC_{24\,h}$ (ng.h/ml)[2]	1016 ±60	3109 ±120	4125 ±178
	[95 % CI]	[893 - 1138]	[2867 - 3352]	[3766 - 4484]
Day	Time (h)	Group D: Wiga - IM		
49	0	61.8 ± 9.1	39.8 ± 9.1	102 ± 17
56	0	65.0 ± 10.5	39.8 ± 6.0	105 ± 16
	4	84.0 ± 26.3	52.4 ± 16.4	136 ± 43
	8	64.0 ± 17.0	43.5 ± 12.4	107 ± 29
	12	54.8 ± 14.8	34.9 ± 12.9	89.7 ± 27.2
	16	60.9 ± 13.4	36.8 ± 8.3	97.8 ± 20.8
57	20	54.8 ± 8.4	31.8 ± 7.3	86.6 ± 13.3
	24	67.4 ± 22.8	42.4 ± 15.5	110 ± 38
56-57	C_{min} (C_{max}/C_{min}-ratio)	54.8 (1.5)	31.8 (1.6)	86.6 (1.6)
	C_{max}	84.0	52.4	136
	9-OH-risp./risp.-ratio		0.6 ± 0.1[1]	
	$AUC_{24\,h}$ (ng.h/ml)[2]	1538 ±51	962 ±36	2501 ±84
	[95 % CI]	[1436 - 1642]	[890 - 1033]	[2330 - 2671]

Day	Time after first sampling (h)	Group F: Hannover - Diet		
		Risperidone (ng/ml)	9-OH-risperidone (ng/ml)	Active Moiety (ng/ml)
49	0	24.0 ± 16.2	78.6 ± 40.0	103 ± 56
56	0	26.3 ± 17.5	81.4 ± 45.9	108 ± 63
	4	15.5 ± 15.6	55.8 ± 42.8	71.4 ± 57.8
	8	26.7 ± 31.4	52.3 ± 37.4	79.0 ± 67.6
	12	26.5 ± 13.7	78.8 ± 37.6	105 ± 51
	16	37.5 ± 24.9	114 ± 62	152 ± 86
57	20	20.3 ± 15.5	77.6 ± 53.7	97.9 ± 68.2
	24	33.8 ± 29.8	87.5 ± 45.7	121 ± 74
56-57	C_{min} (C_{max}/C_{min}-ratio)	15.5 (2.4)	52.3 (2.2)	71.4 (2.1)
	C_{max}	37.5	114	152
	9-OH-risp./risp.-ratio		3.4 ± 1.0[1]	
	$AUC_{24\,h}$ (ng.h/ml)[2]	626 ±64	1853 ±141	2479 ±199
	[95 % CI]	[496 - 756]	[1569 - 2137]	[2078 - 2880]
Day	Time (h)	Group G: Hannover - IM		
49	0	50.8 ± 18.3	26.0 ± 14.9	76.8 ± 32.2
56	0	42.0 ± 16.3	17.8 ± 11.8	59.8 ± 27.4
	4	37.2 ± 10.4	17.4 ± 8.9	54.5 ± 18.8
	8	41.4 ± 8.0	21.7 ± 7.6	63.1 ± 15.0
	12	35.7 ± 8.1	19.3 ± 7.3	55.0 ± 15.1
	16	38.4 ± 20.4	16.2 ± 11.8	54.6 ± 31.7
57	20	43.4 ± 12.2	20.8 ± 8.4	64.2 ± 20.2
	24	42.0 ± 12.3	18.9 ± 7.7	61.0 ± 18.5
56-57	C_{min} (C_{max}/C_{min}-ratio)	35.7 (1.2)	16.2 (1.3)	54.5 (1.2)
	C_{max}	43.4	21.7	64.2
	9-OH-risp./risp.-ratio		0.5 ± 0.1[1]	
	$AUC_{24\,h}$ (ng.h/ml)[2]	952 ±38	455 ±27	1407 ±63
	[95 % CI]	[875 - 1030]	[401 - 509]	[1279 - 1535]



The sponsor noted that plasma levels of the "active moiety" were higher with dietary administration in both Wiga and Hannover animals, and higher in Wiga than in Hannover with both routes of administration. The sponsor also noted that "..in animals of the Hannover substrain, the plasma concentration *versus* time curve of the active moiety

showed much more fluctuation after dietary administration than after intramuscular dosing..." resulting in a larger C_{max}/C_{min} ratio with dietary dosing. According to the sponsor, the ratio of 9-OH-risperidone to risperidone in this study was similar to that observed in Hannover rats in a previous study (Report R064766/FK3715, June 2001).

2. Mechanistic study to evaluate the effects of an increase in time between sacrifice and tissue fixation on immunohistochemical staining for proliferating cell nuclear antigen in the rat kidney (Study EDMS-PSDB-2498049, Exp. No. 5723, Report date: 3/28/03,

Methods: this study was conducted in untreated female Sprague-Dawley rats (20/grp). Animals were sacrificed (exanguinated) ≈30 (Grp A), 60 (Grp B), 120 (Grp D), and 180 (Grp E) min prior to tissue fixation. At necropsy, kidney samples were collected and processed for H & E examination and immunostaining for Proliferating Cell Nuclear Antigen (PCNA).

Results: the PCNA data were summarized in the following sponsor's table:

Table: Mean number of PCNA positive renal corticotubular nuclei per kidney

Group	Mean	SE	Significance p-value 2-tailed	Significance p-value 1-tailed
Group A	89	10		
Group B	60	8	0.0350 *	0.0175 *
Group D	51	7	0.0014 **	0.0007 ***
Group E	25	6	0.0000 ***	0.0000 ***

Significance computed by Mann-Whitney U test: * p<.05 ** p<.01 *** p<.001

The data confirmed that PCNA is not stable postmortem.

3. Alkermes, Inc. Technical Report: Risperdal Consta: Exp. 5626: full factorial design mechanistic study: Renal prolactin receptor mRNA quantitation (Report No. AT-10-02, **non-GLP**).

Objective: the purpose of this analysis was to "Evaluate the expression of short and long form prolactin receptor mRNA in the kidney of Wistar Hannover and Wistar Wiga rats..." in the 8-wk mechanistic study (Exp. 5626). **This part of the report was not included in the ToC of the final study report, nor was it mentioned in any portion of the final study report.**

Methods: renal prolactin receptor mRNA levels (short and long isoforms) were quantitated from total RNA extracts of kidney obtained in animals from the 8-wk mechanistic study (Exp. 5626) using PCR technology. Tissues were collected at necropsy, which was performed between 30 min and 2 hrs following sacrifice. By strain, treated animals were necropsied after necropsies were completed in all control animals. According to the sponsor, "...control and treated groups from the two substrains were necropsied in parallel and at about the same time."

According to the sponsor, "In order to assess the impact of a postmortem delay of this magnitude on the sensitivity of the PCR method for renal prolactin receptor mRNA, a parallel experiment was performed in Sprague Dawley rats to simulate the tissue collection procedures applied to the Wistar Hannover and Wistar Wiga rats in Experiment 5626". In this exploratory study, kidneys were collected in male Sprague-Dawley rats [2/time point] at 30 min, and 1 and 2 hrs (at room temperature) following sacrifice, and renal

prolactin receptor mRNA was quantitated, and the ribosomal protein S16 housekeeping gene "was evaluated".

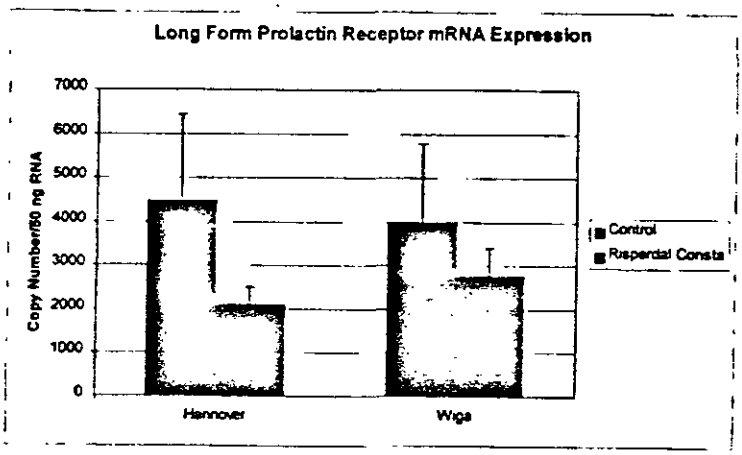
Tissue analyses were conducted by Alkermes, Inc. (Cambridge, MA).

Results:

Exploratory study: there were no significant differences in the short and long isoforms of the renal prolactin receptor mRNA, or in the ribosomal protein S16 housekeeping gene, among the different time points. The long isoform was notably decreased at 1 hr (47-35%), compared to the 30-min and 3-hr sampling times. However, the sponsor considered this due to "intrinsic variability of the test system". No data were provided in Wistar rats (either Hannover or Wiga).

Definitive analysis: the data were summarized in the following sponsor's figures and tables:

Figure 1: Long Form Prolactin Receptor mRNA Quantitation

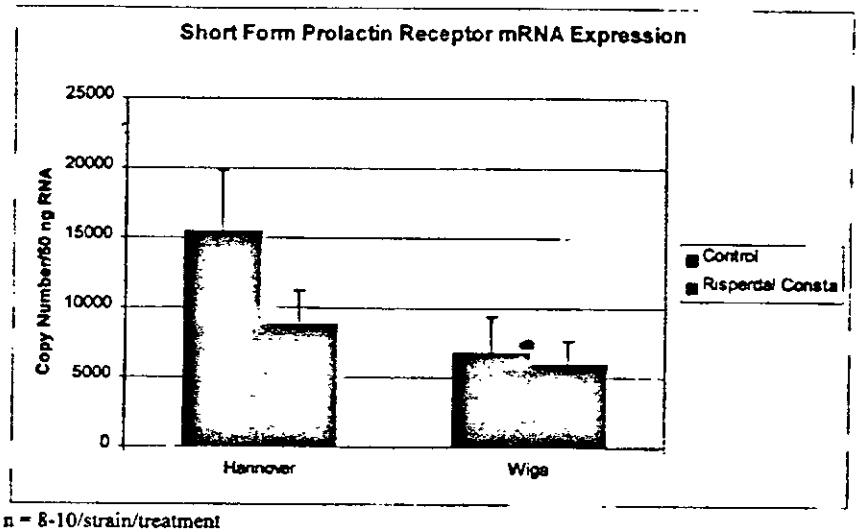


n = 9-10/strain/treatment group

Long Form Prolactin Receptor	Hannover	Wiga
Control Copy Number	4472	3992
Risperdal Consta Copy Number	2099	2771
% Change	53%	31%

Overall Treatment Effect p<0.001
Hannover Treatment Effect vs. Wiga Treatment Effect p= 0.195
Wistar Hannover Treatment Effect p<0.001
Wistar Wiga Treatment Effect p= 0.053
Hannover Control Copy Number vs. Wiga Control Copy Number p=0.448

Figure 2: Short Form Prolactin Receptor mRNA Quantitation



n = 8-10/strain/treatment

Short Form Prolactin Receptor	Hannover	Wiga
Control Copy Number	15491	6781
Risperdal Consta Copy Number	8811	6066
% Change	43%	11%

Overall Treatment Effect $p<0.001$
Hanover Treatment Effect vs. Wiga Treatment Effect $p=0.003$
Wistar Hannover Treatment Effect $p<0.001$
Wistar Wiga Treatment Effect $p=0.572$
Hannover Control Copy Number vs. Wiga Control Copy Number $p<0.001$

Conclusions: the sponsor noted that the data suggest an "enhanced sensitivity of the Hannover strain to risperidone-induced prolactinemia" and "support the concept that the kidney of the risperidone-treated Hannover and Wiga rat may be functioning in distinct prolactin ligand-receptor environments." The sponsor also noted that "...a differential function of these receptors [long and short isoforms] has not been defined..."

Qualification of Impurities

The sponsor responded to FDA's Request #3 (Action Letter of June 29, 2002; Request #3 reproduced below, taken from the sponsor's submission):

3. **You reported that — impurities are present in the risperidone IM depot formulation that are not present in the oral formulations [i.e., tablet, oral solution]. It was stated that impurity —— was qualified in oral nonclinical studies; however, documentation to support this statement was not provided. the impurities ——, were considered to be qualified on the basis that they are rapidly converted to the parent compound when administered. Adequate data were provided to support this statement relative to impurity ——, however, no data were provided for——. Therefore, additional data are needed to address these deficiencies.**

The proposed specification limit for —— impurity) is —, of drug product. In an Expert report provided in the original NDA submission, the sponsor provided a summary of nonclinical studies (cf. sponsor's Table 4-6 below) considered to have qualified this impurity at a level of —. However, in the original NDA submission, the sponsor did not provide the level of —— in the drug substance in each of the studies needed for qualification. The sponsor also did not justify using oral studies to qualify an impurity in an IM dosing formulation.

Table 4-6: Overview of toxicity studies conducted with—

Study	Exp.No.	Doses
Single dose oral toxicity in mice	8623	up to 160 mg kg
Single dose IV toxicity in mice	8622	up to 40 mg kg
Single dose oral toxicity in rats	8520	up to 320 mg kg
Single dose SC toxicity in rats	8625	up to 320 mg kg
Single dose IV toxicity in rats	8523	up to 47.6 mg kg
Single dose oral toxicity in dogs	8624	up to 20 mg kg
Single dose IV toxicity in dogs	8525	up to 40 mg kg
Three-month oral toxicity in rats	1727	0, 0.63, 2.5, 10 mg kg day
Embryofetal development study in rats	-788	0, 0.63, 2.5, 10 mg kg day
Ames test in S. typhimurium	1662	up to 2000 µg plate
Oral micronucleus test in mice	1712	up to 40 mg kg

In the sponsor's response, it was stated that Batch A0101 contained —— at a level of —. Therefore, doses of —— at the high doses tested were as follows:

Study	Exp. No.	High Daily Dose of ——	
		mg/kg	mg/m²
single dose toxicity, p.o., mouse	8623		
single dose toxicity, i.v., mouse	8622		
single dose toxicity, p.o., rat	8520		
single dose toxicity, s.c., rat	8625		
single dose toxicity, i.v., rat	7523		
single dose toxicity, p.o., dog	8624		
single dose toxicity, i.v., dog	8625		
3-month toxicity, p.o., rat	1727		
embryofetal development, p.o., rat	-788		
Ames test in *S. typhimurium*	1662		
in vivo micronucleus assay, p.o., mouse	1712		

*highest concentration of —— in the Ames test.

For comparison, the dose of ——at the maximum recommended human dose of RISPERDAL CONSTA™ (i.e., 50 mg/2 wks), at the proposed specification limit of ——, is — 2 wks or — µg/kg/2 wks. If one assumes a constant daily dose, then the daily human dose would be — µg/kg/day

or — $\mu g/m^2$/day. The sponsor calculated doses of ——— delivered in each of the toxicity studies (cf previous table), assuming 100% oral bioavailability, and concluded that ———, was qualified at up to ———. The sponsor noted that the high-concentration in the Ames test was "...too low to be considered an adequate test".

———

The proposed specification limit for ——— and ——— (——— products) is ———, of drug product. ——— is ———. is ——— The sponsor previously conducted PK studies in rat and dog to demonstrate rapid conversion of ——— to risperidone. Similar studies have now been conducted with ———

1. Study title: The plasma levels and excretion of ———, and——— in the male SPF Wistar rat after a single oral dose of ———, or risperidone at ——— (Study No. FK4366, Report date: 10/18/02, conducting laboratory and location: Johnson & Johnson Pharmaceutical Research and Development, Belgium).

Methods: ———, and risperidone (in 0.5 M tartaric acid solution) were administered to male SPF Wistar rats (Hannover substrain; ———, via gavage at a dose of 0.63 mg/kg. Blood samples were collected (via orbital venous plexus) at 0.25, 0.5, 1, 2, 4, and 8 hrs after dosing. Two consecutive blood samples were collected from each rat, and animals were sacrificed following the 2nd sampling. Urine and fecal samples were collected at 0-8, 8-24, 24-32, and 32-48 hrs postdosing. Urine and methanol-extracted fecal samples were pooled within each time interval. (The number of animals per sampling time [for urine and feces] was not specified.)

Risperidone, ———, and ——— were quantitated in plasma, urine, and methanolic fecal extracts using LC/MS/MS (LLOQ = ———, and — ng/mL, respectively, for all three compounds).

Results: ——— was stated to be stable in spiked samples of plasma; however, data were not provided to support this statement ——— was found not to be stable in urine or fecal samples. In spiked samples, 85 and 15% of added ——— was converted to risperidone in urine and feces, respectively. Therefore, urine and fecal data were not considered reliable.

Following a single 0.63 mg/kg oral dose of ——— was detectable in plasma only at the first sampling point (0.25 hr). PK data were summarized in the sponsor's Table 5-1:

Table 5-1: Plasma concentrations and pharmacokinetic parameters of risperidone [redacted] and [redacted] in pooled plasma samples (n=3) from male SPF Wistar rats after single oral administration of [redacted] at a dose of 0.63 mg/kg.

Time	[redacted] (ng/ml)	[redacted] (ng/ml)	[redacted] (ng/ml)
0.25 h	[redacted]		
0.5 h			
1 h			
2 h			
4 h			
8 h	[redacted]		

Parameter	[redacted]	[redacted]	[redacted]
C_{max} (ng ml)			
T_{max} (h)			
t (h)			
AUC_{0-t} (ng.h ml)			
AUC_{∞} (ng.h ml)			

[1] NQ: not quantifiable by the LC MS MS method ([redacted] ng ml)
[2] calculated from plasma concentrations in the 4-8 h time period
[3] estimated from the [redacted] plasma level at 0.25 h and the quantification limit value of < 0.20 ng ml at 0.5 h
[4] value represents AUC_{0-8h}
[5] value represents AUC_{0-6h}

Plasma exposure following a single 0.63-mg/kg oral dose of risperidone [redacted], were summarized in the following sponsor's Table 5.2:

Table 5-2: Plasma concentrations and pharmacokinetic parameters of risperidone [redacted] in pooled plasma samples (n=3) from male SPF Wistar rats after single oral administration of [redacted] at a dose of 0.63 mg/kg.

Time	[redacted] (ng/ml)	[redacted] (ng/ml)	[redacted] (ng/ml)
0.25 h	[redacted]		
0.5 h			
1 h			
2 h			
4 h			
8 h	[redacted]		

Parameter	[redacted]	[redacted]	[redacted]
C_{max} (ng ml)			
T_{max} (h)			
t (h) [3]			
AUC_{0-8h} (ng.h ml)			
AUC_{∞} (ng.h ml)			

[1] NQ: not quantifiable by the LC MS MS method ([redacted] ng ml)
[2] NC: not calculated
[3] calculated from plasma concentrations in the 4-8 h time period

2. Study title: The plasma levels and excretion of [redacted] and [redacted] (risperidone) in the male beagle dog after a single oral dose of [redacted] or risperidone at 0.31 mg/kg. (Study No. [redacted]4365, Report date: 10/18/02, conducting laboratory and location: [redacted].

Methods: [redacted] (vehicle: [redacted] in water) was administered orally to 3 male beagle dogs (Janssen Animal Breeding Centre). Blood samples were collected (via jugular vein) 2 days prior to dosing (0), and at 0.25, 0.5, 1, 1.5, 2, 4, 6, 8, and 24 hrs postdosing. Urine and fecal samples were collected prior to dosing and at 0-8, 8-24, 24-32, and 32-48 hrs postdosing. Blank urine and fecal samples were spiked with [redacted] in order to "detect possible sample processing artifacts".

Risperidone, [redacted] and [redacted] were quantitated in plasma, urine, and fecal samples using LC/MS/MS. The LLOQ's were as follows:

plasma: [redacted] ng/mL for all three compounds
urine: [redacted] ng/mL for [redacted] and [redacted], [redacted] ng/mL for [redacted].
feces: [redacted] ng/mL for [redacted] and [redacted], ng/mL for [redacted].

Results: [redacted] was reported to be stable in [redacted] plasma samples, although no data were provided to support this statement [redacted] was not stable in feces. The stability of [redacted] in urine was not discussed.

[redacted] was detectable in urine only during the 0-8 hr sampling time, and accounted for [redacted] of dose [redacted] ng): [redacted] ng of [redacted] ([redacted] of dose) was eliminated in urine during the 24-32 hr sampling interval.

Plasma data were summarized in the following sponsor's Table 5.1:

Table 5-1: Plasma concentrations and pharmacokinetic parameters of risperidone [redacted] and [redacted] in pooled plasma samples (n=3) from male beagle dogs after oral administration of [redacted] at a dose of 0.31 mg/kg.

Time	[redacted] (ng/ml)	[redacted] (ng/ml)	[redacted] (ng/ml)
0.25 h			
0.5 h			
1 h			
1.5 h			
2 h			
4 h			
6 h			
8 h			
24 h			

Parameter	[redacted]	[redacted]	[redacted]
C_{max} (ng/ml)			
T_{max} (h)			
$t_{1/2}$ (h)			
AUC_{inf} (ng·h/ml)			
AUC_{0-t} (ng·h/ml)			

[1] NQ: not quantifiable by the LC/MS/MS method ([redacted] ng/ml)
[2] An estimate for plasma $t_{1/2}$ of [redacted] was calculated from plasma levels of [redacted] at 0.5 h, 1 h, and 2 h.
[3] Value represents estimated AUC_{0-2h} with interpolation between 1 h and 2 h (see Figure 5-2 for profile)
[4] Value represents estimate for AUC_{inf} with limited reliability, since extrapolated part represents more than 50 % of total AUC

Conclusions:

[redacted] are degradation products of risperidone. In the original NDA submission, the proposed specification limit in the drug product was [redacted], i.e., at the limit of qualification for a drug product for which the maximum daily dose is >10 mg-2 gm. [Although Risperdal CONSTA™ is to be administered bi-weekly at a dose of 50 mg/dose, there are no data available on the absorption of the degradants from the IM formulation. Therefore, the most conservative approach is to assume that the entire dose of the degradants is absorbed within the first 24 hrs.]

In the resubmission, the specification limit has been reduced to [redacted] This limit is below the qualification threshold; therefore, these degradants do not need to be qualified.

[redacted] is a [redacted] impurity. According to the guidance for impurities in a drug substance, the qualification threshold for a drug for which the maximum daily human dose is ≤2 gm/day is 0.15% or 1.0 mg/day (whichever is lower). The maximum dose of Risperdal CONSTA™ is 50 mg bi-weekly; therefore, the qualification threshold is 0.15%. The sponsor has proposed a specification of [redacted] for this impurity, a level above the qualification threshold. The sponsor provided study information considered to have qualified [redacted], at a level of [redacted] of drug substance. Studies needed to qualify an impurity are as follows: (a) *in vitro* Ames assay and an *in vitro* chromosomal aberration assay in mammalian cells, (b) a subchronic toxicity study of 2-13 wks in duration, and (c) additional studies as considered necessary (in this case, an embryofetal development study in one species). [redacted] was present in the drug batch used in a 3-mo oral toxicity study and an oral embryofetal development study in rat, an *in vitro* Ames assay, and an *in vivo* micronucleus assay in mouse. Since the level of [redacted] in the drug batch used in these studies was less than the proposed specification, the sponsor calculated the actual doses of [redacted] administered in the studies used to qualify this impurity. Although the doses administered in the *in vivo* studies are higher than that at maximum recommended human dose (MRHD), they were calculated assuming 100% oral bioavailability. There are no data to support this assumption. In addition, the maximum amount of [redacted] tested in the Ames test was very low [redacted], a level even the sponsor considered inadequate.

These issues were discussed with the sponsor in a recent telecon (10/21/03). The sponsor acknowledged that the acute studies were inadequate to support qualification, and that there are no bioavailability data to support the use of the oral studies to qualify an impurity in an i.m. depot formulation. (Although bioavailability data are not required to qualify an impurity, nonclinical studies to qualify an impurity should employ the clinical route of administration.) In order to avoid the need for additional nonclinical studies, the sponsor indicated the intent to reduce the specification for [redacted] from [redacted]. The specification for [redacted] in the drug substance for the marketed oral dosage form is [redacted] The doses of [redacted] at the oral and i.m. depot MRHD are [redacted] g/day and [redacted] µg biweekly, respectively. At a specification of [redacted] for [redacted] (a level not requiring qualification), the maximum i.m. depot dose of of [redacted] would be [redacted] biweekly. This difference is not considered sufficient to require additional toxicity testing. However, the genotoxic potential of [redacted] has not been adequately evaluated, either for the clinical oral or i.m. dosage forms. As previously discussed, [redacted], was present at only [redacted] in the drug batch used in the Ames assay. Although this is lower than the specification proposed (i.e., [redacted], the Division has a history of accepting *in vitro* assays conducted with a drug batch containing the impurity at the same or greater level (%) as the intended clinical batch for qualification. Therefore, asking the sponsor to conduct a repeat Ames assay with a drug substance containing [redacted] at a level [redacted] would result in an absolute concentration of [redacted] of [redacted] instead of the [redacted] tested. This difference is not sufficient to warrant a repeat study. However, additional assessment of genotoxic potential is needed. The other genotoxicity assay conducted, i.e., the *in vivo* micronucleus assay, did not adequately assess the genotoxic potential of [redacted]. The study conducted did not use the clinical route and the doses of [redacted] administered (orally) were low [redacted] µg/kg as a single dose). Considering the relative insensitivity of the *in vivo* assay, it is particularly important to maximize systemic exposure.

Whereas one could argue that the toxicity of [redacted] has been tested in oral nonclinical studies (with the reservations noted), in clinical trials, and through extensive postmarketing use, the same cannot be argued for the genotoxic potential of [redacted]. In addition, there would be no justification for allowing a genotoxic impurity in a drug substance since there is no clinical benefit related to an impurity. (The sponsor indicated that [redacted] was not present in the drug batch used in the 2-yr i.m. depot carcinogenicity study.)

Therefore, the sponsor should conduct an *in vitro* chromosomal aberration assay in mammalian cells or an *in vitro* mouse lymphoma assay (with colony sizing) using either a batch of risperidone enriched in [redacted] or the impurity itself. The study may be conducted Phase 4. The recommendation for the study as a Phase 4 commitment does not reflect lack of need for or the importance of the data, but the fact that oral risperidone has been marketed for almost a decade with the same specification for [redacted].

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IX. DETAILED CONCLUSIONS AND RECOMMENDATIONS

CARCINOGENICITY

Renal tumors

In the original NDA submission, the sponsor attributed the renal tubular adenomas/adenocarcinoma in males (5/50 males at the HD of 40 mg/kg/2 wks) to exacerbation of chronic renal disease (CRD). However, based on review of the data and a published study by Dr. Gordon C. Hard (Hard GC *et al.* *Toxicol Path* 26(1):132-143, 1997), it was this reviewer's opinion that the increase in renal tubular adenomas observed in the 2-yr rat i.m. carcinogenicity study was not due to CRD.

At the sponsor's request, Dr. Hard prepared an expert report based on examination of renal tissue sections from the 2-yr rat i.m. carcinogenicity study (12-mo interim, terminal sacrifice) and a 6-mo s.c. toxicity study in rat. Dr. Hard confirmed the incidence of renal tubular adenomas/carcinoma. Based on his analysis, Dr. Hard concluded that the tumors could not be ascribed to advanced CRD because (a) risperidone was not associated with "a biologically significant exacerbation of CPN [CRD]..." and (b) renal tumors were not associated with severe CRD in individual animals. However, Dr. Hard also concluded that there was no microscopic evidence that the renal tumors were drug-related. That is, risperidone is non-genotoxic and there was no evidence of an increase in renal distal tubule hyperplasia or microscopic findings consistent with non-genotoxic mechanisms (e.g., direct cytotoxicity). Dr. Hard recommended that additional analyses be performed to rule out possible direct cytotoxicity.

In response, the sponsor conducted the following additional studies: (a) assessment of cellular proliferation and apoptosis in renal tissue sections from the 2-yr rat i.m. study (12-mo interim and terminal sacrifices), (b) a reexamination of renal tissue in control animals from 4 previous 2-yr carcinogenicity studies (2 in Wiga and 2 in Hannover rats; experimental design of these studies not specified), (c) 8-wk mechanistic study in Wiga and Hannover Wistar rats. The sponsor also provided copies of a number of published studies addressing the issue of strain differences in spontaneous and/or compound-induced tumor susceptibility.

Cellular proliferation/apoptosis (2-yr i.m. carcinogenicity): the sponsor examined renal tissue from (a) 18 CM and 18 HDM sacrificed at Wk 52 and (b) 18 CM, 18 non-tumor bearing HDM and the 5 tumor-bearing HDM sacrificed at the end of the 2-yr dosing period. Tissue sections were stained for the presence of Proliferating Cell Nuclear Antigen (PCNA) or apoptosis. In the tissue from the 12-mo interim sacrifices, there was a statistically significant increase in PCNA-positive renal corticotubular nuclei in HDM, but no difference between CM and HDM in the no. of apoptosis-positive renal corticotubular nuclei. In the terminally sacrificed males, there was a statistically significant increase in PCNA positive renal corticotubular nuclei in tumor-bearing HDM compared to CM and to non-tumor bearing HDM. The mean number of apoptosis-positive nuclei was also increased in tumor-bearing HDM compared to CM and non-tumor bearing HDM. There were no differences in PCNA- or apoptosis-positive nuclei between CM and non-tumor bearing HDM at terminal sacrifice.

These data indicate that risperidone, administered as an i.m. depot, produced an increase in cellular proliferation (after 12 mo of dosing) in males at the dose associated with renal tumors. In tumor-bearing males, increases in cellular proliferation and apoptosis were observed after 2 yrs of dosing. Based on the PCNA data, it was the sponsor's conclusion that "...the suggestion by Dr. Hard that the tumors might be of a spontaneous nature...becomes questionable." As noted by the sponsor, increased cellular proliferation is an acknowledged mechanism underlying tumor induction for non-genotoxic compounds (in animals and human).

Substrain differences/Route of administration: in an attempt to explain the lack of renal tumors in the 2-yr oral carcinogenicity study, the sponsor provided published literature regarding strain differences in tumor susceptibility and the results of a reanalysis of renal tissue from previously conducted carcinogenicity studies and an 8-wk mechanistic study in Wistar Wiga (used in the 2-yr dietary carcinogenicity study; HD = 10 mg/kg/day) and Wistar Hannover (used in the 2-yr i.m. depot carcinogenicity study; HD = 40 mg/kg/2 wks) rats.

Regarding (sub)strain differences in tumor susceptibility, the sponsor provided tumor incidence data from published studies documenting differences in spontaneous and compound-induced tumor incidences among various strains of rats (e.g., Fisher 344, Sprague-Dawley). Interstrain differences in a variety of spontaneous and compound-induced findings are acknowledged, but are not particularly relevant to the issue of such differences between Wistar Wiga and Wistar Hannover rats. The sponsor did cite one published article (Walsh K_M, Poteracki J. *Fund Appl Toxicol* 22:65-72, 1994) that provided spontaneous tumor incidences for the Wistar strain. The authors discussed differences in their own database between the Wiga and Hannover substrains; however, they acknowledged that time and animal suppliers were confounding factors. The sponsor did not provide any published data relevant to differences between the Wiga and Hannover substrains in terms of sensitivity to compound-induced tumors.

In the original NDA submission, the sponsor provided historical control (HC) tumor data from eight 2-yr carcinogenicity studies in Wistar Hannover rats. In the resubmission, the sponsor provided the results of a reanalysis of renal tissue from 4 previously conducted carcinogenicity studies, 2 in Wiga (conducted 1993-1995) and 2 in Hannover (conducted 1997-1999) rats. There were clear differences in the incidence of various renal findings between the two substrains; however, some findings (e.g., related to pelvic mineralization) were observed to a greater extent in Hannover rats whereas others (e.g., related to interstitial fibrosis/inflammation and glomerulopathy) were observed to a greater extent in Wiga rats. Short-comings of this reanalysis were that only 20 males/grp per study were examined (it is presumed that the total number of C animals was at least 50/sex per study), that renal tissue from only 2 studies per substrain were examined, and the studies in Wiga rats were conducted prior to those in Hannover rats (usually HC data from studies conducted within a 5-yr period of the current study are considered the most relevant).

Regardless, it is unclear how the data from the reanalysis aid in the interpretation of the i.m. depot carcinogenicity study. The sponsor's consultant, Dr. Hard, noted that non-neoplastic renal findings in the i.m. depot study were observed in C as well as treated animals, and (more importantly) with a similar incidence and severity in HDM and HDF rats, although renal tumors were not observed in HDF. Therefore, it would not appear that drug-induced non-neoplastic renal findings alone were critical to the development of renal tumors. Even if the data did support such a conclusion, there are no data to indicate which substrain is more like human. It is reasonable to assume that there is a wide variability in susceptibility to drug-induced renal effects in humans due to a variety of factors (e.g., genetic, co-existing disease).

The sponsor did acknowledge that the fact that renal tumors were observed in the 2-yr oral study and not the 2-yr i.m. depot study would "...suggest that there may be an effect of the route of administration..." In order to investigate both substrain and route differences, the sponsor conducted an 8-wk mechanistic study in Wistar Wiga and Hannover rats. This study employed a unique experimental design. Risperidone was administered to male Wistar Wiga and male Wistar Hannover rats (80/grp) via a drug-diet admixture (10 mg/kg) or as an i.m. depot (40 mg/kg, biweekly). Control animals were not treated. Observations included body wt, food consumption, clinical pathology (selected parameters), serum prolactin, TK, gross pathology (selected tissues), histopathology (kidney only; C and HD Hannover rats only), PCNA analysis (kidney sections; C and HD Hannover rats only). Clinical signs consisted of ptosis and decreased activity; the incidences of both signs were greater in Wiga rats (at both doses). Body wt

(relative to C) and body wt gain were reduced in all treatment grps; the effect was greater at the LD for both substrains and tended to be greater in Hannover vs Wiga rats.

Serum prolactin levels (measured at the end of the dosing period) were elevated in all treatment grps, but to a greater extent at the LD (25 and 19% for Wiga and Hannover rats, respectively). That is, oral (dietary) administration resulted in higher serum prolactin levels than did i.m. depot administration in both substrains. Serum prolactin AUCs (0-24 hr) were slightly higher in Wiga rats (28 and 22% at the LD and HD. respectively). However, the baseline AUC was also higher in Wiga rats. The change from baseline was greater in Hannover rats (11- and 9-fold at the LD and HD, respectively) compared to Wiga rats (7- and 6-fold). (Although the sponsor stated that the strong main-effect of route precluded a comparison of LD Wiga to HD Hannover [the most relevant comparison], it is of note that the change from baseline prolactin levels was 7- and 9-fold, respectively. In fact, the sponsor stated that "...the study does not provide an insight into the risperidone-mediated increase in serum prolactin levels in the oral carcinogenicity study with Wiga rats as compared to the IM carcinogenicity study with Hannover rats.")

No treatment-related gross lesions were observed in kidney in Hannover rats. In Wiga rats, dilated renal pelvis was observed in 6-10 rats per grp (including C). Mammary gland stimulation was increased in all treatment grps; the incidence was similar at the LD and HD for both substrains, but was slightly higher in Wiga compared to Hannover rats (in C animals as well). Alterations in prostate (i.e., stippled, yellow appearance) were markedly increased in all treated grps, whereas swollen prostate was observed only in the LD grps (both substrains).

Microscopic and PCNA analysis of kidney was conducted only in Hannover rats (C and HD). (The reason for this was unclear since the sponsor's purpose in conducting this study was to study differences between the two substrains.) Unfortunately, a delay (≈1 to 2 hrs) in processing tissue samples following sacrifice of HDM (CM were processed first) resulted in a high autolysis rate (18/18 HDM) and invalid microscopic assessment. PCNA data indicated a highly significant decrease in PCNA-positive renal corticotubular nuclei in HDM, an unexpected finding considering the results of the 12-mo and 2-yr sacrifice data from the 2-yr i.m. depot carcinogenicity study. The sponsor conducted a separate study to assess the effects of delayed tissue processing on PCNA staining. It was clearly demonstrated that PCNA is not stable postmortem and, therefore, that the lower PCNA values in HDM are an artifact of the delay in tissue processing.

In order to further characterize possible substrain differences in response to prolactin, the sponsor quantitated short and long isoform prolactin receptor mRNA in kidney samples collected at the end of the 8-wk mechanistic study; only C and HD (i.m. depot) of both substrains were examined. (In an exploratory study, the sponsor assessed the effect of postmortem delay (up to 2 hrs) in processing of tissues on quantitation of prolactin receptor mRNA in renal tissue. There was no consistent effect of delay on mRNA quantitation. Levels of the long form were decreased by 50% following 1 hr of delay, but were decreased by only 20% at 2 hrs; the sponsor attributed this to intrinsic variability of measurement rather than a delay effect.) There were no significant differences between substrains in either C levels of mRNA for the long isoform or response (i.e., decrease) to risperidone (i.m. depot) treatment. Control levels of mRNA for the short isoform were ≈2-fold higher in Hannover rats as compared to Wiga rats. In addition, risperidone (i.m.) treatment produced a significant decrease in short form prolactin receptor mRNA in Hannover rats, whereas no effect of treatment was observed in Wiga rats. The sponsor concluded that these data suggested an "enhanced sensitivity of the Hannover strain to risperidone-induced prolactinemia"; however, the sponsor also noted that the differential function(s) of the two isoforms is unknown.

TK data collected during the 8-wk study indicated that plasma exposure (C_{max}, AUC) to risperidone, 9-OH-risperidone, and the "active moiety" was higher in Wiga rats compared to Hannover, regardless of route of administration. In addition, plasma exposure to the "active moiety" was greater at the LD (i.e.,

with dietary administration) than at the HD (i.e., with i.m. depot administration) for both substrains. Also, for both substrains, the 9-OH-risperidone-to-risperidone ratio was higher with dietary dosing (3.3-3.4) than with i.m. depot (0.6-0.5) dosing.

Overall, the data from the 8-wk mechanistic study did demonstrate differences between the Wiga and Hannover substrains of Wistar rats; however, the data do not support a conclusion that the renal tumors observed in Hannover (but not Wiga) rats is substrain-specific and irrelevant to humans. The TK data, however, provide a possible explanation for the differences in tumor profile in the dietary and i.m. depot carcinogenicity studies. That is, the ratio of 9-OH-risperidone was greater following dietary administration than following i.m. depot administration for both substrains. The possibility that risperidone and 9-OH-risperidone have different tumor liability cannot be dismissed.

Adrenomedullary tumors

In the original NDA submission, the sponsor attributed the increase in non-neoplastic (i.e., focal medullary hyperplasia) and neoplastic (pheochromocytomas) adrenal gland lesions observed in the 2-yr i.m. depot carcinogenicity study to D_2 antagonist-induced hyperprolactinemia, which resulted in an exacerbation of chronic renal disease (CRD). CRD-induced hypercalcemia was considered to possibly being contributory. It was this reviewer's opinion that the data provided by the sponsor did not adequately support this position. Most importantly, there was no evidence of risperidone-induced CRD (as discussed in detail in the previous section on renal tumors) in the 2-yr i.m. depot study. In addition, the incidence of pheochromocytomas was not significantly increased in the 2-yr oral carcinogenicity study. Although serum prolactin was not measured during the 2-yr oral carcinogenicity study in rats, serum prolactin was shown in a separate study to be markedly elevated at the doses used in the 2-yr oral study.

In response, the sponsor provided adrenal gland histopathology data from the 2-yr dietary carcinogenicity study in rats to support the position that oral risperidone did produce an increase in pheochromocytomas. In addition, copies of published studies were submitted to address possible mechanisms and the relevance of rat adrenomedullary tumors to human risk.

In the i.m. depot carcinogenicity study, the incidences of benign adrenomedullary tumors were as follows (V = vehicle [microspheres]):

2/50, 2/50, 2/50, and 11/50 in CM, VM, LDM, and HDM, respectively.
0/50, 1/50, 1/50, and 3/50 in CF, VF, LDF, and HDF, respectively.

The increase in HDM was statistically significant compared to both C grps. There was a significant positive trend in adrenomedullary tumors in females in the i.m. depot study when treated grps were compared to "CF" (saline, but not V [vehicle] control).

According to the sponsor, the incidences of benign adrenomedullary tumors in the dietary carcinogenicity study were as follows:

3/50, 7/50, 6/50, and 6/49 in CM, LDM, MDM, and HDM, respectively.
1/50, 3/50, 1/50, and 3/50 in CF, LDF, MDM, and HDF, respectively.

Adrenomedullary tumors in the dietary study were not significantly increased in either males ($p = 0.0687$) or females ($p = 0.2966$). However, the sponsor stated that there was a 2-fold increase in treated males compared to CM, and concluded that these data demonstrated qualitative similarity between the dietary and the i.m. depot study in terms of adrenomedullary tumor induction. In this reviewer's opinion, the incidences of adrenomedullary tumors in the dietary study do not demonstrate even a marginal drug-related effect. There is a clear lack of dose-response in both males and females. In contrast, the

incidences of adrenomedullary tumors in the i.m. depot study were observed only at the HD in both males and females, and benign adrenomedullary tumors were markedly elevated in HDM.

The sponsor concluded that the adrenomedullary tumors represented a rat-specific phenomenon based on the following: (a) morphological and functional differences in adrenomedullary tumors between rats and humans, (b) adrenomedullary tumors may result from hyperprolactinemia and hypercalcemia in rats, but not in humans, and (c) adrenomedullary tumors have not been observed in postmarketing experience with oral risperidone. The fact that adrenomedullary tumors have not been detected during the postmarketing experience is not a compelling reason to dismiss the relevance of the adrenomedullary tumor findings in the i.m. depot study. It is very unlikely that a drug-induced increase in most tumors would be detected postmarketing, particularly for a drug like risperidone that has only been on the market for ≈10 yrs. In addition, the fact that pheochromocytomas in humans may be silent (Tischler AS, DeLellis RA. *J Am Coll Toxicol* 7(1):23-44, 1988) means that the incidence of these tumors in humans is in all likelihood underreported.

The issue of the relevance of drug-induced adrenomedullary tumors in rats to human was discussed in detail in the original NDA review. None of the mechanisms proposed by the sponsor (or in the published articles provided) can clearly be related to the increased adrenomedullary tumors observed in the i.m. depot study. The doses used in the 2-yr oral carcinogenicity study were demonstrated to markedly increase serum prolactin; however, as noted, there was not an increase in adrenomedullary tumors in that study. In fact, the majority of marketed antipsychotic drugs (one exception being clozapine) produce elevations in serum prolactin in rodents. However, no increases in adrenomedullary (or renal) tumors have been reported for any of the marketed antipsychotic medications for which there are adequate data. (Reserpine, a dopamine-depleting agent, does induce adrenomedullary tumors, but this effect is thought to be due to neural stimulation of the adrenal medulla resulting from drug-induced hypotension [Rosol TJ *et al. Toxicol Pathol* 29(1):41-48, 2001]). One finding observed in the i.m. depot, but not the oral, carcinogenicity study was hypercalcemia. Hypercalcemia has been associated with an increase in adrenomedullary tumors for some compounds (sugar alcohols). If the adrenomedullary tumors in the i.m. depot study were related to hypercalcemia, then there might be justification for minimizing the clinical relevance of these tumors since hypercalcemia was not reported in the clinical trials of RISPERDAL CONSTA™ and published literature would suggest that the rat adrenal gland is particularly sensitive to the stimulation effects of hypercalcemia. However, there are insufficient data to do so. For example, the degree of hypercalcemia was similar in HDM and HDF (4 and 6%, respectively, compared to VCs) in the 2-yr i.m. depot study, but the incidence of adrenomedullary tumors was markedly higher in HDM. An analysis of the individual data for serum Ca indicated that serum Ca was not notably elevated (i.e., above the current control range) in HDM in which adrenomedullary tumors were detected. Also, there are no i.m. carcinogenicity data in the mouse, a species suggested as being closer to human in sensitivity to compound-induced adrenomedullary tumors.

Several of the published studies emphasized the greater susceptibility of rats in general, and perhaps Wistar rats in particular, to adrenomedullary tumors, suggesting that compound-induced adrenomedullary tumors may represent an exacerbation of common spontaneous findings. One study (Lynch *et al. Reg Toxicol Pharmacol* 23:256-297, 1996) cited a spontaneous incidence rate of 69% for adrenomedullary tumors in Wistar rats. However, in the 2-yr carcinogenicity studies, the incidence in C grps was low (oral: 3/50 and 1/50 for CM and CF, respectively; i.m. depot: 2/50 and 3/50 in male saline and vehicle controls, respectively, and 0/50 and 1/50 in female saline and vehicle controls, respectively). In addition, the incidences (benign and malignant pheochromocytomas combined) reported in the HC data (from eight 2-yr studies) provided by the sponsor ranged from 1/60 to 3/50 in males and from 0/59 to 1/48 in females.

Conclusions: the sponsor did not provide sufficient data to conclude that the renal tubular and adrenomedullary tumors are rat-specific and, therefore, irrelevant to humans. In fact, PCNA data from the 2-yr i.m. depot carcinogenicity study (12-mo interim and terminal sacrifices) would indicate that the renal

tubular tumors were a result of an increase in risperidone-induced cellular proliferation. An increase in cellular proliferation is a known mechanism underlying drug-induced tumors in animals and humans. Furthermore, plasma exposure to the active moiety at the effect-dose in the i.m. depot study was only 2 times the expected plasma exposure at the i.m. depot maximum recommended human dose (MRHD). (Plasma exposure at the no-effect dose was ≈0.3 times the i.m. depot MRHD.)

The sponsor also did not provide sufficient data to conclude that oral and i.m. depot risperidone are associated with the same tumor liability. The TK data from the 8-week mechanistic study in Wistar Wiga and Wistar Hannover rats would suggest the possibility that differences in the 9-OH-risperidone-to-risperidone ratio between the two routes may have a role.

REPRODUCTIVE AND DEVELOPMENTAL TOXICOLOGY

In the FDA's action letter (6/29/02), the Agency informed the sponsor that an embryofetal development study in rat would need to be conducted using the clinical IM depot formulation and including an oral dose group for comparison. The sponsor was relying on the oral reproductive and developmental toxicology studies to support approval of the i.m. depot formulation. However, differences in tumor profile between the 2-yr oral and i.m. depot carcinogenicity studies and the detection of osteodystrophy in the 2-yr i.m. depot study (a finding not observed in the oral studies) raised the possibility that route may have an effect on the toxicity of risperidone. Therefore, it was considered necessary for the sponsor to assess the i.m. depot formulation in at least one embryofetal development study. Since the differences between the two routes were observed in rat, the sponsor was asked to conduct the study in this species.

At the time of NDA resubmission (4/28/03), the sponsor was in the process of conducting a dose-range finding study in order to select doses for a definitive i.m. depot embryofetal development study in rat. (The design of the definitive study was discussed with the sponsor in a telecon held on 3/25/03 at the sponsor's request.) Considering the patient population for which risperidone is intended, that oral risperidone is currently marketed, and the fact that drug exposure cannot be terminated quickly following an i.m. depot dose (except, perhaps, by excision), it is this reviewer's opinion that the definitive embryofetal development study be required for approval.

IMPURITIES

In the NDA resubmission, the sponsor lowered the specification for — , degradants ——— . and ——— from — to ——. The —— level is below the qualification threshold for impurities in drug product; therefore, these impurities do not need to be qualified.

——— is a , impurity. Although the specification for this impurity was also decreased from —— to ——, the——, still falls above the qualification threshold. The sponsor submitted a list of toxicity and genotoxicity studies conducted with a drug batch containing——. of this impurity. Although the level in the nonclinical drug batch did not exceed the set specification, the actual doses administered in the nonclinical studies did exceed the expected maximum clinical dose. However, the definitive toxicity studies (i.e., 3-month toxicity study in rat, embryofetal development study in rat) were conducted using the oral route. Although the sponsor calculated estimated daily doses of the impurity, these calculations were made assuming 100% oral bioavailability, an assumption with no supportive data. Although systemic exposure to an impurity need not routinely be demonstrated, it is usually expected that the studies to qualify an impurity be conducted using the clinical route, in this case i.m. depot. This issue was discussed with the sponsor in a telecon (10/21/03). During the telecom, the sponsor indicated the intent to lower the specification for ——— , in drug substance to —— the same specification set for the drug substance for the orally marketed formulation. It is unclear why the sponsor does not set the specification for this impurity to —— (below the qualification threshold) since all data available to FDA indicate that none of the drug batches (except for one) contained ——— , at levels ——. Nonetheless,

difference of [redacted] in the level of the impurity would not warrant additional toxicity studies. However, the genotoxic potential of [redacted] has not been adequately assessed. The *in vivo* micronucleus assay is relatively insensitive, and the amount of [redacted] administered was too low to provide an adequate assessment. (Although the Ames assay was conducted using a drug batch containing a percentage of [redacted] lower than the specification set, the difference in the amount of impurity that would be tested at the higher percentage is minimal compared to the level tested.) Therefore, the sponsor needs to conduct an *in vitro* chromosomal aberration assay in mammalian cells or an *in vitro* mouse lymphoma assay (with colony sizing). The study should be conducted using either a drug batch enriched with the impurity or the impurity directly.

Recommendations:

1. The renal tubular and adrenomedullary tumors observed in the 2-yr i.m. depot carcinogenicity study in rat cannot be dismissed as irrelevant to humans, and must be included in labeling.

2. The sponsor is in the process of conducting an i.m. depot embryofetal development study. It is this reviewer's recommendation that this study be completed and reviewed prior to approval.

3. It is recommended that the sponsor be asked to commit to further investigating the osteodystrophy observed in the 1-year i.m. depot toxicity and the 2-year i.m. depot carcinogenicity studies in rat. Additional studies to be conducted Phase 4 should address the exact nature of the bone lesion(s) and possible mechanism(s) underlying this finding.

4. It is recommended that the sponsor conduct Phase 4 an *in vitro* chromosomal aberration assay in mammalian cells or an *in vitro* mouse lymphoma assay (with colony sizing) to assess the genotoxic potential of the [redacted] impurity. [redacted]. The study can either be conducted using a drug batch enriched in [redacted], or directly testing [redacted]

Labeling with basis for findings: proposed revisions (bold, strikeouts) to the sponsor's labeling are indicated below:

"Hyperprolactinemia

III. Carcinogenesis, Mutagenesis, Impairment of Fertility

Carcinogenesis - Oral

Carcinogenesis - IM

Mutagenesis

Impairment of Fertility

IV. Pregnancy
Pregnancy Category C

ANIMAL TOXICOLOGY

APPEARS THIS WAY
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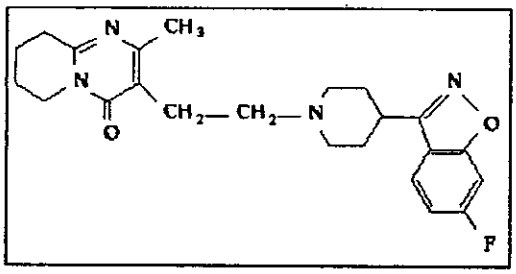
/s/

Lois Freed
10/29/03 04:14:40 PM
PHARMACOLOGIST

PHARMACOLOGY/TOXICOLOGY COVER SHEET

NDA number: **21-346**
Review number: 1
Sequence number/date/type of submission: N-000/August 31, 2001
Information to sponsor: Yes (x) No ( )
Sponsor and/or agent: Janssen Research Foundation
1125 Trenton-Harbourton Rd
Titusville, NJ 08560-0200
Manufacturer for drug substance: Janssen Pharmaceutical Ltd., Little Island, County Cork, Ireland
Reviewer name: Lois M. Freed, Ph.D.
Division name: Neuropharmacological Drug Products
HFD #: 120
Review completion date: 6/25/02
Drug: risperidone long-acting injection
Trade name: RISPERDAL CONSTA™
Generic name (list alphabetically): risperidone
Code name: R064766
Chemical name: 3-[2-[4-(6-fluoro-1,2-benzisoxazol-3-yl)-1-piperidinyl]ethyl]-6,7,8,9-tetrahydro-2-methyl-4*H*-pyrido[1,2-a]pyrimidin-4-one
CAS registry number: n/s
Mole file number: n/s
Molecular formula/molecular weight: 410.49
Structure:



Relevant INDs/NDAs/DMFs: DMF [redacted] (drug substance), I52982 (RISPERDAL i.m. depot, Janssen), N20-272 (RISPERDAL tablets, Janssen)
Drug class: D_2, $5HT_2$ antagonist
Indication: schizophrenia
Clinical formulation: solution for injection [25, 37.5, 50 mg]; excipient: 75:25 polylactide-co-glycolide (PLG); 381 mg risperidone per gm microspheres.
Route of administration: i.m. depot injection
Proposed use: n/a
Studies reviewed within this submission:
Safety Pharmacology (Vol 1.11; two cardiovascular safety reports)
PK/ADME (Vol 1.11-1.12): Reports FK2366, FK2631, FK2632, FK3152, FK3169, FK3170, FK3186, FK3214, FK3715.
Toxicology
Study 4731: 6-mo s.c. toxicity, Wistar rat (Vol 1.14-1.15)
Study 4729A: 12-mo i.m. toxicity, Wistar rat (Vol 1.15-1.16)
Study 4730: 12-mo i.m. toxicity, Beagle dog (Vol 1.19-1.20)
Supplemental Toxicology
Study 3150: single-dose, dermal, albino rabbit (Vol 1.24)

Study 3711: 1-mo, s.c., Beagle dog (Vol 1.24)
Study 3726: 1-mo, i.m., Beagle dog (Vol 1.24)
Study 4891: single-dose, i.m., Beagle dog (Vol 1.25)

Genotoxicity

Study 5414: *in vitro* Ames test (Vol 1.20)

Carcinogenicity

Study 4729: 24-mo i.m., Wistar rat (Vol 1.21-1.23)

Studies previously reviewed:

PK/ADME

Report No. FK24, FK1560, FK1561, FK1975, FK2092, FK1976, FK1260, FK2633, FK2687, FK2762, FK2846, FK2904, FK2932

Toxicology

Study 3095: 6-mo toxicity, Wistar rat (Vol 1.13)
Study 3589: 6-mo toxicity, Wistar rat (Vol 1.13-1.14)
Study 3114: 6-mo toxicity, Beagle dog (Vol 1.17)
Study 3590: 6-mo toxicity, Beagle dog (Vol 1.17-1.18)

Supplemental Toxicology (Vol 1.25-1.26)

Study 4338: single-dose, i.m., Beagle dog
Study 4364: single-dose, i.m., Beagle dog
Study 4337: single-dose, i.m., Beagle dog
Study 4335: single-dose, i.m., Beagle dog
Study 4336: repeated-dose, i.m., Beagle dog
Study 4443: single-dose, i.m., Beagle dog
Study 4460: single-dose, i.m., Beagle dog
Study 4295: repeated-dose, i.m. Beagle dog
Study 4296: repeated-dose, i.m., Beagle dog
Study 4365: single-dose, i.m., Wistar rat
Study 3977: single-dose, i.m., Belgian pig
Study 4114: single-dose, i.m, Belgian pig
Study 4328: single-dose, i.m., Beagle dog

Previous reviews:

Review and Evaluation of Pharmacology/Toxicology Data: Rev 1 [Lois M. Freed, Ph.D., 5/7/97].

Review and Evaluation of Pharmacology/Toxicology Data: Rev 2 [Lois M. Freed, Ph.D., 3/3/98].

Pharmacology/Toxicology Memorandum to IND 52,982 [Lois M. Freed, Ph.D., 3/12/98].

Pharmacology/Toxicology Memorandum to IND 52,982 [Lois M. Freed, Ph.D., 7/8/98].

Review and Evaluation of Pharmacology/Toxicology Data [Lois M. Freed, Ph.D., 3/31/99].

Review and Evaluation of Pharmacology/Toxicology Data [Lois M. Freed, Ph.D., 5/3/99].

Executive Summary

I. Recommendations

A. Recommendation on Approvability: it is recommended that the NDA not be approved until certain issues are adequately resolved: (a) the increases in renal tubular adenomas and adrenomedullary tumors that were observed in male rats in the carcinogenicity study conducted on the i.m. depot formulation, but not in the carcinogenicity studies conducted on oral risperidone. (b) the need for, at a minimum, an embryofetal development study in rat using the i.m. depot formulation. (c) the lack of documentation that impurities present in the i.m. depot formulation have been qualified.

B. Recommendations on Labeling: in the event that the NDA is considered approvable, the following revisions to the sponsor's proposed labeling are recommended:

PRECAUTIONS
General
Hyperprolactinemia

"As with other drugs that antagonize dopamine D_2 receptors, risperidone elevates prolactin levels and the elevation persists during chronic administration....

neither clinical studies nor epidemiologic studies conducted to date have shown an association between chronic administration of this class of drugs and tumorigenesis in humans; the available evidence is considered too limited to be conclusive at this time."

Carcinogenesis, Mutagenesis, Impairment of Fertility

Mutagenesis

Impairment of Fertility

Pregnancy

Pregnancy Category C

II. Summary of Nonclinical Findings

Risperidone i.m. depot was tested in a number of chronic toxicity studies conducted in Sprague-Dawley rat and Beagle dog, including 1-yr studies using the clinical i.m. depot formulation. An i.m. depot carcinogenicity study was conducted in rats. Both vehicle- and drug-related effects were observed in the 1-yr studies and the carcinogenicity study. Vehicle-related findings were evident at the injection site [e.g., microsphere encapsulation, inflammation, fibrous tissue reaction] and at other sites [lymph node, adrenal gland, pancreas, lung]. Microspheres were detected in lung of a few animals in both rat and dog and in mammary gland in 1 rat [in the carcinogenicity study]. Drug-related changes at the injection site consisted of granulocytic infiltration, necrotic cells, and/or muscle degeneration.

The most notable drug-related findings in the i.m. depot studies were (1) osteodystrophy in sternum and/or stifle joint [1- and 2-yr studies in rat] and (2) renal tubular adenomas and adrenomedullary tumors in male rat in the 2-yr carcinogenicity study. Additional tumors were detected [i.e., mammary gland adenocarcinomas, pancreatic islet cell adenomas, and pituitary adenomas]; however, these tumor types were observed in the oral carcinogenicity studies in

mouse and/or rat. The renal and adrenomedullary tumors were not observed in the oral studies. The sponsor conducted mechanistic studies in order to investigate possible factors underlying the difference in the tumor profile with the oral and i.m. depot formulations.

Reproduction studies were not conducted on the i.m. depot formulation.

The genotoxic potential of the i.m. depot formulation was tested in an Ames assay; no increases in revertants were observed with any of the tester strains used.

The sponsor reported the presence of —— impurities in the i.m. depot formulation that were not present in the oral formulations. Documentation of qualification was provided for one of these impurities.

More detailed discussions of the nonclinical studies are provided at the end of the appropriate sections.

TABLE OF CONTENTS - PHARMACOLOGY/TOXICOLOGY REVIEW

PHARMACOLOGY/TOXICOLOGY REVIEW

I. PHARMACOLOGY

No studies were submitted. The sponsor conducted a number of pharmacology studies to support the NDA [20-272] for RISPERDAL™ Tablets.

II. SAFETY PHARMACOLOGY

Cardiovascular effects:

The sponsor submitted two cardiovascular safety study reports.

1. **Synopsis of preclinical experiments on cardiac electrophysiology with risperidone and 9-OH-risperidone - Status on February 14th, 2000** [Author: Fred DeClerck, Janssen Research Foundation, report date: March 2001; Report No. EDMS-BEBE-2515175]. This report was amended on March 6, 2001 to include results of additional cardiovascular studies on risperidone and 9-OH-risperidone and on March 16, 2001 to correct an error.

According to this summary report, the following results have been obtained in the battery of *in vitro* and *in vivo* cardiovascular toxicity studies conducted on risperidone and/or 9-hydroxyrisperidone:

(a) in the HERG assay [transfected HEK293 cells], risperidone and 9-OH-risperidone inhibited the I_{Kr} channel at IC_{50}'s of 6.5×10^{-7} and 1.2×10^{-6} M, respectively. "Similar effects were found on native I_{Kr} with risperidone in isolated feline cardiomyocytes (76% inhibition...at 1×10^{-6} M)".

(b) in isolated canine Purkinje fibers [1 Hz, normal extracellular K^+ (4 mM)], both risperidone and 9-OH-risperidone prolonged APD_{90} [30% and 22%, respectively, at 1×10^{-6} M]. Under higher extracellular [K^+] (5.7 nM), there was no effect of risperidone on APD at concentrations of 9.7×10^{-8} and 7.5×10^{-7} M [higher concentrations were apparently not tested]. At a lower pacing frequency [0.5 Hz, 4 mM KCl], risperidone and 9-OH-risperidone prolonged APD_{90} at concentrations of 1×10^{-6} and 1×10^{-5} M [91 and 73%, respectively]. At a higher pacing frequency [3 Hz], neither compound affected APD_{90} at concentrations up to 1×10^{-5} M.

Under hypokalemic conditions [2.7 mM KCl; 1 Hz], both risperidone and 9-OH-risperidone prolonged APD_{90} at 1×10^{-6} and 1×10^{-5} M [≤24 and ≤39% with risperidone and 9-OH-risperidone, respectively].

(c) in isolated guinea pig cardiac papillary muscle [4 mM KCl, 1 Hz], concentrations of 1×10^{-6} and 1×10^{-5} M of both risperidone and 9-OH-risperidone prolonged APD_{90} by "20% or more". No effect [i.e., <10% prolongation) was observed at lower concentrations]. "Concentration-dependent and rate-dependent effects of risperidone and 9-OH-risperidone, similar to the ones seen in canine Purkinje fibers, were found in isolated guinea-pig papillary muscles."

Under hypokalemic conditions [2.7 mM, 1 Hz], risperidone and 9-OH-risperidone prolonged APD_{90} at concentrations of 1×10^{-6} and 1×10^{-5} M [≤20 and ≤179% with risperidone and 9-OH-risperidone, respectively].

(d) in isolated rabbit Purkinje fibers [4 mM KCl, 1 Hz], risperidone prolonged APD_{90} at concentrations of 1×10^{-6} and 3×10^{-6} M [90 and 103%, respectively]. At a lower pacing frequency [0.2 Hz, 4 mM KCl], risperidone also prolonged APD_{90} at these concentrations [1×10^{-6} M: 128%, 3×10^{-6} M: 175%; solvent: 57% (vs baseline at 1 Hz)]. In addition, risperidone elicited early afterdepolarizations [EAD] in

1-3 of 10 preparations at 1 Hz and in 4-7 of 10 preparations at 0.2 Hz. No delayed afterdepolarizations [DAD] were observed at either pacing frequency.

(e) the sponsor noted that findings similar to those observed in isolated papillary muscle and Purkinje fibers were obtained in isolated feline and rabbit hearts under condutions of normal [K^+]. At 1 x 10^{-6} M, risperidone prolonged QT in feline and rabbit hearts [26%]; 9-OH-risperidone prolonged QT [26%] at 1 x 10^{-5} M.

(f) in anesthetized guinea pigs, risperidone [0.08-5 mg/kg i.v.; total cumulative dose: 7.4 mg/kg i.v.] increased hr at doses ≥0.16 mg/kg i.v. [9-22%], reduced QT_c [Bazett's; -7 to -11%], and had no effect on ECG morphology. The sponsor noted that the basal hr is high in this species [i.e., 230 bpm] and, therefore, Fridericia's correction is inappropriate. The sponsor also noted that (a) this species is "highly sensitive to I_{Kr} blocking compounds" and (b) the α_1-adrenoreceptor antagonist properties of risperidone and 9-OH-risperidone may decrease a QT-prolonging effect by increasing hr and by countering QT-prolonging effects of α_1 stimulation.

(g) 4 early studies were conducted in awaked, acclimated dogs; in these studies, QT_c was calculated using Bazett's correction. In the 1st experiment, an acute dose [0.08 mg/kg p.o.] of risperidone was tested in 7 dogs. Heart rate was not affected at this dose; only a very small [5%] increase in QT was observed from 70-min postdosing on. In the 2nd experiment, multiple doses of risperidone [0.08 mg/kg, 6 days] were administered to 7 dogs. No "consistent changes occurred" in hr, PQ, QRS, QT or QT_c". In the 3rd experiment, an acute dose [0.31 mg/kg p.o.] was tested in 7 dogs. Heart rate increased 10% and QT interval increased from 175 msec to 190 msec [8%]; QT_c increased 11%. In the 4th study, risperidone was tested at doses of 0.02, 0.08, and 0.31 mg/kg p.o. in 7 dogs. QT_c was not affected at any dose [Bazett's, Van de Water]. At 0.31 mg/kg, hr was markedly increased [36%; 30 bpm].

In a recent PK/PD study, doses of 0.08 and 0.31 mg/kg p.o. were administered to 7/grp. [Higher doses were not administered due to the increased hr [and clinical signs (i.e., restlessness, trembling)] observed at 0.31 mg/kg. Heart rate was significantly increased at both doses [37 and 42%, respectively]; no significant effect was noted on QT_c using Bazett's, Fridericia's or Van de Water's corrections. Plasma levels of "active moiety" were 54 and 232 following the 0.08 and 0.31 mg/kg p.o. doses, respectively. [In humans, C_{max} for active moiety following an 8-mg oral dose was 134 ng/mL; the plasma level of active moiety associated with a 70% D_2 receptor occupancy was 18 ng/mL.] [This study is reviewed in more detail as #2 in this section.]

2. **Effects of risperidone, on cardiovascular and behavioural parameters in instrumented, awake dogs: dose 0.08 and 0.31 mg/kg orally** [LMD No. N144705, Study No. CPF 241, Report date: 2/00, study start date: 10/98, Janssen Research Foundation, Belgium, **non-GLP**]

Methods: the cardiovascular effects of risperidone [lot no. ZR064766PUA111; vehicle: demineralized water, tartaric acid] were assessed in awake male Beagle dogs [7/grp] at doses of 0, 0.08 and 0.31 mg/kg p.o. ECG lead II was used to quantitate parameters [at 0, 30, 60, 120, 180, and 240 min postdosing]: PQ, QRS, QT, QT_c [Bazett's, Fridericia, and Van de Water corrections]. Additional parameters recorded were as follows: hr, DAP, SAP, PRP, L dp/dt max, LV dp/dt max/p, LV dp/dt min, Relaxation time constant, CO, SV, TSR. Blood samples were collected at 30, 60, and 240 min postdosing for quantitation of plasma risperidone and 9-OH-risperidone.

Results: restlessness was observed in 2/7 LD and 3/7 HD dogs; trembling was also observed in 1 of the 2 affected LD dogs and 2 of the 3 affected HD dogs. Plasma levels [ng/mL] of risperidone and 9-OH-risperidone are summarized in table below:

TIME (min)	LD		HD	
	median	range	median	range
RISPERIDONE				
30	13		88.4	
60	19.2		45.7	
240	0.7		1.99	
9-OH-RISPERIDONE				
30	18.7		144	
60	35.4		151	
240	33.6		106	

The mean (range) C_{max} at the LD and MD were 29 (7.71-68.8) and 95 (12.4-209) ng/mL, respectively, for risperidone and 44 (23.6-81.4) and 168 (74.9-247) ng/mL, respectively, for 9-OH-risperidone. Plasma levels were highest in HDM #B15916 and HDM #B99672.

The QT interval data were summarized in the following sponsor's table:

Table 3: Comparative effects of risperidone 0.08 and 0.31 mg/kg orally on heart rate, QTc Bazett, QTc Fridericia and QTc Van de Water in awake, trained dogs.

	Change (median % versus premedication#)				
	Heart rate	QTcB	QTcF	QTcV	ng/ml**
Solvent 0.08 mg/kg (n=7)					
30 min	-2	0	0	0	/
60 min	+11	+4	+4	+2	/
240 min	0	0	0	0	/
Risperidone 0.08 mg/kg (n=7)					
30 min	+12	+8	+7	+7	32
60 min	+37*	+5	+3	+2	54
240 min	+9	+2	+0	+0	34
Solvent 0.31 mg/kg (n=7)					
30 min	-17	-4	-3	-4	/
60 min	-4	+3	+1	+1	/
240 min	+26	+5	-3	0	/
Risperidone 0.32 mg/kg (n=7)					
30 min	+26*	+5	+4	+2	232
60 min	+31*	+14	+5	+2	197
240 min	+25	+14	+6	+2	108

Premedication values (median, n=7). Heart rate: solvent 0.08 mg/kg = 89 beats/min; solvent 0.31 mg/kg =75 beats/min;risperidone 0.08 mg/kg = 83 beats/min; risperidone 0.31 mg/kg = 96 beats/min.
QTc Bazett (B): solvent 0.08 mg/kg = 202; solvent 0.31 mg/kg =191; risperidone 0.08 mg/kg = 214; risperidone 0.31 mg/kg = 218.
QTc Fridericia (F): solvent 0.08 mg/kg = 187; solvent 0.31 mg/kg =194; risperidone 0.08 mg/kg = 209; risperidone 0.31 mg/kg = 202.
QTc Van de Water (V): solvent 0.08 mg/kg = 193; solvent 0.31 mg/kg =192; risperidone 0.08 mg/kg = 210; risperidone 0.31 mg/kg = 205.

* $p < 0.05$ versus solvent

** Plasma level of the active moiety (risperidone + 9-OH risperidone; in ng/ml, median values).

Heart rate was significantly increased at both doses. QT_c was not significantly affected regardless of the correction method used. QT_c (% change from baseline) was slightly greater in the treated grps as compared to the solvent controls at both doses; however, the effect was dose-related only with Bazett's correction.

Individual data were provided only in graphic format. The sponsor reported the following findings: (a) dose-related increases in heart rate [37 and 42% at LD and HD, respectively], LV dp/dt max [19 and 44% at LD and HD, respectively], LV dp/dt max/p [18 and 34% at LD and HD, respectively], and CO [33 and 41% at LD and HD, respectively], (b) dose-related decreases in DAP [15% at HD], SAP [12 and 26% at LD and HD, respectively], and TSR [25 and 42% at LD and HD, respectively]. The sponsor noted that no effects on ECG morphology was noted at the LD; however, "Some single extrasystoles" were observed in 2/7 CM and 2/7 HDM.

Safety pharmacology summary and conclusions: the sponsor has conducted a number of *in vitro* and *in vivo* cardiovascular safety studies on risperidone and 9-OH-risperidone, most submitted to NDA 20-272 [RISPERDAL™ tablets]. Data from the *in vitro* studies indicate that risperidone has the potential to increase APD, particularly at higher concentrations [μM]. Risperidone and 9-OH-risperidone both inhibited the I_{Kr} channel [HERG assay; IC_{50} = 650 nM and 1.2 μM, respectively] *in vitro* and increased APD_{90} in a variety of preparations [canine Purkinje fibers, guinea pig cardiac papillary muscle, rabbit Purkinje fibers]. [For comparison, sertindole inhibited the I_{Kr} channel (HERG) at an IC_{50} of 14 nM.] The *in vivo* data did not demonstrate a significant effect of risperidone [i.v., p.o.] on QT_c in either anesthetized guinea pig or awake dog.

The sponsor did not review the published literature on risperidone's effect on cardiac conduction in the synopsis report. For example, Drici *et al.* [Drici M-D *et al. J Clin Psychopharm* 18(6):1998] tested 5 antipsychotic drugs [haloperidol, risperidone, sertindole, clozapine, olanzapine] *in vitro* in isolated feline heart at concentrations of 0.1-20 μM. All drugs tested produced concentration-related increases in QT interval, with risperidone and haloperidol being "...significantly more potent than sertindole, clozapine, and olanzapine..." [Table 1, copied directly from the published article, below].

TABLE 1. Percent change ± SD from baseline in QT intervals of isolated feline hearts perfused with antipsychotic drugs at different concentrations*

	Percent Change from Baseline in QT Interval (μmol/L)						
Compound	0.1	0.5	1.0	5.0	10	20	30
Haloperidol	13 ± 0.1 (N = 2)	26 ± 1 (N = 2)	27 ± 2 (N = 4)	—	50 ± 3 (N = 4)	—	—
Risperidone	10 ± 3 (N = 3)	20 ± 3 (N = 4)	26 ± 10 (N = 4)	36 ± 10 (N = 4)	38 ± 5 (N = 5)	51 ± 9 (N = 5)	—
9-OH risperidone	—	—	5 ± 3 (N = 3)	—	27 ± 3 (N = 3)	33 ± 5 (N = 3)	—
Sertindole	—	9 ± 3 (N = 4)	15 ± 1 (N = 3)	24 ± 5 (N = 4)	31 ± 3 (N = 3)	40 ± 7 (N = 5)	—
Lu 28-092	—	6 ± 2 (N = 4)	13 ± 3 (N = 4)	26 ± 2 (N = 3)	34 ± 13 (N = 4)	38 ± 2 (N = 3)	—
Lu 25-073	—	4 ± 2 (N = 5)	10 ± 1 (N = 4)	9 ± 3 (N = 4)	20 ± 6 (N = 3)	21 ± 2 (N = 3)	—
Clozapine	—	—	0.7 ± 1 (N = 4)	—	9 ± 3 (N = 4)	17 (N = 1)	43 (N = 1)
Olanzapine	—	—	7 ± 1 (N = 4)	12 (N = 1)	27 ± 5 (N = 4)	—	43 ± 2 (N = 3)

*Dashes indicate that concentrations were not measured.

In addition, there have been published reports of QT prolongation with risperidone in humans [cf. Titier *et al. Toxicol Appl Pharmacol* 180:145-149, 2002]. A review of the literature is beyond the time limits [and scope] of this review; however, both *in vitro* and *in vivo* studies have demonstrated that risperidone and/or 9-OH-risperidone have the potential to prolong QT, and therefore, to produce cardiac arrhythmias.

III. PHARMACOKINETICS/TOXICOKINETICS

The sponsor submitted 22 PK/ADME/TK study reports. Of these, 13 have previously been reviewed [5/7/97, 5/3/99]; results are included in the PK/TK summary and conclusions section. The remaining 9 reports are reviewed below.

1. **Plasma concentrations of the active moiety (=sum of risperidone (R064766) and 9-hydroxy-risperidone (R076477)) in the beagle dog after single administration of a risperidone microsphere formulation via 4 different administration routes, either at 0.31 (IV) or at 2.5 mg/kg (SC, IM and injection into adipose tissue).** [Report No. N130128/1, Protocol No. FK2366, report date: 9/23/97, study dates: 10/28/96-12/23/96, conducting laboratory: sponsor, Belgium].

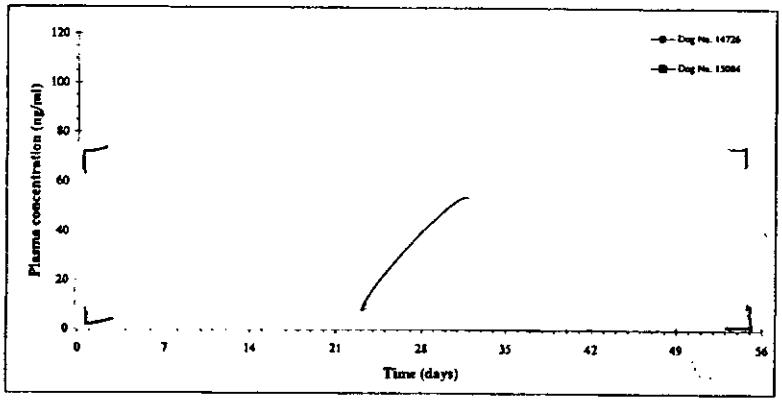
Methods: this study was conducted in female beagle dogs [2/grp, 6-12 mo old]. Risperidone [0.31 or 2.5 mg/kg] was administered as a depot copolymer [dl-lactic and glycolic acid; 75:25] microsphere formulation ——————————s.c., i.m., into adipose tissue, or i.v. Blood samples were collected at 0, 1, 5, 24, and 72 hrs following dosing during Wk 1 and on Days 7,11, 15, 18, 21, 25, 28, 32, 35, 39, 42, 46, 49, 53, and 56 postdosing. The "active moiety" [i.e., risperidone + 9-OH-risperidone] was quantitated in plasma using RIA [LLOQ = ———].

Results: PK parameters are summarized in the following sponsor's table [portion of Table 5-3]:

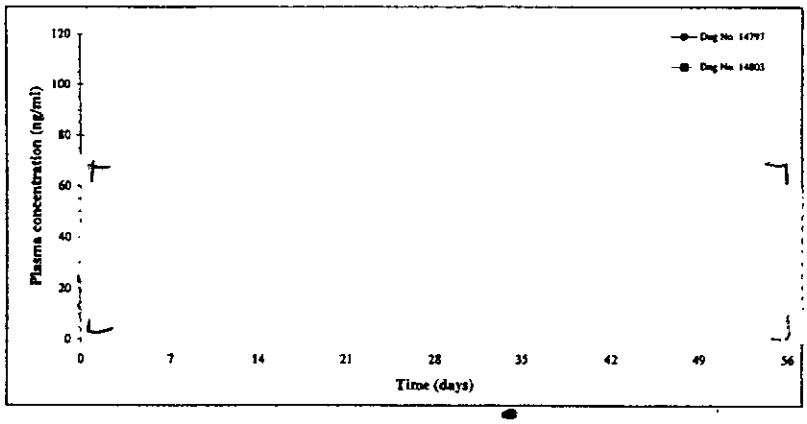
		s.c. (indiv)	(mean)	i.m. (indiv)	(mean)	i.v. (indiv)	(mean)	adipose (indiv)	(mean)
C_{max1}	(ng/ml)		25.0		18.3		17.1		4.53
T_{max1}	(h)		5		1		3		37
C_{max2}	(ng/ml)				34.3		88.6		2.12
T_{max2}	(h)				552		672		672
$t_{1/2}$	(h)				98.0		75.0		
AUC_{0-t}	(ng.h/ml)		18483		14373		22196		925
$AUC_{0-\infty}$	(ng.h/ml)				14498		22226		

The time-concentration curves following each route of administration (provided by the sponsor) are given below:

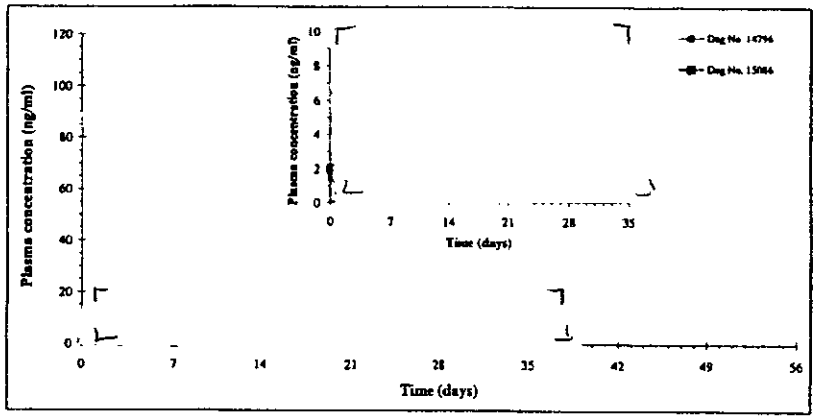
s.c. [2.5 mg/kg]



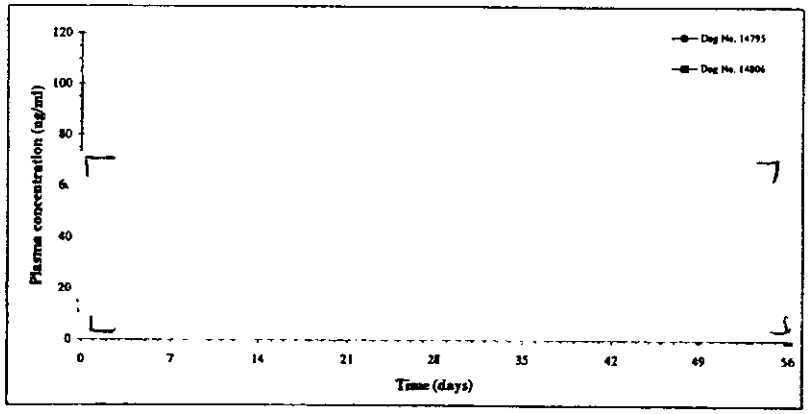
i.m. [2.5 mg/kg]



i.v. [0.31 mg/kg]



adipose tissue [2.5 mg/kg]



Following the i.m. dose, there was an "unexpected peak" on Day 11; the plasma concentration on Day 11 [.[redacted] ng/mL] was the C_{max}. The peak at around Day 35 was expected, and represents disintegration of the microspheres with burst release of residual drug. The s.c. route provided the most stable exposure of the 4 routes examined.

2. **A study on the plasma concentration-time profile of the active moiety (=sum of risperidone (R064766) and 9-hydroxy-risperidone (R076477) in beagle dogs after single intramuscular administration of a risperidone depot microsphere formulation at 5 mg/kg at one injection site, using different injection volumes** [Study No. FK2631; addendum to Toxicity Report Exp No. 4337 (previously reviewed), report date: 11/25/98, conducting laboratory: sponsor, Belgium].

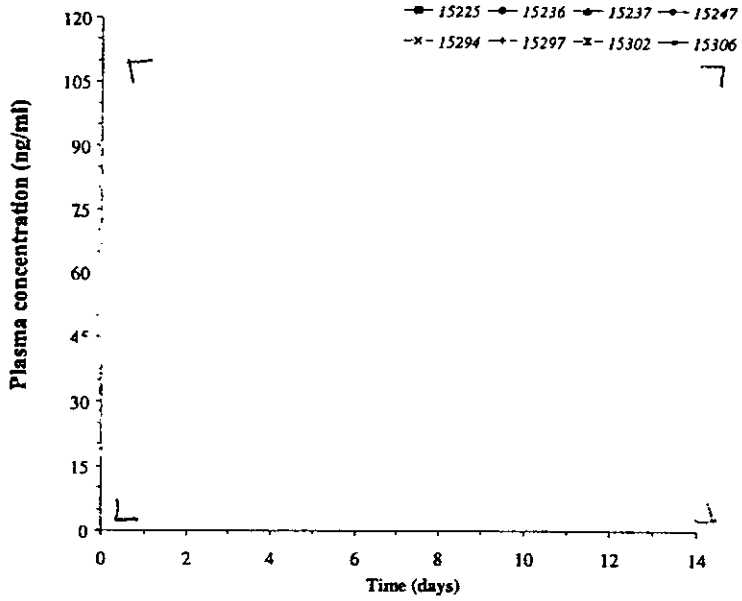
Methods: this study was conducted in order to further investigate the phenemonon of "partial early release" [i.e., prior to Day 14] observed with the i.m. depot formulation. Risperidone depot microspheres [75:25 d,l-lactide-co-glycolide polymer] were administered to female Beagle dogs [4/grp] at a single dose [5 mg/kg], either as a single injection of volumes of 0.1, 0.2, or 0.4 mL/kg. Blood samples were collected at 0, 1, 5, and 24 hrs, Day 3, 6, 7, 8, 9, 10, 13, 17, 20, 24, and 27 postdosing in ½ of the dogs; additional samples were collected on Day 31, 34, 38, 41, 45, 48, 52, and 55 [at 8:00 a.m.] from the remaining dogs. The "active moiety" was quantitated in plasma using RIA [LLOQ = —ng/mL.

Results: extra peaks [i.e., partial early release] were observed 3-9 days following i.m. injection of all volumes tested in 2-3 of 4 dogs. The "main release" was observed "around Day 17 to 20" at all but the lowest volume, with the magnitude of the peak inversely proportional to the magnitude of the partial early release. The sponsor concluded that there might be a "trend that an injection volume of 0.4 mL/kg gives a lower chance of partial early releases and more limited (as far as extent is concerned) partial early releases", however, acknowledged that there was "...no clearcut difference in the occurrence of partial early releases when different injection volumes.." were used.

3. **Plasma concentration of the active moiety (=sum of risperidone (R064766) and 9-hydroxy-risperidone (R076477)) in beagle dogs in a repeated dose intramuscular irritation study (Exp. No. 4336) of a risperidone depot microsphere formulation at 5 mg/kg every two weeks, using the same injection site for either dose administration** [Study No. FK2632, report date: 11/25/98, conducting laboratory: sponsor, Belgium.]

Methods: this study is an addendum to a repeat-dose i.m. depot toxicity study in Beagle dog [#4336, previously reviewed]. The studies were conducted in order to further investigate the occurrence of "partial early release" [i.e., between Days 2 and 15 postdosing] observed in animals [and humans]. This study assessed the effect of repeated doses at one injection site on the occurrence of partial early releases. Risperidone i.m. depot formulation was administered at a concentration of 50 mg/mL [dose: 5 mg/kg] to 8 female Beagle dogs every 2 wks for 6 wks; all injections were given at the same site. The "active moiety" was quantitated in plasma [using RIA, LLOQ =——ng/mL], prepared from blood samples collected on Day 0 [0, 1, 5, and 24 hrs postdosing], 3, 6, 7, 8, 9, 10, 13, and 14 [8:00 a.m.] after each bi-weekly dose. Additional blood samples were collected [at 8:00 a.m.] on Days 17, 20, 24, 27, and 30 following the 4th dose.

Results: Early peaks [consistent with partial early release] were observed in the majority of dogs following the first dose [see following sponsor's Figure 5-1].



Peaks were more difficult to evaluate following the later doses due, according to the sponsor, "...to the obvious accumulation of plasma levels of the active moiety".

Signs of anaphylaxis were observed in 2/8 dogs following the 3rd dose; the sponsor attributed this to aging of the vehicle. Therefore, fresh vehicle was used for the 4th dose.

The sponsor concluded that repeated doses at one site do not increase the risk of a partial early release; therefore, the sponsor concluded that an allergic response to risperidone and/or the microspheres did not develop with repeated i.m. doses.

4. **Toxicokinetics of risperidone, 9-hydroxy-risperidone and of the active moiety (=sum of risperidone and 9-hydroxy-risperidone) in the SPF Wistar rat in a combined intermittent repeated dose intramuscular toxicity study and 24-month carcinogenicity study (Exp. No 4729) on an aqueous depot microsphere formulation of risperidone (Ro64766) at 5 and 40 mg/kg/2 weeks** [Study No. FK3152, study initiation date: 2/11/99, conducting laboratory: sponsor, Belgium, GLP, QA:Y].

Methods: TK data were collected in satellite animals during the 1-yr i.m. depot toxicity/24-mo i.m. depot carcinogenicity study conducted in Wistar rats [6/sex/grp]. In that study, risperidone i.m. depot microspheres [microsphere batch 164-2298, used in Phase 3 trials] was administered at doses of 5 and 40 mg/kg [50 mg/mL] every 2 wks. [The depot formulation contained the following: ——— lactic acid:glycolic acid (3:1) ———.] Blood samples were collected (via orbital venous plexus) from 3/sex/grp at 1, 24, and 192 hrs after the 1st and 13th doses, and from the remaining 3/sex/grp at 5, 24, and 336 hrs following the 6th, 19th and 26th doses and at 5 and 24 hrs following the 52nd dose. Risperidone and 9-hydroxy-risperidone were quantitated in plasma using LC/MS/MS (LLOQ = ——ng/mL for both compounds). The method for calculating the "active moiety" was not specified.

Results: the data were summarized in the following sponsor's tables:

Table 5-2: **Mean (± SD; n = 3) plasma concentrations (ng/ml) and some pharmacokinetic parameters of risperidone (R064766) and its active metabolite 9-hydroxy-risperidone (R076477) in SPF Wistar rats after the first dose administration of an aqueous depot microsphere formulation of risperidone at 5 and 40 mg/kg in a combined intermittent repeated dose intramuscular toxicity study and 24 month carcinogenicity study (Exp. No. 4729).**

		5 mg/kg				40 mg/kg			
		Males		Females		Males		Females	
Time (h)[1]	(day)[2]	R064766	R076477	R064766	R076477	R064766	R076477	R064766	R076477
1	0	2.70 ± 0.19	4.41 ± 0.55	4.83 ± 2.48	1.78 ± 0.94	22.2 ± 2.1	11.0 ± 0.53	33.9 ± 9.8	14.2 ±12.1
5		< 0.50[3]	< 0.50[3]	< 0.50[3]	1.28 [3]	1.04 ± 0.13	5.59 ± 1.05	2.97 ± 1.17	9.24 ± 2.38
24	1	1.45 ± 0.57	1.16 ± 0.67	2.91 ± 1.56	1.50[3]	2.66 ± 1.80	1.10 ± 0.77	4.96 ± 2.02	1.62 ± 1.21
120	5	0.75 ± 0.26	< 0.50[3]	1.18[3]	0.59[3]	3.13 ± 0.57	2.27 ± 0.36	11.2 ± 3.5	5.01 ± 0.58
192	8	< 0.50[3]	< 0.50[3]	< 0.50[3]	< 0.50[3]	1.63 ± 0.62	0.77 ± 0.35	3.19 ± 1.75	1.50[3]
336	14	< 0.50[3]	< 0.50[3]	< 0.50[3]	0.52[3]	5.57 ± 3.37	3.89 ± 2.56	7.83 ± 4.90	3.34 ± 1.78
C_{max}	(ng/ml)	2.70	4.41	4.83	2.10	22.2	11.0	33.9	14.2
T_{max}	(h)	1	1	1	24	1	1	1	1
$C_{plateau}$ [4]	(ng/ml)	< 0.50[3]	< 0.50[3]	< 0.50[3]	1.15[3]	2.81 [62]	2.72 [74]	6.03 [58]	4.25 [73]
$AUC_{0-336\ h}$	(ng.h/ml)	154	66.3	621	383	1060	708	2255	1115

[1] Time after dosing on day 0 of the toxicity experiment.
[2] Day of the toxicity experiment.
[3] Median.
[4] Calculated as the average plasma concentration from 5 h up to 336 h after dosing The coefficient of variation (%) is given between square brackets, when applicable.

Table 5-3: **Mean (± SD; n = 3) plasma concentrations (ng/ml) and some pharmacokinetic parameters of risperidone (R064766) and its active metabolite 9-hydroxy-risperidone (R076477) in SPF Wistar rats after the 13th dose administration of an aqueous depot microsphere formulation of risperidone at 5 and 40 mg/kg/2 weeks in a combined intermittent repeated dose intramuscular toxicity study and 24 month carcinogenicity study (Exp. No. 4729).**

		5 mg/kg				40 mg/kg			
		Males		Females		Males		Females	
Time (h)[1]	(day)[2]	R064766	R076477	R064766	R076477	R064766	R076477	R064766	R076477
1	0	13.2 ± 2.5	8.53 ± 1.15	14.1 ± 3.8	5.19 ± 2.56	96.5 ± 13.8	24.5 ± 0.3	104 ± 27	35.5 ± 23.2
5		4.45 ± 0.99	1.84 ± 0.72	8.50[3]	7.85[3]	77.8 ± 0.8	55.4 ± 9.2	63.0 ± 13.6	43.6 ± 1.0
24	1	8.70 ± 0.75	4.05 ± 0.30	10.3 ± 5.2	3.96 ± 3.03	44.0 ± 14.9	12.9 ± 1.8	52.9 ± 19.9	22.5 ± 12.9
120	5	7.02 ± 3.90	1.69 ± 0.88	12.7[3]	8.01[3]	55.8 ± 24.3	36.4 ± 16.0	76.2 ± 24.4	39.1 ± 2.3
192	8	8.04 ± 1.12	3.98 ± 0.88	8.47 ± 2.87	4.35 ± 2.05	44.6 ± 3.2	16.8 ± 4.0	52.9 ± 27.6	23.9 ± 16.1
336	14	4.03 ± 0.55	0.99 ± 0.19	7.50[3]	4.65[3]	43.8 ± 12.1	25.5 ± 4.8	38.7 ± 13.8	18.9 ± 4.5
C_{max}	(ng/ml)	13.2	8.53	14.1	8.01	96.5	55.4	104	43.6
T_{max}	(h)	1	1	1	120	1	5	1	5
$C_{plateau}$ [4]	(ng/ml)	6.45 [33]	2.51 [56]	9.48 [21]	5.76 [35]	53.2 [28]	29.4 [58]	56.7 [24]	29.6 [37]
$AUC_{0-336\ h}$	(ng.h/ml)	2333	918	3240	1808	16322	8147	18928	9105

[1] Time after dosing on day 182 of the toxicity experiment.
[2] Day of the toxicity experiment.
[3] n = 2.
[4] Calculated as the average plasma concentration from 5 h up to 336 h after dosing. The coefficient of variation (%) is given between square brackets.

Table 5-4: **Mean (± SD; n = 3) plasma concentrations (ng/ml) and some pharmacokinetic parameters of the active moiety (= sum of risperidone (R064766) and 9-hydroxy-risperidone (R076477)) in SPF Wistar rats after the first and 13th dose administration of an aqueous depot microsphere formulation of risperidone at 5 and 40 mg/kg in a combined intermittent repeated dose intramuscular toxicity study and 24 month carcinogenicity study (Exp. No. 4729).**

		1st dose				13th dose			
		5 mg/kg		40 mg/kg		5 mg/kg		40 mg/kg	
Time (h)[1]	(day)	Males	Females	Males	Females	Males	Females	Males	Females
1	0	7.11 ± 0.72	6.60 ± 3.11	33.2 ± 1.9	48.1 ± 21.9	21.8 ± 2.8	19.3 ± 6.2	121 ± 14	139 ± 50
5		< 0.50[2]	1.78[2]	6.63 ± 1.18	12.2 ± 3.6	6.29 ± 1.60	16.3[2]	133 ± 10	107 ± 14
24	1	2.61 ± 1.24	4.47 ± 2.56	3.77 ± 2.57	6.58 ± 2.98	12.7 ± 1.0	14.2 ± 8.3	56.9 ± 16.1	75.4 ± 31.1
120	5	1.25 ± 0.26	1.77[2]	5.39 ± 0.93	16.2 ± 4.0	8.71 ± 4.78	20.7[2]	92.1 ± 40.2	115 ± 27
192	8	< 0.50[2]	< 0.50	2.39 ± 0.97	4.70 ± 2.75	12.0 ± 0.9	12.8 ± 4.8	61.4 ± 6.3	76.8 ± 43.6
336	14	< 0.50[2]	1.16[2]	9.46 ± 5.92	11.2 ± 6.7	5.02 ± 0.36	12.1[2]	69.4 ± 16.6	57.7 ± 17.0
C_{max}	(ng/ml)	7.11	6.60	33.2	48.1	21.8	20.7[4]	133	139
T_{max}	(h)	1	1	1	1	1	120	5	1
$C_{plateau}$ [3]	(ng/ml)	< 0.50[2]	1.82[2]	5.53 [49]	10.2 [45]	8.96 [38]	15.2[23]	82.6 [38]	86.3 [28]
$AUC_{0-336\ h}$	(ng.h/ml)	301	947	1768	3315	3251	5048	24469	28033

[1] Time after dosing on day 0 (1st dose) or day 182 (13th dose) of the toxicity experiment.
[2] Median.
[3] Calculated as the average plasma concentration from 5 h up to 336 h after dosing The coefficient of variation (%) is given between square brackets when applicable.
[4] A first peak was observed at 1 h, a second peak at 120 h.

Table 5-5: **Mean (± SD; n = 3) plasma concentrations (ng/ml) and some pharmacokinetic parameters of risperidone (R064766) and its active metabolite 9-hydroxy-risperidone (R076477) in SPF Wistar rats after the 6th, 19th, 26th and 52nd dose administration of an aqueous depot microsphere formulation of risperidone at 5 and 40 mg/kg/2 weeks in a combined intermittent repeated dose intramuscular toxicity study and 24 month carcinogenicity study (Exp. No. 4729).**

		5 mg/kg				40 mg/kg			
		Males		Females		Males		Females	
Time (h)[1]	(day)[2]	R064766	R076477	R064766	R076477[3]	R064766	R076477	R064766	R076477
5	84	4.33 ± 1.62	3.30 ± 1.38	7.58 ± 1.64	4.09 ± 1.51	39.3 ± 9.9	22.1 ± 4.6	44.5 ± 3.8	25.8 ± 4.3
24	85	6.62 ± 2.91	3.47 ± 1.03	14.0 ± 6.3	6.12 ± 4.88	40.6 ±10.8	15.5 ± 3.1	44.3 ± 4.2	20.6 ± 6.7
336	98	4.69 ± 0.61	2.44 ± 0.35	4.02 ± 0.84	1.93 ± 0.21	32.6 ± 6.2	12.5 ± 2.3	40.4 ±11.8	18.4 ± 7.8
$AUC_{0-336\ h}$[4]	(ng.h/ml)	1879	994	3042	1364	12282	4781	14179	6590
$C_{plateau}$[4]	(ng/ml)	5.21 [24]	3.07 [18]	8.55 [59]	4.05 [52]	37.5 [11]	16.7 [29]	43.1 [5]	21.6 [18]
5	266	5.30 ± 1.23	3.89 ± 0.75	8.55 ± 2.35	5.38 ± 2.40	42.1 ±14.7	19.3 ± 5.9	36.7 ±10.2	23.9 ± 9.2
24	267	9.80 ± 0.70	5.24 ± 0.88	12.0 ± 6.6	5.19 ± 2.61	46.2 ± 6.2	15.3 ± 2.8	40.2 ± 8.8	19.1 ± 6.8
336	280	5.76[3]	3.24[3]	13.2 ± 6.5	4.35 ± 2.03	35.0 ±14.1	12.6 ± 5.7	43.9 ±23.6	21.3 ±15.9
$AUC_{0-336\ h}$[4]	(ng.h/ml)	2584	1420	4145	1602	13611	4717	13948	6772
$C_{plateau}$[4]	(ng/ml)	6.95 [36]	4.12 [25]	11.2 [21]	4.97 [11]	41.1 [14]	15.7 [22]	40.3 [9]	21.4 [11]
5	364	5.42[3]	4.43[3]	8.40 ± 3.50	5.43 ± 3.07	43.3 ± 3.6	18.5 ± 3.7	75.8 ± 3.8	42.5 ±10.4
24	365	8.26[3]	4.61[3]	10.6 ± 4.3	4.47 ± 2.29	33.5 ± 6.3	10.8 ± 2.6	84.1 ±21.5	38.4 ±19.0
336	378	3.32[3]	2.09[3]	6.35 ± 0.86	2.72 ± 1.10	45.8 ±25.2	18.1 ±11.2	46.6 ±13.6	24.8 ±10.1
$AUC_{0-336\ h}$[4]	(ng.h/ml)	1950	1141	2846	1229	13215	4842	22093	10739
$C_{plateau}$[4]	(ng/ml)	5.67 [44]	3.71 [38]	8.45 [25]	4.21 [33]	40.9 [16]	15.8 [27]	68.8 [29]	35.2 [26]
5	728	10.5 ± 4.3	6.70 ± 3.94	12.9[3]	8.35[3]	133 ±143	75.5 ±92.1	76.3 ±57.1	53.7 ±43.2
24	729	14.7 ± 8.3	7.09 ± 5.00	14.1[3]	8.37[3]	83.7 ±49.4	45.6 ±40.1	65.3 ±45.8	34.7 ±28.9
$C_{plateau}$[5]	(ng/ml)	12.6	6.90	13.6	8.36	108	60.6	70.8	44.2

[1] Time after the respective dose administration.
[2] Day of the toxicity experiment.
[3] n = 2.
[4] Calculated as the average plasma concentration from 5 h up to 336 h after dosing. The coefficient of variation (%) is given between square brackets.
[5] Calculated as the average plasma concentration from 5 h and 24 h after dosing (n = 2).

Table 5-6: **Mean (± SD; n = 3) plasma concentrations (ng/ml) and some pharmacokinetic parameters of the active moiety (= sum of risperidone (R064766) and 9-hydroxy-risperidone (R076477)) in SPF Wistar rats after the 6th, 19th, 26th and 52nd dose administration of an aqueous depot microsphere formulation of risperidone at 5 and 40 mg/kg/2 weeks in a combined intermittent repeated dose intramuscular toxicity study and 24 month carcinogenicity study (Exp. No. 4729).**

		5 mg/kg		40 mg/kg	
Time (h)[1]	(day)[2]	Males	Females	Males	Females
5	84	7.63 ± 2.89	11.7 ± 2.8	61.5 ± 12.5	70.4 ± 6.3
24	85	10.1 ± 3.8	20.2 ± 11.2	56.1 ± 13.7	65.0 ± 9.5
336	98	7.13 ± 0.92	5.95 ± 0.81	45.1 ± 8.5	58.8 ± 19.0
$AUC_{0-336\ h}$[4]	(ng.h/ml)	2873	4405	17063	20769
$C_{plateau}$[4]	(ng/ml)	8.28 [19]	12.6 [57]	54.2 [15]	64.7 [9]
5	266	9.19 ± 1.98	13.9 ± 4.8	61.4 ± 20.5	60.6 ± 17.6
24	267	15.0 ± 1.6	17.2 ± 8.9	61.5 ± 8.9	59.3 ± 12.6
336	280	9.00[3]	17.5 ± 7.7	47.6 ± 19.7	65.2 ± 39.5
$AUC_{0-336\ h}$[4]	(ng.h/ml)	4004	5747	18328	20720
$C_{plateau}$[4]	(ng/ml)	11.1 [31]	16.2 [12]	56.8 [14]	61.7 [5]
5	364	9.84[3]	13.8 ± 6.4	61.8 ± 7.3	118 ± 8
24	365	12.9[3]	15.1 ± 6.5	44.4 ± 8.9	123 ± 40
336	378	5.41[3]	9.07 ± 1.73	63.9 ± 36.2	71.4 ± 22.8
$AUC_{0-336\ h}$[4]	(ng.h/ml)	3091	4075	18056	32832
$C_{plateau}$[4]	(ng/ml)	9.37 [40]	12.7 [25]	56.7 [19]	104 [27]
5	728	17.2 ± 8.2	21.3[3]	208 ± 235	130 ± 100
24	729	21.8 ± 13.3	22.5[3]	129 ± 89	100 ± 75
$C_{plateau}$[5]	(ng/ml)	19.5	21.9	169	115

[1] Time after the respective dose administration.
[2] Day of the toxicity experiment.
[3] n = 2.
[4] Calculated as the average plasma concentration from 5 h up to 336 h after dosing. The coefficient of variation (%) is given between square brackets.
[5] Calculated as the average plasma concentration from 5 h and 24 h after dosing (n = 2).

The $AUC_{(0\text{-}336\ hr)}$ data (units: ng•hr/mL) from these tables are summarized in the following table:

DOSE	MALES		FEMALES	
	5 mg/kg	40 mg/kg	5 mg/kg	40 mg/kg
RISPERIDONE				
1st	154	1060	621	2255
13th	2333	16322	3240	18928
6th	1879	12282	3042	14179
19th	2584	13611	4145	13948
26th	1950	13215	2846	22093
9-OH-RISPERIDONE				
1st	66.3	708	383	1115
13th	918	8147	1808	9105
6th	994	4781	1364	6590
19th	1420	4717	1602	6772
26th	1141	4842	1229	10739
ACTIVE MOIETY				
1st	301	1768	947	3315
13th	3251	24469	5048	28033
6th	2873	17063	4405	20769
19th	4004	18328	5747	20720
26th	3091	18056	4075	32832

5. **The plasma levels and excretion of , R076477 (9-hydroxy-risperidone) and R064766 (risperidone) in the male beagle dog after single oral administration of at mg/kg** [Report No. FK3169, conducting laboratory: sponsor, Belgium, study initiation date: 2/16/99].

Methods: the purpose of the study was to determine plasma exposure to the , following direct administration of to 4 male Beagle dogs. The dose used, mg/kg, was the LD of risperidone used in the 12-mo oral toxicity study in Beagle dogs [Exp 1789, R064766/FK516] was dissolved in an aqueous solution of 0.5 M tartaric acid to a final concentration of mg/mL. [Analysis of the solution verified the final concentration to be of intended.] Blood samples were collected prior to dosing, and at 0.25, 0.5, 1, 1.5, 2, 4, 6, 8, and 24 hrs postdosing. Urine and fecal samples were collected at 0-8, 8-24, 24-32, and 32-48 hr postdosing. R064766, R076477, and were quantitated in plasma, urine, and fecal samples using LC/MS/MS; LLOQs for the 3 compounds were and ng/mL in plasma, urine, and feces, respectively. [Analysis of spiked samples verified stability of in dog plasma.]

Results: the AUC for accounted for only of the sum of the plasma AUCs for the three compounds analyzed. The sponsor concluded that was metabolized to R064766 (VC) by intestinal anaerobic bacteria, and noted that this phenomenon was also observed in male Wistar rats following oral administration. Since this activity (i.e., intestinal reduction) is not species-specific, these data have relevance to metabolism in humans. Urinary and fecal concentrations could not be reliability determined due to the poor stability of in these biological media.

6. **The plasma levels and excretion of), R076477 (9-hydroxy-risperidone), and R064766 (risperidone) in the male SPF Wistar rat after single oral**

administration of [redacted] or R064766 at 0.63 mg/kg [Report No. FK3170, conducting laboratory: sponsor, Belgium, study initiation date: 2/15/99].

Methods: [redacted] and R064766 were dissolved in an aqueous tartaric acid solution for dosing; the final drug concentration was 0.063 mg/mL [oral dose 0.63 mg/kg]. [Analyses conducted on solutions confirmed content of both compounds were [redacted] of intended.] The study was conducted in male Wistar rats. Plasma samples were collected in 9/grp; urine and fecal samples were collected in an additional 4/grp. [The data for [redacted] from the urine and fecal samples were not considered reliable due to the poor stability of [redacted] in the biological media.] Blood samples were collected at 0.25, 0.5, 1, 2, 4, and 8 hrs postdosing. Each rat was used only for 2 consecutive blood sampling. Plasma samples collected at each time point were pooled for analysis. Urine and fecal samples were collected at 0-8, 8-24, 24-32, and 32-48 hrs postdosing. Concentrations of [redacted], R076477, and R064766 were quantitated using LC/MS/MS; LLOQs for the compounds were [redacted], and [redacted] ng/mL in plasma, urine, and feces, respectively.

Results: the concentration of [redacted] was very low in plasma, and could be quantitated only at the first sampling time. [redacted] was determined to be stable in plasma by analysis of [redacted] samples.] As in dog, the sponsor attributed this result to conversion of [redacted] to parent compound via the [redacted] activity of intestinal anaerobic bacterial. Plasma exposures (AUCs) for risperidone [R064766] and 9-hydroxy-risperidone [R076477] were similar following administration of "nearly equimolar doses" of [redacted] or R064766: 49.4 and 49.9 ng•hr/mL for risperidone and 143 and 146 ng•hr/mL for 9-hydroxy-risperidone. C_{max} for risperidone and 9-hydroxy-risperidone was also similar following administration of these two compounds: 19.8 and 21.6 ng/mL for risperidone and 40.9 and 42.5 ng/mL for 9-hydroxy-risperidone.

Following oral administration of [redacted], risperidone and [redacted] accounted for 0.44 and [redacted], respectively, of dose excreted in urine and [redacted] and 0.04%, respectively, of dose excreted in feces. Following oral administration of R064766, risperidone and [redacted] accounted for 0.5 and [redacted], respectively, of dose excreted in urine and 0.43 and [redacted], respectively, of dose excreted in feces.

7. **Toxicokinetics of the active moiety (=sum of risperidone and 9-hydroxy-risperidone) in the SPF Wistar rat in a six-month intermittent repeated dose toxicity study (Exp. No. 4731) on an aqueous depot microsphere formulation of risperidone (R064766) when administered subcutaneously once every 2 weeks at 10, 20, and 40 mg/kg for the first two doses, 20, 40, and 80 mg/kg for the third and fourth dose and 40, 80, and 160 mg/kg from the fifth dose onwards** [Study No. FK3186, conducting laboratory: sponsor, Belgium, study initiation date: 3/8/99].

Methods: blood samples were collected in satellite animals [4/sex/grp] dosed during the conduct of a 6-mo s.c. toxicity study. The clinical i.m. depot formulation [37% R064766, 3:1 lactic acid: glycolic acid copolymer, [redacted] was used in the 6-mo study. Risperidone depot formulation was administered s.c. at doses as described in the report title. Dosing volumes were 0.025, 0.05, and 0.1 mL/100 gm for the first 2 doses, 0.05, 0.1, and 0.2 mL/100 gm for the 3rd and 4th doses, and 0.1, 0.2, and 0.4 mL/100 gm for the 5th dose and the remaining doses. Injection sites were alternated between left and right sides. Blood samples were collected from 2/sex/grp at 1, 24, and 192 hrs following the 1st, 5th, 8th, and 13th doses, and at 1, 24, 196 hrs after the 3rd dose, and another 2/sex/grp at 5, 96, and 336 hrs following the 1st, 5th, 8th, and 13th doses and at 5, 144, and 312 hrs following the 3rd dose. The "active moiety" was quantitated using RIA; the LLOQ was [redacted] ng/mL.

Results: the data are summarized in the following table:

DOSE	C_{max} (ng/mL)					$AUC_{(0-336hr)}$ (ng•hr/mL)				
	10 mg/kg	20 mg/kg	40 mg/kg	80 mg/kg	160 mg/kg	10 mg/kg	20 mg/kg	40 mg/kg	80 mg/kg	160 mg/kg
MALES										
1st	13.5	33.9	71.0			794	1262	2578		
3rd		50.4	124	244			6141	10914	16691	
5th			144	566	741			14121	31816	75359
8th			191	1143	1400			30198	65028	132953
13th			366	704	1196			30706	68255	156476
FEMALES										
1st	13.5	55.8	78.2			1139	3478	2261		
3rd		36.5	189	210			5081	19542	16838	
5th			114	552	748			18590	51766	75160
8th			180	406	986			27314	74618	108900
13th			268	968	1148			35126	87160	94813

T_{max} for the first 2 injections was 1 hr for all doses, males and females. For subsequent doses, T_{max} was 1 or 5 hrs, with no clear relationship to dose or gender. Plasma exposure was characterized by an initial burst [1-5 hrs postdosing], followed by a "...sharp decrease up to 5...or 24 hours....after which they tended to remain on a plateau up to 336 h after dosing". The sponsor noted that the "...ratio between the highest and the lowest plasma concentrations ranged between 1.3 and 4.7" from the 3rd injection thereafter. Steady-state levels appear to have achieved "...at least within 4 consecutive administrations of the same dose..." [i.e., for the 40- and 80-mg/kg doses]

8. **Toxicokinetics and tissue distribution of risperidone, 9-hydroxy-risperidone and of the active moiety (=sum of risperidone and 9-hydroxy-risperidone) in the beagle dog in a 12-month intermittent repeated dose intramuscular toxicity study (Exp. No. 4730) on an aqueous depot microsphere formulation of risperidone ——— at 1.25, 2.5, and 5 mg/kg for the first two doses, 2.5, 5, and 10 mg/kg for the third and fourth dose and 5, 10, and 20 mg/kg from the fifth dose onwards** [Study No. FK3214, conducting laboratory: sponsor, Belgium, study initiation date: 4/13/99].

Methods: samples for this study report were collected during the conduct of a 12-mo i.m. toxicity study in Beagle dog [Exp. No. 4730]. In that study, risperidone depot formulation [identical to the clinical formulation using in Phase 3 trials] was administered to Beagle dogs [4/sex/grp] according to the regimen detailed in the title of the study report. [Injection sites (*m. biceps femoris*) were alternated between left and right sides.] Blood samples were collected from 2/sex/grp at 0, 1, 5, and 24 hrs following the 1st dose, at 8:00 a.m. on Days 3; 8, and 14 after the 1st, 3rd, 5th, 13th, and 26th doses, and on Day 112 just prior to the 9th dose. In addition, in these same animals, blood and tissue [injection site (muscle), i.e., site of last injection, and noninjection site (muscle), mesenteric lymph nodes, lung, kidney, brain, and liver] samples were collected at necropsy on Day 364-365, 14-15 days following the final dose. Risperidone and 9-OH-risperidone were quantitated in plasma (drug-treated only) and tissue samples using LC/MS/MS [LLOQ =——— ng/mL for plasma, ——ng/g tissue for both compounds].

Results: the plasma data are summarized in the following table:

	C_{max} (ng/mL)					$AUC_{(0\text{-}336\ hr)}$ (ng•hr/mL)				
	1.25 mg/kg	2.5 mg/kg	5 mg/kg	10 mg/kg	20 mg/kg	1.25 mg/kg	2.5 mg/kg	5 mg/kg	10 mg/kg	20 mg/kg
RISPERIDONE										
1	3.87 ± 1.09	9.32 ± 2.82	30.6 ± 16.1			2.27	606 ± 417	2421 ± 2953		
3		12.4 ± 2.41	31.0 ± 4.42	86.6 ± 21.7			1072 ± 235	2334 ± 539	5907 ± 3085	
5			33.4 ± 7.1	70.1 ± 19.6	249 ± 97			2468 ± 685	4947 ± 1549	34257 ± 24075
13			38.6 ± 8.2	100 ± 33	162 ± 32			5851 ± 1357	11344 ± 941	22919 ± 1567
26			46.2 ± 11.7	94.8 ± 15.0	252 ± 101			6466 ± 660	11662 ± 738	42549 ± 10393
9-OH-RISPERIDONE										
1	4.78 ± 1.78	10.2 ± 1.8	26.4 ± 8.5			458 ± 342	2837 ± 2130	8266 ± 11191		
3		21.9 ± 2.0	47.5 ± 10.3	102 ± 26.3			4626 ± 1666	9849 ± 3089	16762 ± 5585	
5			46.6 ± 9.3	90.3 ± 5.7	333 ± 176			9096 ± 3033	19642 ± 6759	75604 ± 52949
13			80.0 ± 18.3	191 ± 38	261 ± 82			19732 ± 5292	43661 ± 6023	74972 ± 26612
26			129 ± 54	241 ± 52	510 ± 179			26294 ± 5120	56232 ± 10032	126266 ± 26879
ACTIVE MOIETY										
1	8.32 ± 2.21	18.6 ± 4.6	50.6 ± 16.3			594 ± 432	3432 ± 2541	10687 ± 14131		
3		32.5 ± 2.92	74.3 ± 12.7	170 ± 23			5698 ± 1677	12183 ± 3601	22670 ± 8431	
5			73.1 ± 15.3	141 ± 35	522 ± 176			11564 ± 3075	24589 ± 7779	109861 ± 76163
13			115 ± 28.3	247 ± 56	379 ± 87			25583 ± 5349	55004 ± 6901	97891 27432
26			162 ± 51	296 ± 69	710 ± 242			32760 ± 4654	67894 ± 10213	168815 ± 34726

Tissue-distribution data were summarized in the following sponsor's tables:

plasma (ng/mL) and tissue (ng/g) concentrations

	5 mg/kg			10 mg/kg			20 mg/kg		
Tissue	R064766	R076477	Active moiety	R064766	R076477	Active moiety	R064766	R076477	Active moiety
Plasma	31.5 ± 13.0	106 ± 45	138 ± 56	36.0 ± 14.5	195 ± 31	231 ± 45	131 ± 47	407 ± 45	538 ± 77
Lung	149 ± 80	736 ±432	885 ± 506	4272 ±4900	1659 ± 451	5931 ±5333	7528 ± 7121	2783 ± 285	10311 ± 7114
Liver	97 ± 39	1204 ±504	1300 ± 540	136 ± 29	2348 ± 350	2484 ± 372	591 ± 517	3866 ± 797	4456 ± 1245
Kidney	158 ± 63	637 ±287	795 ± 324	161 ± 68	1313 ± 157	1474 ± 207	574 ± 361	2353 ± 368	2927 ± 689
Brain	204 ± 91	70 ± 27	274 ± 76	177 ± 93	127 ± 8	304 ± 99	272 ± 171	217 ± 67	488 ± 186
Lymph nodes	90 ± 18	398 ±132	489 ± 145	153 ± 28	1132 ± 315	1285 ± 324	510 ± 263	2167 ± 379	2677 ± 598
Muscle (last inject. site)	2152110± 2362466	1410 ±856	2153520± 2363223	1542921± 1305933	1177 ± 719	1544098± 1306613	13193786± 9963475	5682 ± 2167	13199468± 9965345
Muscle [2]	8330 ±7042	114 ± 45	8444 ±7039	894 [1]	213 ± 29	1098 [1]	909 ± 1016	349 ± 110	1258 ± 989

[1] Median value.
[2] Different from last injection site

tissue:plasma ratios

	5 mg/kg			10 mg/kg			20 mg/kg		
Tissue	R064766	R076477	Active moiety	R064766	R076477	Active moiety	R064766	R076477	Active moiety
Lung	4.8 ± 1.3	6.9 ± 2.2	6.4 ± 1.9	148 ± 173	8.9 ± 3.3	27.5± 24.6	59.2 ± 56.5	6.9 ± 1.2	18.6 ± 11.4
Liver	3.2 ± 0.6	11.8 ± 2.8	9.7 ± 1.9	4.2± 1.7	12.3 ± 2.6	11.1± 2.7	4.1 ± 2.1	9.6 ± 2.0	8.3 ± 1.8
Kidney	5.2 ± 1.5	6.2 ± 1.3	5.9 ± 0.7	4.6± 1.3	6.9 ± 1.5	6.6± 1.5	4.1 ± 1.1	5.8 ± 0.6	5.4 ± 0.7
Brain	8.3 ± 6.6	0.7 ± 0.1	2.5 ± 1.6	5.4± 2.8	0.7 ± 0.1	1.4± 0.4	2.5 ± 2.5	0.5 ± 0.2	0.9 ± 0.5
Lymph nodes	3.2 ± 1.1	3.9 ± 0.8	3.7 ± 0.7	5.0± 2.5	6.1 ± 2.4	5.8± 2.4	3.8 ± 0.6	5.4 ± 1.0	5.0 ± 0.8
Muscle (last inject. site)	102217± 128319	19.4 ± 19.4	24849±30105	43324±41898	6.0 ± 3.6	6596 ±5913	124000± 104812	14.5 ± 6.9	26550 ± 21380
Muscle [2]	324 ±291	1.2 ± 0.3	81.0 ± 72.8	420 ± 798	1.1 ± 0.3	4.6 [1]	7.8 ± 8.3	0.9 ± 0.3	2.3 ± 1.7

[1] Median value.
[2] Different from last injection site

Clearly, the highest tissue levels were detected at the injection site. The sponsor noted that drug concentrations in muscle "...from other site than the last injection site..." were markedly higher than observed in a previous i.m. depot study in dog [Report No. R064766/FK516; there was some discrepancy between the text and the reference in the description (i.e., doses, duration) of previous study].

9. **LC-MS/MS methods for the determination of risperidone and 9-hydroxyrisperidone in plasma and tissues from different animal species** [Study No. FK3715, conducting laboratory: sponsor, Belgium, study initiation date: 11/00].

Methods: methods validation analyses for risperidone and 9-OH-risperidone were conducted on plasma from rat, dog, rabbit, guinea pig, "pig", and human, and on tissue from dog [liver, lung, kidney, muscle, brain, lymph nodes]. In plasma, concentration ranges of 0.5-10000 [rat], 0.5-1250 [dog, guinea pig], and 0.1-250 [pig] ng/mL were tested. Tissues were examined following homogenation and dilution to 1:10 [100 µL aliquot]; a concentration range of 10-20000 ng/g was tested. Stability at various temperatures and through 4 freeze-thaw cycles was tested on plasma from all species assayed.

Results: acceptable intra-batch accuracy and precision [expressed as CV (%) = 100% x SD/mean] were demonstrated for both compounds in rat [86-109 and 0-15%, respectively], dog [97-110 and 0-20%, respectively; at one concentration, a markedly high value was obtained "...due to the low number of results (n = 2) for which the calculation was conducted"], guinea pig [92-113 and 0.3-7%, respectively], rabbit [86-102 and 0.4-11%, respectively], and pig [86-104 and 0.7-16%, respectively]. Interfering peaks (>20% at LLOQ) were not detected in plasma of any of the species tested for either risperidone or 9-hydroxyrisperidone. The LLOQs were determined to be [redacted] ng/mL in rat plasma (high-range) [redacted] ng/mL in rat (low-range), dog, and guinea pig plasma, and [redacted] ng/mL in rabbit and pig plasma. The ULOQs were determined to be [redacted] ng/mL in rat plasma (high-range), [redacted] ng/mL in rat plasma (low-range), dog, and guinea pig plasma, and [redacted] ng/mL in rabbit and pig plasma.

Acceptable stability was demonstrated for risperidone and 9-OH-risperidone in plasma following 2 hrs at room temperature, at 4 and 37° C, and after 4 freeze-thaw cycles.

The LC-MS/MS method allows for quantitation of both risperidone and 9-hydroxyrisperidone with one method [compared to 2 for RIA] and with an LLOQ of [redacted] ng/mL. The LC-MS/MS method is also required a smaller volume of plasma.

It was noted that the data are preliminary and will be finalized when long-term stability data are available.

10. **Toxicokinetics of risperidone, 9-hydroxy-risperidone and the active moiety (= sum of risperidone and 9-hydroxy-risperidone) in the male SPF Wistar (Hannover and Wiga) rat in a single-dose oral mechanistic toxicity study (Exp. No. 5441) on aqueous solutions of risperidone (R064766) at 0.63 mg/kg** [Study No. EDMS-OSDB-1683869, Exp. No. FK4043, Conducting laboratory and location: [redacted], study initiation date: 7/12/01, non-GLP] [This report was submitted in a supplement to the NDA; 3/25/02]

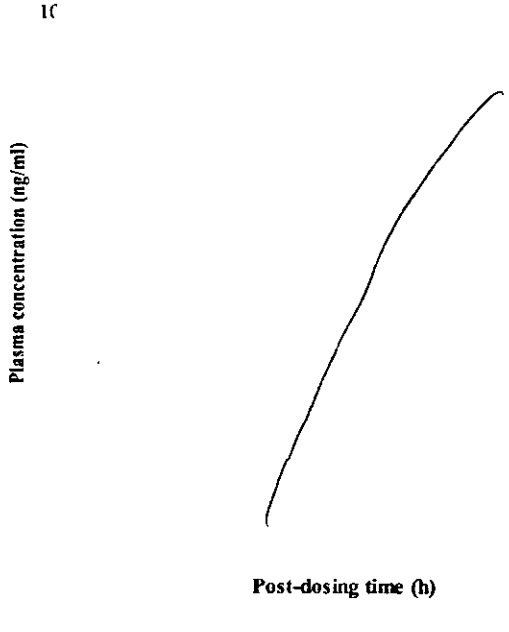
Methods: blood samples for this study were from animals dosed in Exp. No. 5441, an acute oral toxicity study conducted in male Wistar Hannover and Wiga rats. In that study, risperidone was administered orally (aqueous solution) at a dose of 0.63 mg/kg. Animals were fasted from 14 hr predosing to 4 hrs postdosing. Blood samples were collected from 7/grp at 0, 1, 2, 4, 8, and 24 hrs postdosing. Serum prolactin, risperidone, and 9-hydroxy-risperidone were quantitated in plasma. The prolactin data were included in the toxicity study report. Risperidone and 9-hydroxy-risperidone were quantitated using LC/MS/MS [LLOQ = [redacted] ng/mL for each compound].

Results: the data were summarized in the following sponsor's table and figure:

Table 5-2: **Mean (± SD[1]; n = 7) plasma concentrations (ng/ml) and some pharmacokinetic parameters of risperidone (R064766), its active metabolite 9-hydroxy-risperidone (R076477) and the active moiety (R064766 + R076477) in male SPF Wistar Hannover and Wiga rats in a single dose oral mechanistic toxicity study on aqueous solutions of risperidone (R064766) at 0.63 mg/kg (Exp. No. 5441).**

	SPF Wistar Hannover rats			SPF Wistar Wiga rats		
Time after dosing(h)	Risperidone	9-OH-risperidone	Active Moiety	Risperidone	9-OH-risperidone	Active Moiety
0	< 0.50	< 0.50	< 1.0	< 0.50	< 0.50	< 1.0
1	9.90 ± 5.31	56.8 ± 26.2	66.7 ± 31.3	***35.9*** ± 31.6	91.4 ± 43.0	127 ± 71
2	***2.80*** ± 1.03	24.7 ± 14.7	27.5 ± 15.5	***10.3*** ± 2.7	***74.3*** ± 16.4	***84.6*** ± 18.0
4	***1.02***[2]	***14.6*** ± 6.2	***15.3*** ± 6.7	***1.08*** ± 0.62	***26.7*** ± 10.3	***27.7*** ± 10.8
8	< 0.50	***1.65***[2]	***1.65***[2]	< 0.50	***3.35*** ± 1.40	***3.35*** ± 1.40
24	< 0.50	< 0.50	< 1.0	< 0.50	< 0.50	< 1.0
C_{max} (ng/ml)	9.90 ± 5.31	56.8 ± 26.2	66.7 ± 31.3	35.9 ± 31.6	91.4 ± 43.0	127 ± 71
T_{max} (h)	1	1	1	1	1	1
$t_{1/2}$[3] (h)	1.4	1.3	1.2	0.6	1.3	1.3
AUC_{0-t}[4] (ng.h/ml)	15.1	141	157 ± 17	52.5	290	344 ± 31
$AUC_{(w-H)}$[5] (ng.h/ml)	-	-	-	-	-	188 ± 35
[95 % CI]	-	-	-	-	-	[112-263]

[1] For the AUC-values the standard error is given.
[2] Median value.
[3] Calculated with the concentrations in ***bold italics***.
[4] t is the time corresponding to the last concentration above the limit of quantification.
[5] Difference between the mean AUC_{0-t} obtained for the Wiga and Hannover rats. The 95 % confidence interval is given between brackets.



The data indicate that plasma exposure to risperidone and 9-hydroxy-risperidone was higher in Wiga than in Hannover rats; the difference was greater for risperidone [4-fold] than for the metabolite [1.6-fold]. The ratio of 9-hydroxy-risperidone to risperidone was higher in Hannover [5.7 vs 2.5] indicating more extensive metabolism in the Hannover strain.

11. **Toxicokinetics of risperidone (R064766), its metabolite 9-hydroxy-risperidone (R076477) and the active moiety (=sum of risperidone and 9-hydroxy-risperidone) in the male SPF Wistar Hannover rat in a 7-week repeated dose mechanistic toxicity study (Exp. No. 5459) after dietary administration of risperidone at 12.8 mg/kg/day or after repeated intramuscular dose administration of an aqueous depot microsphere formulation of risperidone at 40 mg/kg/2 weeks.** [Study No. EDMS-PSDB-1683960, Exp. No. FK4132, Conducting laboratory and location: [redacted], study initiation date:

Methods: blood samples were collected from animals [male Wistar Hannover] tested in Exp. No. 5459. In that study, risperidone was administered as a drug-diet admixture for 7 weeks or as 4 i.m. microsphere depot injections given bi-weekly [starting on Day 0]. Animals [7/grp/time period] were sacrificed and blood samples were collected on Day 49-50 at 0, 4, 8, 12, 16, 20, and 24 hrs [the first animal was sacrificed at 8:10 on Day 49]. Serum prolactin, risperidone, and 9-hydroxy-risperidone were quantitated in serum. The prolactin data were included in the toxicity study report. Risperidone and 9-hydroxy-risperidone were quantitated using L/MS/MS [LLOQ =—— ng/mL for each compound].

Result: the data were summarized in the following sponsor's table and figure:

	Dietary administration at 12.8 mg/kg/day		
Time after first sampling (h)	Risperidone (ng/ml)	9-OH-risperidone (ng/ml)	Active Moiety (ng/ml)
0	10.2 ± 6.9	40.4 ± 23.8	50.6 ± 30.5
4	11.2 ± 6.7	33.5 ± 16.4	44.8 ± 22.6
8	5.50 ± 2.70	22.3 ± 11.1	27.8 ± 13.8
12	25.4 ± 22.0	65.6 ± 29.3	91.0 ± 50.3
16	27.3 ± 17.3	79.9 ± 38.0	107 ± 54.6
20	45.7 ± 44.3	77.1 ± 37.5	123 ± 59.8
24	14.0 ± 9.5	49.0 ± 31.6	63.0 ± 41.0
$AUC_{24\,h}$ (ng.h/ml)	509 ± 77	1293 ± 99	1802 ± 151
	IM administration of depot formulation at 40 mg/kg/injection		
0	26.6 ± 7.6	17.0 ± 11.1	43.7 ± 18.4
4	54.1 ± 21.1	40.3 ± 19.1	94.4 ± 38.0
8	36.8 ± 10.9	21.5 ± 15.1	58.3 ± 25.2
12	36.5 ± 12.0	23.5 ± 10.6	60.0 ± 21.6
16	38.7 ± 14.5	31.5 ± 14.1	70.2 ± 27.8
20	30.9 ± 11.4	24.1 ± 20.5	55.1 ± 30.6
24	34.2 ± 9.5	20.4 ± 13.7	54.6 ± 23.0
$AUC_{24\,h}$ (ng.h/ml)	910 ± 49	639 ± 56	1548 ± 100
$AUC_{(IM-DI)}$ [2] (ng.h/ml) [95 % CI]	401 ± 91 [220 - 582]	-654 ± 114 [(-882) – (-426)]	-253 ± 181 [(-615) - 109]

[1] For the AUC-values the standard error is given.
[2] Difference between the mean $AUC_{24\,h}$ after intramuscular administration and dietary administration. The 95 % confidence interval is given between brackets.

With dietary administration, the $AUC_{(0\text{-}24\ hr)}$ for risperidone was 0.6 times the $AUC_{(0\text{-}24\ hr)}$ with the i.m. depot; however, the $AUC_{(0\text{-}24\ hr)}$ for 9-hydroxy-risperidone was 2 times higher with dietary administration. The $AUC_{(0\text{-}24\ hr)}$ for the "active moiety" was similar with the two routes.

PK/TK summary and conclusions: the sponsor has conducted a number of PK/ADME/TK studies, using different i.m. depot formulations. As noted by the sponsor, these studies were designed to examine the PK of the i.m. depot, to investigate the possible mechanisms underlying the "early release" observed in dogs with the i.m. depot formulation, and to provide estimates of plasma exposure for the toxicity studies conducted in rat and dog.

In a majority of the PK/ADME/TK studies, only plasma levels of the "active moiety" were quantitated [instead of plasma risperidone and 9-OH-risperidone]. Quantitation of the "active moiety" [= sum of risperidone and 9-OH-risperidone] was performed using an RIA method. Using the RIA method, 2 haptens were used to quantitate risperidone and risperidone + 9-OH-risperidone [i.e., the "active moiety"]. The 9-OH-risperidone metabolite was estimated by subtraction. The hapten used to quantitate the "active moiety" exhibited crossreactivity not only between risperidone and 9-OH-risperidone, but also to 7-OH-risperidone. Therefore, to the extent that 7-OH-risperidone is present in the circulation, the quantitation of the "active moiety" and 9-OH-risperidone may not be accurate.

Rat: distribution of risperidone and 9-OH-risperidone into various brain regions was assessed in Wistar rat following a single 0.02 mg/kg s.c. dose of risperidone. In all brain areas samples [i.e., frontal cortex, striatum, cerebellum, and "rest of brain"], brain levels of risperidone were higher than those of 9-OH-risperidone [≈3-4.5 fold]. The greater brain penetration of the parent compound is somewhat consistent with the slightly more polar nature of the metabolite. [Whether or not this difference would have an impact on the clinical safety and/or efficacy of risperidone as compared to 9-OH-risperidone is unclear; however, it would suggest that attributing effects to the "active moiety" may be ill advised.] Tissue distribution was not assessed using the i.m. depot formulation, except for analysis of the injection site.

TK data were provided for the three 6-mo s.c. studies, the 1-yr i.m. depot [interim for 2-yr] toxicity study, and the 2-yr carcinogenicity study in Wistar rat. In the 6-mo s.c. studies, data were provided only for the "active moiety"; the "active moiety" was quantitated using RIA. In Exp. No.'s 3095 and 3589, dosing was monthly, whereas in Exp. No. 4731, dosing was bi-weekly. In all three s.c. studies, plasma exposure was characterized by a sharp initial peak [T_{max} = 1-5 hr, except for Exp. No. 3589 (T_{max} = 1-24 hrs)] followed by a decline in exposure over subsequent days (usually <1 wk); plasma exposure then remained fairly stable until the next dose. Plasma exposure was difficult to compare among studies due to the differences in sample collection and dosing regimen. An examination of injection site "nodules" in Exp. No. 3095 indicated the presence of a "significant" amount of residual risperidone at the site.

In the 1-yr and 2-yr studies, risperidone was administered bi-weekly as an i.m. depot at doses of 5 and 40 mg/kg. Plasma levels of risperidone and 9-OH-risperidone were quantitated using LC/MS/MS. The active moiety was calculated; however, the method of calculation was not specified. [It did not appear to be a simple summing of means and SEs' for risperidone and 9-OH-risperidone in all cases.] Plasma exposure was characterized by an initial sharp rise, followed by a rapid decline and a slight increase [during the 24-hr period postdosing], then fairly stable plasma levels of risperidone and 9-OH-risperidone for up to 2 wks postdosing. The AUC data indicated a marked increase in exposure [≈11-15 fold in males, ≈5-8 fold in females] between the 1st and subsequent doses [6-26th doses]. However, from the 6th dose on, plasma exposure remained fairly stable.

TK data were also provided for a single-dose in Wiga and Hannover Wistar rats and a 7-wk repeat-dose mechanistic study in Wistar Hannover rat. In the single-dose study, plasma levels of risperidone, 9-OH-risperidone, and the "active moiety" were quantitated in Wiga and Hannover Wistar rats following an

acute oral dose of risperidone [0.63 mg/kg]. The data indicated that plasma exposure to risperidone and 9-OH-risperidone was higher in Wiga than in Hannover rats; the difference was greater for risperidone [4-fold] than for the metabolite [1.6 fold]. The ratio of 9-OH-risperidone to risperidone was greater in Hannover [C_{max}: 5.7 vs 2.5 for Wiga; AUC: 9.3 vs 5.5 for Wiga], indicating more extensive first-pass metabolism in the Hannover strain.

In the 7-wk study, risperidone was administered as a drug-diet admixture [12.8 gm/kg] or an i.m. depot [clinical formulation; 40 mg/kg/2 wks]. Plasma levels were analyzed on Day 49-50, i.e., the end of the dosing period. Risperidone and 9-OH-risperidone were quantitated, and the active moiety was calculated [it would appear, by summing the means and mean SEs']. With dietary administration, the $AUC_{(0\text{-}24\ hr)}$ for risperidone was 0.6 times the $AUC_{(0\text{-}24\ hr)}$ with the i.m. depot; however, the $AUC_{(0\text{-}24\ hr)}$ for 9-OH-risperidone was 2 times higher with dietary administration. The $AUC_{(0\text{-}24\ hr)}$ for the "active moiety" was similar with the 2 routes. These data are consistent with the extent of first-pass metabolism observed following oral dosing. [The data from these mechanistic studies are further discussed in the **Carcinogenicity summary and conclusions** section.]

Dog: early PK studies indicated that administration of various i.m. depot formulations in dog was characterized by an expected early peak followed after some period [e.g., 28-35 days] by a second peak. The first peak represented systemic absorption of risperidone that adhered to the outside of the microspheres, while the second peak represented disintegration of the microsphere particles with release of remaining risperidone. However, an extra peak, or "partial early release", was observed [e.g., Days 2 and 13 postdosing] following acute and multiple dosing. A number of studies were conducted using different formulations and dosing regimens in order to further investigate this phenomenon [which was also observed clinically, but not in rat]. These studies assessed various factors, such as volume/mass and frequency of dosing, as well as the presence of certain solvents. Although there was some indication that local inflammation was associated with an increased incidence of "partial early release", the factor(s) responsible were not definitively determined.

TK data were collected during the 6-mo [2 studies] and 1-yr i.m. depot toxicity studies. Doses were administered 1/mo in the 6-mo studies and bi-weekly in the 1-yr study. Doses were increased during the dosing period in all studies. However, since the dose-escalation regimen was different for each of the studies, it is difficult to compare exposures among studies. Since only in the 1-yr study was the dosing regimen [i.e., bi-weekly] similar to that intended for humans, and since the maximum doses per grp were administered from ≈Wk 10 on, emphasis will be placed on these data for the purposes of this review. In the 1-yr study, doses remained stable from the 5th dose on [5, 10, 20 mg/kg]. Plasma exposure [C_{max}, AUC] to 9-OH-risperidone and "active moiety" increased over time [5th to 26th dose], with the exception of the C_{max} for risperidone which remained stable. The increase in exposure over time was greater at the lower doses [2-3 fold] than at the HD [1.2-1.5 fold]. At Wk 26, C_{max} and AUC for risperidone were fairly dose-proportional between 5 and 10 mg/kg, but increased in a slightly greater-than dose-proportionate manner at 20 mg/kg. Both the C_{max} and AUC for 9-OH-risperidone increased fairly linearly with dose. For the "active moiety", the C_{max} increased in a fairly linear manner and the AUC increased in slightly greater-than dose-proportionate manner between 10 and 20 mg/kg.

Tissue distribution was assessed to a limited extent in one of the 6-mo studies and in the 1-yr study. In the 6-mo study, at final doses of 10, 20, and 80 mg/kg, "activity moiety" was quantitated in plasma, brain, liver, lung, kidney, muscle (non-injection), and at the injection site. Tissue levels were > plasma, except for brain. Tissue levels increased with dose, but in a less-than dose-proportionate manner. Highest levels of "active moiety" were detected at the injection site [>100 fold higher than in other tissues]; of the other tissues sampled, highest levels were in lung. In the 1-yr study [doses: 5, 10, 20 mg/kg], highest levels of risperidone were present at the injection site; the residual levels were 360-10,550 times the highest levels in other tissues. Risperidone and 9-OH-risperidone were detected in all

other tissue examined [lung, liver, kidney, brain, lymph nodes, muscle (non-injection)]. Of these tissues, the highest levels of risperidone were detected in (non-injection site) muscle [at the LD] and lung [MD, HD]. Highest levels of 9-OH-risperidone were detected in liver [all doses]. Hepatic levels of 9-OH-risperidone were 12, 17, and 6.5 times those of risperidone at 5, 10, and 20 mg/kg, respectively. Highest levels of "active moiety" were detected in (non-injection site) muscle [LD] and lung [MD, HD]. Brain levels of risperidone were 3 times higher than those of 9-OH-risperidone at the LD, but only slightly higher at the MD [40%] and HD [25%].

For comparison, the plasma levels [mean ± SD] of risperidone, 9-OH-risperidone, and "active moiety" at a dose of 50 mg i.m. depot in humans [only Study -54 used the to-be-marketed (TBM) formulation] are summarized in the following table ["n" refers to the number receiving the 50-mg dose, not the total n]:

STUDY	C_{max} (ng/mL)			AUC (ng•hr/mL)		
	risperidone	9-OH-risperidone	active moiety	risperidone	9-OH-risperidone	active moiety
RIS-xxx-54	21.6 ± 15.0	19.9 ± 12.0	39.8 ± 15.7	5873 ± 3604	6094 ± 4050	11978 ± 4469
RIS-INT-31	38.9 ± 24.8	34.7 ± 22.3	68.3 ± 29.3	8981 ± 6460	6835 ± 3268	15595 ± 5938
RIS-xxx-072	23.3 ± 18.6	20.9 ± 12.0	43.45 ± 21.01	6841 ± 4827	7146 ± 4896	13999 ± 6282

RIS-xxx-54: single i.m. injection [n = 26], $AUC_{(last)}$. No genotyping.
RIS-INT-31: multiple-dose [5th bi-weekly dose] [n = 9; 3 PM], $AUC_{(0\text{-}336\ hr)}$.
RIS-xxx-072: single i.m. injection [n = 26; 6 heterozygous EM, 11 homozygous EM, 5 PM], AUC_{last}.

The 50-mg dose contains [redacted] mg of microspheres [redacted mg/kg or redacted mg/m^2].

APPEARS THIS WAY
ON ORIGINAL

IV. GENERAL TOXICOLOGY

RAT

1. Study title: Six month intermittent repeated dose subcutaneous toxicity study in the Wistar rat [Study no: EDMS-BEBE-2746990, Exp. No. 4731, Volume #: 1.14-1.15, Conducting laboratory and location: Janssen Pharmaceutica, Belgium, Date of study initiation: 3/8/99, GLP, QA report: Y]

Drug, lot #, and % purity: R064766, batch no. ZR064766EIA221 [one impurity, ———, identified as

Formulation/vehicle: i.m. depot formulation/microspheres [lactic and glycolic acids (3:1), ethyl acetate ———; clinical Phase 3 formulation]. Concentration and homogeneity stated to be documented. Actual concentrations were ——— of intended when tested on three separate occasions; homogeneity data not provided.

Methods
Dosing:

Species/strain: Wistar rat ———
#/sex/group or time point (main study): 20/sex/grp
Satellite groups used for toxicokinetics or recovery: 4/sex per dose grps for TK analysis [designated by the sponsor as "FK" animals].
Initial age: 6 wks
Initial weight: 113-172 gm
Doses in administered units: doses 1-2: 0, 10, 20, and 40 mg/kg; doses 3-4: 0, 20, 40, and 80 mg/kg, doses 5 on: 0, 40, 80, 160 mg/kg.
Frequency of dosing: bi-weekly, injection site alternated between L and R sides.
Route, volume: s.c.; doses 1-2: 0.025, 0.05, 0.1 mL/100 gm; doses 3-4: 0.05, 0.1, 0.2 mL/100 gm; doses 5 on: 0.1, 0.2, 0.4 mL/100 gm

Observations and times:

Clinical signs: animals were observed daily.
Body weights: body wts were recorded at baseline, weekly during the dosing period, and "at the end of the study".
Food consumption: food intake was recorded weekly during the dosing period.
Ophthalmoscopy: ophthalmology examination of conjunctiva, sclera, cornea, iris, lens, and fundus was performed on 10/sex C and HD animals at the start and "towards the end" of the study. Mydriasis was induced by application of 1% atropine sulfate for examination by slitlamp biomicroscopy.
EKG: no
Hematology: blood samples were collected [via orbital venous puncture] "towards the end of the study" for analysis of the following parameters: hct, hgb, rbc ct, wbc ct [toal, differential], thrombocyte ct, normoblasts, MCV, MCH, MCHC.
Clinical chemistry: blood samples were collected [via orbital venous puncture] "towards the end of the study" for analysis of the following parameters: Na, K, Cl,, Ca, P_i, total protein, albumin, glucose, cholesterol, TG, PL, BUN, creatinine, total bilirubin, alkaline phosphatase, AST, ALT.
Urinalysis: blood samples were collected [methods not specified] "towards the end of the study" for analysis of the following parameters: specific gravity, pH, volume, glucose, ketones, urobilinogen, bilirubin, occult blood, proteins, microscopic analysis of sediment.
Gross pathology: a complete necropsy was performed on all animals. According to the sponsor, "During necropsies a pathologist was always available for consultation".
Organs weights: the following organs were weighed in all animals: lungs, spleen, liver, heart,

pancreas, kidneys, brain, thymus, adrenal glands, thyroid/parathyroid glands, testes, ovaries.

Histopathology: the following tissues were examined microscopically in C and HD animals: injection site, adrenal gland, aorta, bone (stifle joint, sternum) with bone marrow, brain (cerebrum, cerebellum, midbrain), coagulating glands, epididymides, esophagus, extraorbital lacrimal gland, external ear, eye, heart, kidneys, large intestine (cecum, colon, rectum), liver, lungs, lymph node(s) (mesenteric), mammary gland, nose, ovaries, pancreas, parathyroid glands, pituitary gland, prostate salivary gland (parotid, submandibular), seminal vesicles, skeletal muscle (psoas muscle), small intestine (duodenum, ileum, jejenum), spinal cord (thoracic), spleen, stomach (forestomach, glandular stomach), testes, thymus, thyroid glands, trachea, urinary bladder, urterus, vagina, gross lesions. In addition, the following tissues were examined in the lower dose grps: injection site, adrenals, coagulating glands, mammary gland, pituitary, prostate, seminal vesicles, spleen, ovaries, uterus, vagina, gross lesions.

Tissues were preserved in 10% buffered formalin, except for eyes and testes which were preserved in 2.5% buffered formalin/1.25% glutaraldehyde solution. Histopathology examination was conducted by [redacted]

Toxicokinetics: methods and data were reported in a separate study report [R064766/FK3186].

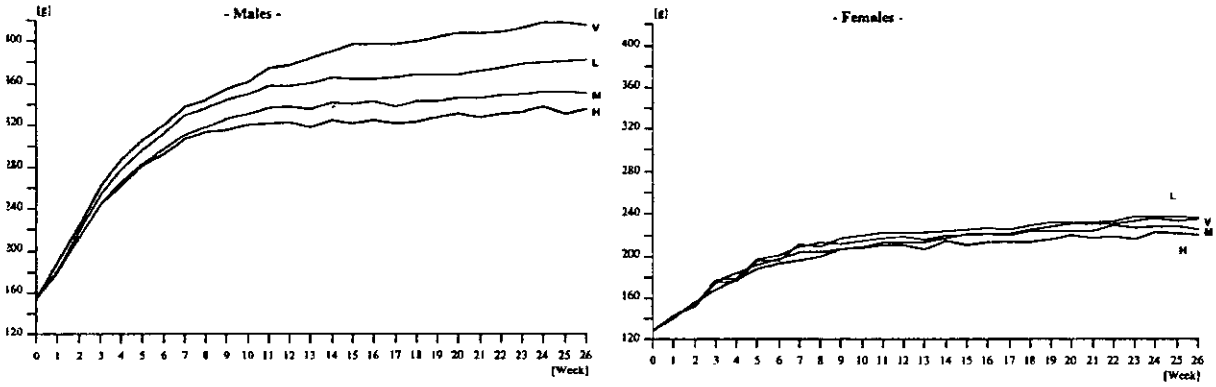
Results:

Mortality: there were 4 unscheduled deaths during the study. In main study animals, 1 LD animal was sacrificed during Wk 21 [malignant lymphoid tumor detected at necropsy] and 1 CF died just prior to terminal sacrifice. In satellite-TK animals, 1 MDM [#42] and 1 HDF [#163] died during "lunar month" 4 and 5, respectively. None of the deaths was considered by the sponsor to be drug-related. [Note: the animal numbers given for the satellite animals that died also correspond to animal no's for main study animals. This needs to be clarified with the sponsor. According to the individual data tables, MDM #42 and HDF #163 were sacrificed on schedule.]

Clinical signs: the primary clinical signs noted were ptosis and sedation. Ptosis was observed in all dosed animals. At the LD, ptosis was noted only after the 1st dose (10 mg/kg), and after 1-2 doses at 20 mg/kg; no ptosis was observed from the 5th injection on even though the dose was increased (to 40 mg/kg). At the MD, ptosis was observed following the 1st dose (20 mg/kg), and "in some animals" at 40 mg/kg; ptosis was observed only in 1 MDF after the increase to 80 mg/kg. At the HD, ptosis was observed following the 1st dose (40 mg/kg), and after 1-2 doses at 80 mg/kg; ptosis was observed following each 160-mg/kg dose. Sedation (characterized as "slight") was detected in all MD and HD animals, but was consistently observed (i.e., after each dose) only at 80 and 160 mg/kg, respectively. The sponsor noted that at each dose affected, ptosis and sedation were observed only during the 1st wk following dosing. Food wastage was noted in 1/20 MDM, 1/20 MDF, 9/20 HDM, and 8/20 HDF; the sponsor considered only the HD to be affected (finding noted only at 160 mg/kg in HDM and HDF). Hardening at the injection site was detected primarily in MD and HD animals; ½ of all MD and all HD animals were affected.

Body weights: in males, body wt was reduced in a dose-related manner, with all dose grps affected. Body wt was consistently reduced (compared to CM) throughout most [or all] of the dosing period at the MD and HD, and from Wk 11 on at the LD. Final mean body wt was reduced by 8, 16, and 20% at the LD, MD, and HD, respectively, compared to CM. In females, body wt was sporadically increased during the first 9 wks of dosing at the LD and MD, and during Wk 3 at the HD. From Wk 15 on, body wt was slightly (5-6%) reduced (compared to CF) at the HD. Final mean body wt was decreased by 6% in HDF (compared to CF). Overall mean body wt gain was reduced by 13, 25, and 31% in

LDM, MDM, and HDM, respectively, and by 13% in HDF. Summary body wt data were illustrated in the sponsor's figures provided below.



Food consumption: food intake was reduced in males during Wks 7-15 at the HD [5-14%], during Wks 10-15 at the MD [7-9%], and during Wks 12-15 at the LD [5-7%]; intake during the last 11 wks of dosing [Wks 16-26] was fairly similar among grps. Food intake in females was fairly similar among grps through most of the dosing period; however, mean intake was increased [at one or more doses during the first 11 wks [8-17%] and the last 6 wks [Wks 21-26; 4-23%] of dosing.

Ophthalmoscopy: no drug-related findings reported.

Hematology: the following findings were of note: (a) small, but significant decreases in rbc ct in males [3, 4, and 5 at the LD, MD, and HD, respectively] and females [2-3%, all doses], (b) increase in thrombocyte ct in MDF and HDF (8-9%), (c) small increases in MCV and MCH in males [2-4%; all doses] and in MCHC in MDM, HDM, and at all doses in females [1-2%], (d) an increase in neutrophil ct in females [42, 24, and 58% at LD, MD, and HD, respectively], (e) decreased lymphocyte ct in males [14-15%, all doses] and females [13-16%, all doses].

Clinical chemistry: the following findings were of note: (a) small, but significant decrease in Na in females [1%, all doses], (b) decreased K in males [6, 8, and 9% at LD, MD, and HD, respectively] and in females [2, 5, and 5% at LD, MD, and HD, respectively], (c) decreased CL in males [1-2%, all doses] and females [3%, all doses], (d) increased Ca in males [4%, all doses] and females [4-6%, all doses], (e) increased P_i in males [14, 14, and 20% at LD, MD, and HD, respectively] and females [23, 30, and 30% at LD, MD, and HD, respectively], (f) a small decrease (5%) in total protein in HDM, (g) decreased glucose in males [6, 9, and 12% at LD, MD, and HD, respectively] and females [12-17%; all doses, but not dose-related], (h) decreased TG in MDM [25%] and HDM [40%], (i) increased PL in males [15-22%; all doses, but not dose-related] and females [10, 16, and 18% at LD, MD, and HD, respectively], (j) decreased BUN in MDM [6%], HDM [10%], and HDF [13%]. (k) decreased AST in HDM [13%], MDF [17%], and HDF [14%], (l) increased ALT in males [18-38%; all doses, but not dose-related] and females [18-250%; all doses, but not dose-related].

Urinalysis: the following findings were of note: (a) a small, but significant decrease in specific gravity in males [1%, all doses], (b) a decrease in pH in males [0.4-0.5 units, all doses], (c) an increase in wbcs in males [220, 700, and 1000% in LDM, MDM, and HDM, respectively], (d) an increase in sperm at the MD [152%] and HD [340%], (e) an increase in squamous epithelial cells in males [73, 230, and 300% at LD, MD, and HD, respectively], (f) an increase in ketones in females [48-60%; all doses, but not dose-related], and (g) an increase in bacteria in females [20, 40, and 50% at LD, MD, and HD, respectively].

Organ weights: the following were noted: (a) an increase in spleen wt [absolute-relative] at all doses in males [13-24, 10-29, and 6-31% at LD, MD, and HD, respectively] and females [27-24, 17-19, and 14-19% at LD, MD, and HD, respectively], (b) an increase in heart wt [absolute-relative] in MDF [3-5%] and HDF [5-10%], (c) an increase in pancreas wt [absolute-relative] in males [10-21, 3-22, and 6-32% at LD, MD, and HD, respectively], (d) a decrease in thymus wt [absolute-relative] in males [11 (A only), 12 (A only), and 33-16% at LD, MD, and HD, respectively] and females [14, 12-18, and 18-13% at LD, MD, and HD, respectively], (e) an increase in adrenal wt [absolute-relative] in males [43-56, 49-75, and 65-103% in LD, MD, and HD, respectively], (f) a decrease in relative thyroid wt in males [14, 18, and 21% at LD, MD, and HD, respectively], (g) a decrease in absolute testis wt [5, 4, and 9% at LD, MD, and HD, respectively]; relative wt was increased at the MD and HD [13%]. (h) ovary wt [absolute-relative] was decreased in MDF [12-11%] and HDF [16-12%].

Gross pathology: selected findings are summarized in the following table:

TISSUE	FINDING	MALES				FEMALES			
		C	LD	MD	HD	C	LD	MD	HD
injection site	powdery deposit s.c.	20/20	19/20	20/20	20/20	20/20	20/20	19/20	19/20
adrenal	swollen	0/20	2/20	3/20	6/20*	1/20	0/20	0/20	0/20
mammary gland	stimulation	0/20	0/20	0/20	0/20	0/20	20/20***	19/20***	19/20***
prostate	gray	1/20	12/20***	15/20***	18/20***				
	swollen	0/20	6/20*	6/20*	11/20***				
testes	swollen	0/20	0/20	0/20	2/20				
ovaries	small					0/20	1/20	6/20*	4/20
uterus	small					0/20	11/20***	14/20***	11/20***

Histopathology: the sponsor expressed the microscopic findings in terms of "Mean scores per dosage group". How these scores were calculated was not described; however, it is assumed that the scores reflect both incidence and severity. Selected findings are summarized in the following table [numbers in parentheses are S.E.]

TISSUE	FINDING	MALES				FEMALES			
		C	LD	MD	HD	C	LD	MD	HD
injection site	chronic inflammation	2.21 (0.16)	1.78 (0.13)	2.21 (0.20)	2.37 (0.17)	1.68 (0.13)	1.84 (0.16)	2.00 (0.17)	1.74 (0.13)
	fibrotic encapsulation s.c.	3.15 (0.16)	2.89*** (0.11)	3.16** (0.14)	3.58 (0.16)	3.42 (0.12)	2.37*** (0.14)	2.84** (0.12)	3.11 (0.11)
	fibrous tissue reaction s.c.	3/42 (0.16)	3.17 (0.19)	3.42 (0.16)	3.00 (0.17)	2.95 (0.18)	3.05 (0.19)	2.53 (0.14)	2.68 (0.22)
	giant cell encapsulation	3.95 (0.05)	4.00 (0.00)	3.84 (0.16)	4.00 (0.00)	4.00 (0.00)	4.00 (0.00)	4.00 (0.00)	3.79 (0.21)
	granulocytic infiltration	0.00 (0.00)	0.00 (0.00)	0.47* (0.23)	0.37* (0.16)	0.00 (0.00)	0.11 (0.07)	0.42* (0.18)	0.26* (0.10)
	necrotic center/cell debris	0.00 (0.00)	0.00 (0.00)	0.32* (0.17)	0.16 (0.12)	0.00 (0.00)	0.05 (0.05)	0.05 (0.05)	0.05 (0.05)
drenal	fibrotic encapsulation	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.05 (0.05)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)
	giant cell encapsulation	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.11 (0.11)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)
	prominent pigmented cells (z. reticularis)	0.05 (0.01)	0.21 (0.10)	0.21 (0.10)	0.26 (0.10)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)

TISSUE	FINDING	MALES				FEMALES			
		C	LD	MD	HD	C	LD	MD	HD
	swollen cortical cells								
	z. fasciculata	0.00 (0.00)	0.63*** (0.11)	0.89*** (0.07)	0.89*** (0.07)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.05 (0.05)
	z. reticularis	0.00 (0.00)	0.11 (0.07)	0.21* (0.10)	0.26* (0.10)	0.00 (0.00	0.00 (0.00)	0.00 (0.00)	0.05 (0.05)
coagulating gland	prominent low epithelium	0.00 (0.00)	0.11 (0.07)	0.21* (0.10)	0.32** (0.11)				
	granulocytes								
	pelvic cavity	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.21 (0.12)	0.00 (0.00)	0.00	--	0.00 (0.00)
	pelvic mucosa	0.16 (0.09)	0.00 (0.00)	0.00 (0.00)	0.26 (0.10)	0.05 (0.05)	0.00	--	0.05 (0.05)
kidney	mineral deposits								
	pelvic cavity	0.05 (0.05)	1.00 (0.55)	0.67 (0.67)	0.21 (0.12)	0.16 (0.12)	0.00	--	0.00 (0.00)
	pelvic epithelium	0.00 (0.00)	0.40 (0.24)	0.00 (0.00)	0.05 (0.05)	0.05 (0.05)	0.00	--	0.11 (0.07)
	female appearance/glandular development	0.00 (0.00)	1.68*** (0.11)	1.76*** (0.14)	1.95*** (0.05)	1.05 (0.09)	3.00*** (0.08)	3.00*** (0.08)	3.00*** (0.08)
mammary gland	focal hyperplasia	0.00 (0.00)	0.05 (0.05)	0.29* (0.11)	0.42** (0.14)	0.00 (0.00)	0.16 (0.12)	0.32* (0.15)	0.37** (0.14)
	prominent secretion	0.00 (0.00)	1.79*** (0.22)	2.47*** (0.21)	2.26*** (0.17)	0.05 (0.05)	2.00*** (0.13)	1.95*** (0.12)	1.79*** (0.14)
	amt of interstitial tissue					2.53 (0.22)	3.11* (0.11)	3.32** (0.11)	3.32** (0.11)
	atretic follicles					1.21 (0.16)	1.63* (0.14)	1.53 (0.16)	1.84** (0.14)
ovary	basophilic corpora lutea					0.74 (0.10)	0.11*** (0.07)	0.05*** (0.05)	0.16*** (0.09)
	clear appearance (interstitial)					0.37 (0.11)	0.68 (0.11)	0.68 (0.15)	1.16*** (0.16)
	eosinophilic corpora lutea					3.11 (0.32)	1.89** (0.11)	1.79*** (0.10)	1.79*** (0.12)
	generations of corpora lutea					3.42 (0.26)	2.95** (0.09)	2.95** (0.09)	2.79** (0.12)
pituitary (H & E)	erythrosinophils (adenohyp)	2.68 (0.11)	2.26* (0.13)	2.00*** (0.00)	2.00*** (0.00)	1.79 (0.10)	1.89 (0.07)	1.89 (0.07)	1.95 (0.05)
	hypertrophy (pars interm)	0.00 (0.00)	0.11 (0.07)	0.17 (0.09)	0.21* (0.10)	0.00 (0.00)	0.05 (0.05)	0.05 (0.05)	0.11 (0.07)
pituitary (PRL stain)	immunoreactive cells	1.68 (0.11)	2.11* (0.15)	2.50*** (0.15)	3.05*** (0.14)	3.00 (0.15)	3.00 (0.13)	2.84 (0.09)	2.95 (0.14)
	focal interstitial fibrosis (dorsolateral)	0.00 (0.00)	1.05*** (0.15)	1.63*** (0.11)	1.84*** (0.09)				
	focal interstitial granulocytes								
	dorsolateral	0.11 (0.07)	0.95*** (0.05)	1.26*** (0.10)	1.42*** (0.12)				
	ventral	0.00 (0.00)	0.00 (0.00)	0.16 (0.12)	0.05 (0.05)				
prostate	focal interstitial round cells								
	dorsolateral	0.16 (0.09)	1.26*** (0.13)	1.74*** (0.10)	1.95*** (0.09)				
	ventral	0.16 (0.09)	0.05 (0.05)	0.21 (0.12)	0.11 (0.11)				

TISSUE	FINDING	MALES				FEMALES			
		C	LD	MD	HD	C	LD	MD	HD
	focal tubuli with granulocytes								
	dorsolateral	0.16 (0.12)	2.05*** (0.22)	2.84*** (0.14)	3.21*** (0.12)				
	ventral	0.00 (0.00)	0.05 (0.05)	0.16 (0.12)	0.11 (0.11)				
seminal vesicles	less prominent secretions	0.00 (0.00)	0.26 (0.21)	0.63*** (0.17)	0.32* (0.13)				
	prominent low epithelium	0.00 (0.00)	0.05 (0.05)	0.16 (0.09)	0.26* (0.10)				
spleen	red pulp								
	diffuse hyperplasia	0.26 (0.10)	0.26 (0.10)	0.32 (0.11)	0.42 (0.12)	0.42 (0.14)	0.37 (0.11)	0.37 (0.11)	0.39 (0.12)
	erythrocytes	1.89 (0.11)	2.11 (0.15)	2.37** (0.11)	2.47** (0.14)	1.79 (0.12)	2.37** (0.11)	2.26** (0.10)	2.39** (0.12)
	hemosiderin pigment	3.32 (0.11)	3.84** (0.24)	4.26*** (0.10)	4.26*** (0.13)	3.37 (0.22)	4.32*** (0.11)	4.42*** (0.12)	4.33*** (0.11)
uterus	granulocytes (endometrium)					1.95 (0.19)	0.37*** (0.11)	0.32*** (0.11)	0.47*** (0.12)
	height of epithelium					2.42 (0.12)	3.11*** (0.20)	3.16*** (0.12)	3.00*** (0.15)
	mitoses (epithelium)					0.74 (0.15)	0.42 (0.14)	0.21** (0.10)	0.21** (0.10)
	resting appearance					0.05 (0.05)	0.74*** (0.10)	0.79*** (0.10)	0.95*** (0.05)
	vacuolated karyorrhectic cells (epithelium)					1.00 (0.26)	0.32* (0.15)	0.32* (0.15)	0.32* (0.17)
vagina	cornification					0.63 (0.17)	0.11** (0.07)	0.00*** (0.00)	0.05** (0.05)
	granulocytic infiltration					1.84 (0.14)	1.26** (0.10)	1.32** (0.13)	1.21** (0.10)
	mucification					0.58 (0.21)	1.53** (0.22)	1.53*** (0.22)	2.11*** (0.25)
	necrotic epithelial cells					0.05 (0.05)	0.84*** (0.21)	0.84*** (0.21)	1.26*** (0.20)
	thickness of epithelium					3.37 (0.23)	2.21** (0.26)	2.00*** (0.23)	2.47* (0.33)

The sponsor considered the microscopic findings in pituitary gland, prostate, coagulating gland, seminal vesicles, mammary gland, and female reproductive organs to be secondary to elevated prolactin, and the spleen findings to be related to the α-lytic effects of risperidone. The adrenal gland findings and the increase in granulocytic infiltration at the injection site were considered drug-related. The other findings at the injection site [e.g., chronic inflammatory fibro-granulomatous encapsulation] were considered to be related to the vehicle.

Toxicokinetics: methods and data provided in a separate report.

2. Study title: 12-month intermittent repeated dose intramuscular toxicity study in the Wistar rat [Study no: EDMS-BEBE-2743275, Exp. No. 4729A, Volume #: 1.14-1.15, Conducting laboratory and location: Janssen Pharmaceutica, Belgium, Date of study initiation: 2/10/99, GLP, QA report: Y]. **This study was run concurrently with the 2-yr carcinogenicity bioassay.** [Additional details on methodology can be found in the review of the 2-yr bioassay.]

Methods

Dosing:

Species/strain: Hannover Wistar rat [Charles River bred; [redacted] (supplier)]

#/sex/group: 20/sex/grp

Initial age: 5-6 wks

Initial body weight: 95-135 gm

Observations and times:

Clinical signs: all animals were observed daily, with attention to injection site.

Body weights: body wts were recorded prior to the start of dosing, weekly during the dosing period, and at the end of the dosing period.

Food consumption: food consumption was recorded weekly during the dosing period.

Hematology/Clinical chemistry: blood samples were collected at 6 and 12 months in survivors.

Hormones: aldosterone and corticosterone concentrations were quantitated at 12 months in survivors (12/sex/grp). [Blood samples were not available in 8 animals.]

Terminal studies: animals were sacrificed after 12 months of dosing. A complete necropsy was performed on all animals, and organ wts and gross and microscopic findings were determined as described for the 2-yr study. Additional procedures: (a) s.c. tumor of HDM (#310) was stained with Von Gieson stain. (b) lung sections from 8 animals [#109, 503, 508, 519, 613, 705, 706, and 716] were stained with 0.5% methylene blue/saline. (c) injection site tumor was immunohistochemically stained [HDM #313]. (d) liver section from LDF #609 was stained with Perls' stain. [The "technical histoprocessing work" was conducted by [redacted].

One per sex/grp were prepared for EM analysis and kidney samples from selected animals (6) were prepared for EM examination; however, EM was not performed.

Results

Mortality: there were 2 unscheduled deaths [1 LDM, 1 HDM]. The LDM was sacrificed moribund during Wk 50. Clinical signs in this animal consisted of bad condition, "vaginal discharge, red", and moderate sedation; the cause of death was determined to be an oligodendroglioma. The HDM [#316] was found dead during Wk 20; the cause of death could not be determined. Neither death was considered drug-related by the sponsor.

Clinical signs: clinical signs consisted of ptosis [HD], sedation [LD, HD], and food wastage [HDF]. Ptosis was observed throughout the dosing period, primarily during the first wk postdosing. Sedation was moderate-to-severe in males following the first dose, but decreased in severity and was no longer observed in LD animals afterward. At the HD, sedation was characterized as slight following subsequent doses, and was not observed in all HD animals after all doses; when observed, sedation was evident primarily during the 1st wk postdosing. Food wastage was not considered drug-related by the sponsor, due to the transient nature of the finding and the fact that it was not observed in the main-study animals used for carcinogenicity testing.

Body weight: in males, body wt was consistently reduced (compared to VCM) at the HD from Wk 3 on; final body wt was 15% lower in HDM than in VCM. Overall body wt gain was reduced by 19% in HDM. In females, body wt was increased at the LD (compared to VCF) from Wk 11 on; final body wt was 12% higher in LDF than in VCF. Overall body wt gain was increased by 22% in LDF.

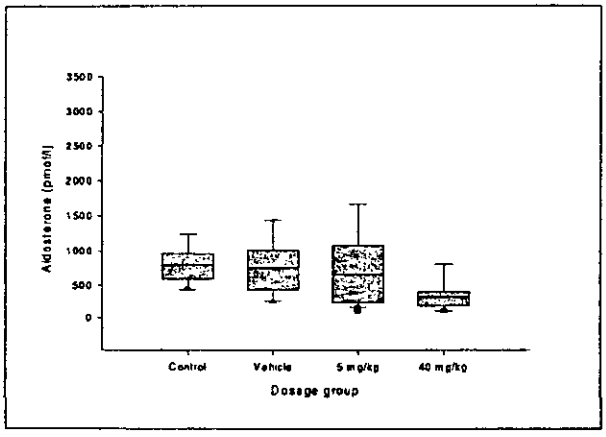
Food consumption: food consumption was reduced [7-9%] in HDM during Wks 12-16 and at Wk 24 and 33, 37; thereafter, food intake was sporadically increased in HDM [7-8%; Wks 37, 46]. Total food intake was similar among grps in males. In females, food intake was increased through most of the dosing period at both doses; the effect was more consistent at the LD; total intake was increased by 8% in LDF and by 5% in HDF.

Hematology: the following findings were of note: (a) small decreases in hct and rbc ct in HDM [1-6%], with small increases in MCV, MCH, and MCHC [2-6%], (b) small decreases in hgb [HD, 5-6%], hct [MD (2-3%), HD (6%)], and rbc ct [MD (2%, Wk 27 only), HD (5-6%)], with a slight increase (1%) in MCHC [MD, HD], (c) a decrease in lymphocytes in HDM [10-17%], (d) increases in monocytes in males [MD (31%, Wk 52), HD (19-38%)] and fmeales [MD (27-54%), HD (64-69%)], (e) an increase in neutrophils in males [MD (38%), HD (55%); Wk 52] and females [MD (57-40%), HD (60-45%)], (f) an increase in thrombocytes in females [MD (12%), HD (8%)], (g) an increase in eosinophils in HDM [44%, Wk 52] and females [MD, HD (71-50%)] All comparisons are to VC.

Clinical chemistry: the following findings were notable: (a) small (1%) decreases in Na in HDM, LDF, and HDF, (b) a transient decrease in K in HDM (4%, Wk 27), and an increase in K in LDF (10% at Wk 27) and HDF (15-14%), (c) a decrease in Cl in HDM (1-3%), LDF (2-3%), and HDF (3%), (d) an increase in Ca in HDM (4%, Wk 52), LDF (4%), and HDF (9-10%), (e) an increase in P_i in LDM (15%, Wk 52), HDM (24-42%), LDF (57-32%), and HDF (69-51%), (f) a small decrease in total protein in HDM (1-4%), (g) a decrease in glucose in LDM (5%, Wk 27), HDM (24%, Wk 27), LDF (17-7%), and HDF (26-16%), (h) an increase in cholesterol in HDM (21%) and HDF (26-15%), (i) a decrease in TG in HDM (28-43%), (j) an increase in PL in LDM (7%, Wk 27), HDM (14%, Wk 27), and HDF (13-10%), (k) a small decrease (6-9%) in BUN in HDM, LDF (Wk 52), and HDF (Wk 52), (l) an increase in total bilirubin in LDM (25%, Wk 52) and HDM (25-100%), and (m) a decrease in creatinine in HDF (6%). All comparisons were to VC.

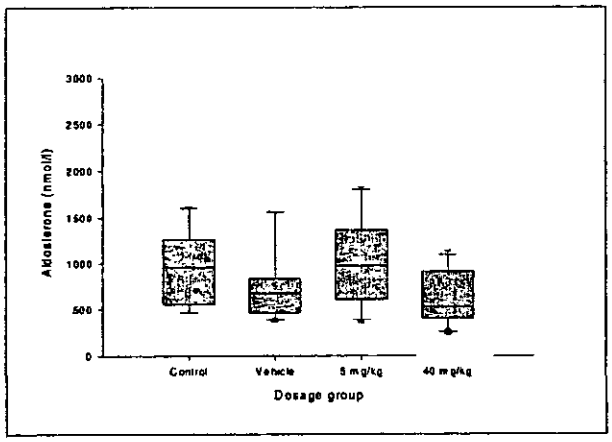
Hormones: serum aldosterone was reduced in HDM (52%) when compared to VCM. Aldosterone was not significantly affected in females when compared with VC; however, aldosterone levels were reduced at the HD when compared to SC [34%]. Serum corticosterone was not affected in either males or females. The data for serum aldosterone were illustrated in the following sponsor's figures:

males



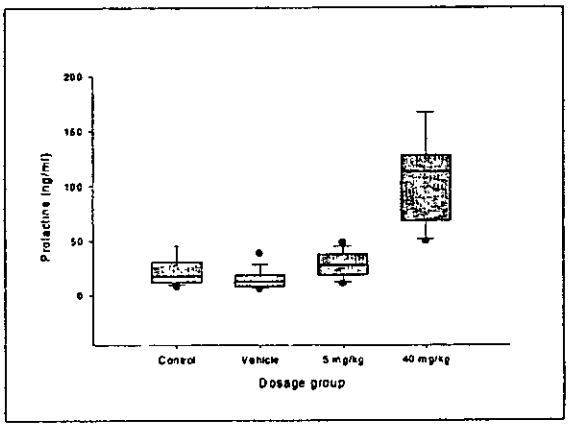
Median, 10, 25, 75, 90 percentiles and outlyers (•) are presented

females

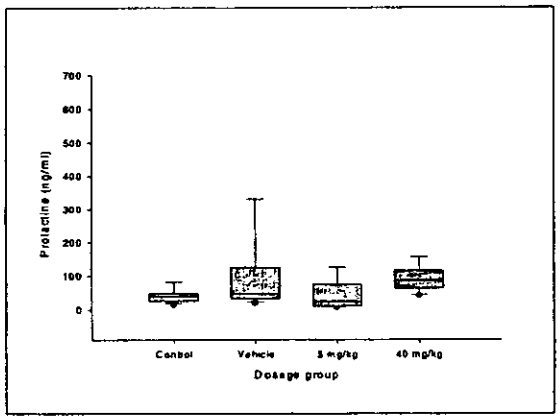


Serum prolactin was significantly increased in HDM [7-fold (600%)] when compared to VC (and SC). Serum prolactin was not significantly affected in females when compared to VC; however, prolactin levels in HDF were significantly higher compared to SC [2.2 fold (120%)]. Serum prolactin was 2.8 fold higher in VCF than in SCF. [This increase (not statistically significant) was due primarily to a high value in 1 F (652 ng/mL); when calculated without this value, the mean was 71 ng/mL for VCF.] The data were illustrated in the following sponsor's figures:

males



females



Median, 10, 25, 75, 90 percentiles and outlyers (•) are presented

The sponsor considered serum prolactin to be increased in LDM, although there was no difference in serum prolactin between SCM and HDM; serum prolactin was 35% lower in VCM as compared to SCM. The sponsor also considered serum prolactin to be elevated in HDF. As noted, this effect was noted only when HDF was compared to SC. Removing the 1 outlier in the VCF grp, the range of values was 15-110 ng/mL in SCF, 19-192 ng/mL in VCF, 6-159 ng/mL in LDF, and 41-223 ng/mL in HDF.

Gross pathology: selected findings are summarized in the following table [comparisons are to VC]:

TISSUE	FINDING	MALES				FEMALES			
		SC	VC	LD	HD	SC	VC	LD	HD
injection site	powdery deposit i.m.	0/20	20/20	20/20	20/20	0/20	20/20	20/20	20/20
	tissue mass, fatty white	0/20	0/20	0/20	1/20	0/20	0/20	0/20	0/20
adrenal gland	swollen	0/20	0/20	0/20	6/20*	0/20	0/20	0/20	3/20
kidney	pale	0/20	0/20	1/20	4/20	0/20	0/20	2/20	6/20*
liver	more pronounced lobulation	0/20	0/20	1/20	0/20	0/20	0/20	2/20	4/20
mammary gland	inspissated secretion	0/20	0/20	0/20	2/20	0/20	0/20	0/20	2/20
	stimulation	0/20	0/20	0/20	1/20	0/20	0/20	19/20***	20/20***
pituitary gland	swollen	1/20	0/20	0/20	3/20	0/20	0/20	2/20	0/20
	stippled, yellow	0/20	0/20	0/20	5/20	0/20	0/20	0/20	0/20
prostate gland	swollen	0/20	0/20	0/20	5/20				
subcutaneous	tissue mass	0/20	0/20	0/20	3/20	0/20	0/20	0/20	0/20
uterus	swollen					8/20	4/20	0/20	0/20
	swollen, watery contents					7/20	4/20	0/20	0/20

*p<0.05, **p<0.01, ***p<0.001

The sponsor considered all findings given in the table to be dose-related, except for the injection site changes that were considered vehicle-related.

Organ weights: the following were notable: (a) an increase in kidney wt [absolute-relative] in HDM [8-29%] and HDF [11-10%], (b) an increase in adrenal wt [absolute-relative] in HDM [71-100%] and HDF [16%], (c) a decrease in absolute testis wt in LDM (9%) and HDM (13%), (d) an increase in spleen wt [absolute-relative] in HDM (13-34%), (e) an increase in liver wt [absolute-relative] in LDF [19-6%] and HDF [12%], (f) a decrease in thymus wt [absolute-relative] in HDF [9-10%], and (g) a decrease in ovary wt [absolute-relative] in LDF [6-15%] and HDF [8-9%].

Histopathology: selected non-neoplastic findings are summarized in the following table [asterisks indicate significance compared to SC; bolded numbers indicate significance (p-value of 0.05-0.001) compared to VC]:

TISSUE	FINDING	MALES				FEMALES			
		SC	VC	LD	HD	SC	VC	LD	HD
injection site	multifocal, chronic inflammation	0/19	20/20***	**13/19***	18/20***	0/19	20/20***	**15/20***	20/20***
	encapsulation	0/19	20/20***	19/19***	20/20***	0/19	20/20***	20/20***	20/20***
	fibro-granulomatous reaction	0/19	20/20***	19/19***	20/20***	0/19	20/20***	20/20***	20/20***
	focal thickening perimysium	0/19	20/20***	16/19***	20/20***	4/19	20/20***	16/20***	20/20***
	giant cells	0/19	20/20***	19/19***	20/20***	0/19	20/20***	20/20***	20/20***
	granulocytic infiltration	0/19	8/20**	8/19**	10/20***	0/19	9/20**	13/20***	13/20***
	necrotic center/cell debris	0/19	2/20	0/19	5/20*	0/19	1/20	2/20	6/20*
	prominent muscular degeneration	0/19	3/20	2/19	5/20*	0/19	4/20	1/20	7/20**
adrenal	congestion	1/19	0/19	0/19	**10/19****	3/19	1/19	1/19	3/19
	clear/vacuolated cells, diffuse (z. glomer)	0/19	2/19	3/19	0/19	1/19	0/19	4/19	**6/19**
	ectasia	0/19	0/19	0/19	3/19	0/19	0/19	1/19	2/19
	foamy cells	0/19	11/19***	**0/19**	6/19*	0/19	4/19	0/19	4/19
	swollen cells								
	zona fasciculata	0/19	0/19	1/19	**16/19*****	0/19	0/19	1/19	3/19
	zona reticularis	0/19	0/19	1/19	**14/19*****	4/19	6/19	6/19	8/19
	cortical hyperplasia, focal	5/19	8/19	**0/19***	**1/19**	2/19	0/19	2/19	1/19
bone, sternum	osteodystrophy	0/19	0/19	0/19	**6/19***	1/19	0/19	2/19	**12/18*****
bone, stifle joint	osteodystrophy	0/19	0/19	0/19	2/19	1/19	0/19	1/19	3/19
	round particles/inflammatory cells								
	surrounding tissue	0/19	18/19***	15/19***	17/19***	0/19	17/19***	13/19***	13/19***
	intra-articular	0/19	2/19	4/19	3/19	0/19	4/19	2/19	4/19
brain	multifocal vacuolation	0/19	1/19	1/19	0/19	1/19	2/19	2/19	4/19
coagulating gland	granulocytes/cellular debris in lumen	0/18	0/19	1/19	3/19				
	granulocytic infiltration	0/18	0/19	1/19	3/19				
heart	fibrosis a/o chronic inflammation	2/19	3/19	4/19	7/19	0/19	1/19	2/19	3/19
	necrotic fibers/inflammatory cells	1/19	2/19	1/19	3/19	0/19	0/19	0/19	0/19
kidney	diffuse dilated tubuli	0/19	0/19	1/19	**6/19***	1/19	1/19	2/19	**13/19*****
	focal dilated tubuli	0/19	1/19	0/19	4/19	2/19	0/19	0/19	2/19
	hyaline casts	8/19	4/19	7/19	5/19	1/19	3/19	4/19	9/19**
	hypertrophic tubules	0/19	0/19	0/19	3/19	1/19	0/19	2/19	4/19
	inflammation pelvis	5/19	5/19	6/19	9/19	6/19	4/19	9/19	7/19
	mineral deposit								
	cortex	3/19	1/19	1/19	**8/19**	0/19	3/19	2/19	2/19
	medulla	0/19	2/19	1/19	0/19	2/19	1/19	3/19	5/19
	papilla	1/19	1/19	2/19	1/19	3/19	4/19	4/19	5/19
	pelvis	10/19	6/19	10/19	**15/19**	14/19	14/19	17/19	17/19
	swollen/vacuolated tubules	0/19	0/19	0/19	**8/19****	8/19	2/19	4/19	8/19
liver	perivascular brown pigment	0/19	0/19	0/19	4/19	1/19	0/19	0/19	0/19
	pigmented sinusoidal lining cells	0/19	1/19	0/19	1/19	2/19	0/19	1/19	**8/19**
	RES-aggregates	7/19	6/19	9/19	6/19	10/19	8/19	3/19*	3/19*
g	granulocytes/perivascular/peribronchial	1/19	2/19	3/19	6/19	8/19	2/19	**10/19**	**12/19**
	foamy cells [lymphoid tissue]	0/19	2/19	0/19	2/19	0/19	2/19	0/19	0/19
	macrophages, focal	12/19	5/19*	6/19	9/19	6/19	3/19	0/19*	**11/19**

TISSUE	FINDING	MALES				FEMALES			
		SC	VC	LD	HD	SC	VC	LD	HD
lymph node	iliac								
	plasmacytosis	10/18	16/19	14/19	19/19**	11/19	14/18	**19/19****	18/19*
	sinus histiocytosis/foamy cells	0/18	19/19***	**7/19****	17/19***	0/19	18/18***	16/19***	19/19***
	inguinal								
	plasmacytosis	1/19	1/19	2/19	2/19	0/19	0/19	1/18	0/19
	sinus histiocytosis/foamy cells	0/19	19/19***	**0/19**	17/19***	0/19	19/19***	**1/18**	18/19***
	mesenteric								
	plasmacytosis	0/19	0/19	0/19	2/19	0/19	0/19	0/19	0/19
	sinus histiocytosis/foamy cells	0/19	11/19***	**0/19**	6/19*	0/19	13/19***	**0/19**	14/19***
	prominent granulocytes	0/19	0/19	0/19	2/19	2/19	0/19	0/19	0/19
	popliteal								
	plasmacytosis	2/18	0/17	3/19	**7/18**	2/18	2/19	5/19	3/19
	sinus histiocytosis/foamy cells	0/18	17/17***	**5/19**	18/18***	0/18	19/19***	**6/19***	18/19***
mammary gland	female appearance/glandular development	1/19	1/18	**9/19****	**19/19*****	19/19	19/19	19/19	20/20
	focal hyperplasia	0/19	0/18	0/19	**5/19***	1/19	0/19	**5/19**	**9/20****
	multifocal inflammatory cells	0/19	1/18	0/19	4/19	0/19	0/19	3/19	1/20
	inspissated material	0/19	0/18	0/19	**5/19***	0/19	0/19	0/19	1/20
	secretion	1/19	1/18	**7/19***	**19/19*****	12/19	11/19	**19/19****	**20/20****
ovary	basophilic corpora lutea					11/19	14/19	**6/19**	**3/19***
	cystic hyperplasia/papillary					6/19	3/19	3/19	0/19*
pancreas	foamy cells	0/19	11/19***	**0/19**	15/19***	0/19	8/19**	**0/19**	7/20**
pituitary	diffuse hyperplasia (pars distalis)	0/19	1/19	0/19	6/19*	0/19	0/19	2/19	1/19
	hypertrophy (pars intermedia)	1/19	3/19	9/19**	**12/19*****	2/19	3/19	6/19	9/19*
	round cells, focal	0/19	0/19	0/19	2/19	0/19	1/19	0/19	0/19
	prolactin immunoreactive cells	19/19	19/19	19/19	19/19	19/19	19/19	19/19	19/19
ostate	dorsolateral								
	fibrosis, interstitial	0/19	0/19	**5/19***	**11/19*****				
	round cells	5/19	4/19	**13/19***	**18/19*****				
	tubuli with granulocytes, focal	2/19	2/19	**14/19*****	**19/19*****				
seminal vesicles	granulocytes/cell debris in lumen	0/19	0/19	1/19	2/19				
	granulocytic inflammation	0/19	0/19	1/19	**6/19***				
	inspissated material	0/19	0/19	1/19	**12/19*****				
spleen	atrophy, white pulp	0/19	0/19	0/20	3/19	0/19	0/19	0/19	2/19
thyroid	high follicular epithelium	5/19	5/19	2/19	4/19	1/19	1/19	4/19	5/19
uterus	dilated lumen					14/19	11/19	5/19**	5/19**
	granulocytes (endometrium)					15/19	18/19	**1/19*****	**1/19*****
	mitosis (epithelium)					14/19	11/19	8/19	6/19*
	vacuolated/karyorrhetic cells (epi)					8/19	12/19	**0/19****	**4/19**
vagina	cornification					7/19	9/19	**1/19***	**1/19***
	mucification					5/19	6/19	**15/19****	**17/19*****
	necrotic cells					2/19	1/19	**9/19***	**11/19****
	round cells					14/19	12/19	**0/19*****	**3/19*****

*p<0.05, **p<0.01, ***p<0.001,

The sponsor also expressed findings as "mean scores". The severity of a finding was scored based on a scale of 1 ["minimal histological change or small quantity of a histological entity"] to 5 ["severe histological change or large quantity of a histological entity"]. Considering the data in this way was primarily useful in interpreting findings of erythrocytes in red pulp and hemosiderin deposits in spleen. Although these findings were detected in all (or almost all) animals, the severity of the finding was significantly increased in HD [erythrocytes] or in MD and HD [hemosiderin] animals. The mean severity of osteodystrophy in sternum in HDM and HDF was 0.53 and 0.83, respectively. However, the mean score takes into account data from both affected and unaffected animals. If only affected animals are considered, the severity score in HDM and HDF

ranged from 1-3 and 1-2, respectively. The mean score in affected HDM and HDF was 1.67 and 1.25, respectively.

There were no clearly drug- or vehicle-related increases in tumors. Tumor findings were summarized in the following sponsor's tables:

MALES

Organ or Tissue - Observation		Dosage group (mg / kg) Control	Vehicle	Low:5	High:40
Administration site	*Number examined:*	*19*	*20*	*19*	*20*
- Sarcoma					1
Brain	*Number examined:*	*19*	*19*	*19*	*19*
- Oligodendroglioma				1	
Lymph node(s), mesenteric	*Number examined:*	*19*	*19*	*19*	*19*
- Hemangio(endothelio)ma			1		
Pancreas	*Number examined:*	*19*	*19*	*19*	*19*
- Adenoma, endocrine		1			1
Pituitary gland	*Number examined:*	*19*	*19*	*19*	*19*
- Adenoma		2	1	1	1
Spleen	*Number examined*	*19*	*19*	*20*	*19*
- Hemangio(endothelio)ma			1		
Subcutis	*Number examined*				*2*
- Fibroma					1
Thyroid glands	*Number examined:*	*19*	*19*	*19*	*19*
- Adenoma, follicular		1			

Significance versus Vehicle computed by the Fisher Exact test (one tailed) : * P < .05 ** P < .01 *** P < .001
Statistics are only performed if more than 50 % of the animals of the group are examined

FEMALES

Organ or Tissue - Observation		Dosage group (mg / kg) Control	Vehicle	Low:5	High:40
Mammary gland	*Number examined:*	*19*	*19*	*19*	*20*
- (Fibro)adenoma					1
- Adenocarcinoma				1	
Pituitary gland	*Number examined.*	*19*	*19*	*19*	*19*
- Adenoma		1		2	2
Thyroid glands	*Number examined:*	*19*	*19*	*19*	*19*
- C-cell adenoma					1
Uterus	*Number examined:*	*19*	*19*	*19*	*19*
- Polyp		1	1		

Significance versus Vehicle computed by the Fisher Exact test (one tailed) : * P < .05 ** P < .01 *** P < .001
Statistics are only performed if more than 50 % of the animals of the group are examined

The sponsor considered the microscopic findings in "adrenal gland, coagulating glands, seminal vesicles, prostate, pituitary gland, mammary gland, female genital tract, kidneys and bones" to be drug-related [except as noted]; these findings were considered "Possible prolactin-mediated effects" by the sponsor. Findings in spleen [i.e., accumulation of erythrocytes, hemosiderin pigmentation] were considered secondary to the α-lytic effects of risperidone. Certain microscopic findings at the injection site [necrosis/cellular debris, muscular degeneration] were considered to be the result of drug-induced irritation.

Microscopic findings related to the vehicle [microspheres] included (a) encapsulation of microspheres at the injection site accompanied by a chronic granulomatous inflammatory response, (b) inflammatory reaction (i.e., plasmacytosis) in iliac and popliteal lymph nodes, resulting from inflammatory reactions at the injection site, (c) granulomatous inflammation in lungs [upon examination, "...the intravascular presence of small

microsphere particles could be detected....[that]...seem to be related to the quantity of microspheres that was injected, increasing the chance for intravascular presence of small microsphere particles". The sponsor noted that in 4 VC, 1 LD, and 3 HD animals, "small microsphere particles" were detected intravascularly in lung. (d) foamy cells in adrenal gland, peribronchial lymphoid tissue, and pancreas, and sinus histiocytosis/foamy cells in lymph nodes [considered secondary to activation of the Mononuclear Phagocyte System (MPS)].

DOG

1. Study title:Twelve month intermittent repeated dose intramuscular toxicity study in the beagle dog [Study no: EDMS-BEBE-2537997, Exp No 4730, Volume #: 1.19-1.20, Conducting laboratory and location: Janssen Pharmaceutica, Belgium, Date of study initiation:4/13/99, GLP, QA report: Y]

Drug, lot #, radiolabel, and % purity: risperidone, batch no. ZR064766EIA221
Formulation/vehicle: i.m. depot [identical to the clinical Phase 3 forumulation]/microspheres. The dosing formulation contained: 37% risperidone, lactic acid:glycolic acid (3:1) —, solvent , polysorbae 20. citric acid, cHCL, NaCl, water]. Concentration and homogeneity stated to have been analyzed prior to the start and at the end of the dosing period. In addition, concentration was analyzed every 3 months during the dosing period. Actual concentrations were found to be — of intended. Stability was not tested since suspensions were prepared fresh for each dosing period.

Methods
Dosing:
Species/strain: Beagle dog [sponsor's outbred colony]
#/sex/group or time point (main study): 4/sex/grp
Satellite groups used for toxicokinetics or recovery: no
Initial Age: 6-7 mo
Initial Weight: not specified in methods.
Doses in administered units [sponsor's table follows]: in this review, V = C [control]

Dosage groups:	V	L	M	H
(mg/kg body weight)				
doses 1-2:	00	1.25	2.5	5
doses 3-4:	00	2.5	5	10
doses 5-26	00	5	10	20
(ml/kg body weight)				
doses 1-2:	0.125	0.031	0.062	0.125
doses 3-4:	0.25	0.062	0.125	0.25
doses 5-26	0.5	0.125	0.25	0.5

Route, volume: i.m., 0.031, 0.062, 0.125, 0.25, 0.5 mL/kg for 1.25, 2.5, 5, 10, and 20 mg/kg; C grps received 0.125, 0.25, and 0.5 mL/kg for doses 1-2, 3-4, and 5+. Injections were administered in the m. biceps femoris on alternating sides.
Dosing frequency: bi-weekly
Observations and times:
Clinical signs: all animals were observed daily. Feces were examined for parasites on 9/30/99 due to the presence of parasites in a concurrent study; giardia was found in the feces of one animal [#143].
Body weights: body wts were recorded at baseline and weekly during the dosing period.
Food consumption: food consumption was recorded in all animals weekly during the dosing

period.

Ophthalmoscopy: an examination of conjunctiva, sclera, cornea, interior chamber, iris, lens, and fundus was conducted in all animals prior to the start of dosing, after 6 mo of dosing, and "towards the end of the dosing period". Eyes were examined by slit lamp and indirect biomicroscopy following induction of mydriasis by application of atropine sulfate (1%).

ECG/bp: ECG and bp [SAP, DAP] were recorded at baseline, and at 3, 6, and 9 mo of dosing, and "towards the end of the dosing period". Methods used to assess these parameters were not described.

Hematology: blood samples were collected twice prior to the start of dosing, and at 2, 4, 8, 12, 16, 20, 24, 28, 32, 36, 40, 44, and 48 wks of dosing, and "towards the end of the study for analysis of the following parameters: hct, hgb, rbc ct, wbc ct [total, differential], MCV, MCH, MCHC, thrombocyte ct, APTT.

Clinical chemistry: blood samples were collected twice prior to the start of dosing, and at 2, 4, 8, 12, 16, 20, 24, 28, 32, 36, 40, 44, and 48 wks of dosing, and "towards the end of the study for analysis of the following parameters: Na, K, Cl, Ca, P_i, total protein, albumin, glucose, cholesterol, TG, PL, BUN, creatinine, total bilirubin, alkaline phosphatase, AST, ALT, GGT.

Urinalysis: urine samples were collected [via catheterization of the urinary bladder] in all dogs at baseline, and at Wks 4, 12, 24, and 40 of dosing, and "towards the end of the study" for analysis of the following parameters: urobilinogen, pH, proteins, glucose, ketones, bilirubin, occult blood, specific gravity, microscopic analysis of sediment.

Gross pathology: animals were sacrificed after 12 mo of dosing. Heparin was administered to prevent blood coagulation prior to exsanguination [carotid artery]. A complete necropsy was performed on all animals

Organs weights: wts of the following organs were recorded: lungs, spleen, liver, heart, pancreas, kidneys, brain, thymus, adrenal gland, thyroid/parathyroid, testes, ovaries, pituitary gland, prostate.

Histopathology: the following tissues were examined microscopically in all animals: injection site [muscle, subcutis, skin], adrenal gland, aorta, bone [distal femur, sternum], bone marrow, brain [cerebrum, cerebellum, midbrain], cranial nerve/optic tract, eipdidymides, esophagus, eye, gall bladder, heart, kidneys, lacrimal gland, large intestine [cecum, colon, rectum], liver, lungs, lymph nodes [iliac (L, R), popliteal (L, R), bronchial, mesenteric], mammary gland, ovaries, pancreas, parathyroid glands, peripheral nerve (sciatic nerve), pituitary gland, prostate, salivary gland [mandibular, parotid], skeletal muscle [psoas], skin, small intestine [duodenum, jejunum, ileum], spinal cord [cervical, lumbar], spleen, stomach, testes, thymus, thyroid glands, tongue, trachea, urinary bladder, uterus, vagina, gross lesions].

Tissues were preserved in 10% buffered formalin, except for eyes [2.5% buffered formalin + 1.25% glutaraldehyde] and testes [Bouin's fluid], and stained with H & E for examination. Additional sections of formalin-fixed lungs stained with 0.5% methylene blue were examined for presence of microspheres, and paraffin sections of pituitary gland from all animals were immunohistochemically stained for PRL-immunoreactive cells

Toxicokinetics: methods and data were provided in a separate report [Study No. R064766/FK3214].

Results:

Mortality: there were no unscheduled deaths.

Clinical signs: the only drug-related clinical signs were sedation [characterized as "slight", and observed in all but 1 (MD) dosed animal] and a decrease in vaginal discharge in all dose grps [only 1 dosed F had vaginal discharge compared to 4/4 CF]. Sedation was transient

at all doses [only observed on day of dosing, and not after Wk 8]. Injection site findings were characterized as slight, and only observed in C animals.

Body weights: there was no clear drug-related effect on body wt.

Food consumption: there were no clear drug-related effects. One LD animal refused food during Wks 12-18, resulting a decrease in mean food intake for that dose grp. The sponsor also noted that dental problems resulted in lower food intake in 2 other LD animals.

Ophthalmoscopy: no drug-related findings were reported.

ECG/bp: there were no clear drug-related effects. Heart rate was not affected; QT_c was significantly increased, but only at the lower doses and only sporadically. The data are provided in a portion of the sponsor's Table T3:

			Dosage group (mg/kg)			
Parameter	Week	Date	Vehicle	Low:5	Medium:10	High:20
QTC m sec	-1	01-APR-1999	221 (7)	227 (7)	229 (3)	229 (4)
	12	02-JUL-1999	244 (4)	236 (6)	235 (8)	246 (5)
	24	27-SEP-1999	224 (6)	240 (6)	241 * (3)	235 (3)
	40	14-JAN-2000	222 (4)	234 * (2)	238 (6)	239 (7)
	52	07-APR-2000	231 (6)	237 (5)	244 (7)	240 (6)

SAP was significantly decreased in the HD grp during Wks 12 [11%] and 52 [16%]; SAP was also decreased during Wk 24 [11%], but the effect was not significant.

Hematology: the following were of note: (a) wbc ct was decreased in all dose grps throughout the dosing period [LD: 15-34%; MD: 9-23%; HD: 22-31%]; however, the effect was not dose-related at any of the sampling times. (b) thrombocyte ct was reduced at the HD prior to the star of dosing [14%] and throughout the dosing period. At Wk 52, thrombocyte ct was 21-24% lower at the HD compared to C. (c) APTT was reduced at the HD during Wks 4-36 [≈10%]; however, this difference was due to an increase in APTT over this time period in the C grp. (d) lymphocyte ct was decreased at all doses [LD: 14-37%; MD: 16-33%; HD: 21-42%] throughout the dosing period; the effect tended to be greater at the HD. (e) neutrophil ct was reduced at all doses from Wk 8 on; however, the greatest effect was observed at the LD. Final values [Wk 52] were reduced by 25, 11, and 12% at the LD, MD, and HD, respectively.

Clinical chemistry: the following were of note: (a) K tended to be lower in dosed grps; however, the effect was greatest at the HD. K was significantly reduced at the HD during Wks 16, 28, and 44-52 [8-12%]. At Wk 52, K was 7, 7, and 8% lower in LD, MD, and HD grps, respectively, compared to C. (b) Ca was slightly, but significantly, increased in the HD grp throughout most of the dosing period [<5%]; however, values did exceed the baseline value to any notable extent. (c) glucose was slightly, but significantly increased in dosed grps compared to C [LD: <10%, Wks 8, 20; MD: <10%, Wks 16, 20, 24; HD: <15%, Wks 2-40]. (d) cholesterol was increased at all doses throughout the dosing period; however, the increases were small, and were significant only at the HD. Final values were increased by 16, 16, and 24% in LD, MD, and HD grps, respectively. (e) PL was increased at all doses during the dosing period; however, the effect was significant only at the HD. Final values were increased by 13, 17, and 21% at the LD, MD, and HD, respectively, compared to C. (f) creatinine was decreased at all doses throughout the dosing period; however, the effect was due to an increase in creatinine in the C grp. Final values in dosed grps were fairly similar to baseline values.

Urinalysis: there were no clear drug-related effects.

Organ weights: the following were of note: (a) increase in spleen wt [absolute-relative] at all doses [48-56, 88-84, 108-106% at LD, MD, HD, respectively], (b) decrease in testis wt [absolute-relative] at the HD [34-43%], (c) a non-dose related decrease in prostate wt [absolute-relative] at all doses [22-53%].

Gross pathology: the only finding of note was white strand formation detected at the injection site in all animals, including C animals.

Histopathology: selected findings are summarized in the following table:

TISSUE	FINDING	C	LD	MD	HD
injection site	cellular debris (encapsulation)	8/8	7/8	8/8	8/8
	chronic inflammation	8/8	8/8	8/8	8/8
	encapsulation (muscle)	8/8	8/8	8/8	8/8
	fibro-granulomatous reaction	8/8	8/8	8/8	8/8
	focal muscular degeneration	8/8	7/8	8/8	8/8
	focal thickened endomysium	8/8	7/8	8/8	8/8
	focal thickened perimysium	8/8	8/8	8/8	8/8
	giant cells (encapsulation)	8/8	8/8	8/8	8/8
adrenal gland	prominent vacuolated (foamy) cells (zona reticularis)	8/8	7/8	8/8	8/8
	vacuolated clear cells (zona glomerulosa)	0/8	0/8	1/8	2/8
epididymides	cellular debris (ductular lumen)	0/4	1/4	1/4	3/4
kidneys	focal basophilic tubuli	1/8	1/8	2/8	3/8
	focal round cells (interstitum)	2/8	3/8	3/8	2/8
	mineral deposits (medulla)	8/8	8/8	8/8	8/8
	round cell infiltration (pelvis)	3/8	2/8	2/8	2/8
lung	microspheres and/or remnants of microspheres	2/8	0/8	3/8	1/8
	focal (foamy) macrophages	7/8	6/8	7/8	6/8
lymph node	iliac (L-R)				
	plasmocytes (medullary cords)	8/8	8-6/8	7-4/8	8-7/8
	sinus histiocytes (foamy cells)	7-8/8	7-5/8	6-4/8	8-7/8
	popliteal (L-R)				
	plasmocytes (medullary cords)	8/8	8/8	8/8	8/8
	sinus histiocytes (foamy cells)	4-6/8	4-7/8	4-6/8	5-7/8
	bronchial				
	sinus histiocytosis (foamy cells)	3/8	2/8	3/8	4/8
	mesenteric				
	sinus histiocytosis (foamy cells)	8/8	7/8	8/8	8/8
ovary	corpora lutea (regressive)	4/4	0/4*	1/4	0/4*
pituitary gland	immunopositive cells (PRL)	8/8	8/8	8/8	8/8
salivary gland	parotid				
	focal atrophic acini	1/8	2/8	0/8	5/8
	focal periductular round cells	2/8	4/8	3/8	5/8
jejunum	dilated crypts with cellular debris	1/8	2/8	2/8	4/8
spleen	erythrocytes (red pulp)	8/8	8/8	8/8	8/8
	hemosiderin pigment	8/8	8/8	8/8	8/8
testis	atrophic tubuli	0/4	1/4	0/4	3/4
	spermatic giant cells	0/4	2/4	1/4	3/4
thyroid gland	focal round cells (interstitum)	0/8	0/8	0/8	1/8
urinary bladder	bleedings (mucosa)	4/8	4/8	3/8	6/8
	chronic inflammation (mucosa)	1/8	1/8	3/8	2/8
	edema (mucosa)	3/8	3/8	3/8	6/8
	focal granulocytes (epithelium)	2/8	2/8	2/8	4/8
	focal granulocytic infiltration	2/8	3/8	2/8	4/8
	lymphoid follicles (mucosa)	0/8	0/8	1/8	2/8
	ulceration(s)	0/8	2/8	2/8	1/8
uterus	resting appearance	0/4	4/4*	3/4	4/4*
vagina	granulocytic infiltration	1/4	1/4	2/4	3/4
	resting appearance	0/4	2/4	3/4	3/4

*$p<0.05$

The sponsor scored findings for severity [i.e., 1 = minimal or small area affected; 5 = severe or large area affected]. Severity scores for selected findings are summarized in the following table:

TISSUE	FINDING	C	LD	MD	HD
injection site (L)	encapsulation (muscle)	3.88(0.13)	2.63(0.38)*	3.13(0.23)*	4.00(0.00)
	fibro-granulomatous reaction	3.88(0.13)	2.88(0.30)*	3.25(0.16)*	4.00(0.00)
	giant cell (encapsulation)	4.88(0.13)	3.75(0.31)**	3.88(0.13)**	5.00(0.00)
injection site (R)	encapsulation (muscle)	3.75(0.16)	2.63(0.32)*	3.00(0.19)*	3.75(0.16)
	fibro-granulomatous reaction	3.88(0.13)	3.00(0.19)**	3.25(0.16)*	3.75(0.16)
	giant cell (encapsulation)	5.00(0.00)	3.50(0.27)**	3.88(0.23)**	4.88(0.13)
epididymes	cellular debris (ductular lumen)	0.00(0.00)	0.25(0.25)	0.25(0.25)	1.00(0.41)
	amount of spermatozoa	4.50(0.29)	3.50(0.87)	4.50(0.29)	3.50(0.65)
lymph node, iliac (L)	sinus histiocytosis (foamy cells)	1.25(0.25)	1.38(0.26)	1.57(0.30)	2.13(0.27)*
lymph node, iliac (R)	sinus histiocytosis (foamy cells)	1.50(0.19)	1.17(0.31)	1.75(0.25)	2.29(0.18)*
ovaries	corpora lutea (regressive)	1.25(0.25)	0.00(0.00)*	0.50(0.50)	0.00(0.00)*
pituitary gland	immuno-positive cells (PRL)	2.13(0.13)	2.75(0.25)	2.75(0.16)*	3.25(0.16)**
prostate	glandular development	4.00(0.41)	3.25(0.63)	2.50(0.29)	2.75(0.25)
spleen	erythrocytes (red pulp)	1.88(0.40)	3.25(0.16)*	3.38(0.18)*	3.63(0.18)**
	hemosiderin pigment	1.88(0.23)	2.38(0.32)	2.38(0.18)	3.00(0.19)**
testes	atrophic tubuli	0.00(0.00)	0.50(0.25)	0.00(0.00)	1.00(0.41)
	spermatic giant cells	0.00(0.00	0.75(0.48)	0.25(0.25)	1.00(0.41)
uterus	glandular development	2.25(0.25)	1.00(0.00)*	1.25(0.25)	1.00(0.00)*
	height of epithelium	3.25(0.48)	2.25(0.25)	2.25(0.25)	2.00(0.00)
	resting appearance	0.00(0.00)	1.25(0.25)*	1.25(0.48)	1.75(0.25)*
vagina	resting appearance	0.00(0.00)	0.50(0.29)	1.00(0.41)	1.00(0.41)

*p<0.05, **p<0.01

The sponsor considered the following findings related to microsphere administration (except as noted): (a) findings at the injection site, (b) presence of foamy cells in the adrenal cortex and sinus histiocytosis in lymph nodes [iliac, popliteal]; however, the increased severity of sinus histiocytosis in iliac lymph node was considered a drug-related effect. and (c) the presence of microspheres (or remnants) in pulmonary tissue [as visualized in frozen sections].

The findings in prostate, pituitary gland, mammary gland [except for the presence of microspheres], and female reproductive organs were considered related to drug, but secondary to prolactin effects. Although the sponsor cited a dose-related increase in the resting aspect of the mammary gland in females; the data were not consistent with such a finding (mammary gland listing from the sponsor's summary table provided below):

Mammary gland *Number examined:*	*8*	*8*	*8*	*8*
- alveolar development (active)	1	0	0	0
- alveolar development (regressive)	3	0	1	0
- bleedings (interstitium)	1	0	0	0
- focal (peri-) folliculitis	1	2	1	0
- focal inflammatory cells (subepithelial)	3	4	2	4
- inspissated secretion	3	0	1	0

Splenic findings were attributed to the "slight α-lytic properties" of risperidone. The sponsor appeared to consider only the testicular findings [i.e., "Slight atrophic testicular changes and a decreased spermatozoa amount in the epididymides..."] at the HD to be a direct drug effect.

Toxicology summary and conclusions: three 6-mo s.c. studies and one 12-mo i.m. depot toxicity study were conducted in Wistar rat; the i.m. depot study was conducted concurrently with the 2-yr i.m. depot

carcinogenicity study. Two 6-mo and one 12-mo i.m. depot toxicity studies were conducted in Beagle dog.

Rat: in the three 6-mo s.c. studies, doses were increased during the dosing period. Doses were as follows:

Exp. 3095: 1st [0, 10, 40, 80 mg/kg], 2nd-5th [0, 30, 80, 160 mg/kg], 6th [0, 80, 320, 640 mg/kg]
Exp. 3589: 1st-2nd [0, 20, 40, 160 mg/kg], 3rd [0, 40, 80, 320 mg/kg], 4th-6th [0, 80, 160, 640 mg/kg]
Exp. 4731: 1st-2nd [0, 10, 20, 40 mg/kg], 3rd-4th [0, 20, 40, 80 mg/kg], 5th on [0, 40, 80, 160 mg/kg]

In 2 of the studies [Exp. No. 3095, 3589], doses were administered once per month; in the 3rd study [Exp. No. 4731], doses were administered bi-weekly. The s.c. studies were reviewed previously [5/7/97]. Exp. No. 3095 was not a definitive study; no histopathology was performed and the n/grp was small [5/sex/grp]. Microspheres [lactic acid-glycolic acid copolymer] were used to suspend risperidone in Exp. No.'s 3589 and 4731; the clinical Phase 3 formulation was used in Exp. No. 4731. [The formulation used in Exp. No. 3095 was not specified.]

Drug-related deaths occurred only in Exp. No. 3589. In that study, deaths occurred at 160 and 640 mg/kg in males and females. [Although similar doses were administered in Exp. No.'s 3095 and 3589, plasma exposure was higher in Exp. 3589. At the lethal doses, C_{max} was 5-12 and ≈2 times higher at 160 and 640 mg/kg, respectively, and the AUC was 3-7 and ≈2 times higher at 160 and 640 mg/kg, respectively, in Exp. No. 3589 compared to values in Exp. No. 3095. Although a direct comparison is difficult (due to differences in route and overlapping plasma exposures at different doses), the plasma exposure at the highest no-effect dose for death in rats was at least 16 and 4 times the anticipated C_{max} and AUC, respectively, in humans at the maximum recommended clinical dose.] The primary drug-related clinical signs were ptosis and sedation. Ptosis was observed at all doses in all 3 studies. Sedation was observed at all doses in Exp. No.'s 3095 and 3589, and at doses ≥20 mg/kg in Exp. No. 4731. In Exp. No. 4731, both ptosis and sedation were transient at the lower doses. Body wt was adversely affected in all 3 studies in males. In Exp. No. 3095, body wt was reduced compared to CM at the HD [12% at 160 mg/kg, 28% at 640 mg/kg, i.e., body wt loss at 640 mg/kg]. In the other experiments, body wt was decreased (compared to CM) at all doses in males; final body wts were 9, 15, and 32% lower than CM at 80, 160, and 640 mg/kg, respectively, in Exp. No. 3589 and 8, 16, and 20% lower than CM at 40, 80, and 160 mg/kg, respectively, in Exp. No. 4731. Body wt was not as adversely affected in females. In Exp. No. 3095, body wt at the lower doses were higher compared to CF, and similar to CF at the higher doses [i.e., 80-640 mg/kg]. In Exp. No. 4731, body wt was transiently increased at all doses; final body wt in HDF [160 mg/kg] was 6% lower compared to CF. In the 3 experiments, effects on food intake were generally fairly consistent with effects on body wt, but perhaps to a lesser degree. No drug-related ophthalmology findings were reported in any of the studies.

Hematology findings [assessed at the end of each study] consisted primarily of small decreases in rbc parameters, increases in neutrophil ct, and decreases in lymphocyte ct; a decrease in total wbc ct was noted only in Exp. No. 3095. For the most part, these findings were observed at all doses [80-640 mg/kg] in males and/or females. A number of clinical chemistry findings [assessed at the end of each study] were observed, particularly in Exp. No. 4731; however, only decreases in glucose and TG [40-640 mg/kg] were observed in all 3 studies. No urinalysis findings were observed in Exp. No. 3095. In Exp. No. 3589, findings consisted of a decrease in urinary volume in males at all doses, a decrease in protein in MDF and HDF, and an increase in sperm at all doses; the sponsor didn't consider any of these to be drug-related. In Exp. No. 4713, decreases in specific gravity and pH, an increase in wbc and squamous cells at all doses [40-160 mg/kg], and an increase in sperm at 80-160 mg/kg were observed in males, and an increase in bacteria was observed in females at all doses [40-160 mg/kg].

Gross pathology findings consisted of injection site changes and mammary gland stimulation in all studies. In Exp. No. 3095, nodules were detected at the injection site, whereas in the other 6-mo studies the local finding was powdery deposits. In Exp. No. 3589, powder deposits were also detected in the abdomen in females [160, 640 mg/kg]. Mammary gland stimulation was only observed in females [all doses, all studies] except in Exp. No. 3095 in which it was also observed in HDM [640 mg/kg]. In that study, small seminal vesicles were also observed at the HD. In Exp. No. 3589, dose-related lung congestion was observed in females. Swollen adrenal [160 mg/kg, M], gray and/or swollen prostate [all doses], swollen testis [160 mg/kg], and small ovaries or uterus [not dose-related] were observed only in Exp. No. 4731. The most consistent organ wt effect was an increase in adrenal wt in males. In Exp. No. 3095, this finding was detected only at 640 mg/kg; however, in the other studies it was noted at all doses [40-640 mg/kg]. Thymus wt was reduced in all 3 studies, but only in Exp. No. 4731 was it noted at all doses [40-160 mg/kg] in both males and females. Pancreas wt was increased in males at all doses [40-160 mg/kg] in Exp. No. 4731. Ovary wt was decreased in Exp. No. 3095 [320-640 mg/kg] and Exp. No. 4731 [80-160 mg/kg].

Histopathology examinations were not performed in Exp. No. 3095 and in Exp. No. 3589 "autolysis" was the most prominent "drug-related" finding (affecting up to 12/20 HDF depending on the organ). Therefore, the most reliable histopathology data were from Exp. No. 4731. However, in Exp. No. 4731, the summary data were expressed as "mean scores". These "scores" represented an average of severity scores in affected animals, but were averaged across the total number of animals per grp. Therefore, it was impossible to tell from the mean score how many animals per grp were affected. [Incidences were not determined from individual line listings due to time constraints and the availability of the 12-mo study.] [In Exp. No. 3589, the data were expressed as incidences.] Microscopic findings were detected in adrenal gland, male and female reproductive organs, pituitary, mammary gland, and spleen. Feminine aspect/alveolar glandular development and prominent secretions were detected in both studies at all doses in males and females [40-640 mg/kg]; focal hyperplasia was only noted in Exp. No. 4731 [females only, 80-160 mg/kg]. Regarding male reproductive organs, only the prostate was affected in both studies. Tubuli with focal granulocytes was a finding common to both studies [all doses, 40-640 mg/kg]. Other prostate findings [i.e., focal inflammation, focal interstitial granulocytes, round cells and/or fibrosis], were observed in only one or the other study. Microscopic findings in coagulating gland [i.e., prominent low epithelium; 40-160 mg/kg] and seminal vesicles [less prominent secretion (80-160 mg/kg), prominent low epithelium (160 mg/kg)] were detected only in Exp. No. 4731. There were no notable effects on testes in either study. The female reproductive organs exhibited a similar "inhibitory or quiescent" effect. Microscopic findings in ovary and uterus in both studies suggested decreased activity/development [e.g., atretic follicles, decreased generation of corpora lutea, decreased basophilic/eosinophilic corpora lutea in ovary; decreased glandular development and epithelial mitosis and a resting appearance in uterus; 40-640 mg/kg]. Pituitary findings [increased degree of PRL-immunoreactive cells, hypertrophy of the pars intermedia] were noted in Exp. No. 4731 [all doses in males]. Adrenal gland was affected in males in both studies [40-640 mg/kg]; swollen cortical cells were detected in both studies, whereas clear vacuolated cells were noted only in Exp. No. 3589. Spleen findings were detected in females in both studies. In Exp. No. 3589, the only finding was diffuse hyperplasia of the red pulp [640 mg/kg]. In Exp. No 4731, diffuse hyperplasia was not observed, but erythrocytes in red pulp and/or hemosiderin pigment were detected at all doses.

There were no no-effect doses in the 6-mo studies; however, even if findings had not been detected at all doses, establishing a NOEL in these studies would have been difficult because no one dose was administered for the entire 6-mo dosing period. Drug-related target organs were numerous, i.e., CNS (sedation), male and female reproductive organs, mammary gland, adrenal gland, pituitary, and spleen. Effects on mammary gland and reproductive organs were, for the most part, consistent with an elevation of serum prolactin [not directly measured in these studies; however, there was evidence of increased PRL-immunoreactivity in pituitary gland]. Microscopic findings in adrenal cortex were fairly

nondescriptive, and no potential mechanism was proposed. Spleen findings were consistent with some degree of rbc destruction; however, only fairly minimal effects on rbc parameters were observed. However, the sponsor attributed the splenic effects to "...the method of anaesthesia and euthanasia..." and to an α-lytic effect of risperidone. [The sponsor provided no data or literature to support this hypothesis.] Injection of the microsphere formulation resulted in microscopic changes at the administration site. These changes reflected inflammatory and fibrous tissue reaction effects and microsphere encapsulation at the site. Fibrotic encapsulation was greatest in those grps receiving the highest dose of microspheres, i.e., the C and HD grps. However, granulocytic infiltration and necrotic cells were detected only in drug-treated grps, thus indicating irritation induced by risperidone itself.

The 12-mo study was the only general toxicity study conducted using the i.m. depot route. Risperidone [5, 40 mg/kg] was administered as the clinical (Phase 3) microsphere formulation and 2 C grps [saline, vehicle (microspheres)] were included in order to assess both drug and vehicle effects. The study was run concurrent with the 2-yr carcinogenicity study.

There were two deaths during the study [1 C, 1HD]. The cause of death in the HD animal [at Wk 20] was not determined; however, the sponsor did not consider it drug-related. The primary clinical signs were ptosis and sedation. Ptosis was observed throughout the dosing period at both doses. Sedation was observed at the LD only after the 1st dose; at the HD the severity decreased with subsequent doses and was not consistently observed (and usually only after the 1st wk of dosing). Body wt was consistently reduced at the HD [compared to vehicle C (VC)] in HDM; final body wt was 15% lower compared to VC. Body wt was increased in females at the LD and not affected at the HD. Final body wt was 12% higher in LDF compared to VC. Overall food intake was not affected in males, but was increased in females primarily at the LD. On hematology parameters, slight decreases in rbc parameters, an increase in neutrophil ct, and a decrease in lymphocyte ct were observed, as also noted in the 6-mo studies. Other findings included increases in monocytes, thrombocytes, and eosinophils. No effects on hematology parameters were observed at the LD. As noted particularly in one of the 6-mo s.c. studies (also using the clinical formulation; Exp. No. 4731), numerous clinical chemistry parameters were affected. The following were noted in Exp. No. 4731 [all doses; 40-160 mg/kg] and the 12-mo [both doses] in males and/or females: (a) small, but significant decreases in Na and Cl, (b) increases in Ca , P_i [the effect on P_i was the most notable effect in the 12-mo study], and PL, and (c) decreases in glucose. Small decreases in total protein [160 and 40 mg/kg in Study 4731 and the 1-yr, respectively] and TG [80-160 and 40 mg/kg in Study 4731 and the 1-yr, respectively] were noted in males in both studies. [No urinalysis was performed in the 1-yr study.] The effects noted in the 1-yr study were potentially drug-related since comparisons were made to VC.

Gross pathology findings related to vehicle consisted only of powdery deposit at the injection site, a finding detected in all animals receiving microspheres [including VC]. Gross findings attributed to drug were detected in adrenal gland [swollen], kidney [pale], liver [pronounced lobulation], mammary gland [inspissated secretion, stimulation], pituitary gland [swollen, yellow stippled], prostate [swollen], subcutaneous [tissue mass; presumed mammary gland tumors], and uterus [decrease in swollen]. Except for the mammary gland findings [observed at both doses in females], all findings were detected primarily or solely at the HD [40 mg/kg]. Findings in adrenal gland, kidney, and mammary gland were noted in both males and females. Pituitary gland was affected in males, and liver was affected in females. Subcutaneous tissue masses were noted only in males. A number of organ wts were affected. That of kidney [40 mg/kg, M and F], adrenal [40 mg/kg, M and F], spleen [40 mg/kg, M], and liver [5-40 mg/kg, F] were increased, where that of testis [5-40 mg/kg], thymus [40 mg/kg, F], and ovary [5-40 mg/kg] were decreased. Histopathology examination revealed both vehicle- and drug-related effects in a number of organs. Vehicle-related changes were detected at the injection site [i.e., multifocal chronic inflammation, encapsulation of microspheres, fibro-granulomatous reaction, focal thickening of the perimysium, giant cells, granulocytic infiltration]; these findings were detected in all grps receiving microspheres. In

contrast, the necrotic center/cellular debris and prominent muscular degeneration noted at the injection site were greater in HD animals (M and F), indicating drug-related effects. Other vehicle-related findings consisted of round particle/inflammatory cells in tissue surrounding the stifle joint and, to a lesser extent, the intra-articular region of the stifle joint [all microsphere-dosed grps], sinus histiocytosis/foamy cells in iliac, inguinal, mesenteric, and popliteal lymph nodes [all microsphere-dosed grps, but to a lesser extent in LD grps], and foamy cells in the adrenal gland and pancreas [VC and HD only]. The distribution of the findings indicates that the lymph node and adrenal findings were primarily observed with the higher dose of microspheres. The sponsor attributed the foamy cell [adrenal, pancreas] and sinus histiocytosis/foamy cell [lymph nodes] findings to activation of the Mononuclear Phagocyte System. The sponsor noted that small microsphere particles were detected in the lung vasculature (as evidenced by granulomatous inflammation and detection of the particles themselves in frozen sections) in VC, LD, and HD grps. The sponsor noted that "In frozen sections prepared from the lungs of [selected animals] the intravascular presence of small microsphere particles could be detected".

Drug-related findings were detected at the injection site (as previously discussed) and in adrenal, bone [sternum, stifle joint], coagulating gland, kidney, spleen, mammary gland, ovary, pituitary, prostate, seminal vesicles, uterus, and vagina. [Except for the microsphere formulation-associated effects (at sites other than the injection site) and the drug-related bone and kidney effects, these findings are generally consistent with those observed in the 6-mo stud(ies).] All of these, with the exception of spleen findings, were considered by the sponsor to be secondary to elevations in serum prolactin. [The severity, but not the incidence, of the spleen findings (i.e., erythrocytes in red pulp, hemosiderin deposits) were dose-related.] Effects on male and female reproductive organs, mammary gland, and pituitary gland were consistent with those observed in other drugs that elevate serum prolactin. However, those on kidney, adrenal gland, and bone are not consistently observed in this class of drugs. The sponsor attributed the kidney effects to a prolactin-mediated exacerbation of chronic nephropathy in male dogs, and the adrenal and bone findings to be a result of altered Ca balance resulting from the exacerbation of chronic nephropathy. These findings are discussed in more detail in the Summary and Conclusions section under **Carcinogenicity**.

TK data were provided for risperidone, 9-OH-risperidone, and the "active moiety". According to the 1-yr data, plasma AUCs for risperidone and 9-OH-risperidone (and, therefore, active moiety) were higher in females than males at both doses [LD: 8-45%; HD: 67-120%]. Also, increases in plasma exposure (AUC) for risperidone, 9-OH-risperidone, and the "active moiety" were fairly dose-proportional in females; in males, the increases were slightly less than dose-proportional. The 9-OH-risperidone-to-risperidone ratio was 0.4-0.6 at both doses. A comparison of the plasma exposure data to the expected human exposure at the maximum recommended dose indicated the following: (a) at the LD used in the 1-yr study, the AUC for risperidone, 9-OH-risperidone, and active moiety were 0.2-0.3, 0.2, and 0.2-0.3 times, respectively, the AUC at the maximum recommended clinical dose. (b) at the HD used in the 1-yr study, the AUC for risperidone, 9-OH-risperidone, and active moiety were 1.5-2.5, 0.7-1.6, and 1.2-2.1 times the AUC at the maximum recommended clinical dose. Clearly, there is no safety margin between the mean exposure at the maximum recommended human dose and the exposure in rats in the 1-yr study.

No NOEL was established for drug-related findings in the 12-mo study. The HD was adequate in males based on the magnitude of the body wt effect. In females, there were no clear dose-limiting findings; sedation was noted, but the incidence and severity decreased with duration of dosing and there were no body wt effects. [This assumes that the premature death in the 1 HDM was not drug-related.]

Dog: in the two 6-mo and the 12-mo i.m. toxicity studies, doses were increased during the dosing period. Doses were as follows:

Exp. 3590: 1st [0, 2.5, 5, 20 mg/kg], 2nd [0, 5, 10, 20 mg/kg], 3rd [0, 10, 20, 40 mg/kg], 4th-6th [0, 10, 20, 80 mg/kg]
Exp. 3114: 1st [0, 0.63, 5, 10, 20 mg/kg], 2nd-5th [0, 5, 10, 20 mg/kg], 6th [0, 20, 40, 80 mg/kg]
Exp. 4730: 1st-2nd [0, 1.25, 2.5, 5 mg/kg], 3rd-4th [0, 2.5, 5, 10 mg/kg], 5th-26th [0, 5, 10, 20 mg/kg]

In the 6-mo studies, dosing was once per month; in the 12-mo study, doses were administered bi-weekly. The 6-mo studies have been previously reviewed [5/7/97]. Exp. 3114 was not a definitive study; no terminal studies were conducted and the n/grp was small [2/sex/grp]. The 6-mo studies were conducted using a microsphere formulation, but the formulation(s) used was not the one used clinically. The Phase 3 clinical formulation was used in the 12-mo study.

In Exp 3114, there were no unscheduled deaths. The only clinical sign, sedation, was observed at doses >0.63 mg/kg. Body wt was reduced (compared to CM) at the HD. There were no significant effects on hr/bp/ECG, ophthalmology, clinical chemistry, or urinalysis parameters. Small decreases in wbc ct, thrombocyte ct, and APTT were noted at doses >5 mg/kg. In Exp 3590, there were no unscheduled deaths or drug-related clinical signs. There were no clear effects on hr/bp/ECG [except for a small but consistent decrease in SAP in HD animals (40-80 mg/kg)], ophthalmology, body wt, or food intake. Rbc parameters [hgb, hct, rbc ct] were reduced at doses of 20-80 mg/kg. Total wbc ct was decreased at all doses, and thrombocyte ct was decreased at doses ≥ 5 mg/kg. Clinical chemistry findings consisted of a decrease in K at doses of 20-80 mg/kg, and an increase in glucose at all doses. ALT was markedly elevated in one MD dog; microscopic findings in liver in that dog consisted of prominent bile ductuli and Kupffer cells, pigmented sinusoidal cells, and swollen sinusoidal cells. Urinalysis findings consisted of decreases in urinary pH [all doses] and slight increases in rbcs [all doses] and wbcs [20-80 mg/kg] in the urine. Spleen [all doses] and pancreas [80 mg/kg] wts were increased; prostate wt tended to be lower at all doses. Microscopic findings were detected at the injection site [focal muscular degeneration (80 mg/kg), encapsulation and fibro-granulomatous reaction (decreased severity at the LD and MD; i.e., vehicle related)], epididymides [focal testicular desquamated cells; all doses], liver [hydropic aspect (centrilobular); all doses], prostate [reduced glandular epithelium], spleen [erythrocytes in red pulp (increased severity, not incidence, at all doses)], uterus/vagina [resting aspect; all doses].

The 12-mo study [Exp. 4730] was the only chronic study in dog that used the clinical formulation and dosing regimen. There were no unscheduled deaths. The only clinical signs were sedation [observed transiently at all doses] and decreased vaginal discharge [all doses]. No drug-related effects were observed on body wt, food intake, ophthalmology, or urinalysis parameters. The only cardiovascular finding was a decrease in SAP; heart rate was not affected and QT_c was not increased in a dose-related manner. There were no clear drug-related findings on hematology parameters, although decreases in wbc, lymphocyte, and neutrophil cts were noted at all doses. Thrombocyte ct was slightly decreased at the HD [and to a lesser extent even prior the start of dosing]. Serum K was decreased and cholesterol and PL were increased at all doses, but significantly only at the HD. Serum Ca was increased at the HD, but values did not exceed baseline values to any notable extent. Spleen wt was increased at all doses. Testis wt was decreased at the HD; prostate wt was decreased at all doses, although the effect was not dose-related. The only macroscopic finding, noted in all grps (including C), was white strand formation at the injection site. Microscopic findings were noted at the injection site [cellular debris (encapsulation), chronic inflammation, encapsulation (muscle), fibro-granulomatous reaction, focal muscle degeneration, focal thickened endomysium, and giant cells (encapsulation)], adrenal gland [prominent vacuolated (foamy) cells (zona reticularis), vacuolated clear cells (zona glomerulosa)], epididymides [cellular debris (ductular lumen), reduced amount of sperm], lung [microspheres and/or remnants of microspheres, focal (foamy) macrophages], lymph nodes [sinus histiocytes (foamy cells)], ovary [regressive CL], pituitary [increased immuno-positive (PRL) cells], spleen [increased degree of erythrocytes in red pulp (all doses) and hemosiderin pigment (particularly HD)], testis [atrophic tubuli, spermatic giant cells], uterus/vagina [resting appearance, decreased glandular develop in uterus].

Vehicle-related findings were detected at the injection site and in lymph node, adrenal gland, and lung as noted by a similar incidence in vehicle and drug-related grps and/or an increase in severity in C and HD grps [i.e., the grps receiving the largest dose of microspheres]. However, in iliac lymph nodes there was an increase in the severity of the sinus histiocytosis at the HD, indicating a drug-related effect. Prominent foamy cells were evident in the adrenal cortex [z. reticularis] in almost all animals; the severity of the effect was similar among grps. However, there was a dose-related increase in vacuolated clear cells in the zona glomerulosa [MD, HD]. The foamy cells and sinus histiocytosis were considered secondary to chronic inflammation at the injection site. The presence of microsphere particles in lung was evidence of the presence of microspheres in the systemic circulation. This finding was also reported in rats [1-yr study], except that in rat the microspheres were detected in the pulmonary vasculature whereas in the dog the microspheres were noted within pulmonary tissue. [The sponsor noted that "The biodegradation of the placebo- and the risperidone-loaded microspheres was slight. They seemed to be microspheres from the last intramuscular injection.] Drug-related effects (in addition to those in lymph node and adrenal cortex) were noted in male and female reproductive organs, pituitary gland, and spleen. All but the spleen effects were attributed to the D_2 antagonist activity of risperidone. Drug-related effects were observed at all doses [final doses: 5, 10, 20 mg/kg]; however, none of the effects appeared dose limiting. [In 6-mo studies, monthly doses up to 80 mg/kg were reported to have been well tolerated.] Therefore, higher doses could have been administered. The sponsor did not justify the HD on the basis of maximum feasible dose.

Plasma exposure data were collected throughout the 1-yr study. Following the 26th dose, plasma exposure in dog compared to anticipated human exposure at the maximum recommended clinical dose [dog:human ratio; based on C_{max} and AUC data from Study RIS-INT-31 (50-mg dose)] was as follows:

C_{max}:

risperidone: ≈1, 2.5, and 6.5 times human at the LD, MD, and HD, respectively.
9-OH-risperidone: ≈4, 7, and 15 times human at the LD, MD, and HD, respectively.
active moiety: ≈2.5, 4.5, and 10.5 times human at the LD, MD, and HD, respectively.

AUC:

risperidone: ≈0.7, 1.5, and 5 times human at the LD, MD, and HD, respectively
9-OH-risperidone: ≈4, 8, and 18.5 times human at the LD, MD, and HD, respectively.
active moiety: ≈2, 4.5, and 11 times human at the LD, MD, and HD, respectively.

General comments and conclusions: vehicle-related effects were observed in both rat and dog. The findings of particular interest were those detected at other than the injection site [lymph node, adrenal gland, and lung (rat, dog), and pancreas (in rat)]. Small microsphere particles or remnants were detected in lung in 1-yr studies in both rat and dog. The foamy macrophages and/or sinus histiocytosis observed at various sites may represent a reaction to chronic inflammation. However, it would seem a possibility that the sinus histiocytosis observed in various lymph nodes might represent phagocytosis of microsphere-associated particles cleared via the lymphatic circulation. The sponsor attributed the lymph node, adrenal gland, and pancreas findings to activation of the mononuclear phagocyte system [MPS], as secondary to chronic inflammation at the injection site, and the presence of microspheres in lung to inadvertent intravascular [in dog, or in the case of the rat stiffle joint, intra-articular] administration. The injection site findings indicate that the vehicle [i.e., microspheres] itself is irritating. In addition, dose-related findings in both rat and dog indicate that risperidone is also irritating. In the rat, drug-related findings consisted of granulocytic infiltration and/or necrotic cells in one 6-mo and the 1-yr [40 mg/kg] studies. In dog, focal muscle degeneration was detected at the 80-mg dose in a 6-mo study.

In the 1-yr rat study, there was no no-effect level for the presence of microspheres in lung [the LD contained 13.2 mg/kg microspheres or 79 mg/m^2, based on 378 mg/gm of microspheres (stated to be

identical to the Phase 3 formulation); according to the sponsor's Summary Report No. EDMS-BEBE-2810462, the dose of microspheres in the VC grp was 65 mg/kg] whereas in the 1-yr dog study, microspheres were not detected in lung at the LD [i.e., 5 mg/kg risperidone and 13.2 mg/kg microspheres (264 mg/m^2)]. There was also no NOEL for vehicle-related injection site findings in the 1-yr rat study. [Although vehicle effects were not directly assessed in the 1-yr dog study, evidence of, for example, sinus histiocytosis in lymph nodes in all grps would suggest non-injection site vehicle-related findings in all grps.] For comparison, the clinical dose provides 132 mg of microspheres per dose or 2.2 mg/kg [or 82 mg/m^2]. [According to the sponsor's summary Report No. EDMS-BEBE-2810462, the maximum clinical dose of microspheres is 1.7 mg/kg.] NOELs for drug-related injection site findings were established in both rat [i.e., 5 mg/kg] and dog [40 mg/kg].

The sponsor attributed both injection and non-injection site vehicle-related findings to the increased amount of microspheres administered in animals. While this may certainly be the case [the lowest dose of microspheres administered in both rat and dog was lower [≈6 times] than at the maximum recommended clinical dose], the fact is that no NOELs were established. The finding of most concern is the presence of microspheres in lung in both rat and dog.

The sponsor provided [upon request] copies of a number of published studies on the degradation properties of various copolymer [lactide:glycolide] formulations. In *in vitro* studies, degradation of the copolymer to component tetramers and trimers, and finally to monomers have been found to depend upon a variety of factors, including the ratio of the two monomers, the method of preparation, and the presence of drug. For example, Giunchedi *et al.* [Giunchedi P *et al. J Control Release* 56:53-62, 1998] demonstrated that copolymer microspheres degraded more rapidly when formulated with diazepam compared to the microspheres alone. Two published *in vivo* studies were provided by the sponsor. Miller *et al.* [Miller RA *et al. J Biomed Mater Res* 11:711-719, 1977] implanted duel-radiolabeled poly(lactic acid/glycolic acid) microspheres [GA:LA ratios ranging from 100/0 to 0/100, including 25:75] into the tibia and abdominal wall of Sprague-Dawley rats; implants remained in place for 1-11 months. The authors noted that the "...radioactivity in the tissues was negligible, with no significant [radioactivity] observed in any of the tissues analyzed" [i.e., liver, spleen, kidney, lung, muscle, and injection site]. Therin *et al.* [Therin M. *et al. Biomaterials* 13(9):594-600, 1992] compared *in vitro* and *in vivo* degradation characteristics of the poly(lactic acid)/poly(glycolic acid) copolymers, including PLA75GA25 polymers. For *in vivo* analysis, copolymer preparations were implanted into lumbar muscle tissue in New Zealand rabbits. Animals were sacrificed at 2-5 wks after implantation. Degradation of the microspheres was examined using light microscopy and size-exclusion chromatography. The data indicated that degradation was more rapid *in vivo* than *in vitro*, although the author concluded that *in vivo* degradation of poly(α-hydroxy acids) derived from LA and/or GA proceeds as *in vitro*, provided that suitable modelling [sic] conditions are selected". The Therin *et al.* [1992] study did not directly address absorption of the microspheres. These studies provide some reassurance that absorption of intact and/or partially intact microspheres is minimal; however, none definitively demonstrate this. The poly(lactic acid)/poly(glycolic acid) microspheres appear to be considered biocompatible and nontoxic due to the aqueous, non-enzymatic hydrolysis of the polymers into endogenous monomers [Park TG. *Biomaterials* 16:1123-1130, 1995; Ramchandani M *et al. J Controlled Release* 43:161-173, 1997]

According to the CMC information, the specifications for particle size distribution are that the median particle size range is [redacted] µm, with [redacted] by volume] of the particles being NMT [redacted] µm and 90% [by volume] of the particles being NLT [redacted] µm. With this particle size distribution, it would seem very unlikely that intact particles would be systemic absorbed. However, it would also seem unlikely that there would be significant intravascular injection of these particles without obvious sequela, e.g., thrombosis. Therefore, it is difficult to reconcile the systemic toxicity findings related to vehicle observed in rat and dog with the sponsor's explanations for their presence in the circulation. One final consideration is that poly(lactic acid)/poly(glycolic acid) microspheres are present in at least four

marketed i.m. depot drugs [Sandostatin LAR depot (189-566 mg copolymer/dose), Lupron depot-PED (66-132 mg copolymer/dose, Lupron depot 3.75 and 7.5 mg (33 and 66 mg/dose), Nutropin depot (69-115 mg/dose), Zoladex (13.3-14.3 mg/dose) and Zoladex 3-month (12.82-14.76 mg/dose)]. According to information provided by the respective review divisions, the microspheres were not studied in a complete battery of nonclinical toxicity studies [i.e., chronic toxicity, reproductive toxicity, carcinogenicity, genotoxicity] for Sandostatin or Nutropin [IND 37,768 and NDA 20-175, respectively] or for Lupron depot or Zoladex [NDA 19-010 and NDA 19-726, respectively]. A 3-mo toxicity study of the Nutropin i.m. depot formulation [containing microspheres] was conducted in monkey. Local effects were noted, but, according to the review, "...examination of tissues other than the injection site showed no apparent drug-related histopathology". Carcinogenicity studies in mouse [72-wk] and rat [2-yr] appear to have been conducted with Zoladex (microsphere formulation); foamy macrophages in lung, considered to be vehicle-related, were detected in both studies.

The primary target organs [other than injection site] in both rat and dog were CNS, male and female reproductive organs], mammary gland, adrenal gland, pituitary, spleen, lung, and lymph node. [Vehicle-related findings were detected in adrenal gland (dog), lung, and lymph node, as previously discussed.] The primary CNS finding was sedation. Changes in male and female reproductive organs, mammary gland, and pituitary were attributed to elevated serum prolactin, and were consistent with effects observed with other drugs elevating serum prolactin. The spleen findings, also observed in the oral toxicity studies with risperidone, were attributed to α-lytic [i.e., α_1 antagonistic] effects and/or the method of sacrifice. One finding observed in rat, but not dog, was osteodystrophy. The changes in bone were not further characterized, but were detected in sternum and [to a lesser extent] in the stifle joint. Osteodystrophy was not detected in the toxicity studies with oral risperidone. Kidney effects, considered by the sponsor to reflect "...an increased tendency towards chronic renal disease...", were noted at the HD in the 1-yr rat study.

No NOEL was established in the chronic toxicity studies in either rat or dog. Dose-limiting toxicity [i.e., reduced body wt compared to C] was noted in male rats, but not in female rats or in dog. Plasma exposure data in the 1-yr rat study indicated no margin of safety between that exposure and the exposure anticipated in humans at the maximum recommended clinical dose. In the 1-yr dog study, the C_{max} at the LD, MD, and HD were 1.2-3.7, 2.4-6.9, and 6.5-14.6 times, respectively, the expected C_{max} at the maximum recommended clinical dose, and the AUC at the LD, MD, and HD were 0.7-3.8, 1.3-8.2, and 5-18 times the expected AUC at the maximum recommended clinical dose for risperidone, 9-OH-risperidone, and the "active moiety".

Table 2 : GLP Study in the absence of a metabolic activation system

Individual and mean number of revertant colonies (His+) per bacterial plate

			TA98			TA1537			TA100			TA1535			TA102					
Dosage µg/plate	Solvent	Group	Indiv. Count	Mean	SD	Indiv. Count	Mean	SD	Indiv. Count	Mean	SD	Indiv. Count	Mean	SD	Indiv. Count	Mean	SD	Indiv. Count	Mean	SD
Solvent contrôl	0	DMSO	13			8			107			11			240					
			17	14	2.6	14	11	3.1	118	103	17.3	14	13	2.1	412	340	89.4			
			12			10			84			15			368					
R064766	25	DMSO	23			4			92			12			312					
			19	24	5.0	6	5	1.2	116	107	13.1	13	14	2.1	268	308	38.2			
			29			6			113			16			344					
R064766	50	DMSO	16			8			117			10			436					
			22	18	3.2	8	7	1.7	112	120	9.8	6	11	5.0	468	472	38.2			
			17			5			131			16			512					
R064766	100	DMSO	28			16			114			9			344					
			16	22	6.0	9	13	3.6	109	113	3.2	5	9	4.5	312	327	16.2			
			23			14			115			14			324					
R064766	250	DMSO	38			9			111			14			416					
			28	31	6.1	12	11	1.7	102	118	19.9	9	12	2.5	368	391	24.1			
			27			12			140			12			388					
R064766	500	DMSO	19 P			10 P			106 P			13 P			408 P					
			46 P	35	14.4	11 P	11	1.0	91 P	101	9.0	10 P	11	2.1	372 P	405	32.1			
			41 P			12 P			107 P			9 P			436 P					
R064766	1000	DMSO	28 P			6 P			97 P			11 P			268 P					
			21 P	24	3.6	11 P	9	2.9	106 P	103	4.9	7 P	9	2.1	348 P	303	41.1			
			23 P			11 P			105 P			10 P			292 P					
R064766	2500	DMSO	19 P			7 P			100 P			5 P			224 P					
			17 P	20	3.1	7 P	6	1.7	99 P	88	19.9	9 P	6	2.6	236 P	227	8.3			
			23 P			4 P			65 P			4 P			220 P					
2-Nitrofluorene	5	DMSO	584																	
			768	683	92.7															
			696																	
SodiumAzide	1	Water							404			324								
									328	375	40.9	264	281	37.2						
									392			256								
9-Amino -Acridine	50	DMSO				212														
						146	167	38.7												
						144														
4 - Nitroqui - noline-N-Oxide	5	DMSO													2576					
															2816	2461	423.8			
															1992					

C: Contamination P: Precipitation *: Thinning of background lawn T: Toxic @: Pinpoints

Mon, 18 Jun 2001

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that microspheres would exhibit direct genotoxic potential in this form. In addition, the composition of the microspheres does not raise a particular concern regarding genotoxic potential. Risperidone was negative in a full battery of genotoxicity tests [including the Ames test, *in vitro* mouse lymphoma, and *in vivo* micronucleus assay in mouse] submitted in support of the tablet formulation.

Labeling recommendations: the following labeling has been proposed by the sponsor:

This labeling is consistent with current labeling for the oral formulations and the data on the risperidone microsphere formulation.

VI. CARCINOGENICITY

Study title: **Twenty-four months intermittent repeated dose intramuscular carcinogenicity study in the Wistar rat** [Study No. EDMS-BEBE-2644186, Exp No. 4729, Volume #1.21-1.23, Conducting laboratory and location: Janssen Pharmaceutica, Belgium, Date of study initiation: 2/99, GLP, QA report:Y]

Drug, lot #, and % purity: risperidone, batch no. ZR064766EIA221 ———
CAC concurrence: Y. However, it was recommended that a 3rd dose grp be added.

Study Type (2 yr bioassay, alternative model etc.): 2-yr bioassay
Species/strain: Hannover Wistar rat [Charles River bred· ——————————
(supplier)]
Number/sex/group; age at start of study: 50/sex/grp, 5-6 wks
Animal housing: individual
Formulation/vehicle: i.m. depot/microspheres [lactic acid/glycolic acid (3:1), ———
———)]. Drug concentrations (and homogeneity) were analyzed prior to the start of dosing and at the end of the study; concentrations were stated to have been within ——— of intended. [No data were provided to document homogeneity.]
Drug stability/homogeneity: microspheres were refrigerated until used [duration not specified]; suspensions were reconstituted just prior to administration.
Methods:
- Doses: 0 [saline], 0 [microspheres], 5, 40 mg/kg
- Basis of dose selection: data from 2 i.m. tolerability studies in rat [Exp No's 4322, 4365].
- Restriction paradigm for dietary restriction studies: n/a
- Route of administration: i.m. The site of injection [left and right hind leg muscle] was alternated every 2 wks.
- Dosing volume: 0.08 mL/100 gm for both controls, and 0.01 and 0.08 mL/100 gm for the LD and HD, respectively.
- Frequency of drug administration: every 2 wks
- Dual controls employed: yes
- Interim sacrifices: yes
- Satellite PK or special study group(s): 12/sex for LD, HD for analysis of TK. [TK animals were observed daily and body wts were recorded as for main-study animals; however, only mortality was reported.]
- Deviations from original study protocol: n/a
- Statistical methods: age-adjusted Peto (trend analysis; one-tailed) for neoplastic findings. For detailed review, please refer to the statistical review [Statistical Review and Evaluation: Review of Carcinogenicity Study, Roswitha Kelly, M.S., HFD-710; appended to this review]

Observations and times:
- Clinical signs: all animals were observed daily, with attention to injection site.
- Body weights: body wts were recorded prior to the start of dosing, weekly during the dosing period, and at the end of the dosing period.
- Food consumption: food consumption was recorded weekly during the dosing period.
- Hematology: blood samples were collected [via orbital venous plexus or carotid artery (in moribund animals)] at 6, 12, and 18 mo and "towards the end of the study" in survivors [with the exception of selected animals due to methodological problems]. The following parameters were assessed: hct, hgb, rbc ct, wbc ct [total, differential], platelet ct, normoblasts, MCV, MCH, MCHC.
- Clinical chemistry: blood samples were collected in survivors at the same sampling times

as for hematology for analysis of the following parameters: Na, K, Cl, Ca, P_i, total protein, albumin, glucose, cholesterol, TG, PL, BUN, creatinine, total bilirubin, alkaline phosphatase, AST, ALT. [Samples were not available for 1 animal during Wk 78-79, or in 1 additional animal that was sacrificed moribund.]

Urinalysis: urine samples were collected in all survivors during Wk 89-90 for analysis of the following parameters: specific gravity, pH, volume, glucose, ketones, urobilinogen, bilirubin, occult blood, proteins, microscopic analysis of sediment. [No sample was available for 1 animal.]

Hormones: due to microscopic findings in adrenal gland in interim sacrifices [1 yr], serum aldosterone and corticosterone [using RIA] were quantitated in animals at scheduled sacrifice. Serum prolactin levels were quantitated [using RIA] in 12/sex/grp at scheduled sacrifice. [Data were not available in 3 animals due to methodological problems.]

Organ weights: wts of the following organs were recorded in all animals terminally sacrificed: lungs, spleen, liver, heart, pancreas, kidneys, brain, thymus, adrenal glands, thyroid glands/parathyroid, testes, ovaries.

Gross pathology: a complete necropsy was performed on all animals, including those found dead or sacrificed moribund.

Histopathology: the following tissues were examined microscopically in all animals: injection sites, adrenal gland, bone [stifle joint (bilateral), sternum]/bone marrow, brain, cervix [except in 35 Fs in which a transverse section of the uterine cervix was prepared], coagulating glands, epididymides, heart, kidneys, liver, lungs, lymph node [mesenteric, poplitea, iliac, inguinal; bilateral], mammary gland, ovaries, pancreas, parathyroid gland, pituitary gland, prostate, seminal vesicles, skeletal muscle (psoas muscle), spleen, testes, thymus, thyroid glands, urinary bladder, uterus, vagina, gross lesions.

Tissues were preserved in 10% buffered formalin [except for eye and testes (2.5% buffered formalin and 1.25% glutaraldehyde)] and stained with H & E for examination. In addition, the following special stains were used (as described by the sponsor): "immunohistochemical stains for S-100 and for Neuron Specific Enolase (NSE) of the adrenal gland..., the pituitary gland ....and the pancreas and psoas muscle...of animal No. 60 and a Fontana Masson stain and stain for NSE on the adrenal gland...of animals Nos. 347 and 362". It was noted that "The technical preparation of the organs and tissues was carried out at Huntingdon Life Sciences (Eye, Suffolk, UK)." Additional sections of pituitary were immunohistochemically stained for prolactin.

Toxicokinetics: blood samples were collected from satellite animals (12/grp). Methods and results were not included in the study report.

Results:

Mortality: the mortality rate data are summarized in the following table:

SEX	SC	VC*	LD	HD
M	6/50 (12%)	10/50 (20%)	13/50 (26%)	15/50 (30)*
F	16/50 (32%)	11/50 (22%)	21/50 (42%)	21/50 (42%)

*$p < 0.05$

Mortality was significantly increased in HDM during the last 3 mo; the sponsor characterized this effect as "slight". Mortality was also increased in females (at both doses) during the last 3 mo when compared to the vehicle control (VC), but not to the saline control (SC). Therefore, the sponsor didn't consider there to be a significant drug-related effect on mortality in females. The sponsor noted that "Mortality in the FK-

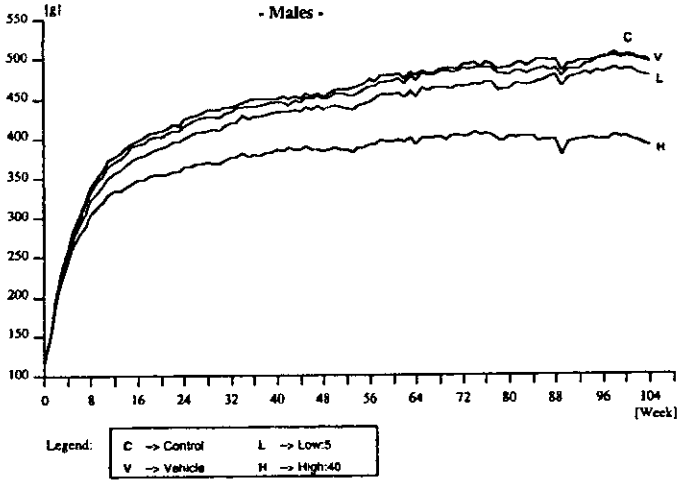
animals [i.e., TK] was comparable..." to that in the main-study animals. There was also no difference in mortality between the C grps.

Clinical signs: selected clinical signs are summarized in the following table [significance compared to SC]:

SIGNS	MALES				FEMALES			
	SC	VC	LD	HD	SC	VC	LD	HD
bad condition	4/50	9/50	8/50	13/50*	10/50	11/50	18/50	16/50
thin	1/50	4/50	1/50	10/50**	2/50	0/50	2/50	4/50
s.c. tissue mass	12/50	13/50	14/50	20/50	10/50	5/50	22/50*	18/50
rough haircoat	0/50	4/50	4/50	18/50***	4/50	3/50	5/50	4/50
turbid eye	2/50	2/50	1/50	0/50	1/50	0/50	1/50	4/50
ptosis	0/50	0/50	0/50	50/50***	0/50	0/50	2/50	50/50***
respiratory difficulties	1/50	0/50	0/50	3/50	1/50	0/50	1/50	3/50
hind limb paralysis	0/50	0/50	0/50	3/50	0/50	0/50	2/50	1/50
sedation								
slight	0/50	0/50	0/50	50/50***	0/50	0/50	5/50	50/50***
moderate	0/50	0/50	50/50***	0/50	0/50	0/50	0/50	15/50***
severe	0/50	0/50	0/50	50/50***	0/50	0/50	0/50	0/50

According to the sponsor, ptosis and sedation were evident at both doses [incidence and severity appeared to be dose-related] following the first dose or two. Thereafter, ptosis was not observed at the LD and only inconsistently at the HD. Sedation was no longer observed at the LD after the first 1-2 doses. At the HD, the severity of sedation decreased over time and after Wk 65-67 was not observed. The subcutaneous masses probably reflected mammary gland tumors.

Body weights: body wt changes are illustrated in the following sponsor's figures:



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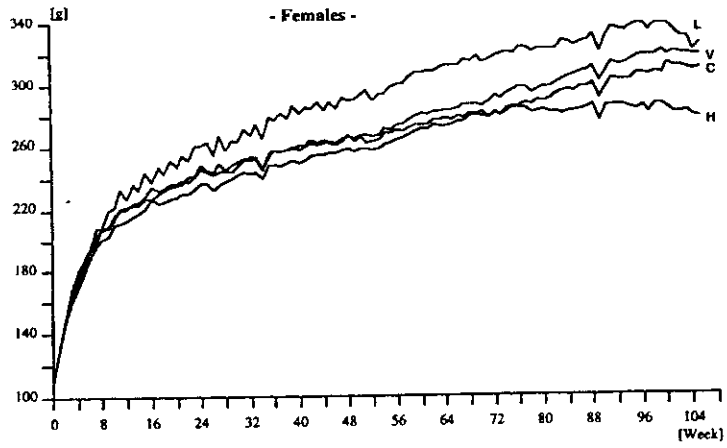


In males, body wt was reduced at both doses. The effect was noted consistently throughout the dosing period at the HD; however, at the LD, mean body wt was not significantly affected from Wk 80 on. Final mean body wt at the HD was 22% lower compared to VC; mean body wts in C grps were similar. Overall mean body wt gain was 28% lower at the HD compared to the VC grp.

In females, mean body wt was increased at the LD throughout most of the dosing period. At the HD, mean body wt was significantly higher (compared to VC) through Wk 44-48, then was significantly reduced from Wk 83 to the end of the dosing period. Final mean body wts in females were similar to VC at the LD and 13% lower than VC at the HD; body wts were comparable in the 2 C grps. Overall mean body wt gain was 20% lower at the HD compared to the VC grp.

Food consumption: food intake was increased (compared to VC) throughout most of the dosing period at the LD in both M and F, and from Wk 74 on in HDM. [Food intake was significantly reduced in HDM to Wk 35.] In HDF, food intake was increased until ≈Wk 54, then remained similar or slightly lower compared to VC thereafter. The sponsor noted that overall food intake at the HD was similar to that in the C grps in both males and females; there were no notable differences between the two C grps.

Hematology: the following were noted: (a) small (<10%), but significant decreases in rbc parameters [↓ hgb in HDF, ↓ hct in HDM and HDF, ↓ rbc ct in M and F (dose-related)], (b) a small (1-3%) increase in MCHC in LDM, MDM, and HDF, (c) small (<10%) increases in MCV and MCH in LDM and HDM (dose-related), (d) a decrease in wbc ct in HDM (8-10%), but an increase in wbc ct in LDF (5-17%) and HDF (8-14%), (e) an increase in platelet ct in LDF (5-10%) and HDF (9-14%), (f) an increase in neutrophil ct in LDM (15% at Wk 104) and HDM (12-28%) and a decrease in lymphocyte ct in LDM (5% at Wk 104) and HDM (14-24%), (g) an increase in neutrophil ct in LDF (14-21%) and HDF (26-47%) and in monocyte ct in HDF (18-27%). In females, decreases in wbc (10-12%) and lymphocyte (14-28%) cts were observed during Wks 27 and 52 in the VC grp (compared to SC), and neutrophil ct (25%) was increased at Wk 104 in VC (compared to SC).

The sponsor also provided hematology data in animals that were sacrificed moribund; however, these data were of minimal value since deaths occurred during Wks 49-103 and animals were, by definition, in poor condition.

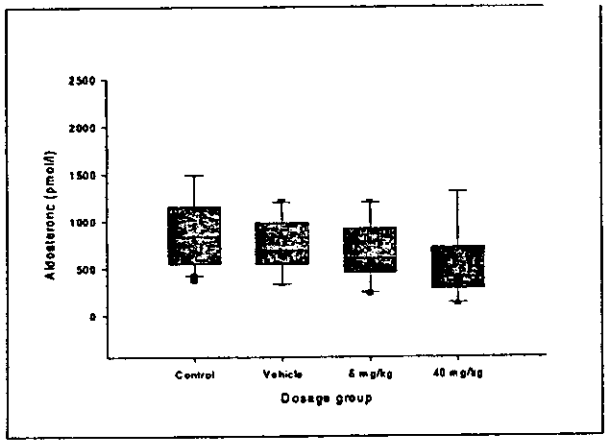
Clinical chemistry: a number of dose-related findings were observed. Findings noted in both males and females were as follows: (a) small (1-2%), but significant, decrease in Na at both doses [transient in females], (b) small (1-4%), but significant, decrease in Cl at both

doses, (c) increases in Ca in HDM (2-4%), LDF (2-5%, transient), and HDF (3-7%), (d) increase in P_i in LDM (6-13%), HDM (24-36%), LDF (8-28%), and HDF (10-30%); in addition, P_i was significantly higher in VC vs SC [Wk 52 in M and F, Wk 104 in F], (e) decrease in glucose in HDM (11-26%, progressive), MDF (16% at Wk 104), and HDF (8-23%), (f) decrease in BUN in LDM (3-9%), HDM (3-13%), LDF (12% at Wk 104), and HDF (3-6%), and (g) decrease in creatinine at Wk 104 in LDM (4%) and HDM (9%), and at Wks 78 and 104 in LDF (5%) and HDF (5-7%). Additional findings observed in males were as follows: (a) decrease in K at the HD (4-10%), (b) an increase in cholesterol at the LD (5-13%) and HD (5-20%) [there was a tendency for cholesterol to be elevated in HDF (9-11%)], (c) decrease in TG at the HD (16-42%) [there was a tendency for TG to be decreased in HDF (14-21%), (d) an increase in PL at the HD (7-13%), (e) an increase in total bilirubin at the LD (22-50%) and HD (22-75%); at both doses the smallest effect was observed at Wk 104, (f) a decrease in alkaline phosphatase at the HD (11-32%); this parameter was also reduced in VC (9-11%) as compared to SC, (g) an increase in ALT at Wk 52 (30 and 54% at LD and HD, respectively) and Wk 104 (18 and 25% at LD and HD, respectively) [ALT was decreased at Wk 104 in females [10 and 24% at LD and HD, respectively].

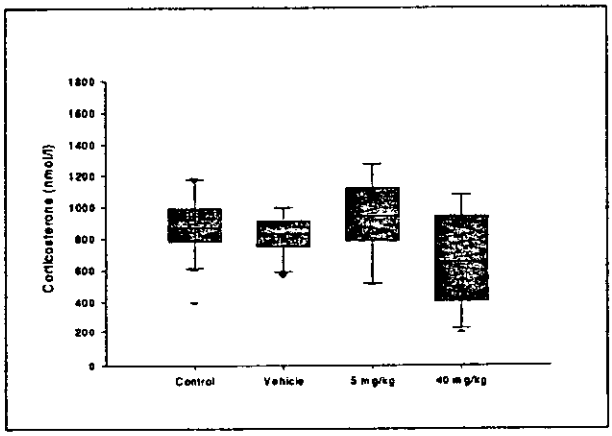
Urinalysis: the following were noted: (a) a small, but significant decrease in specific gravity in LDM and HDM (1%), (b) a decrease in pH in LDM (0.5 units) and HDM (0.7 units) [pH also tended to be lower (0.2 units) in LDF and HDF], (c) increased urinary volume in LDM (28%), HDM (48%), and LDF (31%), (d) decreased urinary protein in LDM (32%) and HDM (62%), (e) an increase in bacteria (100%) and sperm (180%) in HDM, (f) an increase in squamous epithelial cells in LDM (31%) and HDM (54%), and (g) granular casts were detected only urine from HDM, LDF, and HDF. There were no differences between the two C grps.

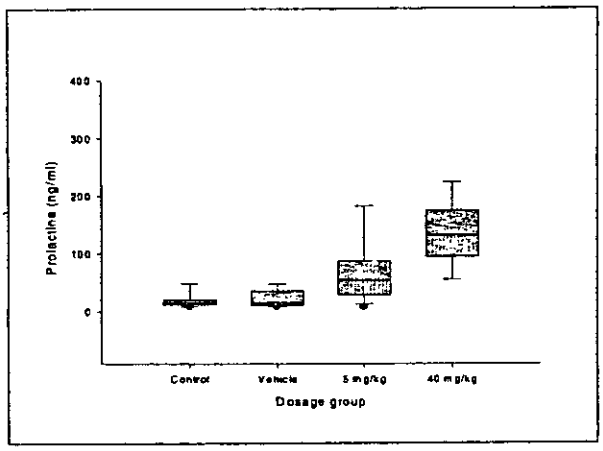
Hormones: the following effects were noted: (a) a decrease in aldosterone at both doses in males [10 and 26% at the LD and HD, respectively]. [In females, aldosterone levels were higher (38%) in VC as compared to SC, but decreased in LDF (39%) and HDF (18%) as compared to VC, but not SC; in addition, the effect was clearly not dose-related.] (b) a decrease in corticosterone in HDM (17%), LDF (16%), and HDF (21%), (c) a dose-related increase in serum prolactin levels in males [230-480%] and females [74-116% compared to VC, 44-78% compared to SC]. These findings were summarized in the following sponsor's figures:

males

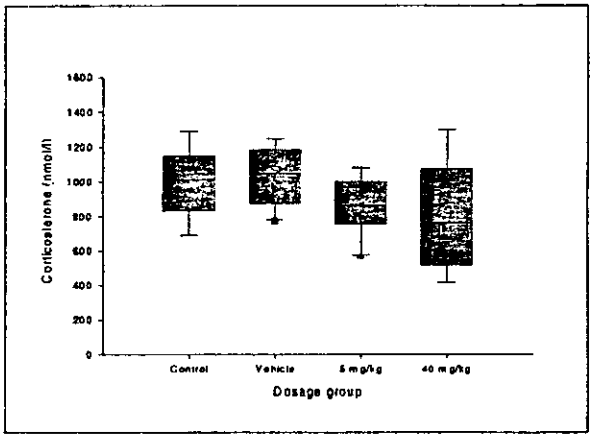


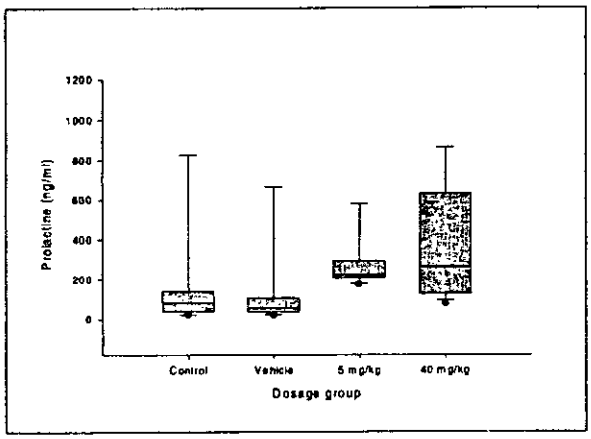
Median, 10, 25, 75, 90 percentiles and outlyers (•) are presented





females





Organ weights: the following were observed: (a) increased spleen wt in LDM (absolute-relative: 13-17%) and HDM (relative: 25%), (b) increased liver wt in LDF (8-4%) and HDF (4-18%), (c) increased heart wt in LDM (7-11%), HDM (6-34%), and HDF (12-27%), (d) increased pancreas wt in HDM (10-40%), (e) increased kidney wt in LDM (14-18%), HDM (29-64%), LDF (15-11%), and HDF (17-33%), (f) decreased thymus wt in LDM (10-7%), HDM (34-17%), and HDF (36-26%), (g) increased adrenal wt in LDF (31-28%) and HDF (24-42%). In males, adrenal wt was decreased at the LD (23-20%), but in HDM, absolute wt was decreased (7%), but relative wt was increased (18%)], (h) decreased ovary wt in LDF (9-13%) and HDF (13-3%), and (i) increased thyroid wt in HDM (68-120%). [In HDM, absolute testis wt was reduced (13%), but relative wt was increased (10%); at the LD, absolute testis wt was reduced (7%, but relative wt was not significantly affected.]

Gross pathology: selected findings are summarized in the following table:

TISSUE	FINDING	MALES				FEMALES			
		SC	VC	LD	HD	SC	VC	LD	HD
general	cachexia	3/50	8/50	5/50	14/50	9/50	9/50	14/50	14/50
	dehydration	0/50	0/50	0/50	2/50	1/50	1/50	2/50	6/50
injection site	bleeding	0/50	0/50	1/50	3/50	1/50	0/50	0/50	2/50
	powdery deposit (i.m.)	0/50	49/50	46/50	49/50	0/50	48/50	48/50	50/50
	tissue mass	0/50	0/50	0/50	1/50	0/50	0/50	0/50	0/50
adrenal gland	dark	1/50	0/50	0/50	6/50	1/50	0/50	3/50	1/50
	swollen	2/50	1/50	6/50	31/50***	3/50	2/50	13/50**	14/50**
	tissue mass	1/50	1/50	1/50	1/50	0/50	0/50	0/50	1/50
hair	rough	0/50	3/50	4/50	16/50**	5/50	1/50	5/50	3/50
kidney	rough surface	1/50	0/50	2/50	4/50	1/50	1/50	1/50	7/50
	white nodule	0/50	0/50	0/50	2/50	0/50	0/50	0/50	0/50
	pale	2/50	1/50	7/50	20/50***	2/50	2/50	2/50	17/50***
	swollen	0/50	0/50	3/50	7/50*	0/50	0/50	0/50	0/50
liver	focus	9/50	8/50	5/50	6/50	1/50	1/50	3/50	8/50*
	dark focus	5/50	3/50	1/50	3/50	0/50	01/50	1/50	4/50
	yellow focus	2/50	4/50	2/50	1/50	0/50	0/50	2/50	4/50
	pronounced lobulation	0/50	0/50	2/50	0/50	0/50	0/50	3/50	3/50
lymph node, medial iliac	swollen	1/50	1/50	0/50	6/50	1/50	0/50	0/50	0/50
	swollen, cystic	1/50	0/50	0/50	3/50	0/50	0/50	0/50	0/50
lymph node, popliteal	swollen	0/50	1/50	2/50	3/50	1/50	0/50	2/50	1/50
mammary gland	inspissated secretion	0/50	0/50	2/50	6/50*	9/50	4/50	15/50*	18/50**
	stimulation	1/50	3/50	5/50	21/50***	34/50	33/50	47/50**	48/50***
	tissue mass	1/50	3/50	0/50	7/50	8/50	4/50	17/50**	18/50**
ovary	cyst					9/50	8/50	0/50**	1/50*
pancreas	nodule	1/50	2/50	1/50	2/50	0/50	0/50	1/50	2/50
	strand formation, hemorrhagic	0/50	0/50	0/50	0/50	0/50	0/50	1/50	3/50
pituitary gland	nodule	2/50	1/50	2/50	9/50	4/50	5/50	4/50	9/50
	nodule, hemorrhagic	2/50	1/50	1/50	8/50	3/50	4/50	3/50	8/50
	swollen	0/50	0/50	5/50	22/50***	3/50	4/50	14/50*	14/50*
prostate	stippled	0/50	1/50	3/50	17/50***				
	stippled, white	0/50	1/50	1/50	4/50				
	stippled, yellow	0/50	0/50	2/50	13/50***				
	swollen	1/50	1/50	4/50	18/50***				
seminal vesicles	dilated	1/50	2/50	3/50	25/50***				
testes	strand formation	0/50	3/50	4/50	11/50*				
	strand formation, white	0/50	3/50	4/50	10/50				
	strand formation, yellow	0/50	0/50	0/50	1/50				
thymus	small	3/50	4/50	6/50	11/50	10/50	7/50	13/50	18/50
uterus	focus					6/50	2/50	1/50	0/50
	focus, swollen					6/50	2/50	0/50*	0/50
	swollen					7/50	6/50	0/50*	0/50*
	tissue mass					5/50	1/50	0/50	0/50
cervix	swollen					6/50	4/50	0/50	1/50

*p<0.05, **p<0.01, ***p<0.001 compared to VC

Histopathology:

Non-neoplastic: the sponsor summarized the significant, non-neoplastic findings in attached Tables T-121 to T-124 [Control = SC, Vehicle = VC (i.e., microspheres)]. Non-significant, but notable findings are summarized in the following table:

TISSUE	FINDING	MALES				FEMALES			
		SC	VC	LD	HD	SC	VC	LD	HD
administration site	muscle atrophy	0/50	0/50	0/50	4/49	0/50	0/49	0/50	0/50
	bleeding	1/50	1/50	0/50	5/50	4/50	0/49	0/50	4/50
	fibrosis	0/50	0/50	0/50	2/50	0/50	0/49	0/50	0/50
adrenal gland	ectasia, focal	4/50	2/50	6/50	11/50	28/50	23/50	26/50	24/50
	small cell foci	3/50	5/50	5/50	10/50	5/50	7/50	2/50	4/50
	cortical cell hypertrophy	1/50	2/50	3/50	5/50	2/50	5/50	4/50	6/50
	vacuolated z. fascic cells	0/50	0/50	0/50	2/50	0/50	0/50	0/50	2/50
	vacuolated z. reticul cells	0/50	0/50	0/50	0/50	0/50	0/50	0/50	2/50
brain	dilated lumen	2/50	7/50	4/50	7/50	9/50	11/50	12/50	12/50
	tumor impression	3/50	8/50	6/50	8/50	13/50	9/50	13/50	10/50
coagulating gland	inflammation	0/50	0/50	1/50	2/50				
epididymides	cellular debris	2/50	1/50	2/50	6/50				
	focal mesothelial hyperplasia	0/50	0/50	0/50	2/50				
heart	fibrosis	16/50	7/50	14/50	22/50	3/50	4/50	8/50	9/50
	mineralized foci	0/50	0/50	0/50	0/50	0/50	0/50	0/50	2/50
	minerals in vessel wall	0/50	0/50	0/50	0/50	0/50	0/50	0/50	3/50
kidney	pelvic hemorrhage	2/50	2/50	2/50	9/50	6/50	2/50	13/50	13/50
	hyperplasia (tubuli), cystic, focal	0/50	0/50	0/50	5/50	0/50	0/50	1/50	0/50
liver	small-cell/basophilic foci	4/50	10/50	4/50	10/50	13/50	19/50	20/50	17/50
	granulomatous inflammation	0/50	3/50	2/50	3/50	4/50	3/50	2/50	1/50
	pigmented macrophages, focus	1/50	3/50	4/50	6/50	3/50	6/50	8/50	4/50
lung	hyperplasia, papillary, focal	0/50	0/50	0/50	1/50	0/50	1/50	0/50	3/50
	encapsulated microspheres	0/50	1/50	0/50	0/50	0/50	3/50	0/50	0/50
lymph nodes, medial iliac	diffuse atrophy	1/46	0/47	1/50	1/48	0/48	0/48	0/49	2/49
lymph nodes, mesenteric	atrophy, diffuse	1/50	4/50	4/50	5/50	3/50	2/50	3/50	14/50
	histiocytic nodules	0/50	5/50	1/50	4/50	1/50	1/50	2/50	2/50
	histiocytosis/foamy macrophages	0/50	5/50	1/50	4/50	0/50	2/50	0/50	5/50
lymph nodes, popliteal	cystic	1/49	3/47	3/46	5/49	0/49	0/45	0/47	0/46
	diffuse atrophy	2/49	2/47	1/46	1/49	0/49	0/45	0/47	2/46
	paracortical hyperplasia	0/49	2/47	1/46	3/49	0/49	1/45	1/47	2/46
mammary gland	secretion present	0/50	1/50	0/50	4/49	38/50	36/49	43/50	47/50
parathyroid gland	focal hyperplasia	6/50	4/49	4/47	8/49	0/49	1/45	4/47	4/50
pituitary gland	diffuse hyperplasia (pars intermedia)	1/48	2/50	4/48	17/49	0/49	0/48	0/50	3/50
prostate	diffuse atrophy	3/50	3/50	2/50	6/50				
skin	diffuse atrophy	1/4	3/5	3/7	16/16	7/8	1/3	5/5	3/4
vascular system	minerals in vessel wall	--	--	--	--	--	--	1/1	3/3

Mean severity scores [scale of 1 (minimal)-5 (severe)] for selected findings [i.e., encapsulated microspheres at injection site, diffuse ectasia of sinusoids in the adrenal cortex, diffuse corticotubular basophilia/dilatation, pelvic mineralization, and diffuse hyperplasia of the pelvis and chronic renal disease in kidney, sinus histiocytosis/foamy cells of lymph nodes (inguinal, medial iliac, popliteal), splenic pigmentation/hemosiderosis and rbc accumulation in red pulp, and thickness of the vaginal epithelium] were summarized in the attached sponsor's tables. The mean severity scores for osteodystrophy in affected animals were 1.2 and 1.5 in HDM and HDF, respectively; the range of severity scores was 1-2 in both grps.

The sponsor grouped non-neoplastic changes into 4 categories:

(1) "changes considered general, aspecific consequences of toxic overdosing observed mainly at 40 mg/kg in male and female rats" [i.e., drug-related]: (a) clear cell/plaque foci in liver in LDM and HDM [considered secondary to body wt effect], (b) diffuse atrophy of mesenteric lymph nodes in HDF [the sponsor

noted that this finding was detected only in moribund sacrifices, and considered this secondary to bad condition], (c) thymic involution in HDF [considered secondary to bad condition]

(2) "changes related to increased serum prolactin...and...to anti-dopaminergic characteristics of the test article": (a) granulocytic inflammation (i.e., acute/exudative inflammation) in dorsolateral prostate in LDM and HDM, (b) secretory material in coagulating gland, prostate, and seminal vesicles in HDM, (c) swollen adrenocortical [zona fasciculata, zona reticularis] cells in LDM, HDM, and HDF; diffuse ectasia and associated pigmentation of the adrenal sinusoids in HDM; hyperplasia of the medulla [also may be related to increased serum Ca due to chronic renal disease] in HDM. (d) renal changes (all those listed in the summary tables). (e) osteodystrophy detected in sternum and stifle joint bones [due to "acceleartion of chronic nephropathy"] in HDM and HDF, (f) thyroid C-cell hyperplasia in HD grps, (g) testicular atrophy and mineralization in HDM (and, perhaps, to body wt changes), (g) mammary gland findings in all dosed grps, (h) female reproductive organ findings, and (i) diffuse hyperplasia of the pars distalis of the pituitary gland in all dosed grps. The hyperplasia of the pars intermedia observed in dosed males "...was not directly prolactin mediated but has been described to result form interference with dopaminergic control mechanisms of the pituitary gland..."

(3) "changes related to the α-lytic effect of the test article": hemosiderosis (pigmentation/hemosiderosis) in the spleen in HDM (also associated with aging); the sponsor noted that this finding was discussed in an Expert Report for oral risperidone .

(4) "changes related to...the vehicle": (a) encapsulated microspheres at the injection site (with stifle joint); the sponsor noted that "...For one [HDM, #357] multifocal fibrosis with small nests of histiocytic cells scattered in the fibrotic foci were observed in the soft tissue of the mammary gland". (b) prominent sinus histiocytosis/foamy cells in inguinal, medial iliacal and popliteal lymph nodes in VC and HD animals. The sponsor also stated that the "inflammatory changes, especially plasmacytosis," detected in LDM and HDM "...are probably related to the stimulation of the mammary gland with an activated lymph draining and more activity in the draining lymph nodes", (c) swelling of the adrenomedullary sinusoidal lining cells in VCM and HDM.

MALES

Organ or Tissue - Observation	Dosage group (mg / kg)			
	Control	Vehicle	Low:5	High:40
Administration site - *Number examined:*	*50*	*50*	*50*	*49*
- chronic reactive inflammation, focal	0	7 *	6 *	9 **
- encapsulated microspheres	0	50 ***	49 ***	49 ***
Adrenal glands *Number examined:*	*50*	*50*	*50*	*50*
- ectasia of sinusoids, diffuse	13	14	27 ** @	46 *** @@@
- focal cellular changes, eosinophilic	14	11	2 ** @	4 *
- focal cellular changes, fatty-vacuolated	25	23	11 ** @	11 ** @
- medullary hyperplasia, focal	8	9	9	33 *** @@@
- pigmentation in sinusoidal cells	18	22	23	35 ** @
- swollen zona fasciculata cells	2	4	18 *** @@	38 *** @@@
- swollen sinusoidal-lining cells (medulla)	0	32 ***	0 @@@	28 ***
- swollen zona reticularis cells	2	4	11 *	34 *** @@@
Bone, sternum *Number examined:*	*50*	*50*	*50*	*50*
- osteodystrophy	0	1	1	33 *** @@@
Bone, stifle joint *Number examined:*	*50*	*50*	*50*	*50*
- encapsulated microspheres	0	17 ***	19 ***	22 ***
- osteodystrophy	0	1	0	23 *** @@@
Coagulating glands *Number examined:*	*50*	*50*	*50*	*50*
- accumulated content	0	1	0	23 *** @@@
Heart *Number examined:*	*50*	*50*	*50*	*50*
- fibrosis	16	7	14	22 @@
Kidneys *Number examined:*	*50*	*50*	*50*	*50*
- basophilic tubuli, focal	22	16	11 *	7 **
- basophilic/dilated tubuli, diffuse	8	6	20 * @@	44 *** @@@
- hyaline cast(s)	16	15	13	3 ** @@
- hyperplasia (transitional epithelium), diffuse	5	8	9	19 ** @
- minerals (pelvis)	28	29	40 * @	49 *** @@@
Liver *Number examined:*	*50*	*50*	*50*	*50*
- clear cell plaques/foci	39	31	22 ***	8 *** @@@
- focal cellular changes, eosinophilic	16	9	4 **	8
Lungs *Number examined:*	*50*	*50*	*50*	*50*
- foamy macrophages	17	15	8	5 ** @
Lymph node(s), inguinal *Number examined:*	*50*	*49*	*49*	*50*
- histiocytosis/foamy macrophages	0	46 ***	3 @@@	45 ***
- inflammation, chronic	0	0	5 *	1
Lymph nodes, medial iliac *Number examined:*	*46*	*47*	*50*	*48*
- cystic	14	16	11	28 ** @
- erythrophagocytosis	4	5	13 *	10
- histiocytosis/foamy macrophages	3	44 ***	39 *** @	48 ***

Significance computed by the Fisher Exact test (two tailed) : versus Control : * P < .05 ** P < .01 *** P < .001
versus Vehicle : @ P < .05 @@ P < .01 @@@ P < .001
Statistics are only performed if more than 50 % of the animals of the group are examined

MALES

Organ or Tissue - Observation		Dosage group (mg / kg)			
		Control	Vehicle	Low:5	High:40
Lymph nodes, medial iliac	*Number examined:*	*46*	*47*	*50*	*48*
- paracortical hyperplasia		9	10	5	3 @
- pigmented macrophages		28	38 *	43 **	40 *
- plasmacytosis		11	11	15	24 * @
Lymph nodes, popliteal	*Number examined:*	*49*	*47*	*46*	*49*
- histiocytosis/foamy macrophages		1	47 ***	24 *** @@@	46 ***
- pigmented macrophages		10	23 **	18	29 ***
- plasmacytosis		8	5	12	14 @
Mammary gland	*Number examined:*	*50*	*50*	*50*	*49*
- accumulated content		1	4	23 *** @@@	46 *** @@@
- acute/exudative inflammation		0	0	1	5 * @
- female aspect (tubulo-acinar development)		2	7	31 *** @@@	48 *** @@@
- fibrosis		0	0	4	20 *** @@@
- galactocoele		0	0	2	17 *** @@@
- granulocytes in lumen		0	1	4	6 *
- hyperplasia, focal		0	1	10 ** @@	25 *** @@@
- inspissated material		1	3	19 *** @@@	40 *** @@@
Pancreas	*Number examined:*	*50*	*50*	*49*	*50*
- atrophy/inflammation		22	19	10 *	9 ** @
- inflammatory round cells, focal		5	6	2	0 @
- pigmented macrophages		12	11	8	1 ** @@
Pituitary gland	*Number examined:*	*48*	*50*	*48*	*49*
- hyperplasia, diffuse (pars distalis)		1	0	14 *** @@@	32 *** @@@
- hyperplasia, diffuse (pars intermedia)		1	2	4	17 *** @@@
- hyperplasia, focal (pars intermedia)		1	3	5	10 ** @
Prostate	*Number examined:*	*50*	*50*	*50*	*50*
- accumulated content		0	1	0	17 *** @@@
- acute/exudative inflammation		17	15	35 *** @@@	43 *** @@@
- inspissated material		39	39	36	27 * @
Seminal vesicles	*Number examined:*	*50*	*50*	*50*	*50*
- accumulated content		1	3	3	28 *** @@@
- inflammation		1	2	6	15 *** @@@
- inspissated material		1	3	8 *	33 *** @@@
Testes	*Number examined:*	*50*	*50*	*50*	*50*
- atrophy, diffuse		15	8	16	29 ** @@@
- mineralization		9	10	15	29 *** @@@
Thymus	*Number examined:*	*47*	*45*	*43*	*47*
- prominent epithelial (cystic) elements		14	3 **	10 @	14 @@
Thyroid glands	*Number examined:*	*49*	*50*	*50*	*50*
- C-cell hyperplasia, diffuse		26	21	22	10 *** @
- C-cell hyperplasia, focal		13	8	13	5 *

FEMALES

Organ or Tissue - Observation		Dosage group (mg / kg)			
		Control	Vehicle	Low:5	High:40
Administration site	*Number examined:*	*50*	*49*	*50*	*50*
- encapsulated microspheres		0	49 ***	47 ***	50 ***
Adrenal glands	*Number examined:*	*50*	*50*	*50*	*50*
- cortical hyperplasia		17	24	15	10 @@
- focal cellular change (zona glomerulosa)		1	6	1	0 @
- focal cellular changes, fatty-vacuolated		8	11	8	3 @
- medullary hyperplasia, focal		8	5	17 @@	13
- swollen zona fasciculata cells		3	3	8	15 ** @@
- swollen zona reticularis cells		5	8	8	16 *
Bone, sternum	*Number examined:*	*50*	*50*	*50*	*50*
- osteodystrophy		7	4	8	21 ** @@@
Bone, stifle joint	*Number examined:*	*50*	*50*	*50*	*50*
- encapsulated microspheres		0	26 ***	12 *** @@	14 *** @
- osteodystrophy		6	4	6	17 * @@
Cervix	*Number examined:*	*42*	*43*	*40*	*38*
- muscular hypertrophy		7	4	0 *	2
Kidneys	*Number examined:*	*50*	*50*	*50*	*50*
- basophilic tubuli, focal		11	10	9	3 *
- basophilic/dilated tubuli, diffuse		13	17	30 ** @	42 *** @@@
- chronic disease		4	3	11 @	14 * @@
- hemorrhages (pelvis)		6	2	13 @@	13 @@
Liver	*Number examined:*	*50*	*50*	*50*	*50*
- proliferation of ducts		11	19	12	5 @@
Lungs	*Number examined:*	*50*	*50*	*50*	*50*
- blood in lumen		1	0	5	6 @
Lymph node(s), inguinal	*Number examined:*	*50*	*49*	*50*	*50*
- histiocytosis/foamy macrophages		0	47 ***	5 @@@	49 ***
- inflammation, chronic		1	0	7 @	1
Lymph node(s), mesenteric	*Number examined:*	*50*	*50*	*50*	*50*
- atrophy, diffuse		3	2	3	14 ** @@
Lymph nodes, medial iliac	*Number examined:*	*48*	*48*	*49*	*49*
- erythrophagocytosis		9	3	16 @@	9
- histiocytosis/foamy macrophages		0	46 ***	39 *** @	48 ***
- pigmentation, dark, focal		14	10	5 *	5 *
- pigmented macrophages		38	44	47 *	45
Lymph nodes, popliteal	*Number examined:*	*49*	*45*	*47*	*46*
- histiocytosis/foamy macrophages		0	42 ***	16 *** @@@	46 ***
- pigmentation, dark, focal		12	9	19 @	8
- plasmacytosis		4	1	8 @	7

FEMALES

Organ or Tissue - Observation	Dosage group (mg / kg)			
	Control	Vehicle	Low:5	High:40
Mammary gland *Number examined:*	*50*	*49*	*50*	*50*
- accumulated content	25	28	38 *	38 *
- fibrosis	5	7	13	25 *** @@@
- glandular development	41	41	48	50 ** @@
- hyperplasia, focal	27	24	45 *** @@@	44 *** @@@
- hyperplasia, papillary, focal	2	1	8 @	16 *** @@@
- inspissated material	16	16	24	30 ** @@
- nodular hyperplasia with prominent fibrosis	4	1	4	9 @
- secretion present	38	36	43	47 * @@
Ovaries *Number examined:*	*50*	*50*	*50*	*50*
- clear interstitial tissue	35	37	45 *	47 ** @
- prominent sex cord cells	26	27	14 * @	15 * @
Pancreas *Number examined:*	*50*	*49*	*50*	*50*
- atrophy/inflammation	11	12	4 @	8
Pituitary gland *Number examined:*	*49*	*48*	*50*	*50*
- hyperplasia, diffuse (pars distalis)	1	1	27 *** @@@	20 *** @@@
- hyperplasia, focal (pars intermedia)	5	2	4	0 *
Spleen *Number examined:*	*50*	*49*	*50*	*50*
- granulopoiesis	2	0	3	6 @
- hyperplasia of the red pulp	1	0	3	6 @
Thymus *Number examined:*	*47*	*49*	*47*	*49*
- involution	37	42	41	48 **
Thyroid glands *Number examined:*	*50*	*48*	*50*	*50*
- C-cell hyperplasia, diffuse	18	17	13	3 *** @@@
- C-cell hyperplasia, focal	12	14	10	3 * @@
Uterus *Number examined:*	*50*	*50*	*50*	*50*
- atrophy, diffuse	2	0	7 @	5
- cystic	20	18	3 *** @@@	0 *** @@@
- dilated lumen (lumina)	14	6	5 *	6
- glandular development	29	22	6 *** @@@	8 *** @@
Vagina *Number examined:*	*50*	*50*	*50*	*50*
- infiltrating granulocytes	17	15	20	30 * @@
- mucified aspect	30	36	42 *	39

MALES

Organ or Tissue - Observation	Dosage group (mg / kg)			
	Control	Vehicle	Low:5	High:40
Administration site *Number examined:*	*50*	*50*	*50*	*49*
- encapsulated microspheres	0.00 (0.00)	2.56 *** (0.07)	1.54 *** @@@ (0.08)	2.51 *** (0.07)
Adrenal glands *Number examined:*	*50*	*50*	*50*	*50*
- ectasia of sinusoids, diffuse	0.28 (0.07)	0.28 (0.06)	0.64 ** @@ (0.09)	1.28 *** @@@ (0.09)
Kidneys *Number examined:*	*50*	*50*	*50*	*50*
- basophilic/dilated tubuli, diffuse	0.16 (0.05)	0.12 (0.05)	0.44 ** @@ (0.08)	1.30 *** @@@ (0.10)
- chronic disease	0.40 (0.09)	0.44 (0.10)	0.42 (0.11)	0.50 (0.10)
- hyperplasia (transitional epithelium), diffuse	0.10 (0.04)	0.18 (0.06)	0.18 (0.05)	0.38 ** @ (0.07)
- minerals (pelvis)	0.66 (0.09)	0.66 (0.09)	1.02 ** @@ (0.09)	1.42 *** @@@ (0.08)
Lymph node(s), inguinal *Number examined:*	*50*	*49*	*49*	*50*
- histiocytosis/foamy macrophages	0.00 (0.00)	1.49 *** (0.09)	0.06 @@@ (0.03)	1.44 *** (0.10)
Lymph nodes, medial iliac *Number examined:*	*46*	*47*	*50*	*48*
- histiocytosis/foamy macrophages	0.07 (0.04)	1.81 *** (0.08)	1.02 *** @@@ (0.10)	1.81 *** (0.06)
Lymph nodes, popliteal *Number examined:*	*49*	*47*	*46*	*49*
- histiocytosis/foamy macrophages	0.02 (0.02)	1.77 *** (0.07)	0.54 *** @@@ (0.08)	1.49 *** @ (0.09)
Spleen *Number examined:*	*50*	*50*	*50*	*50*
- pigmentation / hemosiderosis	1.30 (0.10)	1.32 (0.09)	1.36 (0.09)	1.74 *** @@@ (0.08)
- red blood cells (red pulp)	1.10 (0.08)	1.18 (0.08)	1.18 (0.10)	1.20 (0.10)

Significance computed by Mann-Whitney U test (two tailed) : versus Control : * P < .05 ** P < .01 *** P < .001
versus Vehicle : @ P < .05 @@ P < .01 @@@ P < .001

Standard Error is shown between brackets
Statistics are only performed if more than 50 % of the animals of the group are examined

FEMALES

Organ or Tissue - Observation	Dosage group (mg / kg)			
	Control	Vehicle	Low:5	High:40
Administration site *Number examined:*	*50*	*49*	*50*	*50*
- encapsulated microspheres	0.00 (0.00)	2.31 *** (0.08)	1.24 *** @@@ (0.08)	2.32 *** (0.07)
Adrenal glands *Number examined:*	*50*	*50*	*50*	*50*
- ectasia of sinusoids, diffuse	0.66 (0.07)	0.64 (0.07)	0.66 (0.08)	0.76 (0.08)
Kidneys *Number examined:*	*50*	*50*	*50*	*50*
- basophilic/dilated tubuli, diffuse	0.30 (0.08)	0.36 (0.07)	0.70 *** @@ (0.09)	1.22 *** @@@ (0.10)
- chronic disease	0.10 (0.05)	0.10 (0.06)	0.22 @ (0.06)	0.44 ** @@ (0.11)
- hyperplasia (transitional epithelium), diffuse	0.18 (0.06)	0.08 (0.04)	0.18 (0.05)	0.18 (0.05)
- minerals (pelvis)	0.92 (0.10)	0.98 (0.09)	1.22 * (0.10)	1.10 (0.08)
Lymph node(s), inguinal *Number examined:*	*50*	*49*	*50*	*50*
- histiocytosis/foamy macrophages	0.00 (0.00)	1.47 *** (0.08)	0.10 * @@@ (0.04)	1.50 *** (0.08)
Lymph nodes, medial iliac *Number examined:*	*48*	*48*	*49*	*49*
- histiocytosis/foamy macrophages	0.00 (0.00)	1.83 *** (0.07)	0.96 *** @@@ (0.09)	1.82 *** (0.06)
Lymph nodes, popliteal *Number examined:*	*49*	*45*	*47*	*46*
- histiocytosis/foamy macrophages	0.00 (0.00)	1.42 *** (0.09)	0.36 *** @@@ (0.08)	1.54 *** (0.07)
Spleen *Number examined:*	*50*	*49*	*50*	*50*
- pigmentation / hemosiderosis	1.60 (0.09)	1.73 (0.08)	1.74 (0.08)	1.68 (0.09)
- red blood cells (red pulp)	0.80 (0.09)	0.94 (0.08)	0.84 (0.09)	0.98 (0.09)
Vagina *Number examined:*	*50*	*50*	*50*	*50*
- thickness epithelial layer	1.90 (0.10)	1.72 (0.09)	1.36 *** @@ (0.07)	1.46 ** @ (0.09)

Significance computed by Mann-Whitney U test (two tailed) : versus Control : * P < .05 ** P < .01 *** P < .001
versus Vehicle : @ P < .05 @@ P < .01 @@@ P < .001

Standard Error is shown between brackets
Statistics are only performed if more than 50 % of the animals of the group are examined

Neoplastic: trend analyses of total tumor-bearing animals indicated a positive trend in males when dose grps were compared to SC and VC grps for incidental tumors and for fatal and/or incidental tumors, and in females when dose grps were compared to VC for fatal, incidental, and fatal + incidental tumors. In males, the only fatal tumor exhibiting a postive trend was pituitary adenomas. In females, positive trends were noted for fatal mammary tumors (adenocarcinomas).

Selected neoplastic findings are summarized in the following tables:

TISSUE	FINDING	MALES				sign	FEMALES				sign
adrenal	pheochromocytoma										
	benign	2/50	2/50	2/50	11/50	### **	0/50	1/50	1/50	3/50	#
	malignant	1/50	1/50	1/50	1/50		0/50	0/50	1/50	0/50	
	total	**3/50**	**3/50**	**3/50**	**12/50**	## **	**0/50**	**1/50**	**2/50**	**3/50**	
kidney	renal tubular										
	adenocarcinoma	0/50	0/50	0/50	1/50		0/50	0/50	0/50	0/50	
	adenoma	0/50	0/50	0/50	4/50	## **	0/50	0/50	0/50	0/50	
	total	**0/50**	**0/50**	**0/50**	**5/50**	## **	**0/50**	**0/50**	**0/50**	**0/50**	
mammary gland	adenocarcinoma	0/50	1/50	0/50	2/49		4/50	2/49	14/50	14/50	## ***
	fibroadenoma	0/50	0/50	0/50	1/49		5/50	4/49	8/50	7/50	
	total	**0/50**	**1/50**	**0/50**	**3/49**	#	**9/50**	**6/49**	**20/50**	**21/50**	## ***
pancreas	islet cell tumor										
	adenoma	2/50	3/50	1/49	8/50	## *	0/50	0/49	1/50	7/50	### **
	carcinoma	1/50	0/50	0/49	2/50		0/50	0/49	0/50	0/50	
	adenoma mixed islet cell-acinar	0/50	0/50	1/49	0/50		0/50	0/49	0/50	0/50	
	total	**3/50**	**3/50**	**2/49**	**10/50**	## *	**0/50**	**0/49**	**1/50**	**7/50**	
pituitary	adenoma	**11/48**	**12/50**	**14/48**	**23/49**	## *	**32/49**	**27/48**	**28/50**	**32/50**	
thyroid	follicular										
	adenocarcinoma	3/49	0/50	1/50	1/50		1/50	0/48	0/50	0/50	
	adenoma	7/49	0/50	6/50	7/50	**	1/50	1/48	3/50	5/50	# *
	total	**10/49**	**0/50**	**7/50**	**8/50**	**	**2/50**	**1/48**	**3/50**	**5/50**	*

$^{\#}p<0.05$, $^{\#\#}p<0.01$, $^{\#\#\#}p<0.001$ compared to SC; $^{*}p<0.05$, $^{**}p<0.01$, $^{***}p<0.001$ compared to VC; **statistic: trend test**

TISSUE	FINDING	MALES				FEMALES			
		SC	VC	LD	HD	SC	VC	LD	HD
adrenal	pheochromocytoma								
	benign	2/50	2/50	2/50	11/50$^{\#\#\ **}$	0/50	1/50	1/50	3/50
	malignant	1/50	1/50	1/50	1/50	0/50	0/50	1/50	0/50
	total	**3/50**	**3/50**	**3/50**	**12/50$^{\#\#\ *}$**	**0/50**	**1/50**	**2/50**	**3/50**
kidney	renal tubular								
	adenocarcinoma	0/50	0/50	0/50	1/50	0/50	0/50	0/50	0/50
	adenoma	0/50	0/50	0/50	4/50	0/50	0/50	0/50	0/50
	total	**0/50**	**0/50**	**0/50**	**5/50$^{\#\ *}$**	**0/50**	**0/50**	**0/50**	**0/50**
mammary gland	adenocarcinoma	0/50	1/50	0/50	2/49	4/50	2/49	14/50$^{\#\#\ **}$	14/50$^{\#\#\ **}$
	fibroadenoma	0/50	0/50	0/50	1/49	5/50	4/49	8/50	7/50
	total	**0/50**	**1/50**	**0/50**	**3/49**	**9/50**	**6/49**	**20/50$^{\#\#\ ***}$**	**21/50$^{\#\#\ ***}$**
pancreas	islet cell tumor								
	adenoma	2/50	3/50	1/49	8/50$^{\#}$	0/50	0/49	1/50	7/50$^{\#\#\ **}$
	carcinoma	1/50	0/50	0/49	2/50	0/50	0/49	0/50	0/50
	adenoma mixed islet cell-acinar	0/50	0/50	1/49	0/50	0/50	0/49	0/50	0/50
	total	**3/50**	**3/50**	**2/49**	**10/50$^{\#\ 8}$**	**0/50**	**0/49**	**1/50**	**7/50$^{\#\#\ **}$**
pituitary	adenoma	**11/48**	**12/50**	**14/48**	**23/49$^{\#\ *}$**	**32/49**	**27/48**	**28/50**	**32/50**
thyroid	follicular								
	adenocarcinoma	3/49	0/50	1/50	1/50	1/50	0/48	0/50	0/50
	adenoma	7/49	0/50	6/50*	7/50**	1/50	1/48	3/50	5/50
	total	**10/49**	**0/50**	**7/50****	**8/50****	**2/50**	**1/48**	**3/50**	**5/50**

$^{\#}p<0.05$, $^{\#\#}p<0.01$, $^{\#\#\#}p<0.001$ compared to SC; $^{*}p<0.05$, $^{**}p<0.01$, $^{***}p<0.001$ compared to VC; **statistic: Fishers Exact Test**

The following neoplastic findings were considered drug-related by the sponsor: (a) increase in mammary tumors (particularly adenocarcinomas) in LDF, (b) increase in pancreatic islet cell tumors (particularly adenomas) in HDM and HDF, (c) increase in pituitary adenomas and adrenomedullary pheochromocytomas in HDM, (d) increase in mammary gland tumors (particularly adenocarcinomas) in HDF, (e) decrease in ovarian polyps and absence of ovarian tumors in females, (f) "marginal" increase in solid renal corticotubular tumors in HDM [considered related to exacerbated chronic nephropathy], (g) a significant trend in mammary gland tumors in males [compared to SC], (h) a significant trend in adrenal pheochromocytoma in females [compared to SC]. The sponsor ascribed increases in all drug-related tumors to elevated serum prolactin levels.

The following neoplastic findings were considered incidental: (a) thyroid follicular adenomas in M and F [the sponsor noted that this finding was absent in VCM, and the incidence in females was within the HC range (i.e., 0/49-7/50)]. (b) fibrohistiocytic sarcoma at the injection site (animal #645; LDF).

For reference, historical control data provided by the sponsor for adrenal gland and kidney tumors in males are provided in the following tables [taken directly from the sponsor's HC data tables]:

MALES Exp.No.: Start date: Duration (weeks): Mortality (terminal): Incidences:	3592 16 JAN 96 108-109 36%	3770a 18 JAN 96 109 32%	3770b 18 JAN 96 109 30%	4032a 24 OCT 96 106 36%	4032b 24 OCT 96 106 22%	3817 25 OCT 96 104 20%	4044 17 MAR 97 108 20%	4101 12 DEC 96 105 45%
• Adrenal glands								
– Adenoma, cortical	1/50	0/50	0/50	0/50	1/50	0/50	0/60	0/60
– Adenocarcinoma, cortical	0/50	0/50	0/50	0/50	0/50	0/50	1/60	0/60
– Ganglioneuroma	0/50	0/50	0/50	1/50	0/50	0/50	0/60	0/60
– Phaechromocytoma	3/50	2/50	2/50	2/50	2/50	0/50	1/60	1/60
• Phaechromocytoma, benign	1/50	2/50	0/50	1/50	2/50	0/50	1/60	1/60
• Phaechromocytoma, malignant	2/50	0/50	2/50	1/50	0/50	0/50	0/60	0/60
• Kidneys								
– Adenoma	0/50	0/50	0/50	0/50	0/50	1/50	0/60	0/60
– Carcinosarcoma	0/50	0/50	1/50	0/50	0/50	0/50	0/60	0/60
– Lipoma	0/50	0/50	0/50	0/50	0/50	0/50	1/60	0/60
– Liposarcoma	0/50	0/50	0/50	0/50	0/50	0/50	1/60	0/60

FEMALES Exp.No.: Start date: Duration (weeks): Mortality (terminal): Incidences:	3592 16 JAN 96 105 46%	3770a 18 JAN 96 109 46%	3770b 18 JAN 96 109 46%	4032a 24 OCT 96 106 38%	4032b 24 OCT 96 106 40%	3817 25 OCT 96 104 40%	4044 17 MAR 97 106 37%	4101 12 DEC 96 105-106 48%
• Adrenal glands								
– Adenoma, cortical	0/50	0/50	0/48	0/49	0/50	1/50	1/60	0/59
– Ganglioneuroma	0/50	0/50	0/48	0/49	0/50	0/50	1/60	0/59
– Phaechromocytoma	1/50	0/50	1/48	1/49	1/50	1/50	1/60	0/59
• Phaechromocytoma, benign	1/50	0/50	0/48	1/49	1/50	1/50	1/60	0/59
• Phaechromocytoma, malignant	0/50	0/50	1/48	0/49	0/50	0/50	0/60	0/59

The tumor pattern [for the drug-related tumor types] and the incidence of osteodystrophy in individual animals is presented in the following tables:

MALE #	ADRENAL	KIDNEY	MG	PANCREAS	PITUITARY	OSTEODYSTROPHY	
						STIFLE JT	STERNUM
322	x				x	x	x
324			x				
326					x	x	x
328					x		
329					x	x	x
330	x			x	x		x
331					x		
332	x						x
333						x	x
334					x		
336		x				x	x
337					x	x	x
338			x			x	x
339		x		x			x
341			x				
342					x		
343							x
344				x	x	x	x
345	x	x			x	x	x
346				x	x	x	x
347					x		
348							x
349					x	x	x
350				x		x	x
351	x						x
352				x		x	x
353	x					x	x
354	x						x
355	x					x	x
356	x					x	x
357				x			x
358					x		x
359				x		x	x
360	x			x	x	x	x
361		x					x
362					x		
363					x		
364					x	x	x
366		x			x		
367					x	x	x
368					x	x	x
369	x			x	x	x	x
370	x					x	x

FEMALE #	ADRENAL	MG	PANCREAS	OSTEODYSTROPHY		CRD*
				STIFLE JT	STERNUM	
721				x	x	N
722		x				N
723		x				N
724		x				N
725				x	x	Y/S
729		x				N
730		x	x	x	x	Y/S
731			x			N
733			x		x	Y/S
735				x	x	Y/Se
736		x				N
738		x		x	x	N
740			x		x	N
742		x		x	x	N
745		x		x	x	Y/M
747		x				N
748		x				N
749	x			x	x	N
750		x				N
751		x		x	x	Y/S
752		x				N
753				x	x	N
754				x	x	N
756		x	x		x	N
757				x	x	Y/M
758		x		x	x	Y/M
759	x		x	x	x	N
761		x				N
762				x	x	N
763				x	x	Y/S
764	x	x	x			N
767		x			x	N
768		x				Y/S
770		x		x	x	N

*chronic renal disease (as listed in histopathology tables): Y/N [S = slight, M = moderate, Se = severe]

Toxicokinetics: data were submitted in a separate study [reviewed in the PK/ADME section].

Carcinogenicity summary and conclusions: a 2-yr bioassay was conducted in Wistar rats. Risperidone was administered bi-weekly as a microsphere suspension at doses of 0 (saline), 0 (vehicle microspheres), 5, and 40 mg/kg i.m. [The formulation used was stated to be the same as that used clinically in Phase 3 studies.] Additional (satellite) animals were dosed according to the same regimen in order to assess plasma exposure. The mortality and tumor data from this study were independently analyzed by Roswitha Kelly, M.S. [HFD-710]. [The 52-wk i.m. depot toxicity study was run concurrent with the carcinogenicity study.]

There were 44 and 69 unscheduled deaths in males and females, respectively. According to the sponsor, there was a positive dose-related trend for mortality in males; however, according to Ms. Kelly's analyses, there were no dose-related effects on mortality in either males or females. Clinical signs were evident at both doses. At the LD, the most prominent drug-related clinical sign [observed in all LDM] was sedation [characterized as "moderate"]; clinical signs in LDF were minimal, consisting of ptosis and slight sedation in a few LDF. The primary finding in LDF was an increase in s.c. tissue masses [considered to reflect mammary tumors]. A number of clinical signs [e.g., bad condition, thin, rough haircoat, ptosis, sedation (slight-severe) in males; s.c. tissue masses, ptosis, and sedation in females] were evident at the HD. Ptosis and sedation were transient at both doses, being absent at the LD after the first 1-2 doses and decreasing in severity and frequency with repeated dosing at the HD. Body wt was

reduced at both doses in males. At the LD, the effect was transient [up to Wk 80] and the final body wt was similar to the C grps. At the HD, body wt was reduced (compared to Cs) throughout the dosing period, and final body wt was 22% lower compared to VC [vehicle: microsphere]. In females, body wt was increased at the LD throughout most of the dosing period; however, final body wt was similar to VC. At the HD, body wt was increased through Wks 44-48, then reduced (compared to VC) from Wk 83 to the end of the dosing period; final body wt was 13% lower compared to VC. Overall food consumption was similar in C and HD grps. On hematology parameters, the most consistent finding was small, but significant decreases in rbc parameters in males and females. Changes were noted on numerous clinical chemistry parameters. The most notable findings were increases in Ca [HDM, LDF, HDF] and P_i [LD, HD] and decreases in glucose [HDM, LDF, HDF]. Total bilirubin was increased at the LD and HD in males; however, liver enzymes were not elevated except for small increases in ALT at Wks 52 [30-54%] and 104 [18-25%]. Urinalysis findings [e.g., decreases in specific gravity and pH, increase in urinary volume, increase in bacteria, sperm, and squamous epithelial cells] were noted primarily in males [both doses were affected].

Circulating levels of aldosterone, corticosterone, and prolactin were quantitated at the end of the dosing period. In males, aldosterone was decreased at both doses [10 and 26% at LD and HD, respectively], corticosterone was reduced at the HD [17%], and serum prolactin was elevated at both doses [230-480%]. In females, corticosterone was reduced [16 and 21% at LD and HD, respectively] and serum prolactin was elevated [74 and 116% at LD and HD, respectively] at both doses. There was no clear drug-related effect on aldosterone in females.

A number of organ wt effects were observed, however, the most notable were increases in kidney [LD, HD], adrenal [LDF, HDF], and thyroid wts [HDM]. The primary drug-related gross findings consisted of the following: (a) swollen adrenal gland [LD, HD], (b) rough hair [HDM], (c) pale and/or swollen kidney [HD; the incidence was also somewhat elevated in LDM], (d) swollen medial iliac lymph nodes [HDM], (e) mammary gland development and/or secretion [HDM, LDF, HDF], (f) a decrease in ovarian cysts [LD, HD], (g) swollen pituitary gland [LD, HD], (h) stippled, swollen prostate [HD], (i) dilated seminal vesicles [HD], (j) strand formation in testes [HD], and (k) a decrease in swollen uterus [LD, HD]. Powdery deposit at the injection site was detected in the majority of VC and dosed animals.

Non-neoplastic findings were detected in a number of tissues. Significant findings related to injection of microspheres consisted of chronic, reactive focal inflammation (M only) and encapsulated microspheres at the injection site, encapsulated microspheres at the stifle joint (bone), sinus histiocytosis/foamy macrophages in inguinal, medial iliac, and popliteal lymph nodes, and pigmented macrophages in medial iliac and popliteal lymph node (M only). [Pigmented macrophages in medial iliac lymph node was common in saline C grps as well.] In addition, swelling of the sinusoidal-lining cells of the adrenal medulla was clearly microsphere-related in males. The incidence of these findings were either equally increased in VC and dosed grps, or to a greater extent in VC and HD grps. [These grps received the same amount of microspheres; the LD grp received a lesser amount]. Although not a statistically significant effect, histiocytosis/foamy macrophages were detected in the mesenteric lymph node in some VC and HD males and females [and in 1 LDM]. The sponsor also noted that "...multifocal fibrosis with small nests of histiocytic cells scattered in the fibrotic foci were observed in the soft tissue of the mammary gland" in 1 HDM. This finding was not further described and it is unknown whether or not this reflected encapsulated microsphere in mammary tissue in this animal. It is of note, however, that microspheres were detected in lung [as was observed in the 1-yr studies in rat and dog] in a few animals [1/50 VCM, 3/50 VCF]. [The issue of possible absorption of intact or partially intact microspheres from the injection site was discussed in detail in the General Toxicology "summary and comments" section.]

Non-neoplastic findings related to administration of risperidone were detected in liver, lymph nodes, bone (sternum, stifle joint), male reproductive organs [coagulating gland, prostate, seminal vesicles,

testes], female reproductive organs [ovaries, uterus, vagina], mammary gland, kidney, adrenal, pituitary, thymus, and thyroid [decrease in C-cell hyperplasia]. The sponsor considered findings in liver [a decrease in clear cell plaques/foci (LDM, HDM)], mesenteric lymph nodes [diffuse atrophy (HDF)], and thymus [involution (HDF)] to be due to "General, aspecific consequences of toxic overdosing". The sponsor noted that diffuse atrophy of the mesenteric lymph nodes was detected only in those animals that died spontaneously or that were sacrificed moribund. However, even if it is assumed that this was the case for all grps, the incidence [expressed per grp as the number of affected animals per number of deaths] was still highest in HDF: 1/6 SCM, 4/10 VCM, 4/13 LDM, 5/15 HDM; 3/16 SCF, 2/11 VCF, 3/21 LDF, 14/21 HDF. [There was no significant increase in mortality.] The liver finding was considered by the sponsor to reflect decreases in body wt; however, body wt was not notably affected in LDM. Also, body wt was reduced in HDF but a decrease in clear cell plaques/foci was not observed. Thymic involution is commonly observed in toxicity studies and was not clearly associated with other signs of immunotoxicity. The sponsor attributed all other dose-related non-neoplastic findings to increases in serum prolactin except for the spleen findings [pigmentation/hemosiderosis] in HDM. [The spleen findings were attributed to the α-lytic activity of risperidone.]

Regarding neoplastic findings, there was overall agreement between the sponsor's statistical analysis and an independent analysis of the data conducted by the FDA [Roswitha Kelly, M.S., HFD-710]. Ms. Kelly performed trend analyses on the data, but did not conduct pair-wise comparisons (except for comparison of SC and VC grps). The following significant positive trends were identified: (a) adrenal gland pheochromocytoma (benign, combined benign/malignant) in males [compared to SC and VC] and benign pheochromocytoma in females [compared to SC], (b) renal tubular tumors (adenoma, combined adenoma/adenocarcinoma) in males [compared to SC and VC], (c) mammary gland adenocarcinomas in females [compared to SC and VC] and in combined adenocarcinoma/fibroadenoma in males [compared to SC], (d) pancreatic islet cell adenoma/carcinoma (combined) in males [compared to SC and VC] and pancreatic islet cell adenoma in females [compared to SC and VC], (e) pituitary adenoma in males [compared to SC; marginally significant compared to VC], (f) thyroid follicular tumor (adenoma, combined adenoma/adenocarcinoma in males) [compared to VC]. [There were no drug-related neoplastic findings at the administration site in either males or females. The sponsor noted only a fibrohistiocytic sarcoma at the injection site in 1 LDF [#645].]

Taking into account both the sponsor's and the FDA's statistical analyses, increases in the following tumors were considered statistically significant:

> In males: (a) pituitary adenoma [HD] when compared to SC, and approached significance when compared to VC, (b) adrenomedullary pheochromocytoma [benign, combined] [HD], (c) pancreatic islet cell tumor [adenoma, combined] [HD] when compared with SC, pancreatic islet cell tumor [combined] when compared to VC, and pancreatic islet cell adenoma approached significance when compared to VC, (d) renal tubular tumor [adenoma, combined] [HD], (e) mammary gland [combined] when compared to SC [significant trend], (f) thyroid tumor [follicular cell adenoma, combined] [LD, HD] when compared to VC.
>
> In females: (a) pancreatic islet cell adenoma [HD], (b) mammary gland adenocarcinoma [LD, HD], (c) benign adrenomedullary pheochromocytoma [significant trend] when compared to SC.

The increase in mammary tumors in males was significant only when the incidence of adenocarcinomas and fibroadenomas were combined. According to McConnell *et al.* [McConnell EE *et al. JNCI* Vol 76(2):283-289, 1986], fibroadenomas, adenomas, and adenofibromas may be combined and "carcinomas - various types" may be combined; however, the authors do not directly address whether or not fibroadenomas and adenocarcinomas may be combined for statistical analysis. However, mammary gland tumors (of any type) are rare in males. Therefore, the biological importance of these tumors cannot be

dismissed regardless of the statistical significance, particularly considering the significant incidence of mammary gland hyperplasia at both doses. [This issue was discussed with Dr. Terry Peters (Veterinary Pathologist, Acting Team Leader, HFD-520) who concurred with this conclusion. However, Dr. Peters did indicate that fibroadenomas and adenocarcinomas should not be combined for statistical analysis.] The sponsor did not list historical control (HC) data for mammary gland tumors in males in the HC data tables.

The increase in benign adrenomedullary pheochromocytomas in females was significant only when compared to SC. However, this tumor type is identified as rare in females compared to both concurrent controls and the sponsor's HC data (sponsor's table provided below).

FEMALES Exp.No.: Start date: Duration (weeks): Mortality (terminal): Incidences:	3592 16 JAN 96 105 46%	3770a 18 JAN 96 109 46%	3770b 18 JAN 96 109 46%	4032a 24 OCT 96 106 38%	4032b 24 OCT 96 106 40%	3817 25 OCT 96 104 40%	4044 17 MAR 97 106 37%	4101 12 DEC 96 105-106 48%
• Adrenal glands								
– Adenoma, cortical	0/50	0/50	0/48	0/49	0/50	1/50	1/60	0/59
– Ganglioneuroma	0/50	0/50	0/48	0/49	0/50	0/50	1/60	0/59
– Phaechromocytoma	1/50	0/50	1/48	1/49	1/50	1/50	1/60	0/59
♦ Phaechromocytoma, benign	1/50	0/50	0/48	1/49	1/50	1/50	1/60	0/59
♦ Phaechromocytoma, malignant	0/50	0/50	1/48	0/49	0/50	0/50	0/60	0/59

Focal medullary hyperplasia [possibly a preneoplastic finding] was significant increased in LDF [compared to VC, but not SC] and tended to be increased in HDF. The incidences of focal medullary hyperplasia and adrenal tumors in females are presented in the following table:

FINDING	SC	VC	LD	HD
focal medullary hyperplasia	8/50	5/50	17/50	13/50
pheochromocytomas	0/50	1/50	2/50	3/50
both	0/50	0/50	1/50	1/50

The fact that adrenomedullary tumors were increased in HDM made it difficult to dismiss this finding in HDF.

The sponsor considered the thyroid tumor findings to be "irrelevant" due to the lack of statistical significance compared to SC in males and the fact that the incidences did not exceed the HC range [provided below; from the sponsor's report]. This conclusion is reasonable.

MALES Exp.No.: Start date: Duration (weeks): Mortality (terminal): Incidences:	3592 16 JAN 96 108-109 36%	3770a 18 JAN 96 109 32%	3770b 18 JAN 96 109 30%	4032a 24 OCT 96 106 36%	4032b 24 OCT 96 106 22%	3817 25 OCT 96 104 20%	4044 17 MAR 97 108 20%	4101 12 DEC 96 105 45%
• Thyroid glands								
– Follicular tumour	6/50	8/50	8/50	3/49	6/50	4/50	14/60	5/60
♦ Adenocarcinoma, follicular	2/50	1/50	2/50	0/49	1/50	0/50	2/60	2/60
♦ Adenoma, follicular	5/50	7/50	8/50	3/49	5/50	4/50	13/60	4/60
– C-cell adenoma	5/50	4/50	4/50	1/49	3/50	4/50	2/60	4/60
FEMALES								
• Thyroid glands								
– Adenoma, follicular	3/49	2/50	5/48	0/49	3/50	7/50	2/59	3/59
– C-cell tumours	5/49	3/50	2/48	4/49	5/50	4/50	1/59	4/59
♦ C-cell adenoma	4/49	3/50	1/48	4/49	5/50	2/50	1/59	3/59
♦ C-cell carcinoma	1/49	0/50	1/48	0/49	0/50	2/50	0/59	1/59

Therefore, the following tumors were considered drug-related and toxicologically significant [consistent with the sponsor's conclusions]: (a) pituitary adenoma, adrenomedullary pheochromocytoma, pancreatic

islet cell tumors, mammary gland tumors, and renal tubular tumors in HDM, (b) mammary gland tumors in HDM and in adenocarcinomas in LDF and HDF, (c) pancreatic islet cell adenoma and benign adrenomedullary pheochromocytoma in HDF.

[The carcinogenicity data were reviewed by the Exe-CAC [4/23/02]. The ExeCAC did not concur that the positive trends in mammary gland tumors in males or in benign adrenomedullary pheochromocytomas in females constituted significant tumor findings. [Minutes of the ExeCAC meeting are appended.]

The sponsor attributed all tumor findings [except for thyroid tumors] to elevations in serum prolactin.

Microscopic changes [including neoplasms] in mammary gland is a known and common effect of drugs which elevate serum prolactin. Such drugs also have been demonstrated to produce neoplastic and non-neoplastic changes in pituitary gland and pancreas, and non-neoplastic changes in organs of the male and female reproductive tract. [There is some question as to the site of the pituitary adenomas, i.e., pars distalis vs pars intermedia; the sponsor has been asked to provide a summary of the pituitary adenoma data by site.] However, effects on kidney, adrenal gland, bone, and thyroid have not routinely been observed as a result of drug-induced increases in serum prolactin. Non-neoplastic and neoplastic findings in these organs and their possible relation to hyperprolactinemia are discussed in greater detail.

Kidney, bone, and thyroid: According to the Pharmaco-Toxicological Expert report provided by the sponsor, elevations in serum prolactin resulted in an exacerbation of spontaneous "chronic renal disease" [presumed to refer to "chronic progressive nephropathy" (CPN)], which resulted in (a) adverse effects on Ca^{2+} homeostasis resulting in osteodystrophy [observed in sternum and stifle joint in HDM and HDF], (b) thyroid effects [i.e., a decrease in focal/diffuse C-cell hyperplasia in HDM and HDF]. The Expert report noted that the thyroid findings are "....in accordance with the disturbed 'Ca^{2+}' balance and the osteodystrophy". and (c) renal tumors. The Expert report did, however, point out that "The chronic nephropathy [observed with risperidone i.m. depot] was not associated with the development of severe renal failure as indicated by the normal serum urea and creatinine levels".

The sponsor discussed [in the carcinogenicity study report] the following as evidence for risperidone i.m. depot-induced exacerbation of CPN of chronic renal disease (CRD):

(a) "a clear-cut, dose-related increase in diffuse tubular basophilia with dilatation..." in LD and HD grps [both male and female].

(b) "a dose related increase in chronic disease..." in LDF and HDF. The listing, "chronic disease", was "...scored when a multifocal presence of basophilic tubuli and hyaline casts were obvious".

(c) increases in incidence and severity of pelvic mineralization in LDM and HDM and a "slightly increased" severity of pelvic mineralization in females, particularly at the LD.

(d) "an increase in the diffuse hyperplasia of the pelvic transitional epithelium..." in HDM.

(e) "an increase in haemorrhages in the pelvis..." in LDF and HDF.

(f) "an increase in rats with a focus of tubular hyperplasia..." in HDM. "Focal cystic tubular hyperplasia was observed in..." 5 HDM.

As described by Montgomery and Seely [Montgomery CA, Seely JC. Chapter 10: Kidney. In: *Pathology of the Fischer Rat: Reference and Atlas*. Boorman GA *et al.* (Eds). Academic Press Inc., NY, 1990, pg. 132-134], the progression of nephropathy is as follows:

> "The first changes seen microscopically consist of a few scattered foci of tubular cell regeneration. These tubules have an increased number of cells with more intense staining of the cytoplasm and nucleus. Basement membranes in glomeruli and around tubules are slightly thickened... In chronic studies, chemically-related lesions are sometimes characterized by increased severity and more rapid progression of the changes seen in spontaneous nephropathy; other morphologic features specifically related to chemical exposure may also be present....As nephropathy progresses, the thickness of basement membrane around regenerative tubules is increased. Some dilated tubules with thickened basement membranes may have multiple layers of regenerative epithelium...; others have flattened atrophic epithelium. In addition, interstitial fibrosis and inflammatory infiltrates become more prominent and protein casts fill dilated tubules. Hemosiderin and lipochrome pigments can be seen in the cytoplasm of tubular epithelium and mineralization is commonly present....Marked nephropathy may be manifested as an "end-stage" renal disease with sufficiently impaired renal function to result in secondary hyperparathyroidism with the chief cells of the parathyroid becoming hyperplastic. Secondary mineralization may be seen in several tissues, including kidney, lung, gastrointestinal tract, and media of large arteries. Fibrous osteodystrophy may also be present...Rats with marked nephropathy may have proteinuria, hypoalbuminemia, and hypercholesterolemia, or nephrotic syndrome. Serum urea nitrogen and creatinine are minimally affected until the disease is advanced; serum calcium, phosphorus, or alkaline phosphatase can also be markedly altered."

Alden and Frith [Alden CL, Frith CH. Chapter 15: Urinary System. In: Haschek WM, Rousseaux CG (Eds). *Handbook of Toxicologic Pathology*, Academic Press, San Diego, CA, 1991] note the "hallmarks of chronic progress [sic] nephropathy" as "...including hyaline casts..., thickened basement membrane..., and regenerative proximal tubular epithelium...Interstitial inflammatory cells are present."

Greaves [Greaves P. Chapter IX. Urinary Tract. *Histopathology of Preclinical Toxicity Studies*. 2nd Ed, Elsevier Press, Amsterdam, The Netherlands, 2000, pgs 545-626.] noted that CPN "In its established form....is easy to recognize. Affected kidneys are enlarged, pale with a microcytic cut surface visible to the naked eye...Histopathological changes extend to the renal tubules where casts are found along with tubular basement membrane thickening, tubular sclerosis, basophilia, dilatation and atrophy. In very advanced cases tubules show a variety of cytological changes including the accumulation of intracytoplasmic protein droplets, pigmentation as well as hypertrophy and hyperplasia....The morphological changes are accompanied by functional alterations, notably evidence of increased glomerular basement membrane permeability and loss of selectivity. This is shown by proteinuria that increases with advancing age along with the severity of the histological changes".

There is support for an association between CPN and renal tumors in published literature. Hard *et al.* [Hard GC *et al. Toxicologic Pathology* 25(2):132-143, 1997] investigated the relationship between CPN and renal tumors produced by hydroquinone. As discussed by Hard *et al.* [1997], the primary finding in previously conducted 2-yr carcinogenicity studies in rat was an increase in renal tubular adenomas in males. One of these studies was conducted by NTP [cf. Kari FW *et al. Food Chem Toxicol* 20:737-747, 1992]. Hard *et al.* [1997] examined the tissues from that study for renal tumors, preneoplastic changes, other toxicities, and evidence of CPN. CPN was graded as "...minimal, mild, low-moderate, and high-moderate...[as]...characterized by increasing numbers of discrete focal lesions of basophilic tubules with

thickened basement membranes in the cortex and hyaline casts in the cortex and/or medulla. Severe grade of CPN represented an extension and coalescence of the foci into contiguous areas, and end-stage was characterized by involvement of almost all of the outer zones of the kidney by the disease process with very little or no normal-staining parenchyma remaining". According to the authors, no evidence of nephrotoxicity was detected in animals sacrificed at 66 wks of dosing. After 2 yrs of dosing, hydroquinone produced "A clear enhancement of CPN...In the high-dose males, 49% of the rats surviving into the last 10 wk [sic] of the study had end-stage CPN and 40% of the animals had severe CPN..." Renal tubular tumors were detected in 4 LD [3 adenomas] and 8 HD [7 adenomas, 1 atypical hyperplasia] males. The authors noted that, with one exception [LDM], "Every one of these lesions occurred in rats with either high severe or end-stage grades of CPN and within areas of CPN alteration"; they further stated that "...bona fide atypical hyperplasias and adenomas only occurred in animals with severe or end-stage CPN". The authors concluded that "...we consider that the results of the rat carcinogenicity bioassay with hydroquinone, where a minimal renal tumor response is linked to an interaction with a rodent-specific spontaneous renal disease, may have little relevance for humans, especially at low exposures..."

There is also support in the published literature for an association between hyperprolactinemia, exacerbation of CPN, and renal tubular tumors. For example, Richardson and Luginbuhl [Richardson B, Luginbuhl H. *Virchows Arch A Path Anat and Histol* 380:13-19, 1976; article provided by the sponsor] reported the effects of exogenously administered prolactin [40 IU ovine prolactin, s.c. depot, 1/wk for 10 wks] on the development of CPN in male OFA Sandoz SPF rat. No effects were observed on kidney wt or on gross lesions of the kidney. Microscopic findings were described as follows:

> "Changes in the nephrons of rats treated with exogenous prolactin included thickening of the parietal layer of Bowman's capsule, the presence of protein casts in dilated tubular lumen, as well as desquamation and regeneration of tubular epithelium with hyperplasia, hypertrophy and disorganization. Most kidneys had some degree of focal or diffuse chronic inflammatory cell infiltration, with fibrosis of the intersititium...Occasionally some proliferation of the pelvis epithelium was also observed...Scoring the lesions showed that prolactin administration had produced a significantly increased severity of lesions involving the nephrons...Interstitial and pelvic changes showed similar trends."

The authors concluded that exogenous prolactin resulted in "...a significantly increased severity of progressive nephropathy..." and that the results "...suggest that prolactin is a very important etiological component in the development of [CPN] in rats". They discussed the possible relevance of this finding to humans and concluded that it is "not clear". However, they briefly discussed published associations of renal disease and elevated serum prolactin levels and concluded that "Thus in man prolactin may also have significant renal action". Also, Greaves [2000] noted that "...a number of hormonal factors, including...prolactin...may also influence this condition..." [i.e., CPN] and that "...a wide variety of xenobiotics including pharmaceutical agents are also reported to influence the development of the condition in high-dose toxicity and carcinogenicity studies".

Relevant renal findings observed in the 1-yr chronic toxicity and the 2-yr carcinogenicity studies [with the i.m. depot formulation] are summarized in the following table ["x" indicates notable effect, not simply presence]:

STUDY	PARAMETER	MALES		FEMALES	
		LD	HD	LD	HD
1-yr	↑ pale kidney	x	x	x	x*
	↑ kidney wt	--	x	--	x
	↑ diffuse dilated tubuli	--	x*	--	x*
	↑ hyaline casts	--	--	--	x*
	↑ hypertrophic tubules	--	x	--	x
	↑ mineral deposit				
	cortex	--	x	--	--
	pelvis	--	x	--	--
	↑ swollen/vacuolated tubules	--	x*	--	--
2-yr	↓ urinary protein	x*	x*	--	--
	↑ pale kidney		x*	--	x*
	↑ swollen kidney	x	x*	--	--
	↑ kidney wt	x*	x*	x*	x*
	↑ focal, cystic tubular hyperplasia	--	x	--	--
	↓ focal basophilic tubuli	x*	x*	--	x*
	↑ diffuse basophilic/dilated tubuli	x*	x*	x*	x*
	↓ hyaline casts	--	x*	--	--
	↑ diffuse, transitional epithelium hyperplasia	--	x*	--	--
	↑ minerals, pelvis	x*	x*	--	--
	↑ hemorrhage, pelvis	--	--	x	x
	↑ chronic disease#	--	--	x*	x*

*statistically significant; in the 2-yr study, statistically significant compared to one or both Cs. **Bold** indicates significantly increased severity. #refers to observation in which "...multifocal presence of basophilic tubuli and hyaline casts were obvious".

Unquestionably, there are numerous drug-related effects on kidney, in both males and females. However, based on the finding from the 1-yr and 2-yr studies and literature descriptions of renal changes associated with CPN, it is not clear that CPN [CRD] was exacerbated in HDM. For example, the decreases in urinary protein and renal hyaline casts in males are not consistent with CPN, and the sponsor's own designation of "chronic disease" was not increased in males at either dose. Also, according to Hard *et al.* [1997], renal adenomas were associated with severe or end-stage CPN, conditions not observed with risperidone i.m. depot. Finally, "chronic disease" was significantly increased in females, whereas renal tubular adenomas were not. An increase in renal tubular adenomas associated with a drug-induced exacerbation of CPN, particularly if noted only in male rats, would probably be of little relevance to human risk. However, without a more clear association between these two findings with risperidone i.m. depot, it cannot be assumed that the increase in renal tubular tumors is not relevant to humans.

The lack of convincing evidence that risperidone i.m. depot exacerbated CPN also has implications for interpreting the bone findings [i.e., osteodystrophy]. It is the sponsor's opinion that the osteodystrophy detected in sternum and, to a lesser extent, in stifle joint in both males and females that the HD was a result of Ca^{2+} disturbances secondary to exacerbation of CPN. As for renal tumors, there is support in published literature for a relationship between CPN and osteodystrophy. According to Greaves [2000, pg 166], renal osteodystrophy refers to microscopic changes in bone that "....accompany chronic renal failure" and that include "...osteitis fibrosa, hyperosteoidosis, osteosclerosis and osteoporotic-like changes". Leininger and Riley [Leininger JR, Riley MGI. Chapter 14: Bones, Joints, and Synovia. In: Boorman GA *et al. Pathology of the Fischer Rat.* Academic Press, Inc., San Diego, CA, 1990] also noted that fibrous osteodystrophy is associated with hyperparathyroidism and spontaneous chronic renal disease. Boorman *et al.* [1990] described the pathogenesis of osteodystrophy associated with renal

disease as "complex". According to these authors, the process "probably begins with hyperphosphatemia due to impaired renal excretion of phosphorus. This results in a relative hypocalcemia, which in turn leads to increased secretion of parathyroid hormone. Parathormone acts on osteocytes and osteoclasts to stimulate the resorption of calcium to maintain a proper blood calcium/phosphorus ratio." If renal activation of vitamin D is impaired, then absorption of Ca from the gut is decreased and the resulting hypocalcemia may further stimulate parathyroid hormone [PTH] secretion. If renal metabolism of PTH is also impaired, then this exacerbates the resorption of Ca from bone. Although risperidone i.m. depot does not appear to exacerbate CPN, there were clearly alterations in Ca and P_i balance, i.e., serum Ca and P_i were significantly increased in male and female rats. The effect on serum P_i was the most consistent effect, being increased in both the 1-yr and 2-yr studies in rat at both doses in males and females. According to Duncan *et al.* [Duncan JR *et al. Veterinary Laboratory Medicine*, 3rd Ed, Iowa State University Press, Ames, Iowa, 1994], renal failure and osteolytic lesions of bone are conditions that may underlie elevations in both serum Ca and P_i. No drug-related microscopic findings were detected in the parathyroid in either males or females in the 1-yr study. In the 2-yr study, focal hyperplasia was noted in males and females; however, the effect was not statistically significant in either sex [M: 6/50, 4/49, 4/47, and 8/49 in SC, VC, LD, and HD, respectively; F: 0/49, 1/45, 4/47, and 4/50 SC, VC, LD, and HD, respectively].

Although the data do not clearly support the role of exacerbation of CPN in the etiology of osteodystrophy, there is the possibility that these findings are secondary to an elevation of serum prolactin alone, with no involvement of CPN. There have been a number of published studies investigating the possible effect of hyperprolactinemia on bone mineral density. Ataya *et al.* [Ataya K *et al. Fertil Steril* 50:876-881, 1988] were, by their admission, the first published study to demonstrate a decrease in bone density in patients on neuroleptic therapy. Serum prolactin was markedly elevated in all 10 patients; however, bone density was not correlated with serum prolactin. Keely *et al.* [Keely EJ *et al. Endocr Pract* 3:209-213, 1997] reported a significant decrease in bone mineral density in 3 [lumbar spine, femoral neck, and trochanter] of 4 site sampled in male schizophrenic patients on long-term neuroleptic treatment. It was notable that serum prolactin was elevated, but values above the normal range occurred only in 3 of the 16 patients. Both Ataya *et al.* [1988] and Keely *et al.* [1997] cite previous studies suggesting an association between hyperprolactinemia and reduced bone density. For example, Keely *et al.* [1997] noted that "Neuroleptic use has been associated with a twofold increase in the risk of hip fracture in both sexes [Ray WA *et al. N Engl J Med* 316:363-369, 1987].." and discussed studies that have reported that this effect may be due to either to increased serum prolactin (or other hormone) levels or to a direct effect of neuroleptics on bone [e.g., directly on cellular components or through second messenger systems]. Greenspan *et al.*[Greenspan SL *et al. Ann Int Med* 104:777-782, 1986] reported a decrease in bone density in men with chronic hyperprolactinemia caused by prolactin-secreting pituitary adenomas. These studies certainly do not represent a thorough review of the literature; however, they do suggest a possible role for serum prolactin in bone mineral status in humans. They also suggest that the osteodystrophy observed with risperidone i.m. depot, even if secondary to elevated serum prolactin, may be relevant to human risk. A study conducted in prolactin receptor knockout mice [Clement-Lacroix P *et al. Endocrinology* 140:96-105, 1999] demonstrated that the "...absence of PRL receptors leads to a decrease in bone formation rate...". This study, of course, does not address the issue of hyperprolactinemia; however, it does provide evidence that bone formation may [at some stage(s) of development] be prolactin-responsive.

Regarding the thyroid findings, the sponsor stated that "The reduction in focal and diffuse C-cell hyperplasia in the 40 mg/kg dosed male and female groups is considered secondary to the exacerbated chronic renal pathological changes and is in accordance with the disturbed 'Ca^{2+}' balance and the osteodystrophy". Focal [nodular] and diffuse C-cell hyperplasia is a spontaneous, age-related finding in rat [Capen CC. Thyroid and Parathyroid Toxicology. In: Harvey PW *et al* (Eds), *Endocrine and Hormonal Toxicology*, John Wiley & Sons, Ltd, West Sussex, England, 1999, pgs 52-56, Capen CC *et al.*

Chapter 21: Endocrine System. In: Haschek WM, Rousseaux CG. *Handbook of Toxicologic Pathology*, Academic Press, Inc., San Diego, CA, 1991, pgs 705-711.]. Increases in C-cell hypertrophy and hyperplasia have been associated with prolonged hypercalcemia [e.g., high-calcium diet] [*ibid*]. Serum Ca levels were increased with risperidone i.m. depot; therefore, it is difficult to explain the decrease in C-cell hyperplasia on the basis of altered Ca balance.

This reviewer has no information on a possible direct relationship between serum prolactin and the thyroid C-cell findings and the renal tumors.

Adrenal gland: according to the carcinogenicity study report, the non-neoplastic [i.e., focal medullary hyperplasia] and neoplastic [i.e., benign pheochromocytoma] lesions observed in adrenal gland were considered to be a result of D_2 antagonism-induced hyperprolactinemia, with the possibility that CRD [and associated increases in serum Ca] may have been contributory. According to the Pharmaco-Toxicological Expert Report on Risperidone depot microspheres, the increase in serum prolactin resulted in an "acceleration of chronic nephropathy". Therefore, the sponsor has concluded that the adrenal gland findings were the result of increases in serum prolactin, either directly and/or indirectly via exacerbation of CRD. The lack of data to support an increase in incidence or severity of CRD in males with risperidone i.m. depot has been discussed previously. The possibility that hyperprolactinemia may be associated with an increased incidence of adrenomedullary non-neoplastic and neoplastic changes has been suggested in a number of published articles [Tischler AS *et al. Int J Devl Neuroscience* 7(5):439-448, 1989; Tucker MJ. The Adrenal Medulla as a Toxicological Target Organ. In Harvey PW (Ed), *The Adrenal in Toxicology*, pgs 165-178, London:Taylor & Francis, 1990; Rosol TJ *et al. Toxicologic Pathology* 29(1):41-48, 2001]. However, Tischler *et al.* [1989] stated that any involvement of serum prolactin on adrenomedullary tumors is "...likely to be indirect...because the normal rat adrenal medulla has no demonstrable prolactin receptors..." and that they "...have also been unable to demonstrate prolactin binding to PC12 pheochromocytoma cells (M. Kaplan and A.S. Tischler, unpublished data), which are representative of spontaneously occurring and drug-induced adrenal medullary tumors in many respects..." Tischler *et al.* [1989] also noted that pituitary factors other than serum prolactin [e.g., acidic fibroblast growth factor] may underlie an association between the pituitary gland and adrenomedullary proliferation.

Adrenomedullary pheochromocytomas are common spontaneous and drug-induced tumors in rats. The prevalence of spontaneous pheochromocytomas is, to some extent, genetic as evidenced by notable differences among strains; the incidence is particularly high in Wistar rats [Tischler AS *et al. Fundam Appl Toxicol* 35:216-220, 1997]. [In contrast, pheochromocytomas are relatively rare in mouse (*ibid.*) and other animals species and human (Nyska *et al. Toxicologic Pathology* 27(4):456-462).] In addition, the incidence is greater in male than in female rats, and spontaneous pheochromocytomas are rarely observed at <1 yr of age. Tischler *et al.* [1997] noted that drug-induced adrenomedullary tumors appear to represent an exacerbation of the spontaneous incidence rate resulting from stimulation of chromoffin cell proliferation. With i.m. depot risperidone, the incidence of pheochromocytoma was somewhat consistent with this type of process in that it was primarily males that were affected and that the tumors were late-occurring. [In the 1-yr study conducted concurrently with the 2-yr carcinogenicity study, no drug-related adrenomedullary findings (including tumors) were detected.]

Whether or not there was an adrenal gland effect in females is not as clear as in males. The sponsor considered there to be a positive trend in pheochromocytomas in females. According to the FDA's analysis, there was a positive trend only when compared to SC and only if pheochromocytoma is considered a rare tumor. As noted previously, this tumor type is rare based on the sponsor's HC and concurrent control data. The ExeCAC did not consider pheochromocytomas to be a drug-related finding in females. However, the incidence in HDF did exceed the sponsor's HC range. Considering the significant finding in males, it is difficult to ignore the finding in females. Also, preneoplastic changes

(i.e., focal medullary hyperplasia) were significantly increased in LDF (compared to VC, but not SC) and tended to be increased in HDF. The sponsor postulated that hypercalcemia secondary to an exacerbation of CRD may have had a role in the formation of adrenal tumors. In males, as noted previously, there was no compelling evidence of a drug-related exacerbation of CRD. In females, the incidence (but not severity) of CRD was significantly increased at both doses, and serum Ca was elevated at the HD. However, "chronic disease" was not listed as a finding for any of the 3 HDF with pheochromocytoma.

One possibility not discussed by the sponsor is that risperidone may have had a direct or an indirect effect on the adrenal gland without involvement of prolactin. Several published studies have demonstrated effects of dopaminergic agents on the function of the adrenal gland. For example, Fuller and Snoddy [Fuller RW, Snoddy HD. *Neuropharmacology* 23(12A):1389-1394, 1984] demonstrated that the dopamine agonist, pergolide, elevated serum corticosterone in rats, an effect that was antagonized by a number of D_2 receptor antagonists. Similarly, Borowsky and Kuhn [Borowsky B, Kuhn CM. *Neuropharmacology* 31(7):671-678, 1992] reported that the dopamine D_2 agonist, quinpirole, elevated ACTH and corticosterone in rat, an effect that was antagonized by sulpride, a D_2 antagonist. These results presumably reflect a direct or indirect effect of dopamine agonists and antagonists on the adrenal cortex. However, Pupilli *et al.* [Pupilli C *et al. J Clin Endocrinol Metab* 79(1):56-61, 1994] demonstrated the presence of D_2 receptors in the adrenal medulla and in pheochromocytomas, and Mannelli *et al.* [Mannelli M *et al. Am J Hypertens* 3(6 Pt 2):22S-24S, 1994] detected D_2 receptors in human pheochromocytoma cells; what, if any, function these receptors have in the adrenal gland is unclear. These data do raise the possibility of a prolactin-independent D_2 receptor-modulated effect on the adrenal gland.

One additional consideration is the potential importance of drug-induced adrenomedullary tumors in rodent in an assessment of human risk. According to Greaves [Greaves P *Histopathology of Preclinical Toxicity Studies*, 2nd Ed. Amsterdam: Elsevier], an increase in adrenal pheochromocytomas in rats may have little or no relevance to humans due to (a) the increased sensitivity of the male rat, (b) the lack of a direct human counterpart, and (c) the ubiquitous nature of the agents inducing pheochromocytomas, e.g., lactose. Others have suggested that pheochromocytomas in rat and human are fundamentally different; however, according to Tucker [1990], more recent studies argue against this position. For example, Tischler and DeLellis [1988] pointed out that it is "A common misconception...that rat adrenal medullary nodules differ significantly from human pheochromocytomas in that the latter are almost invariably associated with clinical or biochemical evidence of excess catecholamine secretion and/or hypertension...in a report from the Mayo Clinic in which a 50-year autopsy experience with pheochromocytoma was evaluated...76% of the tumors were clinically unsuspected". They further noted that "The major morphological distinctions between rat adrenal medullary nodules and human pheochromocytomas are the consistently small size and sparse distribution of secretory granules in the former. However, human pheochromocytomas exhibit enormous variability in size and number of secretory granules. Sparsely granulated cells occurring in human pheochromocytomas that contain relatively small amounts of stored catecholamines very closely resemble the cells in rat adrenal medullary nodules..." One difference noted between rat and human was "...the clinical context in which the lesions arise", i.e., either as solitary (usually unilateral) tumors or as part of a "multiple endocrine neoplasia syndrome" [MEN]. Tischler and DeLellis [1988] pointed out that >90% of human pheochromocytomas are solitary tumors, whereas in the rat, adrenomedullary tumors usually arise as part of a MEN syndrome. They noted that "The mechanisms stimulating the formation of adrenal medullary nodules in such rats [with MEN syndrome] are likely to be different from those in almost all human patients". In the 2-yr rat study, 6 of the 12 HDM with pheochromocytoma had no other tumors. Of the 3 HDF with pheochromocytoma, all 3 had additional tumors [#749: pituitary adenoma, thyroid C-cell adenoma, hepatocellular adenoma; #759: pancreatic islet cell adenoma, pituitary adenoma, thyroid C-cell adenoma; #764: mammary gland tumor, pancreatic islet cell adenoma, hepatocellular adenoma]. This

would suggest that the adrenomedullary tumors in female rats may have less relevance to humans than those in male rats.

High-dose toxicity: the ExeCAC concluded that the HD may have exceeded the MTD in males based on body wt effects and clinical signs. [The greater effect in HDM compared to HDF cannot be explained on the basis of greater plasma exposure since AUCs were higher in females.] Concern was expressed by the ExeCAC that the renal and adrenal tumors observed in HDM may have been secondary to general toxicity and may, therefore, be of less importance to human risk. The body wt effect in HDM did exceed the 10% decrease (compared to CM) that is considered prospectively to be a maximally acceptable effect; however, the primary concern related to excessive effects on body wt is not an induction of tumors, but the possibility of a decrease in sensitivity of the animals to induced tumors. As for clinical signs [summarized in the following table, along with individual incidence of adrenal pheochromocytoma and renal tumors], only in 2 of the 16 HDM that exhibited one or both tumors was there clear evidence of generalized excessive toxicity. Therefore, there would seem to be no basis for dismissing the adrenal and renal tumor findings in males simply on the basis of the body wt effect and the clinical signs observed at the HD.

HDM #	TUMORS		CLIN SIGNS	FATE*
	ADRENAL	KIDNEY		
322	x		ptosis [Wks 0, 2, 44] sedation, slight [Wks 2, 4, 10, 36] sedation, severe [Wk 0]	TS
330	x		ptosis [Wks 0, 2, 8, 14, 26, 28, 42, 50] sedation, slight [Wks 2, 4, 10-11, 14, 16, 20, 32, 36] sedation, severe [Wk 0]	TS
332	x		chromodacryorrhea [Wks 19-25, 81-82, 90-91, 102-103] ptosis [Wks 0, 2, 46, 48, 89-105] sedation, slight [Wks 2, 4, 10, 32, 38, 44, 46, 58, 64] sedation, severe [Wk 0]	TS
336		x	ptosis [Wks 0, 2, 8, 10, 12, 16, 24, 30, 44, 50, 52] sedation, slight [Wks 2, 4, 8, 10-11, 12, 14, 24, 28, 44] sedation, severe [Wk 0]	TS
339		x	rough haircoat [Wk 81-87] ptosis [Wks 0, 2, 8, 12, 14, 30, 32, 36, 40, 42, 48, 50, 60, 62, 66, 99-104] sedation, slight [Wks 2, 4, 8, 10, 14, 22, 34, 36, 40, 44, 46, 48, 54] sedation, severe [Wk 0]	TS
345	x	x	rough haircoat [Wk 105] ptosis [Wks 0, 2, 103-104] sedation, slight [Wks 2, 4, 10] sedation, severe [Wk 0]	TS
351	x		ptosis [Wks 0, 2, 6, 8, 10, 12, 15, 22-23, 28, 30, 34, 40, 48, 50, 52] sedation, slight [Wks 2, 4, 6-7, 8, 10-11, 12, 15, 16, 22-23, 28, 30, 44, 52, 64] sedation, severe [Wk 0]	TS
353	x		rough haircoat [Wks 91-97] ptosis [Wk 0, 2, 8, 12, 14, 22, 28, 32, 42, 48, 50] hindlimb paralysis [Wk 91-97] swollen paws [Wk 97] sedation, slight [Wks 2, 4, 6-7, 10-11, 12, 14, 24, 28, 32, 44] sedation, severe [Wk 0]	SM [Wk 97]
354	x		ptosis [Wks 0, 2, 6, 8, 10, 12, 14, 28, 40, 50, 54] sedation, slight [Wks 2, 4, 6-7, 8, 10-11, 12, 18, 20, 36, 38, 40, 54] sedation, severe [Wk 0]	TS
355	x		ptosis [Wks 0, 2, 8] sedation, slight [Wks 2, 4, 8] sedation, severe [Wk 0]	TS
356	x		rough haircoat [Wk 106] ptosis [Wks 0, 2, 8, 10, 12, 14, 22, 30, 36, 42, 46, 48, 50] sedation, slight [Wks 2, 4, 8, 10, 12, 14-15, 20, 22, 30, 32, 36, 42, 46, 48, 58] sedation, severe [Wk 0]	TS
360	x		bad condition [Wk 94] thin animal [Wk 94] ptosis [Wks 0, 2, 8, 10, 30, 50, 52, 60, 62, 91-93] hind-leg paralysis [Wk 94] long nails [Wk 94] sedation, slight [Wks 2, 4, 8, 10, 34, 44, 52, 60, 62] sedation, severe [Wk 0]	SM [Wk 94]
361		x	rough haircoat [Wk 106] ptosis [Wks 0, 2, 8] sedation, slight [Wks 2, 4, 8] sedation, severe [Wk 0]	TS
366		x	ptosis [Wks 0, 2, 6, 8, 10, 42, 50] sedation, slight [Wks 2, 4, 6, 8, 10, 42, 60] sedation, severe [Wk 0]	TS
369	x		ptosis [Wks 0, 2, 8, 30, 50, 101-103] sedation, slight [Wks 2, 4, 10] sedation, severe [Wk 0]	TS
370	x		ptosis [Wks 0, 2, 6, 8, 22, 40, 48] sedation, slight [Wks 2, 4, 6-7, 8, 10-11, 16, 18, 24, 44, 46, 60] sedation, severe [Wk 0]	TS

Comparisons to other antipsychotic drugs and to oral risperidone: as noted previously, tumors of the mammary gland, pituitary gland, and pancreas have been reported in carcinogenicity studies conducted on other antipsychotic drugs which elevate serum prolactin. Adrenal and renal tumors were not observed with olanzapine, sertindole, ziprasidone, or seroquel, or in the 2-yr rat carcinogenicity study with oral risperidone.

Tischler and DeLellis [Tischler AS, DeLellis RA. *J Am Coll Toxicol* 7(1):23-44, 1988] listed neuroleptics as one class of agents associated with rat adrenomedullary tumors. However, they stated that "The specific neuroleptics that produce adrenal medullary lesions have not been disclosed". The suggestion of a link between adrenomedullary tumors and elevations in serum prolactin was not supported by the information available to the reviewer. One non-neoplastic finding of concern, osteodystrophy, is also not a common findings in nonclinical studies of antipsychotic drugs and was not observed with oral risperidone.

The sponsor addressed the difference in the tumor profiles obtained with oral and i.m. depot risperidone in special toxicity studies. According to the sponsor's data, it would not appear that the difference in the profiles can be explained by differential effects on serum prolactin. As discussed in the "Special Toxicology" section of this review, in a 7-wk mechanistic study the AUC for serum prolactin was higher following administration of risperidone at the HD used in the oral carcinogenicity study than following administration of risperidone at the HD used in the i.m. depot carcinogenicity study. Also, at no time during the 24-hr measurement period did the mean serum prolactin level with oral risperidone fall below that with i.m. depot risperidone.

Although it is the additional target organs that are of greatest concern, it is interesting to note that the effects on mammary gland, pituitary gland, and pancreas also differed between the oral and i.m. depot studies. The rat tumor data for oral risperidone are summarized in the table below:

TISSUE	FINDING	MALES				FEMALES			
		C	LD	MD	HD	C	LD	MD	HD
mammary gland	adenocarcinoma	0/50	0/48	3/50	13/49	3/49	14/50	16/50	13/50
	adenoma, adenofibroma, fibroadenoma	0/50	3/48	4/50	3/49	20/49	21/50	27/50	15/50
	carcinoma	0/50	0/48	0/50	2/49	--	--	--	--
pancreas	islet cell adenoma	9/49	9/49	14/49	14/49	3/50	4/50	4/50	3/50

There are several differences between the i.m. depot and oral studies that might explain the difference in tumor profile and other toxicities [i.e., osteodystrophy] between the two studies. Most obvious is the difference in route. Another may be a difference in plasma exposure. Good estimates of plasma exposure were not available from the oral 1-yr chronic toxicity or the 2-yr carcinogenicity studies in rat. The sponsor conducted an acute-dose study comparing plasma exposure in Wiga and Hannover Wistar rats. The Wiga substrain was used in the oral 2-yr study, whereas the Hannover substrain was used in the i.m. depot 2-yr study. Following an oral dose [0.63 mg/kg], plasma exposure to risperidone and 9-OH-risperidone was higher in the Wiga Wistar rat. The difference in the ratio of 9-OH-risperidone to risperidone indicated more extensive metabolism in the Hannover substrain. The sponsor also collected TK data in the 7-wk mechanistic study, comparing i.m. depot and oral administration in Hannover Wistar rats. Following administration of the HDs used in the oral and the i.m. depot 2-yr studies, the plasma AUC for the "active moiety" were similar; however, the ratio of 9-OH-risperidone to risperidone was higher following dietary administration, consistent with more extensive first pass metabolism. Therefore,

it would appear that differences in plasma exposure to the "active moiety" cannot explain the differences in tumor profile, since one would assume that more findings could be expected with higher plasma exposure. However, the difference in the 9-OH-risperidone-to-risperidone ratio between the two routes of administration might be a factor.

Conclusion: neoplastic findings with i.m. depot risperidone included those observed with [e.g., mammary gland, pituitary, pancreas] and those not routinely observed with other antipsychotic drugs or in the 2-yr carcinogenicity study of oral risperidone [i.e., renal and adrenomedullary tumors]. In addition, osteodystrophy, also observed in the 1-yr chronic toxicity study in rat, was a finding not routinely observed with other antipsychotic drugs or with oral risperidone. The sponsor attributed these findings to species-specific effects of hyperprolactinemia. The data did not convincing support the mechanisms proposed. They did, however, support the conclusion that the tumor findings were epigenetic, implying a threshold. The renal and adrenal tumors and the osteodystrophy were observed only at the HD. The plasma AUCs for risperidone, 9-OH-risperidone, and the "active moiety" were only 0.2-0.3 times the anticipated AUC at the maximum recommended dose of 50 mg. Therefore, there is no safety margin between the effect-dose in rat and the maximum recommended clinical dose.

Labeling Recommendations: the sponsor proposed the following labeling:

"Carcinogenesis

The following revisions to the sponsor's labeling are recommended:

"Carcinogenesis

[redacted]

Addendum/appendix listing:

CAC report

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VII. REPRODUCTIVE AND DEVELOPMENTAL TOXICOLOGY

The sponsor did not conduct reproductive and developmental toxicology studies with the i.m. depot formulation. The data from the battery of reproductive studies conducted on oral risperidone are being used to support the safety of the new formulation. The sponsor also briefly discussed Segment II studies (rat, rabbit) of PLGA-containing suture material submitted in NDA 17-472 [1972]. According to the sponsor, no teratogenic effects were observed following implantation of Vicryl® suture [doses up to 1000 mg/kg] into "...female rats 23±3 days prior to mating, so as to obtain maximum systemic levels of absorbed suture materials during days 6 to 15 of pregnancy". In the rabbit study, similar doses of the suture produced no maternal toxicity or teratogenic effects. [The study reports were not available for this review.] It was also noted that the microspheres were tested in a formulation of tetanus toxoid. According to the published article [Chandrasekaran R *et al.*, *Human Exp Tox* 15:349-351, 1996], the tetanus toxoid-microsphere formulation was administered i.m. to pregnant Wistar rats on Days 5, 8, 11, and 14 of gestation. The tetanus toxoid [TT] was administered at doses up to 10 time the human equivalent dose; however, the authors did not specify the amount of microspheres either in the polymer control or included in the various doses of TT. No adverse effects on dams or fetuses were reported. However, this study cannot be considered an adequate assessment of the effects of the microspheres on embryofetal development since animals were dosed only on selected days of gestation and only one dose level [that dose level was not specified] of microspheres was used. There is no indication that the microspheres have been tested during stages of reproduction other than embryofetal development. [The sponsor referenced two study reports for Segment II studies (rat, rabbit) submitted to NDA 17-472 [for XLG sutures]. However, even though it was noted that these report could be found in "the Literature volume(s)", no volume or page number was given and no such volume(s) could be found.]

A review of the i.m. depot chronic toxicity and 2-yr carcinogenicity studies in rat indicated that the incidence of osteodystrophy was significantly and markedly elevated in males and females at 40 mg/kg. The exact nature of the osteodystrophy was not clear. The severity ranged from 1-3 on a scale of 1-5 and did not appear to increase with duration of dosing [from 1-2 yrs] in rat. However, this finding was not observed in the nonclinical studies of oral risperidone. There is a concern that this finding may have implications for the effects of risperidone i.m. depot on the developmental process, particularly considering that the plasma exposure to risperidone, 9-OH-risperidone, and the active moiety at the no-effect dose [i.e., 5 mg/kg] was only 0.2-0.3 times the expected exposure at the maximum recommended clinical dose. In addition, the fact that some toxicities [osteodystrophy, renal and adrenomedullary tumors] were observed with the i.m. depot but not with the oral formulation raises the concern that differential reproductive toxicities may be observed with the i.m. depot formulation. Therefore, it is recommended that an embryo-fetal development study be conducted in rat using the i.m. depot formulation prior to approval.

Labeling recommendations: the sponsor proposed the following labeling:

"Impairment of Fertility

Pregnancy
Pregnancy Category C

The sponsor's proposed labeling does not reflect the revisions recommended by the Division following review of a cross-fostering study [cf. required as a Phase 4 commitment [N20-272]. The following recommended revisions to the sponsor's proposed labeling reflect those and additional revisions:

Impairment of Fertility

Pregnancy
Pregnancy Category C

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VIII. SPECIAL TOXICOLOGY STUDIES

1. Study title: Single dose tolerance study in albino rabbits [Exp No. 3150, Conducting laboratory and location: Janssen Research Foundation, Belgium, Date of study initiation: 10/3/95, **non-GLP**, QA report: N]

Purpose: to assess the dermal irritant and/or corrosive effects of risperidone.

Drug, lot #, and % purity: risperidone, batch no. ZR064766PUA051, purity = [redacted]
Formulation/vehicle: patch/propylene glycol + 5% oleic acid

Methods: risperidone was administered dermally to albino rabbits [redacted] as 24-, 48-, and 72-hr patches [1/sex/grp]. Each rabbit received a drug patch [1 mL/patch; 2.9 mg/kg] and a vehicle patch. Patches were applied to shaved areas of the dorsal trunk [one L, one R] and covered with plastic to prevent evaporation. Observations consisted of the following: clinical signs, dermal irritation [erythema, edema (ratings of 0-4 for each), "Attendant dermal reactions" (i.e., atonia, desquamation, fissures, necrosis, coriaceous skin, thickened skin, blanching, rough haircoat, little hair-growth/baldness, wrinkled skin, pustulae, crust formation; scores: (-) = absent to (+++) = severe present)], body wt, TK.

Results: there was no evidence of drug-induced irritation. The dose used produced no clinical signs or effects on body wt.

2. Study title: One month subcutaneous tolerance study in beagle dogs [Exp No. 3711, Conducting laboratory and location: Janssen Research Foundation, Belgium, Date of study initiation: 8/95, **non-GLP**, QA report: N]

Methods: this study, conducted in Beagle dogs (1/sex/grp), tested a number of different formulations of risperidone, including a risperidone-microsphere suspension and two formulations of haloperidol. Only a single dose of each formulation was administered [route: s.c.]. The risperidone-microsphere formulation [nos] was administered as a single 2.5 mg/kg dose. Observations consisted of clinical signs and microscopic examination of injection sites.

Results: palpable nodules were detected in both dogs at the injection site. It was noted that "The appearance of this local reaction most likely coincided with the time of release of risperidone from the microspheres; the nodule was detected on Day 10 in the M and on Day 25 in the female. Microscopic findings [conducted only in 1 of the 2 dogs] at the injection site consisted of the following: extravasation rbcs, granulomatous inflammation, necrotic collagen bundles, fibrinous exudate, granulocytic infiltration, lymphocytic infiltration, amorph material present, abscess formation, and giant cells. The overall score was 4 on a scale of 0-5 [1 = slight, 5 = severe].

3. Study title: One month intramuscular tolerance study in beagle dogs [Exp No. 3726, Conducting laboratory and location: Janssen Research Foundation, Belgium, Date of study initiation: 9/95, **non-GLP**, QA report: N]

Methods: risperidone was administered i.m. either as a pamoate suspension or as a microsphere suspension at a single dose of 2.5 mg/kg. Observations consisted of clinical signs, hematology, and clinical chemistry.

Results: no drug-related clinical signs, changes at the injection site, or effects on hematology parameters were noted. Creatine kinase was increased 4 hrs following injection of the risperidone-microsphere formulation [76% compared to C], and again during Wks 1 and 2 [76-96%].

4. Study title: Single dose intramuscular tolerance study in the beagle dogs [Study No. EDMS-BEBE-2651343, Exp No. 4891, Conducting laboratory and location: not specified, Date of study initiation: not specified, **non-GLP**, QA report: N]

Methods: risperidone was administered to Beagle dogs [2/sex/grp] i.m. as three different solutions [2 mL/site]. Injections were delivered i.m., alternating sites at each dosing time for 4 weeks [one dose/wk]. Three different diluents were tested. The compositions of these diluents were as follows:

95L07/F65 (F65/95) and 98D15/F65 (F65/98): sodium camellose , Polysorbate 20 , , NaOH (water. F65/95 [A] was prepared on 12/7/95; F65/98 [B] was prepared on 4/15/98.

98E25/F101: Polysorbate 20 (, sodium carboxymethylcellulose , sodium dihydrogen phosphate monohydrate (, disodium hydrogen phosphate anhydrous (, citric acid , NaCl , NaOH ( ), water. This diluent [D] was prepared on 5/25/98.

Controls received NaCl [C]. Observations consisted of clinical signs and examination of the injection sites [ultrasonography, gross pathology, histopathology]. The experimental design (including dosing regimen) was poorly described. From the table notes, it would appear that each dog received all formulations and C injections, but in a different order [depending upon the group].

Results: there were no clinical signs related to drug. At the injection site, the primary findings observed following administration of C, B, or D was slight red spots on the skin. Following administration of A, however, the following were noted (only for 24 hrs postdosing): slight red spts [6/16], moderate red spots [1/16], slight scratching [2/16], moderate scratching [2/16], slight skin lesions [2/16], slight swelling of the head [2/16], moderate swelling of the head [4/16], and slight swelling of the paws [5/16]. The sponsor noted that the polysorbate 20 component {only) in formulation A resulted in an acute anaphylactic reaction. Ultrasonography indicated some acute reaction to all risperidone formulations; however, the reaction tended to be greater with formulation A and B. There was no difference between reactions to A and B as detected by ultrasonography. No effects were noted in gross examination of the injection site. There were also no significant differences in the incidence or severity of microscopic findings.

Mechanistic studies to support carcinogenicity findings

1. **Single dose oral mechanistic study in the rat** [Study No. EDMS-BEBE-3095114, Exp. No. 5441, Conducting laboratory and location: Janssen Pharmaceutica N.V., Belgium,

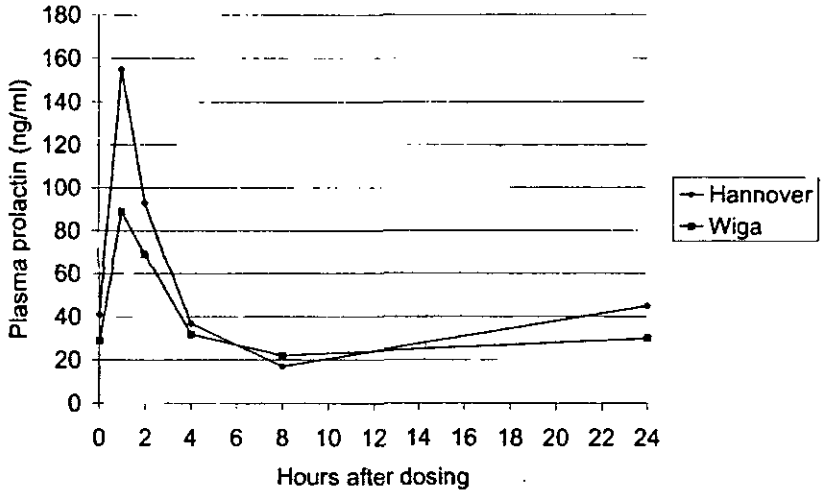
Methods: this study was conducted in male SPF Wistar Hannover and Wiga rats [initial age: ≈12 wks]. Risperidone [batch no. ZR064766PUA111, conversion factor: 1.0] was administered orally via gavage [aqueous solution] as a single dose [0.63 mg/kg; 0.063 mg/mL; 1 mL/100 gm]. [However, the text table under Section 2.2.4 listed the dose as 0.16 mg/kg.] The sponsor stated that the oral route was used since "The oral route is a route of human exposure to the test article". Animals were fasted from 14 hrs predosing to 4 hrs postdosing. Blood samples were collected at 0, 1, 2, 4, 8, and 24 hrs [7/grp/time point] for quantitation of serum prolactin, risperidone, and 9-hydroxyrisperidone. [The TK data were provided in a separate report.] Serum prolactin was quantitated using a rat-specific RIA.

Results: serum prolactin was elevated in both rat strains. The data [units = ng/mL] are summarized in the following table:

SAMPLING TIME (hr)						$AUC_{(0\text{-}24\ hr)}$ (ng•hr/mL)
0	1	2	4	8	24	
Wistar HANNOVER						
41	155*	93	37	17	45	950 ± 88**
Wistar WIGA						
29	89	69	32	22	30	761 ± 66

*p = 0.002 compared to WIGA at 1 hr; **mean ± SEM

The data were illustrated in the following sponsor's figure:



Serum prolactin was higher in Wistar Hannover rats, but the effect was significant only at the 1-hr sampling time. $AUC_{(0\text{-}24\ hr)}$ was not significantly different between strains, but AUC(0-4 hr) was significantly greater in the Hannover strain.

2. **Seven-week mechanistic study in the rat** [Study No. EDMS-PSDB-1677701, Exp. No. 5459, Conducting laboratory and location: Johnson & Johnson Pharmaceutical, Belgium, study initiation date: 9/25/01, GLP, QA:Y] **[Individual data were not provided (except for some histopathology), but were noted to be "available upon request.]**

Purpose: to provide data to explain differences in tumor profile observed in the oral [conducted in Wistar Wiga] and the i.m. depot [conducted in Wistar Hannover] carcinogenicity studies in rat. The HDs used in those studies were 10 mg/kg [oral] and 40 mg/kg [i.m.].

Methods: risperidone [batch no. ZR064766PUA401 (dietary), ZR064766E1A701 (microspheres)] was administered to male Wistar Hannover rats either as a drug-diet admixture [10 mg/kg; continuous access] or an i.m. microsphere depot [40 mg/kg; 4 injections given bi-weekly starting on Day 0] for 7 wks. [There were no control grps.] Drug concentration ——— of intended] and homogeneity ——— were documented in the drug-diet admixture. The concentration of drug in the i.m. depot formulation was slightly higher than intended ———. Observations consisted of the following: mortality, clinical signs [daily], body wt/body wt gain [baseline, weekly, at end of study], food consumption [baseline, weekly, at end of study], hematology ["towards the end of the study"; wbc (ct, differential), hct, hgb, rbc ct, reticulocytes, thrombocytes, normoblasts, MCV MCH, MCHC], clinical chemistry ["towards the end of the study"; Na, K, Cl, Ca, P_i, total protein, albumin, glucose, cholesterol, TG, PL, BUN, creatinine, total bilirubin, alkaline phoshatase, AST, ALT], urinalysis [over a 12-hr period during Wks 2 and 6; specific gravity, volume, pH, glucose, ketones, urobilinogen, bilirubin, occult blood, proteins, microscopic analysis of sediment, Na, K, Cl, Ca IPH (phosphate), glucose, urea N, creatinine, creatinine clearance ((urinary creatinine x urinary volume)x 1000/serum creatinine x body wt x collection time),

hormone analysis [serum prolactin; using rat-specific RIA], TK [data provided in separate report], and terminal studies [gross pathology, organ wts, histopathology]. Animals were sacrificed in grps of 7/grp at each of the following sampling times: 0 (morning), 4, 8, 12, 16, 20, and 24 hr. It was noted that "Rats of both groups were killed alternated (as much as possibly on the same time of day) to balance for diurnal variations". Gross pathology was characterized as "limited", involving macroscopic examination of adrenal glands, kidneys, mammary gland, pituitary gland, prostate, seminal vesicles, pancreas, spleen and coagulating gland. The wts of spleen, adrenal glands, and pancreas were recorded. The following tissues were examined microscopically in all animals: coagulating glands, kidneys, mammary gland, pituitary gland (pars distalis, pars intermedia), prostate (dorsolateral, ventral lobes), seminal vesicles. For microscopic examination, tissues were preserved in 10% buffered formalin and, within three months of necropsy, embedded, then sectioned and stained with H & E.

[The sponsor noted that the study was conducted under GLP, except for "...determination of the necessary amount of solvent to be added to the 75 mg-vials of Risperidone depot."]

Results: there were no unscheduled deaths during the study. Clinical signs were greater in rats dosed via the diet [Grp A] as noted in the following sponsor's table:

Males		
Dosage Group (mg/kg):	Group A Food:10 X/N	Group B IM.40 X/N
Ptosis	10 / 49	0 / 49 **
Eye damage due to blood collection	1 / 49	1 / 49
Waste of food	5 / 49	6 / 49
Malformed incisors	1 / 49	0 / 49
Sedation	3 / 49	0 / 49

Significance level computed with Fisher Exact probability test (two tailed): * p < .05 ** p < .01 *** p < .001
(Significance computed versus the Group A dosage group)
X: Number of positive animals N: Total number of animals
Food:10 -> Dietary:10 mg/kg IM:40 -> Depot IM:40 mg/kg

The sedation and ptosis were observed only in Grp A animals. The sedation was characterized as "slight" and was noted only on Day 2. Ptosis was observed during the 1st wk of dosing. Mean body wt was significantly higher [3-9%] in the i.m. depot grp [Grp B] during Wks 1-5 of dosing, but were similar between grps during the last 2 wks. Food consumption was significantly higher in Grp B animals throughout most of the dosing period [3-18%], except for the last wk during which it was 15% lower in Grp B than in Grp A. Overall food intake was only 4% higher in Grp B. Based on food intake data, the actual doses administered were estimated to be 9.25-12.8 mg/kg/day [overall mean: 10.6 mg/kg/day].

On hematology findings, the following were noted: (a) rbc parameters [hct, hgb, rbc ct] were slightly decreased and MCH and MCV were slightly increased [3-4%] in Grp A [1-5%] compared to Grp B, (b) wbc, lymphocyte, and reticulocyte cts were lower in Grp B [5, 8, and 37%, respectively]. The sponsor considered the differences in hct, hgb, and wbc ct (total, differential) to be "irrelevant". On clinical chemistry parameters, Na, Ca, P_i, PL, BUN, alkaline phosphatase, AST, and ALT were lower in Grp B [1, 2, 9, 8, 14, 12, 9, and 19%, respectively]; only glucose was higher in Grp B [4%]. The sponsor considered the differences in all but P_i and BUN to be "irrelevant"; the higher P_i in Grp A was considered secondary to transient body wt effect and the BUN was retained as a relevant finding since the same effect was observed in a previous i.m. depot study. On urinalysis parameters, findings consisted of the following: (a) specific gravity was lower [<1%] and pH [0.8 units]and urinary volume [50%] were reduced in Grp A during Wk 2. In addition, there was an increase in squamous epithelial cells [90%] in

Grp A. Occult blood was detected only in Grp B animal and triphosphate crystals were increased (4-fold; 300%] in Grp B. (b) during Wks 5 and 6, specific gravity [1-3%], protein [≈8-fold; 700%], and ketones [100-42%] were increased and pH [-1, -1.8 units] and volume [52-60%] were decreased in Grp B. (c) in terms of clearance, the following were noted [expressed as Grp B relative to Grp A]:

Wk 2: increases in K [13%], glucose [8%], creatinine [mg; 25%]
Wk 5: increases in P_i [380%], urea N [48%], glucose [20%] and decreases in Ca [50%]
Wk 6: increases in P_i [100%], urea N [24%] and decreases in Ca [17%], Na [52%], Cl [36%], K [24%], creatinine [mg; 31%], and creatinine clearance [33%]

No differences between grps were evident in spleen, pancreas, or adrenal wts. Macroscopic findings were summarized in the following sponsor's table:

Organ or tissue : observation	Males — Dosage group (mg/kg) GROUP A:Food:10 X/N	GROUP B:IM:40 X/N
Adrenal glands : pale	2/49	0/49
Eyes : lesion	1/49	1/49
Jaw : malformed incisors	1/49	0/49
Kidneys : pale	1/49	3/49
Liver : more pronounced lobulation	1/49	0/49
Lymph node(s),pancreatic : dark	1/49	0/49
Lymph node(s),pancreatic : swollen	1/49	0/49
Mammary gland : stimulation	22/49	1/49***
Prostate : stippled, yellow	22/49	9/49**
Seminal vesicles : small	1/49	0/49
Spleen : swollen	2/49	4/49
Thymus : hemorrhagic	0/49	1/49
Urinary bladder (content) : hemorrhagic	0/49	1/49
Urinary bladder (content) : plug, white	0/49	1/49

Significance computed by Chi-square test (two tailed) : * P < .05 ** P < .01 *** P < .001

The mammary gland effect was characterized as "slight".

Selected microscopic findings are summarized in the following table. The data are expressed as incidence and as "mean scores" [scores are based on severity ranging from 0 = minimal or small area change to 5 = severe or large area of change]. [$^{*}p<0.05$, $^{**}p<0.01$, $^{***}p<0.001$

TISSUE	FINDING	INCIDENCE		SCORE	
		GRP A	GRP B	GRP A	GRP B
coagulating gland	low epithelium	6/49	38/49***	0.12 (0.05)	0.78 (0.06)***
kidney	dilated tubuli (ctx), diffuse	4/49	0/49	0.08 (0.04)	0.00 (0.00)*
mammary gland	female appearance	47/48	46/48	1.44 (0.08)	1.67 (0.10)
	periductular fibrosis	8	32***	0.17 (0.05)	0.69 (0.07)***
	inspissated material	3/48	31/48***	0.06 (0.04)	0.69 (0.08)***
	secretion present	43/48	23/48***	1.46 (0.11)	0.63 (0.11)***
pituitary gland	hypertrophy/hyperplasia (pars intermedia)	28/48	24/47	0.58 (0.07)	0.53 (0.08)
prostate	dorsolateral lobe				
	acute/exudative inflammation	45/49	47/49	1.80 (0.13)	1.65 (0.11)
	dilated glands	11/49	45/49***	0.24 (0.07)	1.51 (0.11)***
	low epithelium	9/49	44/49***	0.18 (0.06)	0.90 (0.04)***
	ventral lobe				
	focal inflammation	1/49	9/49	0.02 (0.02)	0.20 (0.07)**
	low epithelium	0/49	1/49	0.00 (0.00)	0.02 (0.02)
seminal vesicles	low epithelium	6/49	42/49	0.12 (0.05)	0.86 (0.05)***

[Note: summary incidence data were not provided for ventral lobe of the prostate or seminal vesicles; the data were taken from the individual data tables. Mean scores were calculated by summing the severity scores and dividing by the total number of animals examined, i.e., it is not a mean severity score for affected animals. Also, note that significance was expressed in relation to Grp B, not to direction.]

The sponsor attributed findings all findings in male reproductive organs, pituitary gland, and mammary gland to serum prolactin. The sponsor did not consider the kidney affected by drug in either grp. The sponsor concluded that "...there is a clear difference in prolactin-mediated histological changes between rats dosed orally...and rats administered a depot formulation..."

Serum prolactin data were summarized in the following sponsor's table and figure:

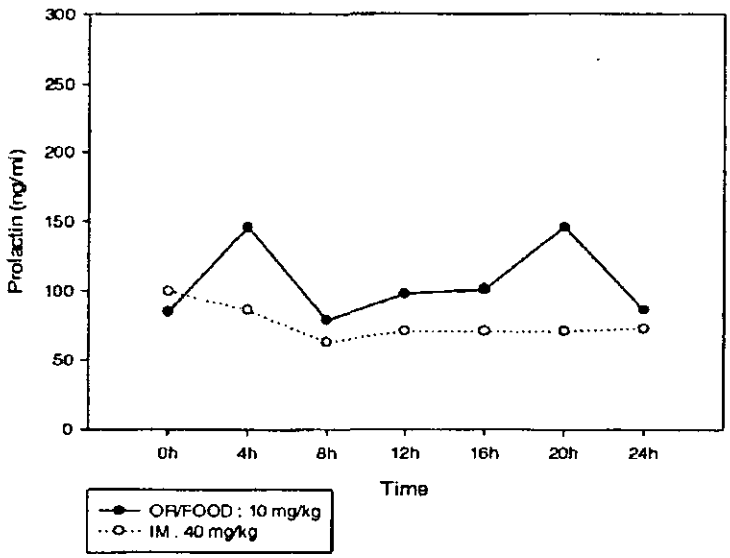
Group A: OR/FOOD 10 mg/kg

	Time						
	0h	4h	8h	12h	16h	20h	24h
Absolute values	85 (13)	146 (32)	79 (18)	98 (13)	101 (9)	146 (48)	86 (12)

Group B:IM: 40 mg/kg

Absolute values	100 (8)	86 (14)	63 (7)	71 (9)	71 (9)	71 (19)	73 (6)

Standard error is shown between brackets



The sponsor noted that there were no statistically significant differences in mean prolactin levels; however, the $AUC_{(0\text{-}24\ hr)}$ for serum prolactin was significantly lower in Grp B. The sponsor also noted that serum prolactin levels were more variable with dietary administration. This variability was considered due to "...the irregular food intake." It was further noted that "This is in contrast to the steady profile after intramuscular dosing with the depot formulation."

Summary and conclusions: the following special toxicology studies were conducted: a dermal irritation study in albino rabbits, 3 single-dose tolerance studies in Beagle dog [one s.c., 2 i.m.], and 2 mechanistic studies in Wistar rat.

No evidence of drug-induced irritation was detected in rabbits at a dermal dose of 2.9 mg/kg. In the s.c. study in dog, s.c. injection of risperidone (2.5 mg/kg) resulted in clear signs of irritation [e.g.,

granulomatous inflammation, necrotic collagen bundles, fibrinous exudate, and abscess formation] in 1 of 2 dogs. In one i.m. study in dog, creatine kinase was increased for up to 2 wks in animals receiving a single i.m. injection [2.5 mg/kg] of a risperidone-microsphere formulation; this would suggest some muscle damage. In the 2nd i.m. study in dog, injection site changes were assessed following 4 different i.m. formulations. Some degree of irritation was observed with all formulations. In none of these studies was the clinical formulation used; however, the data in dog do suggest that the risperidone microsphere formulation has the potential to induce local reaction and/or damage.

Two mechanistic studies were conducted in Wistar rat in order to investigate the possibility that substrain differences may have contributed to the differences in tumor profile observed in the oral and i.m. depot carcinogenicity studies in rat. In the acute dose study, risperidone was administered orally as a single 0.63-mg/kg dose to male Wistar Hannover and Wiga rats. [The Hannover and Wiga substrains were used in the i.m. depot and the oral carcinogenicity study, respectively.] TK and serum prolactin data were collected. The peak serum prolactin level was higher in Hannover rats [74%]; however, the $AUC_{(0\text{-}24\ hr)}$ for serum prolactin was similar between the two substrains. The TK data [previously discussed in the PK/ADME/TK section] indicated that plasma exposure to risperidone and 9-OH-risperidone was higher in Wiga than in Hannover rats, with the difference being greater for risperidone [4-fold] than for the metabolite [1.6 fold]. The ratio of the metabolite-to-parent was greater in Hannover [5.7 vs 2.5], indicating more extensive first-pass metabolism in the Hannover substrain. In the 7-wk study [individual data were not provided for this study, except for some histopathology], male Hannover rats received risperidone either as a drug-diet admixture [10 mg/kg] or as an i.m. microsphere depot [40 mg/kg biweekly]. [There was no C grp.] Observations consisted of clinical signs, body wt, food consumption, hematology, clinical chemisry, urinalysis, limited gross pathology, microscopic examination of selected tissues, TK, and serum prolactin. Clinical signs [slight sedation, ptosis] were observed only in the orally dosed animals; however, there was a similar incidence of food wastage in both grps. Body wt was lower in the orally dosed animals during the first 5 wks of dosing, but was similar between grps thereafter. Food intake was significantly lower in the orally fed animals through most of the dosing period. [Based on food intake data actual doses received were estimated to be 9.25-12.8 mg/kg/day.] The clinical pathology data were difficult to evaluate due to the lack of a C grp. There were differences between grps on a number of parameters. The sponsor considered the higher P_i and BUN values in the orally dosed animals to be the notable findings. The higher serum P_i was attributed to the body wt effect. On urinary parameters, findings of note included a decrease in volume (specific gravity was increased) and increased P_i and urea N clearance in i.m. depot animals during the last 2 wks of dosing. There were no differences between grps in spleen, pancreas, or adrenal wts. At necropsy, the incidences of mammary gland stimulation [characterized as slight] and stippled, yellow prostate were greater in the orally dosed animals. The differences between grps in terms of microscopic findings were interesting. Mammary gland secretion was greater [incidence and severity] in orally dosed animals, although there was no difference in terms of female appearance. However, effects on male reproductive organs [incidence, severity] [i.e., coagulating gland (low epithelium), prostate (low epithelium, dilated glands), seminal vesicles (low epithelium)] were greater in i.m. depot animals. The incidence and severity of hyperplasia/hypertrophy of the pituitary gland [pars intermedia] were similar between grps. Dilated renal tubuli (diffuse) was observed only in orally dosed animals. Serum prolactin was more variable in orally dosed animals; however, the $AUC_{(0\text{-}24\ hr)}$ for serum prolactin was greater in orally fed animals. More importantly, at no time during the 24-hr sampling period did the mean value for serum prolactin in the orally fed animals fall below that in i.m. depot animals. Therefore, the sponsor's notion that exposure to elevated serum prolactin levels is more prolonged with i.m. depot than oral risperidone was not supported by the data, and therefore, would not appear to be a factor in the different tumor profiles with the two routes.

IX. QUALIFICATION OF IMPURITIES

The sponsor noted that mpurities have been identified by HPLC analysis of the risperidone i.m. microsphere formulation, and considers the specification limits of to be acceptable "...from a toxicological point of view" for all compounds.

s an impurity produced during the and and are degradation products. The structures of these compounds were provided by the sponsor and are presented below [taken directly from the submission]:

was stated to have been present [at a level of in the batches of risperidone used in the following oral toxicity studies [sponsor's table]:

Table 4-6: Overview of toxicity studies conducted with A0101

Study	Exp.No.	Doses
Single dose oral toxicity in mice	8623	up to 160 mg/kg
Single dose IV toxicity in mice	8622	up to 40 mg/kg
Single dose oral toxicity in rats	8520	up to 320 mg/kg
Single dose SC toxicity in rats	8625	up to 320 mg/kg
Single dose IV toxicity in rats	8523	up to 47.6 mg/kg
Single dose oral toxicity in dogs	8624	up to 20 mg/kg
Single dose IV toxicity in dogs	8525	up to 40 mg/kg
Three-month oral toxicity in rats	1727	0, 0.63, 2.5, 10 mg/kg/day
Embryofetal development study in rats	-788	0, 0.63, 2.5, 10 mg/kg/day
Ames test in S. typhimurium	1662	up to 2000 µg/plate
Oral micronucleus test in mice	1712	up to 40 mg/kg

According to the sponsor, the dose of administered at the no-toxic effect level [i.e., 2.5 mg/kg] in the 3-mo oral toxicity and the embryofetal development studies in rats was 3 µg/kg. The dose of n humans at the "maximum therapeutic dose of 50 mg every two weeks" is mg/kg/day, corresponding to in terms of drug concentration. The sponsor concluded that "The qualified concentration for , therefore, is . The sponsor did not list any i.m. depot studies in which there was exposure to

The sponsor noted that and are and . The sponsor considers these as qualified "...based upon the fact that they are converted rapidly to the parent drug *in vivo*. The [illustrated in the sponsor's figure below] was stated to occur in various

body compartments [e.g., intestinal cells, hepatocytes, rbcs] in animals and humans. Hepatocytes and rbcs would be relatively more important in conversion of risperidone administered i.m. as compared to oral.

The sponsor conducted 2 PK/ADME studies of [redacted]. The data from these studies indicated that oral administration of [redacted] resulted in negligible plasma levels of [redacted] in rat [redacted] and dog [redacted]. In rat, plasma levels of [redacted] were [redacted] of the sum of the plasma AUCs for [redacted], risperidone, and 9-OH-risperidone. In dog, [redacted] was quantifiable only at the first sampling time. The sponsor did not demonstrate that there is no significant exposure to [redacted] in either rat or dog following administration of the i.m. depot formulation. The sponsor did not appear to have tested the PK/ADME of [redacted]

According to the review chemist [] [redacted], is present in both marketed oral dosage forms [tablet, oral solution], with a specification limit of [redacted] Therefore, the specification limit set by the sponsor for this impurity in the i.m. depot formulation is acceptable. However, CMC documentation for the presence of [redacted] in batches used in the nonclinical studies listed by the sponsor [sponsor's Table 4-6 above] was not provided, nor was there any indication that [redacted], was qualified during the development of the marketed oral formulations.

Conclusions: the sponsor has not provided sufficient information to support the specification limits proposed for [redacted] impurities. [redacted] The sponsor needs to provide documentation that these impurities were qualified in appropriate nonclinical studies, or, for [redacted], to provide documentation that there is no significant exposure to [redacted] following administration of the i.m. depot formulation (or other relevant route).

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X. DETAILED CONCLUSIONS AND RECOMMENDATIONS

Conclusions: conclusions are discussed in detail in the "summary and conclusions" for each section.

General Toxicology Issues: issues are discussed in detail in the "comments and conclusions" section under "General Toxicology:".

[The review of this NDA required extensive examination of individual line listings in order to correlate numerous findings in individual animals. The availability of electronic datasets would have made for a much more efficient review.]

Recommendations: unless risperidone i.m. depot represents a significant clinical benefit, it is recommended that the NDA not be approved until additional information/data are provided. The deficiencies are as follows:

(a) the tumor profile obtained in the 2-yr i.m. depot carcinogenicity study was different than that observed in the 2-yr oral study [NDA 20-272, RISPERDAL tablets]. The sponsor concluded that the renal tubular adenomas and adrenomedullary tumors observed in the i.m. depot study [but not the oral study] were related to elevations in serum prolactin. However, the information/data provided in support of this mechanism were not convincing. In addition, the mechanistic studies conducted in rats did not provide adequate data for dismissing the possibility of a unique tumor profile [with the i.m. depot formulation] on the basis of substrain differences or differential effects on route on serum prolactin. In the i.m. depot study, there was no safety margin between plasma exposures at the no-effect doses for renal and adrenomedullary tumors and that expected at the maximum recommended clinical dose.

If the sponsor has additional data or information that would better support the conclusion that the renal tubular adenomas and adrenomedullary tumors are irrelevant in terms of human risk, such data/information should be submitted for review.

Regardless of whether or not the i.m. depot formulation is approved, the findings of the i.m. depot carcinogenicity study should be added to labeling.

(b) the sponsor was not asked to conduct reproductive toxicology studies on the i.m. depot formulation. However, findings observed in the 1-yr chronic and the 2-yr carcinogenicity studies in rat using the i.m. depot formulation suggest that the i.m. depot formulation may have different toxicities than the oral formulations [for which a complete battery of reproduction studies were conducted]. Specifically, the osteodystrophy detected in the 1-yr and 2-yr studies and the additional tumor types observed with the i.m. depot formulation raise a concern that the oral reproductive toxicity studies may not provide an adequate test of the potential for the risperidone i.m. depot formulation to produce reproductive toxicity. At a minimal, it is recommended that the sponsor conduct an embryofetal development study in rat using the clinical i.m. depot formulation.

(c) the sponsor reported that —impurities are present in the risperidone i.m. depot formulation that are not present in the oral formulations. The sponsor stated that impurity, ———, was qualified in oral nonclinical studies; however, documentation to support this statement was not provided. , impurities [———— ————were noted to be qualified on the basis that they are rapidly converted to the parent compound when administered. Adequate data were provided to support this statement relative to impurity ————, however, no data were provided for

Recommended Labeling: the following revisions to the sponsor's proposed labeling are recommended in the event that this NDA is considered "Approvable":

PRECAUTIONS
General
Hyperprolactinemia

Carcinogenesis, Mutagenesis, Impairment of Fertility

Mutagenesis

Impairment of Fertility

Pregnancy
Pregnancy Category C

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XI. APPENDIX/ATTACHMENTS

Addendum to review: ExeCAC meeting minutes for the 2-yr i.m. depot carcinogenicity study in rat.

Executive CAC
Date of Meeting: 4/23/02
Rat Carcinogenicity Study

Committee: Joseph Contrera, Ph.D., HFD-900, Acting Chair
Jeri El Hage, Ph.D., HFD-510, Alternate Member
Robin Huff, Ph.D. HFD-570, Alternate Member
Barry N. Rosloff, Ph.D., HFD-120, Supervisory Pharmacologist
Lois M. Freed, Ph.D., HFD-120, Presenting Reviewer

Author of Draft: Lois M. Freed, Ph.D.

The following information reflects a brief summary of the Committee discussion and its recommendations. Detailed study information can be found in the individual review.

NDA #**21-346**
Drug Name: risperidone i.m. depot
Sponsor: Janssen Pharmaceutica

Rat Carcinogenicity Study: a 2-yr carcinogenicity study was conducted in Wistar rats at doses of 0, 5, and 40 mg/kg. The study included both saline and vehicle controls. The following tumors were identified by the sponsor as significant drug-related findings: (a) increase in mammary adenocarcinomas in LDF, (b) increase in pancreatic islet cell tumors (particularly adenomas) in HDM and HDF, (c) increase in pituitary adenomas and adrenomedullary pheochromocytomas in HDM, (d) increase in mammary gland tumors (particularly adenocarcinomas) in HDF, (e) decrease in ovarian polyps and absence of ovarian tumors in females, (f) "marginal" increase in solid renal corticotubular tumors in HDM, (g) a significant trend in mammary gland tumors in males (compared to SC), (h) a significant trend in adrenal pheochromocytoma in females (compared to SC). No vehicle- or drug-related findings were detected at the injection site.

Executive CAC Recommendations and Conclusions: the ExeCAC concurred with the following tumor findings: (a) mammary gland adenocarcinomas in LDF and HDF, (b) pancreatic islet cell tumors [adenoma, combined adenoma/carcinoma] in HDM and pancreatic islet cell adenomas in HDF. (c) adrenal pheochromocytomas [benign, combined benign/malignant] in HDM, (d) renal tubular tumors [adenoma, combined adenoma/adenocarcinoma] in HDM, (e) pituitary adenomas in HDM. The Committee noted that the HD may have exceeded the MTD in males, based on body weight/clinical signs data.

Joseph Contrera, Ph.D.
Acting Chair, Executive CAC

cc:\
/Division File, HFD-120
/BRosloff, HFD-120
/LMFreed, HFD-120
/SHardeman, HFD-120
/ASeifried, HFD-024

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

/s/

Lois Freed
6/25/02 04:09:02 PM
PHARMACOLOGIST

Barry Rosloff
6/25/02 05:08:17 PM
PHARMACOLOGIST
Concur with recommendations. See my Memo to File dated 6/25/02.

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