CENTER FOR DRUG EVALUATION AND RESEARCH

APPLICATION NUMBER:

22-173

PHARMACOLOGY REVIEW(S)

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

PHARMACOLOGY/TOXICOLOGY REVIEW AND EVALUATION

NDA NUMBER/ SERIAL NUMBER:	**22-173 / N-0000**
DATE RECEIVED BY CENTER:	**4/30/2007**
PRODUCT:	**Olanzapine long-acting injection**
INTENDED CLINICAL POPULATION:	**adults with schizophrenia**
SPONSOR:	**Eli Lilly and Company**
DOCUMENTS REVIEWED:	**Electronic submission**
REVIEW DIVISION:	**Division of Psychiatry Drug Products (HFD-130)**
PHARM/TOX REVIEWER:	**Sonia Tabacova, Ph.D.**
PHARM/TOX SUPERVISOR:	**Barry Rosloff, Ph.D.**
DIVISION DIRECTOR:	**Thomas Laughren, M.D.**
PROJECT MANAGER:	**Keith Kiedrow, Pharm.D. /Kimberly Updegraff, R.Ph.**

Date of review submission to Division File System (DFS):

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EXECUTIVE SUMMARY

I. Recommendations

A. Recommendation on approvability: Approvable

B. Recommendation for nonclinical studies: Adequate

C. Recommendations on labeling: We generally agree with the proposed labeling. The safety factor under 13.1 "Carcinogenesis, Mutagenesis, Impairment of Fertility" (1st paragraph, line 12) needs to be changed from (b) (4) (b) (4) "…equivalent to 0.3 (males) and 0.8 (females) times the maximum recommended human dose of 300 mg every 2 weeks on a mg/m2 basis."

II. Summary of nonclinical findings

A. Brief overview of nonclinical findings

Pharmacokinetics: The pharmacokinetic evaluation of olanzapine pamoate (OP Depot, the sustained release salt form of olanzapine with pamoic acid allowing long-term exposure to olanzapine) was focused on the absorption of the salt form, as other aspects of olanzapine disposition (distribution, metabolism, excretion, etc) had been previously evaluated for the oral and the rapid acting IM form and were expected "to remain unchanged once the compound is absorbed". The disposition of pamoic acid, particularly its absorption, metabolism, and elimination, was evaluated in selected studies as pamoate represented a new component in the administration of olanzapine.

In the animal species studied (rat, dog, and rabbit), OP Depot produced initial peak plasma concentrations of olanzapine and pamoate followed by a gradual decline in concentrations for up to 28 days post-dose. Exposure was sustained over a period of weeks and increased with increasing dose in all species. There were no gender differences in exposure, and olanzapine did not accumulate following repeated administration of OP Depot. The bioavailability of olanzapine from OP Depot in rats was estimated to be 37%, similar to the 47% bioavailability for oral olanzapine.

The absorption of pamoic acid administered either alone or from the OP Depot formulation in mice, rats, and dogs was likewise initially rapid with Tmax $\leq$ 8 hours and sustained over weeks, with higher exposure seen when pamoic acid was administered alone than as part of OP Depot. Pamoic acid did not undergo metabolism (as shown by in vitro and in vivo studies in the rat) and was rapidly excreted unchanged via the feces, suggesting that biliary excretion is the primary route of elimination. Specific studies on the metabolism, distribution, excretion, and pharmacokinetic drug interactions of olanzapine were not conducted with olanzapine pamoate monohydrate. Summaries of pertinent data from the marketing application for oral olanzapine (NDA 20-592) and for Rapid Absoption IM (RAIM) olanzapine (NDA 21-253) are included in the present application.

General toxicology:
The single- and repeat-dose toxicity of OP Depot was evaluated in rats and beagle dogs. In repeat-dose toxicity studies, OP Depot was given intramuscularly to rats once every 4 weeks for 3 months and to dogs once every 2 weeks for 6 months. Due to limitations in dose volume and suspendability, systemic toxicity was not elicited with OP Depot. In the OP Depot nonclinical studies, lower doses of olanzapine were administered as compared to the oral dosage studies.
The key general toxicology findings are as follows:
– Injection site reactions indicative of chronic inflammation were the major finding in all toxicology studies with OP Depot, in both rats and dogs. The reaction in dogs was more pronounced, appearing within a few days after administration and diminishing in a week or 2 thereafter. Histologic evidence of chronic inflammation and fibrosis was present at necropsy; the inflammation persisted, though significantly reduced, after a 2-month recovery period.
– Pamoic acid (the formulation agent) did not exert systemic toxicity. Injection site reactions from the pamoic acid-treated animals were less frequent and less severe than those from animals treated with OP Depot.
Genetic toxicology: Olanzapine had been previously tested for genetic toxicity (under NDA 20-592) and was negative in a full range of standard tests that included bacterial mutation tests and in vitro and in vivo mammalian tests.
Pamoic acid was negative in the Ames test, the mouse lymphoma assay, and the chromosome aberration assay in human lymphocytes and was also negative in 2 in vivo assays (the mouse micronucleus test and the mouse bone marrow chromosome aberration assay). Reproducibly positive results were obtained in an in vitro chromosome aberration assay in Chinese hamster ovary (CHO) cells (at concentrations >500 μg/ml, about 1000-fold greater than the peak plasma concentrations in humans), however, the absence of oncogenicity in the 2-year carcinogenicity study in rats supports a lack of genotoxic hazard to humans.
Carcinogenicity: In the 2-year study to evaluate the carcinogenic potential of a sustained-release formulation of olanzapine administered by once per 4 weeks intramuscular injections of olanzapine pamoate monohydrate (OPM) to Fischer 344 rats (60/sex/dose) at doses of 0 (vehicle), 0 (pamoic acid), 5, 10, and 20 mg /kg for males and 0, 0, 10, 25, and 50 mg /kg for females, there was no carcinogenic effect attributable to OPM or pamoic acid since there was no dose-related effect on incidence and distribution of neoplastic lesions and they were similar among groups. Effect of pamoic acid alone at i.m. doses similar to those administered in the high-dose OPM group was assessed in parallel in additional groups of rats. The doses were selected in accordance with the Executive CAC recommendations (IND 60701, Executive CAC Meeting Minutes of 3/11/2003). A MTD was achieved in this study based on dose-related injection site adverse effects (chronic inflammatory reactions and residual test substance accumulation in the injection site affecting nearly all animals of both genders at HD). Olanzapine $AUC_{0\text{-}336h}$ values achieved at HD were lower or equal than those in humans at MRHD (300 mg every 2 weeks or 405 mg every 4 weeks). Exposures to pamoic acid ($AUC_{0\text{-}336h}$) achieved at HD were equal to or higher than those in humans at MRHD. Dose-limiting factors were the amount of test article feasible to be injected i.m. in the rat and local injection site reaction.

Reproductive and developmental toxicology:
- Fertility and Early Embryonic Development: No fertility studies were conducted with OP Depot.
- Embryo-Fetal Development: Embryo/fetal studies in rats and rabbits from dams treated with OPM Depot formulation during gestation [i.m. doses of 10, 25 and 75 mg/kg on gestation day 6 (rat) or 7 (rabbit) with plasma exposures maintained throughout the period of organogenesis] showed no OPM- or pamoic acid-related maternal systemic toxicity, embryo/fetotoxicity (as indicated by the lack of effect on embryo/fetal intrauterine growth and survival) or increased incidence of structural malformations up to the maximum feasible dose tested (75 mg/kg).
- Pre- and Postnatal Toxicity: A prenatal/postnatal study with OPM Depot was conducted in rats at i.m. doses of 0, 10, 25, and 75 mg/kg given to dams on gestations days 6 and 16 and again on post-partum day 4. Changes in behavioral development of offspring were observed at the highest dose (a delay in negative geotaxis early in the development of the F1 pups, a lack of habituation to the startle response in F1 males and a reduced performance on memory trials in water maze). These effects are qualitatively similar to the transient decrease in Figure-8 maze activity observed in the 2-generation study conducted with oral olanzapine (NDA 20-592, as cited by the sponsor), and therefore suggest no new risk due to olanzapine pamoate monohydrate use.
Plasma exposure and safety margins in developmental toxicology studies: Pamoic acid exposures at the HD in these studies were about 4x (rabbits) to 40x (rats) the human exposures at MRHD; Olanzapine exposure multiples relative to the MRHD were small due to limitations in dose volumes that could be administered. Based on plasma AUC values, olanzapine exposure multiples in both rat and rabbit vs. human exposure at MRHD were all less than 1. The highest OPM i.m. dose (75 mg/kg) used in the embryofetal (Segment II) studies in rats and rabbits, as well as in the prenatal/postnatal (Segment III) study in rats is the highest dose that could be administered based on the maximum achievable concentration of the test article in suspension and the maximum tolerated intramuscular dose volume for the test animals used.

Impurities

Pamoate-Related Impurities: Compounds (b) (4) and (b) (4) (the (b) (4) (b) (4) of pamoic acid) were at or above their proposed acceptance criteria in Lot 325SB8 (used in the single-and repeat-dose toxicology studies) and Lot CTM00881 (used in the reproduction and carcinogenicity studies). In all these studies, no systemic adverse findings were observed with OP Depot or pamoic acid. The highest tested dose levels used in rat 3-month and dog 6-month toxicity studies provided multiples from about 2- to 3-fold above the acceptance criteria for these 2 impurities when compared to MRHD based on dose (in mg/m2) and multiples from 1.5 to 4.8x the MRHD based on plasma exposure (AUC). Since "drug products derived from the 2 lots of drug substance mentioned above (Lot 325SB8 and ML114 which was re-labeled as CTM00881) were used in all toxicology studies and also in clinical trials", the impurity exposures tested and qualified in toxicology studies were also qualified by their use in clinical studies.

Olanzapine-Related Impurities: Compound (b) (4) (a (b) (4) (b) (4) product of olanzapine that may form during OPM drug substance manufacturing process) has oral qualification data cross-referenced from NDA 20-252, that can be applied in the light of the similar or greater exposures that are expected by the oral vs. the i.m. route.

Olanzapine containing elevated levels of Compound (b) (4) as well as 2 other impurities found as degradation products in oral olanzapine drug (Compounds (b) (4) and (b) (4) were tested and found to be negative for genotoxicity in the Ames test and in vivo mouse micronucleus test, as well as for general toxicity in rats at doses about 100x higher than the MRHD of oral olanzapine in humans (NDA 20-252).

B. Pharmacologic activity: Pharmacology section is not included with this submission. Please refer to the NDA 20-592 for all relevant pharmacology information

C. Nonclinical safety issues relevant to clinical use

- Pamoic acid genotoxicity testing showed reproducibly positive results in an in vitro chromosome aberration assay in Chinese hamster ovary (CHO) cells (at concentrations >500 μg/ml, about 1000-fold greater than the peak plasma concentrations in humans). However, the absence of oncogenicity in the 2-year carcinogenicity study in rats supports a lack of genotoxic hazard to humans.
- Changes in behavioral development of offspring were observed in the prenatal/postnatal study with OPM Depot the at the highest dose tested (75 mg/kg given to dams on gestations days 6 and 16 and again on post-partum day 4), i.e., a delay in negative geotaxis early in the development of the F1 pups, a lack of habituation to the startle response in F1 males and a reduced performance on memory trials in water maze. These effects are qualitatively similar to the transient decrease in Figure-8 maze activity observed in the 2-generation study conducted with oral olanzapine (NDA 20-592, as cited by the sponsor), and therefore suggest no new risk due to olanzapine pamoate monohydrate use.
- Due to dose-limiting factors (i.e., the amount of test article feasible to be injected i.m., maximal concentration achievable in the suspension formulation and local injection site reaction), relatively low olanzapine exposures could be achieved with OP Depot in the nonclinical studies, which resulted in low nonclinical-to-clinical exposure multiples. Due to limitations in dose volume and suspendability, systemic toxicity was not elicited with OP Depot, although maximal suspension concentrations were used in all animal studies. However, this does not constitute a safety issue since the safety of systemic olanzapine was adequately evaluated by previous nonclinical studies with the approved olanzapine oral and rapid-acting IM formulations.
- Impurities: (b) (4) (b) (4) were at or above their proposed acceptance criteria in Lot 325SB8 (used in the single-and repeat-dose toxicology studies) and Lot CTM00881 (used in the reproduction and carcinogenicity studies). In all these studies, no systemic adverse findings were observed with OP Depot or pamoic acid. The highest tested dose levels used in rat 3-month and dog 6-month toxicity studies provided multiples from about 2- to 3-fold above the acceptance criteria for these 2 impurities when compared to MRHD (based on dose in mg/m2) and multiples from 1.5 to 4.8x the MRHD (based on plasma exposure, AUC). Since drug products derived from the 2 lots of drug substance mentioned above were used in all toxicology studies and also in clinical trials, the impurity exposures tested and qualified in toxicology studies were also qualified by their use in clinical studies.

2.6 PHARMACOLOGY/TOXICOLOGY REVIEW

2.6.1 INTRODUCTION AND DRUG HISTORY

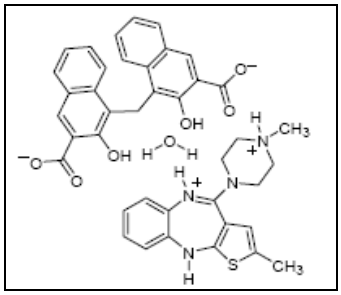
NDA number: 22-173
Review number: 1
Sequence number/date/type of submission: N-0000/ 4/30/2007
Information to sponsor: Yes ( ) No (x)
Sponsor and/or agent: Eli Lilly
Manufacturer for drug substance: Eli Lilly

Reviewer name: Sonia Tabacova
Division name: Psychiatry Drug Products
HFD #: 130
Review completion date: January 31, 2008

Drug:

Trade name: Zyprexa Adhera
Generic name: Olanzapine Pamoate Monohydrate, i.m. depot formulation
Code name: LY170053, Compound 426906
Chemical name: *10H-thieno [2, 3-b] [1, 5] benzodiazepine, 2-methyl-4-(4-methyl-1-piperazinyl)-, 4, 4'-methylenebis [3-hydroxy-2-naphthalene-carboxylate] (1:1), monohydrate*
CAS registry number: 221373-18-8

Molecular formula/molecular weight: C17H22N4S•C23H14O6•H2O/ 718.8
Structure:



Relevant INDs/NDAs/DMFs: NDA 20-592 (oral olanzapine), NDA 21-253 (Rapid Absoption IM olanzapine), IND 60701
Drug class: Antipsychotic

Intended clinical population: Schizophrenia in adults

Clinical formulation: i.m. depot

Route of administration: i.m.
Disclaimer: Tabular and graphical information is from sponsor's submission unless stated otherwise.

Studies reviewed within this submission:

Pharmacokinetics:

Absorption (Single-dose pharmacokinetics)

Study 007R02, ADME Report 02: Plasma Pharmacokinetics of Radioactivity and Pamoic Acid in Fischer 344 Rats Following a Single 10-mg/kg Intramuscular Dose of [14C]Pamoic Acid

Distribution

Study 003M02, ADME Report 01: Radiocarbon Concentrations in Plasma and Bone Marrow of Male ICR Mice Following a Single 4.7-mg Intramuscular Injection of [14C]Pamoic Acid

Metabolism in hepatic S9 fractions

ADME Report 04: Assessment of the In Vitro Metabolism of [14C] pamoic acid (Lilly Serial Number 015784) in Rat Hepatic S9 Fractions by HPLC with Radio-detection and LC/MS

Excretion and metabolism in blood, urine, and feces

Study 007R02, ADME Report 03: Elimination and Metabolism of Radioactivity in Male Fischer 344 Rats Following a Single Intramuscular Dose of 10 mg/kg [14C]Pamoic Acid

General Toxicology:

Single-dose toxicity

Study R08999, Toxicology Report 83: A Study to Evaluate Injection Site Reaction in Male Fischer 344 Rats Given Olanzapine Pamoate Monohydrate (LY170053 Pamoate Monohydrate) as a Single Intramuscular Injection

Study D03899, Toxicology Report 84: A Subchronic Toxicity Study in Beagle Dogs Given a Single Intramuscular Injection of Olanzapine Pamoate Monohydrate (LY170053 Pamoate Monohydrate)

Carcinogenicity

Study R03603: An Oncogenicity Study in Fischer 344 Rats Given Intramuscular Injections of Olanzapine Pamoate Monohydrate Once Monthly for 2 Years

Reproductive and Developmental Toxicity

Embryo-fetal

Study R07403: An Embryo-Fetal Development (Segment II) and a Companion Toxicokinetic Study of Olanzapine Pamoate Monohydrate Administered by Intramuscular Injection to CD Rats

Study B00006: An Embryo-Fetal Development and Companion Toxicokinetic Study in Female New Zealand White Rabbits Given a Single Intramuscular Injection of Olanzapine Pamoate Monohydrate

Pre/postnatal

Study WIL-353058: A Prenatal and Postnatal Development Study, Including Maternal Function of Olanzapine Pamoate Monohydrate Administered by Intramuscular Injection to Female CD Rats

Studies not reviewed within this submission:

Pharmacology Studies – not submitted: Reference NDA #20-592

Toxicology studies: The following studies were previously reviewed by Lois Freed, Ph.D. under IND 60701:

General toxicology studies:

- A subchronic toxicity study in Fischer 344 rats given olanzapine pamoate monohydrate (Compound 426906) by intramuscular injection once a month for 3 months (Study R07100)
- A chronic toxicity study in beagle dogs given multiple doses of olanzapine pamoate monohydrate (LY170053 Pamoate Monohydrate) for 6 months followed by a 2-month reversibility phase (Study D00200)

Genetic toxicology studies [pamoic acid] including:

Ames assay
In vitro chromosomal aberration assay in CHO cells
In vitro chromosomal aberration assay in human lymphocytes
In vitro mouse lymphoma
In vivo micronucleus assay in mice
In vivo chromosomal aberration assay in mice

As no new repeat-dose toxicity studies have been submitted since, and the present reviewer has no reason to disagree with Dr. Freed's assessments, the review data for the above studies are directly reproduced from Dr. Freed's review. The "Key study Findings" are summarized by this reviewer.

2.6.2 PHARMACOLOGY

Pharmacology section is not included with this submission. NDA 20-592 is referenced for all relevant pharmacology information.

2.6.2.1 Brief summary

2.6.2.2 Primary pharmacodynamics

2.6.2.3 Secondary pharmacodynamics

2.6.2.4 Safety pharmacology

2.6.2.5 Pharmacodynamic drug interactions

2.6.3 PHARMACOLOGY TABULATED SUMMARY

A Pharmacology Tabulated Summary is not included with this submission. NDA 20-592 is referenced for all relevant pharmacology information.

2.6.4 PHARMACOKINETICS/TOXICOKINETICS

2.6.4.1 Brief summary

Specific studies on the metabolism, distribution, excretion, and pharmacokinetic drug interactions of olanzapine were not conducted with olanzapine pamoate monohydrate, "as the disposition of olanzapine, once absorbed, was expected to be similar regardless of route of administration". Summaries of pertinent data from the marketing application for oral olanzapine (NDA 20-592) and for Rapid Absoption IM (RAIM) olanzapine (NDA 21-253) were included in present application. [Note: During a pre-NDA discussion with the Division on 17 July 2006, the sponsor proposed and the Division agreed that for the application regarding the OP Depot formulation, the sponsor would briefly describe the results of nonclinical reports previously submitted to NDA 20-592 (oral olanzapine) and NDA 21-253 (RAIM olanzapine), rather than submit the full reports for those studies].
Studies to characterize the absorption and exposure of olanzapine and/or pamoic acid following administration of OP Depot in rats, rabbits, and dogs were conducted in support of the present application. The disposition of pamoic acid, particularly its absorption, metabolism, and elimination, was evaluated in selected studies as pamoate represented a new component in the administration of olanzapine.

Key findings from the evaluation of OP Depot and pamoic acid include:
– Bioavailability of olanzapine when administered as OP Depot in rats and dogs was estimated to be 37% and 77%, respectively, as compared to 47% and 73% when dosed orally.
– Single-dose pharmacokinetics of OP Depot indicated rapid absorption and sustained exposure to olanzapine and pamoic acid in rats, with slower absorption but sustained exposure to both olanzapine and pamoic acid in dogs. Exposure to olanzapine peaked within the first few days and was sustained over a period of weeks.
– Repeat-dose toxicokinetics for both olanzapine and pamoic acid indicate no consistent sex difference and no accumulation of either olanzapine or pamoic acid.
– In rats, rabbits, and dogs, plasma concentrations of olanzapine following IM administration of OP Depot increased with increasing dose. OP Depot produced initial peak plasma concentrations of olanzapine followed by a gradual decline in concentration for up to 28 days postdose, thus demonstrating a sustained release profile.
– The plasma profile of pamoic acid following administration of OP Depot was similar to that of olanzapine. When administered IM as a single agent, pamoic acid was also readily absorbed, underwent very little metabolism, and was excreted largely unchanged via bile into the feces.
– On a monthly basis, oral exposure of rats and dogs to olanzapine was 6 to 13 times greater than that for animals receiving the OP Depot on a once- or twice monthly basis at doses which were limited by suspendability and maximum feasible dose volumes.

2.6.4.2 Methods of Analysis

Plasma concentrations of olanzapine in all studied species were determined using a validated, reversed-phase high performance liquid chromatography (HPLC) method with

electrochemical detection. The same method was used for the measurement of olanzapine in the nonclinical studies performed for the previously approved NDAs 20-592 and 21-253. Assay methodology and validation information are summarized in the following sponsor's tables. The limits of quantitation in the assays were appropriate for the animal studies and allowed full evaluation of the pharmacokinetics of both olanzapine and pamoic acid.

PK: Analytical Methods and Validation Reports

Document ID	Assay Type	Method # and Development Date	Specificity	Species	Sample Fluids	Sample Preparation	Type of Chromatography
820-0212	LC/EC	820-0212 Revision 000 December 1997	Olanzapine	Dog	Plasma	SPE columns	Reverse phase/ isocratic
820-0212	LC/EC	820-0212 Revision 001 December 1999	Olanzapine	Dog	Plasma	SPE columns	Reverse phase/ isocratic
820-0420	LC/EC	820-0420 Revision 000 April 2000	Olanzapine	Rat	Plasma	SPE columns	Reverse phase/ isocratic
820-0420	LC/EC	820-0420 Revision 002 May 2004	Olanzapine	Rat	Plasma	SPE columns	Reverse phase/ isocratic
820-0279	LC/EC	820-0279 Revision 001 April 2004	Olanzapine	Rabbit	Plasma	SPE columns	Reverse phase/ isocratic
820-0425	HPLC/fluorescence	820-0425 Revision 000 June 2000	Pamoic acid	Rat	Plasma	SPE columns	Reverse phase/ isocratic
820-0425	HPLC/fluorescence	820-0425 Revision 001 November 2000	Pamoic acid	Rat	Plasma	SPE columns	Reverse phase/ isocratic
820-0425	HPLC/fluorescence	820-0425 Revision 002 July 2002	Pamoic acid	Mouse	Plasma	SPE columns	Reverse phase/ isocratic
820-0425	HPLC/fluorescence	820-0425 Revision 003 December 2003	Pamoic acid	Rabbit	Plasma	SPE columns	Reverse phase/ isocratic
820-0397	HPLC/fluorescence	820-0397 Revision 001 May 2000	Pamoic acid	Human or dog	Plasma	SPE columns	Reverse phase/ isocratic

Abbreviations: # = number, HPLC = high performance liquid chromatography, LC/EC = liquid chromatography with electrochemical detection.

PK:Analytical Methods and Validation Stability

Approximate Temperature	Mouse Plasma	Rat Plasma	Rabbit Plasma	Dog Plasma
Olanzapine				
-20°C	NA	352 days	17 days	NR
-80°C	NA	NR	17 days	9 months
Method number	NA	820-0420 revision 002[a]	820-0279 revision 001[b]	820-0212 revision 001[c]
Pamoic acid				
-20°C	NR	3 months	NR	NR
-60°C	20 days	3 months	4 months	5 months
Method number	820-0425 revision 002[d]	820-0425 revision 001[e]	820-0425 revision 003[f]	820-0397 revision 001[g]

Abbreviations: NA = not applicable, NR = stability not assessed at that temperature using the cited method.
[a] Long-term stability and dilution stability in rat plasma at the 10-fold concentration was 397 days at -60°C.
[b] Dilution stability in rabbit plasma at the 10-fold concentration was 17 days at -80°C.
[c] Dilution stability in dog plasma at the 10-fold concentration was 55 days at -60°C and 9 months at -80°C.
[d] Dilution stability in mouse plasma at the 3000-fold concentration was 20 days at -60°C.
[e] Dilution stability in rat plasma at the 200-fold concentration was 3 months at -20°C and -80°C.
[f] Dilution stability in rabbit plasma at the 600-fold concentration was 4 months at -60°C.
[g] Dilution stability in dog plasma at the 10-fold concentration was 5 months at -60°C.

2.6.4.3 Absorption

The absorption and bioavailability of olanzapine were previously evaluated in rats and dogs following single oral administrations of ^{14}C-labeled olanzapine (NDA 20-592) and

in dogs following single intravenous and IM doses of ^{14}C-labeled olanzapine (NDA 21-253).

For the present application, the absorption of olanzapine, and in some cases pamoic acid, from the OP Depot formulation was evaluated in rats, gravid rats, gravid rabbits and dogs as part of the toxicology studies (see under individual study reviews). In addition, ^{14}C-labeled pamoic acid was administered to mice and rats (Study 003M02, ADME Report 01: Radiocarbon Concentrations in Plasma and Bone Marrow of Male ICR Mice Following a Single 4.7-mg Intramuscular Injection of [14C]Pamoic Acid and Study 007R02 ADME Report 02: Plasma Pharmacokinetics of Radioactivity and Pamoic Acid in Fischer 344 Rats Following a Single 10-mg/kg Intramuscular Dose of [14C]Pamoic Acid). In repeat-dose studies, the first dose was used to characterize the single-dose pharmacokinetics.

Olanzapine absorption in rats and rabbits following a single dose of OP Depot was initially rapid, with the time to maximum concentration (T_{max}) generally occurring within the first day. Absorption was slower in dogs with T_{max} occurring after approximately 3 to 6 days. Exposure was sustained over a period of weeks and increased with increasing dose in all species. There were no gender differences in exposure, and olanzapine did not accumulate following repeated administration of OP Depot.

The absorption of pamoic acid administered either alone or from the OP Depot formulation in mice, rats, gravid rats, gravid rabbits, and dogs was likewise initially rapid with $T_{max} \leq 8$ hours and sustained over weeks, with higher exposure seen when pamoic acid was administered alone than as part of OP Depot.

2.6.4.3.1. Bioavailability

Rats: As a specific single-dose pharmacokinetic study in rats was not conducted with OP Depot, Day 1 data from the 3-month rat study (Study R07100, Toxicology Report 87) was compared to previously reported olanzapine exposure data from the oral bioavailability study ADME 29 (approved NDA 20-592), as summarized in the sponsor's table on the next page. The bioavailability of olanzapine from OP Depot in rats was estimated to be 37%, similar to the 47% bioavailability for oral olanzapine. As stated by the sponsor, "These estimates should be viewed in light of the extensive metabolism in rats; in the oral bioavailability study ADME 29, concentrations of both olanzapine and total radioactivity were available. Oral bioavailability in rats as measured by total radioactivity, and thus olanzapine and its metabolites, indicated a bioavailability at 79%, considerably higher than that calculated based on olanzapine alone (47%). Thus, the bioavailability estimate of olanzapine from OP Depot in rats would be anticipated to be much higher if total radioactivity concentrations were used to do the calculation. No radiocarbon PK data is available for the OP Depot formulation, but there is no reason to expect that the extent of metabolism would be different between the 2 forms of olanzapine, as the fundamental metabolism characteristics of the molecule would be anticipated to be substantially identical after absorption. Therefore, comparisons based upon olanzapine concentrations only are appropriate for purposes of this submission, so that similar measurements can be compared. Further, release of olanzapine from the injection site of OP Depot is prolonged, which is not represented by the standard calculation of bioavailability."

Mean Plasma Pharmacokinetic Parameters after Administration of Olanzapine to Fischer 344 Rats; Bioavailability of Oral and OP Depot Olanzapine

Parameter			
Species	Rat	Rat	Rat
N	3	3	8
Dose (mg/kg)	8	8	20
Route of administration	Oral	Intravenous	Intramuscular, OP Depot
Gender	Male	Male	Male
C_{max} (ng/mL)	518	3164	115
T_{max} (h)	0.5	0.083	4
Half-life (h)	2.8[a]	2.0[a]	NC
AUC_{0-t} (ng*h/mL)	3073	6498	6002
Report number or	ADME29	ADME29	Tox87
Document ID and source	NDA 20-592	NDA 20-592	Current application
Bioavailability	47% oral/IV[b]	NC	37% OP Depot/IV

Abbreviations: IV = intravenous, NC = not calculated.

[a] Range 3-12 hours.

[b] Bioavailability of radiocarbon in ADME29 was 79%. However, lower bioavailability was calculated using olanzapine exposure only (as for this comparison) suggesting extensive metabolism in rats.

Dogs: The sponsor's table below compares the results from previously reported olanzapine exposure data from the oral bioavailability study and TK parameters from the single-dose subchronic OPM study (Study D03899 Toxicology Report 84). The absolute bioavailability of olanzapine following administration of oral olanzapine (NDA 21-253) was approximately 73% relative to intravenously administered olanzapine. Following administration of OP Depot in dogs, the bioavailability of olanzapine was estimated to be approximately 77%, on the basis of TK parameters from the single-dose subchronic study. The Cmax was lower and AUC was extended with plasma concentrations measurable for 13 days.

Mean Plasma Pharmacokinetic Parameters after Administration of Olanzapine to Beagle Dogs; Bioavailability of Oral and OP Depot Olanzapine

Parameter				
Species	Dog	Dog	Dog	Dog
N	4	3	3	4
Dose (mg/kg)	5	5	5	5
Route of administration	Oral	Intramuscular, Rapid acting	Intravenous	Intramuscular, OP Depot
Gender	Female	Female	Female	Female
C_{max} (ng/mL)	172	744	871	15.44
T_{max} (h)	3.25	0.70	0.08	45
Half-life (h)	9.24	10.70	6.00	NC
AUC[a] (ng*h/mL)	1923	3818	2633	2037
	[0.5-t]	[0.5-t]	[0.83-t]	[0-t]
Report number or	ADME18	ADME58	ADME60	Tox84
Document ID and source	NDA 20-592	NDA 21-253	NDA 21-253	Current application
Bioavailability	73% oral/IV	NC	NC	77% OP Depot/IV

Abbreviations: IV = intravenous, NC = not calculated.

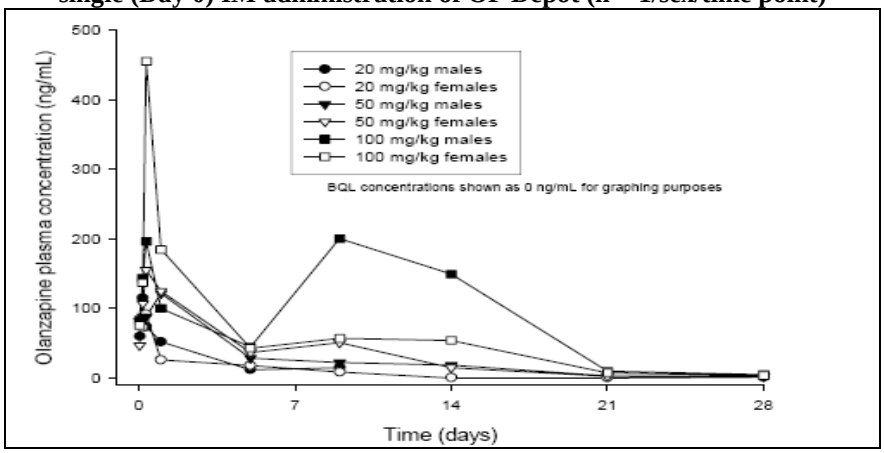
[a] Range over which AUC was calculated is shown in brackets; t is the last measurable time point.

2.6.4.3.2. Single-Dose Pharmacokinetics of Olanzapine

Rats

No separate single-dose PK study for OP Depot was conducted in rats. Single-dose toxicokinetic parameters were determined on Day 0 of the 3-month rat toxicology study with OPM i.m. administration once every 4 weeks (Study R07100, Toxicology Report 87). Peak plasma concentrations occurred within the first day in most cases, and exposure as measured by AUC increased proportionally between the low and mid dose and greater than dose proportionally between the mid and high dose. Plasma concentrations, in general, gradually decreased during the first 2 to 3 weeks and remained near or below the quantifiable limit for the final 1 to 2 weeks (as shown in the sponsor's figure below).

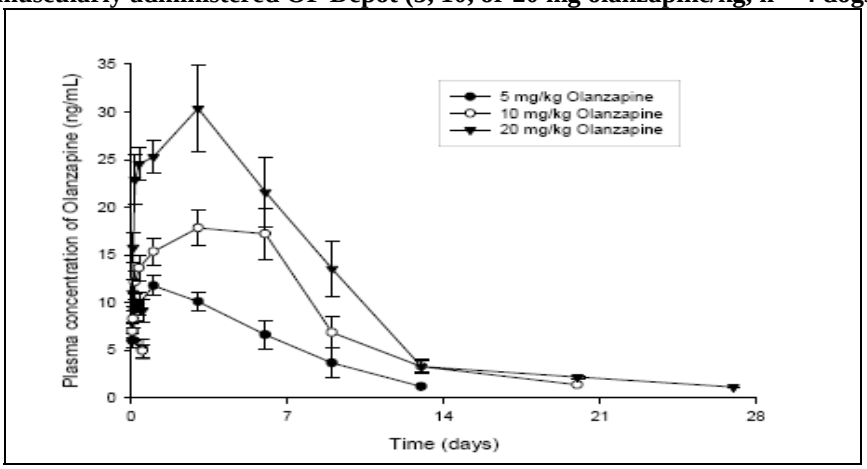
Figure: Plasma concentrations of olanzapine in Fischer 344 rats (M and F combined) following a single (Day 0) IM administration of OP Depot (n = 1/sex/time point)



Dogs

A single-dose pharmacokinetic study was conducted in male and female beagle dogs at OP Depot i.m. doses of 5, 10, or 20 mg/kg (Study D03899, Toxicology Report 84). Single-dose plasma concentrations are shown in the sponsor's figure below. Exposure was dose proportional. Mean T_{max} values were within 1 week, and concentrations declined over the course of the study. These data are similar to the Day 0 TK data from the 6-month dog study. In general, there were no gender differences in exposure.

Figure: Mean (± SEM) plasma concentrations of olanzapine versus time in beagle dogs (M and F) intramuscularly administered OP Depot (5, 10, or 20 mg olanzapine/kg, n = 4 dogs/sex)



2.6.4.3.3. Single-Dose Pharmacokinetics of Pamoic Acid

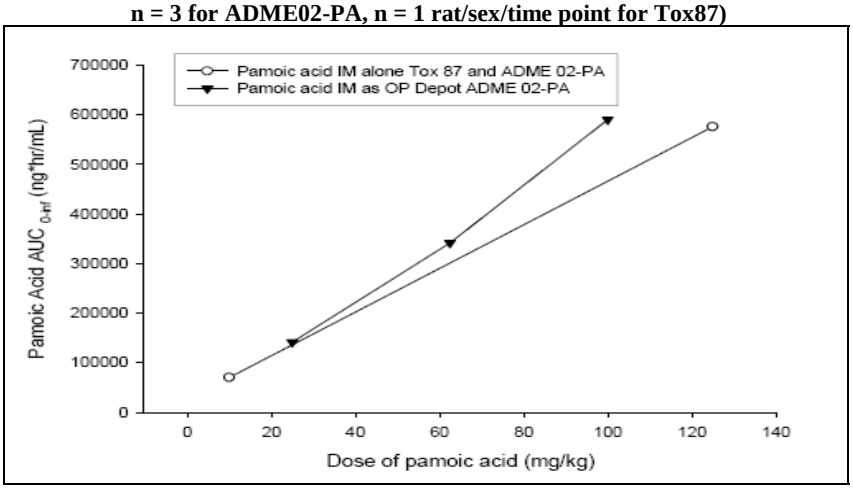
Rats

Pamoic acid exposure was evaluated in a single-dose pharmacokinetic study in rat following IM administration (Study 007R02, ADME Report 02-PA: Plasma Pharmacokinetics of Radioactivity and Pamoic Acid in Fischer 344 Rats Following a Single 10-mg/kg Intramuscular Dose of [14C]Pamoic Acid) and from single-dose IM data from a toxicokinetic study (Day 0 of the 3-month rat toxicology study with OPM i.m. administration once every 4 weeks (Study R07100, Toxicology Report 87).

In the PK study, the plasma pharmacokinetics of pamoic acid were evaluated following a single 10-mg/kg intramuscular administration of [14C]pamoic acid to male Fischer 344 rats. Radioactivity in plasma was determined by liquid scintillation counting and plasma concentrations of pamoic acid were determined by HPLC with fluorescence detection. The mean C_{max}, AUC and half-life values are shown in the sponsor's table under the subheading "Metabolism". The time to peak plasma concentration (T_{max}) for both plasma radioactivity and pamoic acid was 0.5 hours after dosing.

In the TK component of the 3-month rat toxicology study, pamoic acid doses of 0, 50, 100, or 125 mg/kg were evaluated; pamoic acid was administered by IM injection every 4 weeks either alone (125 mg/kg) or with olanzapine in the depot formulation. Single dose TK parameters were determined on Day 0 (tabular data shown in Toxicology section of this review). Peak plasma concentrations occurred within the first day after dosing and the exposure as measured by AUC to pamoic acid increased with increasing dose; T_{max} for pamoic acid dosed alone was 8 hours, suggesting the 12-fold higher dose in the toxicokinetic study (as compared to the pharmacokinetic study) took longer to be absorbed. Plasma concentrations gradually decreased during the first 2 to 3 weeks and remained near or below the quantifiable limit for the final 1 to 2 weeks. Higher Cmax of pamoic acid were observed when pamoic acid was administered alone versus with olanzapine in the depot formulation, but overall exposures as measured by AUC were similar (see sponsor's figure below) when dosed alone or as OP Depot. The administered dose and T_{max} values were similar between the two groups.

Figure: Mean single-dose pamoic acid plasma exposure as measured by AUC in male rats following IM administration of pamoic acid alone or as OP Depot (Data from ADME Report 02-PA* and Tox Report 87*) n = 3 for ADME02-PA, n = 1 rat/sex/time point for Tox87)

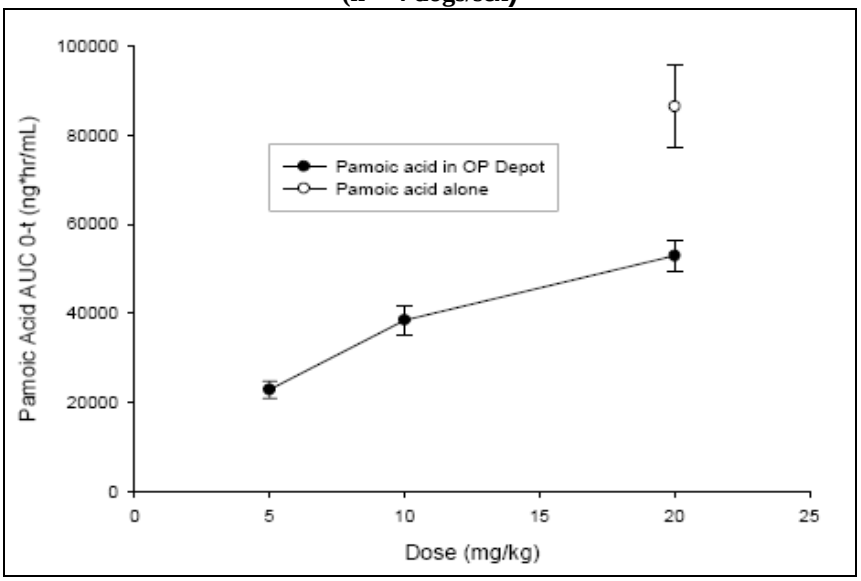


- ADME Report 02-PA: Study 007R02 Plasma Pharmacokinetics of Radioactivity and Pamoic Acid in Fischer 344 Rats Following a Single 10-mg/kg Intramuscular Dose of [14C]Pamoic Acid;
- Toxicology Report 87: Study R07100 3-month rat toxicology study with OPM i.m. administration once every 4 weeks

Dogs

Pamoic acid single-dose data are available from the 6-month dog study (Study D00200, Toxicology Report 88: A Chronic Toxicity Study in Beagle Dogs Given Multiple Doses of Olanzapine Pamoate Monohydrate for 6 Months Followed by a 2-Month Reversibility Phase). Pamoic acid T_{max} values ranged from 104 to 145 hours after the first dose. Plasma levels declined gradually for at least 14 days after the injection. Plasma exposure (AUC) values increased less than dose proportionally with increasing dose. Pamoic acid administered alone resulted in higher peak plasma concentrations and higher AUC than those at the high-dose OP Depot group, which received a similar pamoic acid dose (see sponsor's figure below). There were no gender differences, but inter-animal variability was high.

Mean (± SEM) plasma concentrations of pamoic acid versus time in beagle dogs (male and female) following a single i.m. dose of OP Depot (5, 10, or 20 mg olanzapine/kg) or pamoic acid alone (n = 4 dogs/sex)

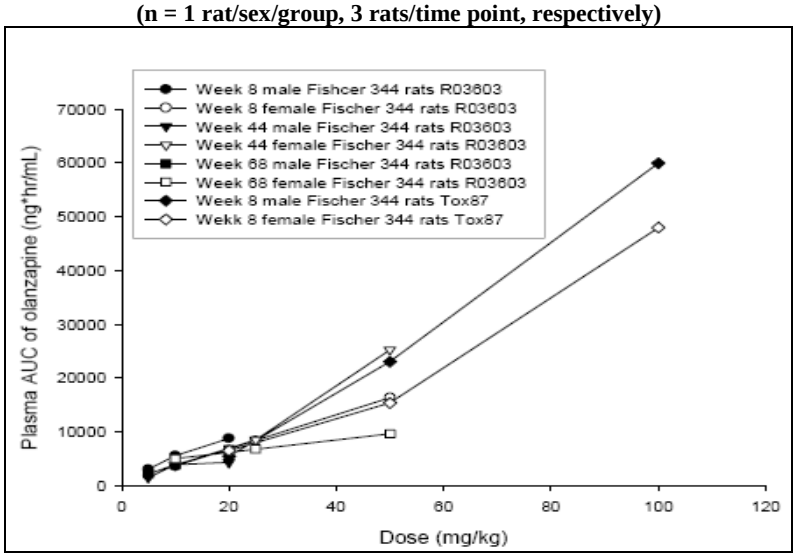


2.6.4.3.4. Repeat-Dose Pharmacokinetics of Olanzapine

Rats:

TK of Olanzapine upon repeat-dose OP Depot i.m. administration at 4-week intervals were determined in two studies: the 3-month toxicology study and the 2-year oncogenicity study in Fischer 344 rats (Document ID: R03603), and plasma concentrations were determined for up to 3 months and up to 18 months, respectively (tabular TK data are available under the individual studies in the toxicology section of this review). Peak plasma concentrations of olanzapine generally occurred within the first day after dosing and declined over the subsequent 28 days. Olanzapine did not accumulate following multiple doses of OP Depot. In general, AUC_{0-t} values for olanzapine increased with increasing dose (see sponsor's figure on the next page), but C_{max} values did not increase with dose. There was a trend to higher exposure in males.

Mean olanzapine AUC0-t vs. dose in Fischer 344 rats (male and female) after IM administration of OP Depot for 3 or 18 months (Data fromTox87 and R03603)*
(n = 1 rat/sex/group, 3 rats/time point, respectively)



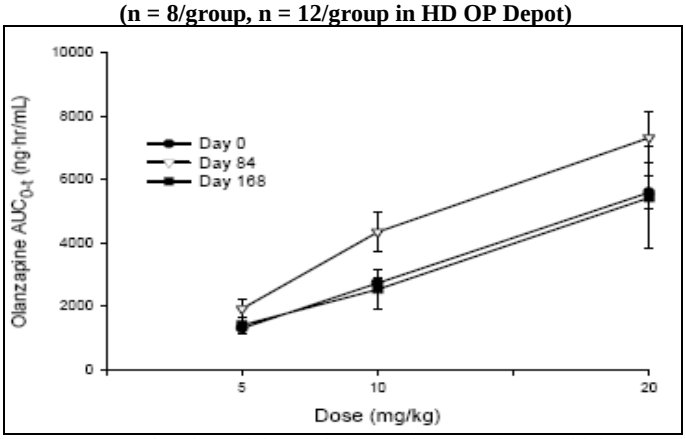
- Toxicology Report 87: Study R07100 3-month rat toxicology study with OPM i.m. administration once every 4 weeks
- R03603: 2-year oncogenicity study in Fischer 344 rats (Document ID: R03603)

Dogs

Olanzapine TK parameters were determined in beagle dogs following multiple IM doses of OP Depot once every 2 weeks over 6 months (Study D00200, Toxicology Report 88: A Chronic Toxicity Study in Beagle Dogs Given Multiple Doses of Olanzapine Pamoate Monohydrate for 6 Months Followed by a 2-Month Reversibility Phase).

Mean olanzapine Tmax values ranged from 120 to 139 hours after the 7th dose (Day 84) and from 8 to 120 hours after the 13th dose (Day 168) for both genders in all dosage groups. Plasma concentrations of olanzapine gradually decreased following Cmax and were measurable in all dose groups for at least 14 days after each injection. In general, AUC_{0-t} values for olanzapine increased dose-proportionally (see sponsor's figure below). Fluctuations in exposure over the study were attributed to a high inter-animal variability. No consistent gender differences were seen.

Mean (± SEM) olanzapine AUC0-t vs. dose in beagle dogs (M and Fcombined) after IM administration of OP Depot for 6 months*
(n = 8/group, n = 12/group in HD OP Depot)



***** Study D00200, Toxicology Report 88: A Chronic Toxicity Study in Beagle Dogs Given Multiple Doses of Olanzapine Pamoate Monohydrate for 6 Months Followed by a 2-Month Reversibility Phase)

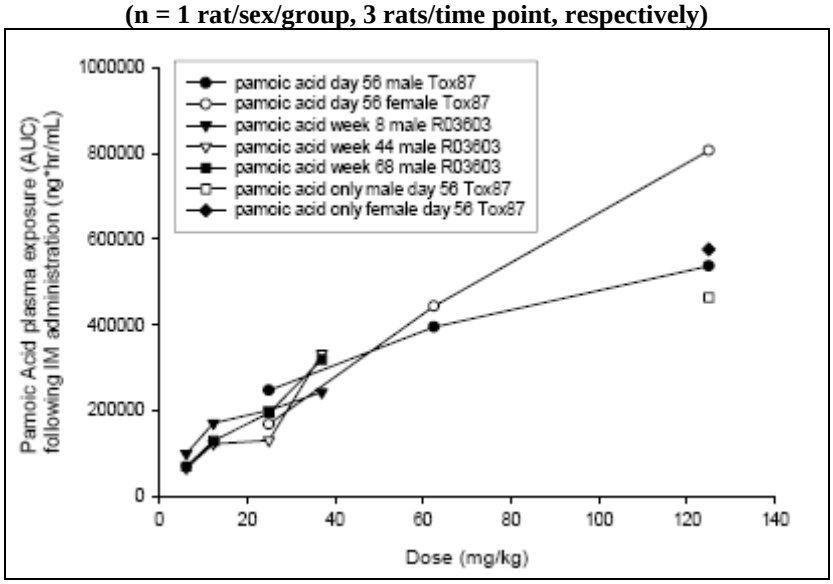
2.6.4.3.5. Repeat-Dose Pharmacokinetics of Pamoic Acid

Rats

Pamoic acid exposure upon repeat-dose OP Depot or pamoic acid alone i.m. administration at 4-week intervals were determined in two studies: the 3-month toxicology study and the 2-year oncogenicity study in Fischer 344 rats (Document ID: R03603), and plasma concentrations were determined for up to 3 months and up to 18 months, respectively (tabular TK data are available under the individual studies in the toxicology section of this review).

Peak plasma concentrations of pamoic acid were generally reached within the first day after OPM injection but the C_{max} values did not increase with increasing dose, while AUC values increased with increasing dose (see sponsor's figure below). There was no consistent gender difference in exposure. Pamoic acid did not accumulate following multiple doses of OP Depot. Plasma concentrations of pamoic acid gradually decreased during the first 2 to 3 weeks after each injection then remained near or below the quantifiable limit for the final 1 to 2 weeks. When administered alone, pamoic acid resulted in much higher peak plasma concentrations but similar AUC values as compared to those observed in the high-dose OP Depot group, which received a similar pamoic acid dose; plasma concentrations of pamoic acid rapidly declined during the first 2 weeks after each injection.

Mean pamoic acid AUC0-t vs. dose in Fischer 344 rats (male and female) after IM administration of OP Depot for 3 or 18 months (Data fromTox87 and R03603 studies)*
(n = 1 rat/sex/group, 3 rats/time point, respectively)



- Toxicology Report 87: Study R07100 3-month rat toxicology study with OPM i m. administration once every 4 weeks
- R03603: 2-year oncogenicity study in Fischer 344 rats (Document ID: R03603)

Dogs

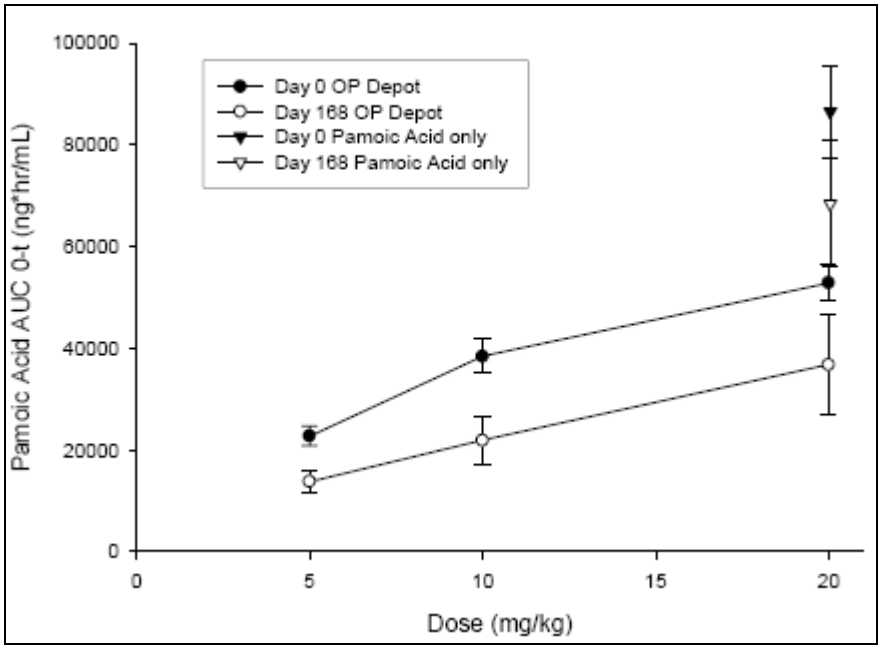
Pamoic acid TK upon repeat-dose administration were determined in beagle dogs following IM doses of OP Depot or pamoic acid alone once every 2 weeks for 6 months (Study D00200, Toxicology Report 88: A Chronic Toxicity Study in Beagle Dogs Given Multiple Doses of Olanzapine Pamoate Monohydrate for 6 Months Followed by a 2-

Month Reversibility Phase). Tabular TK data are available in the toxicology section of this review.

Pamoic acid C_{max} values following OP Depot administration were reached between 3 and 120 hours after the dose for both genders; plasma concentrations gradually decreased thereafter and were measurable for at least 14 days after each injection. AUC_{0-t} values for pamoic acid increased less than dose proportionally with increasing dose (see sponsor's figure below). Pamoic acid administered alone resulted in a much higher peak plasma concentration, a higher plasma exposure (AUC) and a shorter Tmax than those observed in the high-dose OP Depot group, which received a similar pamoic acid dose. Plasma concentrations of pamoic acid in animals receiving only pamoic acid rapidly declined following C_{max}.

There was high inter-individual variability and no consistent gender differences in pamoic acid exposure.

Mean (± SEM) pamoic acid AUC0-t and dose in beagle dogs (male and female combined) after IM administration of OP Depot or pamoic acid alone for 6 months*
(n = 8/group, n = 12/group in high-dose OP Depot)



***** Study D00200, Toxicology Report 88: A Chronic Toxicity Study in Beagle Dogs Given Multiple Doses of Olanzapine Pamoate Monohydrate for 6 Months Followed by a 2-Month Reversibility Phase)

2.6.4.4 Distribution

No distribution studies were conducted with olanzapine pamoate monohydrate as the distribution of olanzapine following IM administration was thought to be "substantially identical" to that observed for oral olanzapine following absorption. The distribution of olanzapine after oral administration was reported in NDA 20-592 and summarized by the sponsor as follows:

> " Briefly, single and repeat oral doses of ^{14}C-labeled olanzapine in rats demonstrated that olanzapine is widely distributed throughout the body and is cleared relatively quickly; by 96 hours postdose, most tissues had minimal levels of radioactivity

(ADME 6, 26, and 25; NDA 20-592). As expected, tissues containing the highest residues after 24 hours (liver, kidney) were associated with excretion of olanzapine and metabolites. Olanzapine was shown to have a fairly high degree of binding to plasma proteins in each of the species examined (ADME 33; NDA 20-592). Using an ultracentrifugation method with radiochemical detection at a concentration of 100 ng/mL, radioactivity associated with 14C-olanzapine was bound at 81%, 83%, 81%, 91%, and 93% in the plasma of mice, rats, dogs, monkeys, and humans, respectively. Pregnant rats given oral doses of 14C-olanzapine on Gestation Day 12 or 18 had high levels of radioactivity in most maternal tissues, but very low levels (≤ 0.04% of the dose) were detected in fetal tissues (ADME 24 and 2; NDA 20-592). These studies indicate that neither olanzapine nor its metabolites pass freely across the rat placenta".

Standard distribution, protein binding, or placental transfer studies for pamoic acid were not conducted, as "these studies were not needed to support toxicology or clinical endpoints". However, a 14C-pamoic acid distribution study was conducted in mice to support genetic toxicology studies (Study 003M02, ADME Report 01: Radiocarbon Concentrations in Plasma and BoneMarrow of Male ICR Mice Following a Single 4.7-mg Intramuscular Injection of [14C]Pamoic Acid). This study determined specifically pamoic acid distribution to bone marrow which was approximately 20.7% of that in the plasma at 4 hours post injection (see sponsor's table below). Bone marrow concentrations fell near or below the detection limit by 18 h post injection, although radiocarbon was detectable in plasma at both 18 and 42 hours post-injection.

Pamoic Acid Organ Distribution After a Single Dose of 14C-Pamoic Acid

Species/strain: Mouse/ICR
Sex/number of animals[a]: Male/12
Fed/fasted: Fed
Route: Intramuscular injection
Dose (mg): 4.7
Radionuclide: ^{14}C
Specific activity: 0.75 µCi/mouse
Sampling time: 4, 18, and 42 hours postinjection
Document ID: ADME01-PA

	Concentration ± SEM			
Tissues/Organs	4 hours	18 hours	42 hours	$t_{1/2}$
Plasma (µg-eq/mL)	86.5 ± 8.8	3.9 ± 0.8	0.8 ± 0.2	NA
Bone marrow (µg-eq/g)	17.9 ± 1.9	BQL ± NC	7.2[b] ± NC	NA

Abbreviations: BQL = below quantifiable limit (<6 DPM, approximately 4.59 µg-eq/g); eq = equivalents of radioactivity as compared to parent compound, 14C-pamoic acid; NA = not applicable; NC = not calculated; SEM = standard error of the mean.

[a] Number of animals reflects total animals in the entire study for which results were described (4 mice/time point).

[b] n = 2.

2.6.4.5 Metabolism

No preclinical studies of olanzapine metabolism following i.m. administration of OP Depot were conducted. However "in humans, the metabolic profile following intramuscular administration of RAIM olanzapine (NDA 21-253) and OP Depot was shown to be similar to that after oral administration".

Pamoic acid in vitro and in vivo metabolism studies were conducted in rats.

The in vitro metabolism of [14C] pamoic acid was assessed in activated rat hepatic S9 fractions upon 4-hour incubations with 14C-pamoic acid, as shown in sponsor's table on the next page. Verapamil was used as a positive control. The retention time and LC/MS profile of the only peak observed in 14C-pamoic acid containing samples was consistent

with that of authentic pamoic acid. Thus, no metabolites of pamoic acid were observed following the incubation of 14C-pamoic acid with activated rat S9 fractions.

Pamoic Acid Metabolism In Vitro: Relative Concentrations of 14C-Pamoic Acid in Rat Hepatic S9

Study system: Hepatic S9 fractions
Species: Rat
Sample time: 4 hours
Test concentration: 1000 µg/mL pamoic acid

Relative concentration of ^{14}C-pamoic acid (parent) in the supernatant: ≥97% of total radioactivity[a]

[a] Essentially all radioactivity was recovered in the supernatants of the samples; no metabolites were observed.

[Data from ADME Report 04: Assessment of the In Vitro Metabolism of [14C] pamoic acid (Lilly Serial Number 015784) in Rat Hepatic S9 Fractions by HPLC with Radio-detection and LC/MS]

In vivo, the plasma pharmacokinetics of pamoic acid were evaluated following a single 10-mg/kg intramuscular administration of [14C]pamoic acid to male Fischer 344 (F344) rats . Radioactivity in plasma was determined by liquid scintillation counting and plasma concentrations of pamoic acid were determined by HPLC with fluorescence detection. Mean peak plasma concentrations (C_{max}), the area under the plasma concentration-time curve values (AUC) and half-life are shown in the following sponsor's table.

PK of pamoic acid following a single 10-mg/kg i.m. administration of [14C]pamoic acid to male Fischer 344 (F344) rats

Parameter	Plasma Pamoic Acid	Plasma Radioactivity
$AUC_{0-\infty}$ (ng•hr/mL or ng-eq•hr/mL)	69916.4	71573.6
β Half-life (hr)[a]	9.7	10.5
γ Half-life (hr)[b]	NC	65.6
C_{max} (ng/mL or ng-eq/mL)	44386.4	39333.3

[a]beta half-life representing the majority of the AUC;
[b]gamma half-life, apparent true elimination half-life representing a small fraction of the AUC;
AUC = area under the plasma concentration-time curve; a user-defined value of 0 ng/mL or 0 ng-eq/mL was used at time zero to calculate AUC values;
C_{max} = maximum plasma concentrations; ng-eq = ng-equivalents of radioactivity; radioactivity calculations are presented as ng-eq or ng-eq.hr/mL values, NC = not calculated.

[Data from ADME Report 02: Plasma Pharmacokinetics of Radioactivity and Pamoic Acid in Fischer 344 Rats Following a Single 10-mg/kg Intramuscular Dose of [14C]Pamoic Acid (Study 007R02)]

Based on the nearly identical plasma exposures and pharmacokinetic profiles of total radioactivity and pamoic acid, it was concluded that pamoic acid did not undergo metabolism when administered i.m. to rats. Residual radioactivity in urine, feces, cage wash, and carcass from the rats indicated that all radioactivity in plasma and feces was pamoic acid. Thus, there was no evidence of any meaningful metabolism of pamoic acid in Fischer 344 rats.

2.6.4.6 Excretion

No specific studies on olanzapine excretion were conducted for the OP Depot, as the excretion characteristics of 14C-labeled olanzapine had been previously determined for

oral olanzapine and RAIM olanzapine and they were "not expected to change once the compound was absorbed".

The elimination of pamoic acid was evaluated following a single 10 mg/kg i.m. administration of 14C-pamoic acid to male Fischer 344 rats by determination of residual radioactivity in urine, feces, cage wash, and carcass (see sponsor's table on the next page). The mean total recovery of radioactivity was approximately 98.2% of the administered dose after 168 hours, with 97.8% of the dose recovered after 72 hours. The majority of radioactivity (approximately 97.5% of the dose) was recovered in feces after 72 hours. Urine, cage wash, and carcass accounted for only approximately 0.3%, 0.1%, and 0.1%, respectively, of the administered radioactivity. The data indicate that after IM administration to male Fischer 344 rats, radioactivity associated with pamoic acid is well absorbed and is rapidly excreted unchanged via the feces, suggesting that biliary excretion is the primary route of elimination.

Pamoic Acid Excretion in Conjunction with Metabolism of 14C-Pamoic Acid

Species: Rat
Sex/number of animals[a]: Male/6 Document ID: ADME03-PA
Feeding condition: Fed
Route: Intramuscular injection
Dose: 10 mg/kg
Vehicle: 0.75% Carboxymethylcellulose sodium, 5% mannitol, and 0.1% polysorbate 80
Formulation: Suspension
Analyte: Total radioactivity, percent of dose recovered, ^{14}C-pamoic acid
Assay: LSC[b]; LC/MS and HPLC radiochemical detection[c]

	Percent of Dose		
Excretion route:	Urine	Feces	Total
0 to 360 hours	0.30	97.80	98.32

Abbreviations: HPLC = high performance liquid chromatography, LC/MS = liquid chromatography with mass spectrometry detection, LSC = liquid scintillation counting.
[a] Number of animals reflect total animals used in the entire study for which results were described.
[b] LSC used to determine percent radioactivity recovered.
[c] LC/MS and HPLC radiochemical detection used for metabolic profiling.

(Data from Study 007R02 ADME Report 03: Elimination and Metabolism of Radioactivity in Male Fischer 344 Rats Following a Single Intramuscular Dose of 10 mg/kg [14C] Pamoic Acid).

2.6.4.7 Pharmacokinetic drug interactions

No preclinical PK studies were conducted to evaluate drug-drug interactions for this form of olanzapine. The sponsor considered that the clinical drug interaction studies previously performed with oral and RAIM olanzapine were "a reliable characterization of what to expect with OP Depot in similar situations due to similarities in metabolism and elimination".

2.6.4.8 Other Pharmacokinetic Studies

No other preclinical PK studies were conducted for this form of olanzapine.

2.6.4.9 Discussion and Conclusions

The pharmacokinetic evaluation of olanzapine pamoate (the sustained release salt form of olanzapine with pamoic acid allowing long-term exposure to olanzapine) was focused on

the absorption of the salt form, as other aspects of olanzapine disposition (distribution, metabolism, excretion, etc) had been previously evaluated for the oral and the rapid acting IM form and were expected "to remain unchanged once the compound is absorbed".
In the animal species studied (rat, dog, and rabbit), OP Depot produced initial peak plasma concentrations of olanzapine and pamoate followed by a gradual decline in concentrations for up to 28 days post-dose. Plasma concentrations of olanzapine following the administration of OP Depot increased with increasing dose. Plasma concentrations of pamoic acid were greater following the administration of pamoic acid alone compared to the administration of OP Depot. The plasma profiles of pamoic acid following administration of OP Depot were qualitatively similar to those obtained following administration of pamoic acid alone.

Conclusions:
- Studies with the OP Depot demonstrated absorption and sustained plasma exposure in all tested animal species.
– Single-dose pharmacokinetics of OP Depot indicated rapid absorption and sustained exposure to olanzapine and pamoic acid in rats, with slower absorption but sustained exposure to both olanzapine and pamoic acid in dogs.
– Repeat-dose toxicokinetics for both olanzapine and pamoic acid indicate no consistent sex difference and no accumulation of either olanzapine or pamoic acid.
– In rats, rabbits, and dogs, plasma concentrations of olanzapine following IM administration of OP Depot increased with increasing dose. OP Depot produced initial peak plasma concentrations of olanzapine followed by a gradual decline in concentration for up to 28 days postdose, thus demonstrating a sustained release profile.
– The plasma profile of pamoic acid following administration of OP Depot was similar to that of olanzapine. When administered IM as a single agent, pamoic acid was also readily absorbed and was excreted largely unchanged via bile into the feces.

2.6.4.10 Tables and figures to include comparative TK summary

The following sponsor's tables compare the maximum exposure to olanzapine in rats and dogs following administration of oral olanzapine (daily) or OP Depot either once/4 weeks in rats or once/2 weeks in dogs. As narrated by the sponsor, "during an 8-week period, male rats given oral olanzapine were exposed to 9.1 times more olanzapine and female rats were exposed to 13.4 times more olanzapine than rats given 3 IM injections of the highest feasible dose of OP Depot. Additionally, male dogs in the oral study following 6 months of treatment were exposed to 9.9 times more olanzapine and female dogs were exposed to 6.4 more times olanzapine than the dogs in the 6-month olanzapine pamoate monohydrate study following 13 injections of IM OP Depot."

In summary, the data indicate that the exposure to olanzapine following administration of OP Depot is lower than the exposure following oral administration of the compound, for the same administered dose. Thus, the relative lack of systemic toxicity in the nonclinical toxicology studies with olanzapine pamoate monohydrate was to be expected based on the lower exposures achievable upon OP Depot IM vs. oral administration.

Comparison of Olanzapine Plasma Exposure in Rats
Following Administration of Oral Olanzapine versus IM OP Depot

				IM OP Depot after 13 Injections	
		Oral Olanzapine after 6 Months		Every 2 Weeks	
	Dose[a]	10 mg/kg Olanzapine		20 mg/kg Olanzapine	
	Sex	Male	Female	Male	Female
AUC_{0-t} (ng*h/mL)[b]		19410	22914	59992	48010
C_{max} (ng/mL)		1280	2257	209	169
Normalized AUC[c]		543480	641592	59992	48010
Ratio of Oral/OP Depot AUC[d]		9.1	13.4		

Abbreviations: AUC = area under the plasma concentration-time curve, C_{max} = maximum plasma concentration, IM = intramuscular, OP = olanzapine pamoate.

a Doses reflect highest doses administered in the studies (Oral > OP Depot).

b $AUC_{0-24\ h}$ for oral, $AUC_{0-672\ h}$ for OP Depot.

c Normalized AUC was calculated by multiplying the oral AUC by 28.

d Ratio was calculated by dividing the normalized oral AUC by the OP Depot AUC.

Comparison of Olanzapine Plasma Exposure in Dogs
Following Administration of Oral Olanzapine versus IM OP Depot

				IM OP Depot after 13 Injections	
		Oral Olanzapine after 6 Months		Every 2 Weeks	
	Dose[a]	10 mg/kg Olanzapine		20 mg/kg Olanzapine	
	Sex	Male	Female	Male	Female
AUC_{0-t} (ng*h/mL)[b]		2276	3492	3204	7655
C_{max} (ng/mL)		218	380	29	54
Normalized AUC[c]		63728	97776	6408	15310
Ratio of Oral/OP Depot AUC[d]		9.9	6.4		

Abbreviations: AUC = area under the plasma concentration-time curve, C_{max} = maximum plasma concentration, IM = intramuscular, OP = olanzapine pamoate.

a Oral olanzapine was given once a day, IM OP Depot was given once every 14 days, doses reflect highest doses administered in the studies.

b $AUC_{0-24\ h}$ for oral, $AUC_{0-336\ h}$ for OP Depot.

c Normalized AUC was calculated by multiplying the oral AUC by 28 and the IM AUC by 2.

d Ratio was calculated by dividing the normalized oral AUC by the OP Depot AUC.

Exposure Multiples

Relatively low olanzapine exposures could be achieved with OP Depot in the nonclinical studies, which resulted in low nonclinical-to-clinical exposure multiples (as shown in the sponsor's tables below). However, this should not be considered a safety issue since the safety of olanzapine was established by previous nonclinical studies with the approved oral and rapid-acting IM olanzapine formulations. The sustained long-term systemic

plasma exposure to olanzapine upon OP Depot administration was not associated with systemic toxicity, and monitorable clinical signs were observed in all nonclinical species. Systemic exposure to the pamoate ion (assayed as pamoic acid) at the highest doses administered in the animal studies (resulting primarily in injection site reactions only), approximated or exceeded human levels of pamoic acid. Exposure multiples (C_{max}) for pamoic acid ranged from 0.5 to 0.7 (dogs) and from 5 to 6 (rats) in repeat-dose studies; 4 and 39 in rabbit and rat embryo-fetal studies, respectively; and 4 to 10 in the 2-year rat carcinogenicity study.

Exposure Multiples Based on Cmax or AUC for 300 mg/2 weeks

		C_{max} (ng/mL)	AUC (ng•h/mL)	Exposure Multiple Based on C_{max} M	Based on C_{max} F	Based on AUC M	Based on AUC F
Human	300 mg/2 weeks[a]	49.7	13600				
Rat	3-Month repeat-dose 100 mg/kg on Day 56	M: 209 F: 169	M: 59992 F: 48010	4.2	3.4	4.4	3.5
Rat	Embryo fetal 75 mg/kg on GD 6	153	8448	–	3.1	–	0.6
Rabbit	Embryo fetal 75 mg/kg on GD 7	51	9703	–	1.0	–	0.7
Rat	2-Year carcinogenicity Max dose at 68 weeks	M: 73 F: 187	M: 6647 F: 9597	1.5	3.8	0.5	0.7
Dog	6-Month repeat-dose 20 mg/kg on Day 168	M: 29.14 F: 53.53	M: 3204 F: 7655	0.6	1.1	0.2	0.6

Exposure Multiples Based on Cmax or AUC for 405 mg/4 weeks

		C_{max} (ng/mL)	AUC (ng•h/mL)	Exposure Multiple Based on C_{max} M	Based on C_{max} F	Based on AUC M	Based on AUC F
Human	405 mg/4 weeks[a]	54.0	26100				
Rat	3-Month repeat-dose 100 mg/kg on Day 56	M: 209 F: 169	M: 59992 F: 48010	3.9	3.1	2.30	1.84
Rat	Embryo fetal 75 mg/kg on GD 6	153	8448	–	2.8	–	0.32
Rabbit	Embryo fetal 75 mg/kg on GD 7	51	9703	–	0.9	–	0.37
Rat	2-Year carcinogenicity Max dose at 68 weeks	M: 73 F: 187	M: 6647 F: 9597	1.4	3.5	0.25	0.37
Dog	6-Month repeat-dose 20 mg/kg on Day 168	M: 29.14 F: 53.53	M: 3204 F: 7655	0.5	1.0	0.12	0.29

Abbreviations: F = female, GD = Gestation Day, M = male, Max = maximum.
a Human exposure values obtained from Study F1D-EW-LOBE.

2.6.5 PHARMACOKINETICS TABULATED SUMMARY

Pharmacokinetics: Overview

Type of Study	Test System	Route	Testing Facility	Document ID
Absorption				
Single-dose pharmacokinetics	Rat	Intramuscular injection	Eli Lilly and Co	ADME02-PA
Distribution				
Tissue distribution	Mouse	Intramuscular injection	Eli Lilly and Co	ADME01-PA
Metabolism				
Metabolism in hepatic S9 fractions	Rat	In vitro	Eli Lilly and Co	ADME04-PA
Excretion and metabolism in blood, urine, and feces	Rat	Intramuscular injection	Eli Lilly and Co	ADME03-PA
Excretion				
Excretion in urine and feces	Rat	Intramuscular injection	Eli Lilly and Co	ADME03-PA
Pharmacokinetic Drug Interactions - Not Applicable				
Other Studies - Not Applicable				

Pharmacokinetics: Absorption after a Single Dose of 14C-Pamoic Acid in the Rat

Sample: Plasma
Radionuclide: ^{14}C
Species: Rat
Route: Intramuscular injection
Document ID: ADME02-PA
Feeding condition: Fed
Dose: 10 mg/kg
Vehicle/formulation: 0.75% Carboxymethylcellulose sodium, 5% mannitol, and 0.1% polysorbate 80/suspension

	Analyte: ^{14}C-Pamoic Acid	Analyte: ^{14}C-Equivalents
Sex/number of animals:	Male/36	Male/36
Assay:	HPLC/fluorescence	LSC
PK parameters:		
$AUC_{0-\infty}$ (ng•h/mL)	69916.4 ng•h/mL	71573.6 ng-eq•h/mL
ß Half-life (h)[a]	9.7	10.5
γ Half-life (h)[b]	NC	65.6
C_{max} (ng/mL)	44386.4 ng/mL	39333.3 ng-eq/mL

Abbreviations: $AUC_{0-\infty}$ = total systemic exposure (area under the plasma concentration-time curve from 0 to infinity), C_{max} = maximal observed plasma concentration, HPLC = high performance liquid chromatography, LSC = liquid scintillation counting, NC = not calculated.

[a] Beta (ß) half-life representing the majority of the AUC.

[b] Gamma (γ) half-life, apparent true elimination half-life representing a small fraction of the AUC.

Pharmacokinetics: Organ Distribution after a Single Dose of 14C-Pamoic Acid

Species/strain: Mouse/ICR
Sex/number of animals[a]: Male/12
Fed/fasted: Fed
Route: Intramuscular injection
Dose (mg): 4.7
Radionuclide: ^{14}C
Specific activity: 0.75 µCi/mouse
Sampling time: 4, 18, and 42 hours postinjection
Document ID: ADME01-PA

Tissues/Organs	Concentration ± SEM			
	4 hours	18 hours	42 hours	$t_{1/2}$
Plasma (µg-eq/mL)	86.5 ± 8.8	3.9 ± 0.8	0.8 ± 0.2	NA
Bone marrow (µg-eq/g)	17.9 ± 1.9	BQL ± NC	7.2[b] ± NC	NA

Abbreviations: BQL = below quantifiable limit (<6 DPM, approximately 4.59 µg-eq/g); eq = equivalents of radioactivity as compared to parent compound, ^{14}C-pamoic acid; NA = not applicable; NC = not calculated; SEM = standard error of the mean.

[a] Number of animals reflects total animals in the entire study for which results were described (4 mice/time point).

[b] n = 2.

Pharmacokinetics: Metabolism In Vivo: Relative Radiocarbon Concentrations in Fischer 344 Rats After Administration of 14C-Pamoic Acid

Species: Rat
Sex/number of animals: Male/6
Feeding condition: Fed
Radionuclide: ^{14}C
Specific activity of dose: 5 µCi/mg
Vehicle/formulation: 0.75% Carboxymethylcellulose sodium, 5% mannitol, and 0.1% polysorbate 80/suspension
Route: Intramuscular injection
Dose: 10 mg/kg
Document ID: ADME03-PA

	Relative Concentrations of Radioactivity in Plasma (% of dose recovered)	Relative Concentrations of Radioactivity in Feces (% of dose recovered)
Sample time	0 to 2 hours	0 to 24 hours
Pamoic acid[a]	92.8[b]	107.2[b]

[a] Based on the nearly complete recovery of ^{14}C-pamoic acid from plasma and complete recovery from feces, there is no evidence of metabolism of pamoic acid.

[b] Analytical extraction recovery.

Pharmacokinetics: Excretion in Conjunction with Metabolism of 14C-Pamoic Acid

Species: Rat
Sex/number of animals[a]: Male/6
Feeding condition: Fed
Route: Intramuscular injection
Dose: 10 mg/kg
Vehicle: 0.75% Carboxymethylcellulose sodium, 5% mannitol, and 0.1% polysorbate 80
Formulation: Suspension
Analyte: Total radioactivity, percent of dose recovered, ^{14}C-pamoic acid
Assay: LSC[b]; LC/MS and HPLC radiochemical detection[c]
Document ID: ADME03-PA

Excretion route:	Percent of Dose		
	Urine	Feces	Total
0 to 360 hours	0.30	97.80	98.32

Abbreviations: HPLC = high performance liquid chromatography, LC/MS = liquid chromatography with mass spectrometry detection, LSC = liquid scintillation counting.

[a] Number of animals reflect total animals used in the entire study for which results were described.

[b] LSC used to determine percent radioactivity recovered.

[c] LC/MS and HPLC radiochemical detection used for metabolic profiling.

2.6.6 TOXICOLOGY

2.6.6.1 Overall toxicology summary

General toxicology:
The single- and repeat-dose toxicity of OP Depot was evaluated in rats and beagle dogs. In repeat-dose toxicity studies, OP Depot was given intramuscularly to rats once every 4 weeks for 3 months and to dogs once every 2 weeks for 6 months. Due to limitations in dose volume and suspendability, systemic toxicity was not elicited with OP Depot in most instances. In the OP Depot studies, lower doses of olanzapine were administered in the nonclinical studies as compared to the oral dosage form.
Key findings include:
– Injection site reactions indicative of chronic inflammation were the major finding in all toxicology studies with OP Depot, in both rats and dogs. The reaction in dogs was more pronounced, appearing within a few days after administration and diminishing in a week or 2 thereafter. Histologic evidence of chronic inflammation and fibrosis was present at necropsy; the inflammation persisted, though significantly reduced, after a 2-month recovery period.
– Pamoic acid (the formulation agent) did not exert systemic toxicity. Injection site reactions from the pamoic acid-treated animals were less frequent and less severe than those from animals treated with OP Depot.

Single-Dose Toxicity

In rats, a single IM injection of OP Depot produced a granulomatous inflammation surrounding the injected material, most severe in 4- and 7-day-old injection sites. Subsequently, the chronic inflammation subsided, but was still evident up to 42 days post-injection. The inflammatory response to pamoic acid alone was of a shorter duration (i.e., by Day 42, about 30% (3 of 10) rats had an observable inflammatory response). The inflammatory response to the vehicle alone was milder and dissipated by Day 21.

In dogs given OPM at single i.m. doses of 5, 10, or 20 mg/kg, injection site reactions occurred approximately 1 week after the injection and lasted for 2 to 12 days in males and for 1 to 8 days in females. By the end of the 6-week observation period, no significant injection site reactions were observed clinically. All reactions were of "minimal or slight" severity and there was no apparent dose dependence in incidence or severity among the dose groups. Histologically, the reaction was characterized by focal or multifocal areas of chronic inflammation with fibrosis with vacuolated macrophages present within the fibrous inflammatory tissue. Changes in hematology and clinical chemistry parameters, associated with inflammation, were limited to moderate transitory increases in neutrophils and monocytes and a slight transitory increase in serum globulin.

Repeat-Dose Toxicity

OP Depot was administered intramuscularly to rats (once every 4 weeks for 3 months) and dogs (once every 2 weeks for 6 months).

In the 3-month rat study with OP Depot, administered intramuscularly once every 4 weeks at doses of 20, 50 and 100 mg/kg, body weights and weight gain were reduced at MD and HD (males only). Treatment-related clinical signs included lacrimation, red

ocular and/or nasal discharge, and soiling of fur (chin, perineal, anal, and urogenital). Generally, these effects were dose related in incidence, showed a slightly higher incidence in females, occurred mainly within 0 to 2 days postdose, and were seen more predominantly after the second or third injection. Treatment-related gross and microscopic changes in rats treated with OP Depot and pamoic acid were limited to injection site reactions consistent with a chronic inflammatory response to a foreign body and increased in severity with the increase of OPM dose. Injection site reactions from the pamoic acid-treated rats were less frequent and less severe than those from rats treated with OP Depot.

In the 6-month dog study with OP Depot, administered intramuscularly once every 4 weeks at doses of 5, 10 and 20 mg/kg, clinical signs were limited to injection site reactions consisting of swelling, firmness, redness, ulceration, and scabs ("scab" was defined as "an open area of skin with drainage"). All dogs given OP Depot developed injection site reactions but their incidence, severity and duration were dose-dependent. The incidence and severity of the reaction did not appear to change over the course of the study and generally resolved within 1 to 2 weeks. In dogs given pamoic acid alone, the injection site reactions were mild and occurred at a low incidence. Grossly, the injection sites from dogs treated with OP Depot were characterized primarily by "accumulations of yellow foreign material within the biceps femoris muscle and adjacent muscles and connective tissues". Histologically, the injection site reactions in dogs given OP Depot were characterized by "focally extensive areas of myocyte loss with replacement by fibroblasts, collagen, and variable numbers of inflammatory cells consistent with chronic inflammation in response to tissue injury and injection of foreign material". The injection site reactions from pamoic acid-treated dogs were histologically similar.
Because of limitations of dose volumes that could be humanely administered and the concentrations that could be achieved with OP Depot, higher doses could not be administered in the nonclinical studies.

Genetic toxicology: Olanzapine was previously tested for genetic toxicity (under NDA 20-592) and was negative in a full range of standard tests that included bacterial mutation tests and in vitro and in vivo mammalian tests.
Pamoic acid was negative in the Ames test, the mouse lymphoma assay, and the chromosome aberration assay in human lymphocytes and was also negative in 2 in vivo assays (the mouse micronucleus test and the mouse bone marrow chromosome aberration assay). Although reproducibly positive results were obtained in an in vitro chromosome aberration assay in Chinese hamster ovary (CHO) cells (at concentrations >500 μg/ml, about 1000-fold greater than the peak plasma concentrations in humans), the absence of oncogenicity in the 2-year carcinogenicity study in rats supports a lack of genotoxic hazard to humans.

Carcinogenicity: In the 2-year study to evaluate the carcinogenic potential of a sustained-release formulation of olanzapine administered by once per 4 weeks intramuscular injections of olanzapine pamoate monohydrate (OPM) to Fischer 344 rats (60/sex/dose) at doses of 0 (vehicle), 0 (pamoic acid), 5, 10, and 20 mg /kg for males and 0, 0, 10, 25, and 50 mg /kg for females (dose range equivalent to 0.1-1.2x the MRHD of 405 mg/ 4 weeks on a mg/m2 basis), there was no carcinogenic effect attributable to OPM or

pamoic acid since there was no dose-related effect on incidence and distribution of neoplastic lesions and they were similar among groups. Effect of pamoic acid alone at i.m. doses similar to those administered in the high-dose OPM group (37 mg/kg in males and 92.5 mg/kg in females) was assessed in parallel in additional groups of rats. The doses were selected in accordance with the Executive CAC recommendations (IND 60701, Executive CAC Meeting Minutes of 3/11/2003). A MTD was achieved in this study based on dose-related injection site adverse effects in both genders (chronic inflammatory reactions and residual test substance accumulation in the injection site affecting nearly all animals of both genders at HD). Olanzapine $AUC_{0\text{-}336h}$ values achieved at HD were lower or equal than those in humans at MRHD (300 mg every 2 weeks or 405 mg every 4 weeks). Exposures to pamoic acid ($AUC_{0\text{-}336h}$) achieved at HD were equal to or higher than those in humans at MRHD. Dose-limiting factors were the amount of test article feasible to be injected i.m. in the rat and local injection site reaction.

Reproductive and developmental toxicology:

- Fertility and Early Embryonic Development: No fertility studies were conducted with OP Depot.
- Embryo-Fetal Development

Embryo/fetal studies in rats and rabbits from dams treated with OPM Depot formulation during gestation [i.m. doses of 10, 25 and 75 mg/kg on gestation day 6 (rat) or 7 (rabbit) with plasma exposures maintained throughout the period of organogenesis] showed no OPM- or pamoic acid-related maternal systemic toxicity, embryo/fetotoxicity (as indicated by the lack of effect on embryo/fetal intrauterine growth and survival) or increased incidence of structural malformations up to the maximum feasible dose tested (75 mg/kg).

- Pre- and Postnatal Toxicity

A prenatal/postnatal study with OPM Depot was conducted in rats at i.m. doses of 0, 10, 25, and 75 mg/kg given to dams on gestations days 6 and 16 and again on post-partum day 4. Changes in behavioral development of offspring were observed at the highest dose (a delay in negative geotaxis early in the development of the F1 pups, a lack of habituation to the startle response in F1 males and a reduced performance on memory trials in water maze). These effects are qualitatively similar to the transient decrease in Figure-8 maze activity observed in the 2-generation study conducted with oral olanzapine (NDA 20-592, as cited by the sponsor), and therefore suggest no new risk due to olanzapine pamoate monohydrate use.

Plasma exposure and safety margins in developmental toxicology studies:

- Pamoic acid exposure

Systemic exposure to the pamoate ion assayed as pamoic acid was monitored in OP Depot developmental toxicity studies in rats and rabbits. The pamoate exposures observed at the HD in these studies were about 4x (rabbits) to 40x (rats) the human exposures at MRHD.

- Olanzapine exposure

Olanzapine exposure multiples in the evaluation of OP Depot developmental toxicity relative to the MRHD were small due to limitations in dose volumes that could be administered. Based on plasma AUC values, exposure multiples in both rat and rabbit vs.

human exposure at MRHD are all less than 1. The highest OPM i.m. dose (75 mg/kg) used in the embryofetal (Segment II) studies in rats and rabbits, as well as in the prenatal/postnatal (Segment III) study in rats is the highest dose that could be administered based on the maximum achievable concentration of the test article in suspension and the maximum tolerated intramuscular dose volume for the test animals used. Maximal suspension concentrations were used in all animal studies.

Special toxicology: Studies on Impurities
Olanzapine Pamoate Monohydrate-Related Impurities: Compounds (b) (4) and (b) (4) (the (b) (4) (b) (4) of pamoic acid) were at or above their proposed acceptance criteria in Lot 325SB8 (used in the single-and repeat-dose toxicology studies) and Lot CTM00881 (used in the reproduction and carcinogenicity studies). In all these studies, no systemic adverse findings were observed with OP Depot or pamoic acid. The highest tested dose levels used in rat 3-month and dog 6-month toxicity studies provided multiples from about 2- to 3-fold above the acceptance criteria for these 2 impurities when compared to MRHD (based on dose in mg/m2) and multiples from 1.5 to 4.8x the MRHD (based on plasma exposure, AUC). Since "drug products derived from the 2 lots of drug substance mentioned above (Lot 325SB8 and ML114 which was re-labeled as CTM00881) were used in all toxicology studies and also in clinical trials", the impurity exposures tested and qualified in toxicology studies were also qualified by their use in clinical studies.
Olanzapine-Related Impurities: Compound (b) (4) (a (b) (4) (b) (4) product of olanzapine that may form during OPM drug substance manufacturing process) has oral qualification data cross-referenced from NDA 20-252, that can be applied in the light of the similar or greater exposures that are expected by the oral vs. the i.m. route. Olanzapine containing elevated levels of Compound (b) (4) as well as 2 other impurities found as degradation products in oral olanzapine drug (Compounds (b) (4) and (b) (4) were tested and found to be negative for genotoxicity in the Ames test and in vivo mouse micronucleus test, as well as for general toxicity in rats at doses about 100x higher than the MRHD of oral olanzapine in humans (NDA 20-252).

2.6.6.2 Single-dose toxicity
Study title: A Study to Evaluate Injection Site Reaction in Male Fischer 344 Rats Given Olanzapine Pamoate Monohydrate (LY170053 Pamoate Monohydrate) as a Single Intramuscular Injection

Key study findings: A single intramuscular injection of OPM in the gastrocnemius muscle of male Fisher rats (N=45) at a dose of 10 mg/rat, suspended in a vehicle of 2% carboxymethylcellulose sodium, 5% mannitol, and 0.1% Tween 80 in Sterile Water, a total injection volume of 0.1 ml, did not affect survival and produced a local granulomatous inflammation at the injection site that surrounded the injected material and was most severe 4- and 7-days post- injection. After that, the chronic inflammation subsided, but was still evident up to 42 days post-injection. Crystals of foreign material were present in 1-, 4-, and 7-day-old injection sites; from the 14-day on, small foci of crystalline structures were present only sporadically, being replaced by numerous cleft-like vacuolar structures within macrophages or free in the inflammatory reaction.

The inflammatory response to pamoic acid (the formulating agent) alone injected i.m at a dose of 12.5 mg to rats (n=25) was similar in character, but resolved earlier. All rats had a subacute reaction of a moderate degree at Day 1 characterized by lymphocytes and occasional neutrophils. By Day 4, the inflammation was still moderate but more chronic, characterized by infiltration of macrophages and lymphocytes. By Day 14, only 3 of 5 rats had an observable chronic inflammation; whereas, by Days 28 and 42, only 3 of 10 rats had an observable inflammatory response. Similar responses were observed in injection sites which received only the vehicle (in the contra-lateral gastrocnemius muscle), but these reactions had all dissipated by Day 21.
In conclusion, the reaction to intramuscular injection of OPM was consistent with that expected as a response to a foreign material. Tissue damage due to the injection process (vehicle only) resolved quickly, and no fibrosis developed. The reaction could be expected to resolve with no permanent tissue alteration.

Study no: R08999
Conducting laboratory and location: Eli Lilly and Co, Greenfield, IN
Date of study initiation: 4 October 1999
GLP compliance: Yes
QA report: Yes
Drug, lot #, radiolabel, and % purity: Olanzapine pamoate monohydrate (LY170053 pamoate monohydrate, compound 426906). Lot number; potency: 325SB8; 43.4% olanzapine.
Pamoic acid (compound 015784), lot 686W02 (relabeled as lot PPD04597), potency 98.8%.
Formulation/vehicle: suspension/ 2% carboxymethylcellulose sodium, 5% mannitol, and 0.1% Tween 80 in Sterile Water for Injection, USP; 0.1 ml per rat

Methods
Dosing:

Species/strain: Rat, Fischer 344
#/sex/group: 40 males (group 01) and 25 males (group 02)
Age: 16-17 wks
Weight: 270.0 to 306.5 g
Doses in administered units: 0 [VC], 0 [PC], and 10 mg/kg olanzapine.

Treatment Group(s)	Dose (0.1 mL)	Injection Site[a]	Number of Animals
01	10 mg olanzapine	Left leg	40
02	12.5 mg pamoic acid	Left leg	25
01 and 02	Vehicle	Right leg	65

[a] Gastrocnemius muscle of right or left hind leg.

For Treatment Group 01, a single dose of olanzapine pamoate monohydrate (10 mg of olanzapine) was injected into the gastrocnemius muscle of the left hind leg. A single dose of 12.5 mg of pamoic acid was similarly administered to Treatment Group 02 rats. For both groups, a single dose of the vehicle was injected into the gastrocnemius muscle of the right hind leg. The left leg, treated with either OPM or pamoic acid, was recorded as "injection site." The right leg of each rat served as control tissue.

Route, form, volume, and infusion rate: Single IM injection, 0.1 ml per rat.

Observations and times:

- Survival, general physical condition and behavior (daily).
- Body weights were collected on Day -3 for randomization purposes only.

Necropsy: Injection and control sites were examined sequentially through 42 days from 5 rats/group euthanized on each of Days 1, 4, 7, 14, 21, 28, 35, and 42 for rats given OPM and on Days 1, 4, 14, 28, and 42 for rats given pamoic acid. Histopathology: H/E staining, light microscopy (an independent peer review evaluation was performed).

Results:

Survival: There was no drug-related mortality (1 rat was euthanized because of trauma not related to the test compound).

Morphologic pathology:

Treatment Group 01 (OPM):

"In 1-day-old injection sites (Phase 01 rats), there was a minimal-to-moderate foreign body reaction associated with deposition of closely packed, anisotropic, refractile crystals of cuboidal to rectangular shape. There was, in general, only mild inflammatory reaction of subacute nature, characterized by infiltration of lymphocytes and occasional neutrophils. Focal necrosis of moderate degree was present adjacent to the crystals.

In 4-day-old injection sites (Phase 02 rats), the inflammatory reaction was characterized by a thick band of organizing granulomatous inflammation, composed of macrophages, lymphocytes, fibrocytes, and early fibrosis. This band of inflammation was peripheral to and surrounded the crystalline material, thus separating and walling the injected substance off from the surrounding muscle. In 1 rat, the deposit of crystals was invaded by macrophages and lymphocytes and there was apparent breakdown of the crystals; the term "resolving" was used when this occurred.

In 7-day-old injection sites (Phase 03 rats), the reaction was still granulomatous, organizing, and peripheral. The resolving nature of the crystalline deposit (influx of macrophages and lymphocytes with apparent breakdown of the crystals) was more advanced than in Phase 02 rats. The crystalline deposit was thus smaller and less distinct within the periphery of organizing inflammation.

In 14-day-old injection sites (Phase 04 rats), the refractile crystalline bodies had vanished. Instead, there were numerous cleft-like vacuolar structures. Some appeared to be free and some were within macrophages. The clefts were not refractile or anisotropic.

The surrounding inflammation was composed of lymphocytes, macrophages, and numerous plasma cells.

In 21-day-old injection sites (Phase 05 rats), the presence of foci of cleft-like vacuolar structures was similar to that seen in the 14-day-old injection sites. However, the areas of vacuolation were, in general, smaller and more condensed and the chronic inflammatory cells did not form a peripheral wall between the deposit and the skeletal muscle, but had diffusely invaded the vacuolar area. Another difference was a marked diminution in the prominence of lymphocytes and plasma cells.

In 28-day-old injection sites (Phase 06 rats), the presence of foci of cleft-like vacuolar structures was similar to that seen in the 21- and 14-day-old injection sites, but the areas of vacuolation were, in general, smaller and more condensed. In addition, residual foci of anisotropic crystals were visible (they were not evident in the 14- or 21-day-old injection

sites) within the areas of vacuolation. The inflammation was chronic, multifocal, and mild.
In 35-day-old injection sites (Phase 07 rats), foci of vacuolar areas were similar to those in the 21-day-old sites, but were smaller and the chronic inflammatory reaction was slight. There was no evidence of anisotropic crystals.
In 42-day-old injection sites (Phase 08 rats), the appearance was similar to the 35-day-old injection sites. In 1 rat, a remnant of crystal deposition was noted."

Treatment Group 02 (Pamoic acid):
"In 1-day-old injection sites, all rats had a reaction of moderate degree characterized by mild inflammation (lymphocytes and occasional neutrophils) and moderate necrosis.
By 4 days postinjection, the inflammation was more chronic, characterized by infiltration of macrophages and lymphocytes, but still moderate in degree.
By 4 days postinjection, 3 of the 5 rats had chronic inflammation (one minimal, one slight, and one moderate), and 2 rats had no evidence of a reaction.
In the 28- and 42-day-old injection sites, only 3 of the 10 rats had minimal reactions; no lesions were seen in the other 7 rats."

Reaction produced by vehicle:
Vehicle was injected into the opposite gastrocnemius muscle of Treatment Group 01 and Treatment Group 02 rats.
"In 1-day-old sites injected with vehicle, there was minimal-to slight inflammation of a subacute nature characterized by influx of lymphocytes and occasional neutrophils.
In 4- to 14-day-old sites, the inflammation was of minimal-to slight severity and composed of lymphocytes, macrophages, and fibrocytes.
In 21-day and older sites, no lesions were observed."

In conclusion, the reaction to a single intramuscular injection of OPM was consistent with a response to a foreign material. Tissue damage due to the injection process (vehicle only) resolved quickly, and no fibrosis developed. The reaction resolved with no permanent tissue alteration.

Study title: A Subchronic Toxicity Study in Beagle Dogs Given a Single Intramuscular Injection of Olanzapine Pamoate Monohydrate (LY170053 Pamoate Monohydrate)
Key study findings: Dogs (4/sex/group) received a single intramuscular injection of 5, 10, or 20 mg olanzapine pamoate monohydrate /kg body weight. Additional dogs (4/sex) received either vehicle (5% Na CMC/2% mannitol/0.1% Tween 80) or pamoic acid alone equivalent to that given to the 20-mg/kg olanzapine group. All dogs were terminated 6 weeks after dose administration. There was no mortality. Drug-related clinical signs were limited to injection site reactions in 5/8, 6/8, and 8/8 dogs at LD, MD and HD, respectively, that occurred approximately 1 week after the injection and lasted for 2 to 12 days in males and 1 to 8 days in females. Histologically, the reaction was characterized by minimal or slight focal or multifocal areas of chronic inflammation with fibrosis. There was no substantial difference in incidence or severity between the OPM dose groups. Changes in hematology and clinical chemistry parameters associated with inflammation were limited to moderate transitory increases in neutrophils, monocytes, and slight transitory increases in serum globulin. There were no compound-related

changes in body weight, food consumption, ophthalmic examination, neurological examination, urinalysis, organ weight or any effects on cardiac rate, rhythm, conduction, or repolarization. Treatment with pamoic acid alone resulted in minor swelling at injection site in 1 dog and histologically, a focal chronic reaction of minimal degree within the injection site of another dog.

Olanzapine plasma exposure (C_{max} and AUC_{0-t} values) increased with increasing dose. Mean peak plasma concentrations occurred between 45 and 126 hours and were 13.7, 21.6, or 35.2 ng/ml and AUC_{0-t} values were 1759, 3797, or 6200 ng.hr/ml at LD, MD and HD respectively. Quantifiable plasma concentrations of olanzapine were detected for up to 13, 20, and 27 days after dosing at LD, MD, and HD, respectively.

In conclusion, a single i.m. administration of olanzapine pamoate monohydrate at doses of 5, 10, or 20 mg/kg to male and female dogs resulted in measurable olanzapine plasma concentrations for at least 13 days. Treatment-related findings were limited to an inflammatory response to the olanzapine pamoate monohydrate formulation in the injection site with a similar incidence and severity across the dose groups.

Study no: R03899
Conducting laboratory and location: Eli Lilly and Co, Greenfield, IN
Date of study initiation: 23 September 1999
GLP compliance: Yes
QA report: Yes
Drug, lot #, radiolabel, and % purity: Olanzapine pamoate monohydrate (LY170053 pamoate monohydrate, compound 426906). Lot number; potency: PPD04598/200 mg olanzapine/vial.

Pamoic acid (compound 015784), lot 686W02 (relabeled as lot PPD04597), potency 98.8%.

Formulation/vehicle: suspension/ 2% carboxymethylcellulose sodium, 5% mannitol, and 0.1% Tween 80 in Sterile Water for Injection, USP.

Methods - Dosing:

Species/strain: Dogs/Beagle
#/sex/group: 4
Age: 5-8 months
Weight: Males: 8.0 to 11.0 kg; Females: 6.2 to 9.6 kg
Doses in administered units: 0 [VC], 0 [PC], and 5, 10, or 20 mg OPM/kg b. wt.
Treatment groups:

Group	Dose of Olanzapine (mg/kg)	Dose Volume (mL/kg)
01	0[a]	0.2
02	0[b]	0.2
03	5	0.05
04	10	0.10
05	20	0.2

[a] Vehicle control, 2% Na CMC, 5% Mannitol, 0.1% Tween 80.
[b] 125 mg pamoic acid/mL.

Route, form, volume, and infusion rate: Single intramuscular injection, 0.05 to 0.2 ml/kg per dog.

Observations and times:

Survival and Clinical Observations: general activity and response to environmental stimuli, posture and gait, body conformation, condition of skin and haircoat, appearance of the eyes, and character of feces were observed "frequently" on the first day of dose administration and once to several times daily on subsequent test days.

Body Weight: recorded the day before dose administration and at weekly intervals during the live phase and near live-phase termination.

Food consumption: a qualitative assessment of food consumption was made daily by visual estimation.

Ophthalmic examination: prior to treatment start and near the end of the live phase.

The eyelids, membrana nictitans, anterior chamber, lens, conjunctiva, cornea, sclera, and iris were examined in each dog's eyes using a focal light source and 2.5X magnification immediately after pupillary responses were evaluated. The adnexa, cornea, sclera, anterior chamber, and lens were examined by biomicroscopy following dilation of the pupil. The fundus of each eye was then evaluated by binocular indirect ophthalmoscopy.

Electrocardiographic evaluation: ECGs (10-second Lead II electrocardiograms) were recorded prior to starting treatment and on Days 1, 14, and 36 and were "subjectively evaluated" for presence of rhythm disturbances, ectopic beats, and P-R, Q-T and QRS durations. Heart rate data at each time point were analyzed in two separate runs using a two-factor analysis of variance model with factors for treatment, sex, and treatment-by-sex interaction.

Hematology: Erythrocyte count, Hemoglobin, Hematocrit, Mean corpuscular volume, Mean corpuscular hemoglobin, Reticulocyte count, Blood cell morphology, Total leukocyte count, Leukocyte differential, Platelet count, Coagulation: Activated partial thromboplastin time, Prothrombin time; Bone marrow: smears were prepared, but microscopic evaluation was not performed "since peripheral blood findings were sufficient for assessment".

Clinical chemistry: Glucose, Blood urea nitrogen, Creatinine, Total bilirubin, Alkaline phosphatase, ALT, ALP, Gamma glutamyltransferase, Creatine phosphokinase, Calcium, Inorganic phosphorus, Sodium, Potassium, Chloride, Cholesterol, Triglycerides, Total protein, Globulin

Urinanalysis

Morphologic pathology: Scheduled necropsies were conducted on Days 37 and 38.

The following tissues were collected from each dog and were preserved in 10% neutral buffered formalin, except for the eyes which were preserved in Davidson's fixative:

Kidney	Duodenum	Mammary gla
Urinary bladder	Jejunum	Lacrimal glan
Liver	Ileum	Skeletal musc
Gallbladder	Cecum	Bone
Heart	Colon	Bone marrow
Aorta	Rectum	Adrenal
Trachea	Ovary	Thyroid
Lung	Uterus	Parathyroid
Spleen	Cervix	Pituitary
Lymph node	Vagina	Cerebrum
Thymus	Testis	Cerebellum
Salivary gland	Epididymis	Brain stem
Pancreas	Prostate	Spinal cord
Tongue	Skin	Sciatic nerve
Esophagus	Injection site	Eye
Stomach		
Note: Mammary glands were not collected from males.		

Organ Weights: Kidneys, Testes, Thyroids with parathyroids, Liver, Prostate, Pituitary, Heart, Adrenals, Brain, Ovaries.
Histopathology: H/E staining, light microscopy (an independent peer review evaluation was performed).
Results: There was no mortality. Clinical signs were limited to injection site reactions (seen in 5/8, 6/8, and 8/8 dogs LD, MD and HD, respectively). Injection site reactions occurred approximately 1 week after the injection and lasted for 2 to 12 days in males and 1 to 8 days in females, and appeared to be recovered by the end of the live phase of the study. Treatment with pamoic acid alone resulted in "minor swelling" at injection site in 1 dog. Changes in hematology and clinical chemistry parameters associated with inflammation were limited to moderate transitory increases in neutrophils, monocytes, and slight transitory increases in serum globulin (see sponsor's table below).

	Administered Dose of Olanzapine (mg/kg/day)					
Parameter (% Change[a])	5		10		20	
Sex	M	F	M	F	M	F
NEUTS:						
Day 7		76		35	40	10
MONOS:						
Day 7		64*		54*	35	87*

Abbreviations: M = male; F = female; * = p<.05.
[a] % change values are relative to the control group.

There were no compound-related changes in body weight, food consumption, ophthalmic examination, neurological examination, ECG parameters, urinalysis, or organ weight.
At necropsy, "a small circumscribed yellow focus was noted within the injected muscle or situated in the overlying fascia in most of the dogs given olanzapine pamoate monohydrate". Histologically, the reaction was characterized by "minimal or slight focal or multifocal areas of chronic inflammation with fibrosis". There was no substantial difference in incidence or severity of injection site reaction between the OPM dose groups. Pamoic acid alone resulted in a focal chronic reaction of minimal degree within the injection site of 1 of 8 dogs.
Plasma exposure (C_{max} and AUC_{0-t} values) increased with increasing dose. T max occurred between 45 and 126 hours and the mean peak plasma concentrations were 13.7, 21.6, or 35.2 ng/ml at LD, MD and HD, respectively. Quantifiable plasma concentrations of olanzapine were detected for up to 13, 20, and 27 days at LD, MD and HD, respectively. AUC_{0-t} values were 1759, 3797, or 6200 ng.hr/ml for dogs given 5, 10, or 20 mg olanzapine/kg, respectively. Mean peak plasma concentrations (C_{max}) and AUC_{0-t} values are shown in the following sponsor's table.

PK parameters for olanzapine, Single-dose OPM study in dogs

Administered Dose (mg LY170053/kg)	5		10		20	
Parameter	M	F	M	F	M	F
C_{max} (ng/mL)	12.00	15.44	19.02	24.27	29.56	40.94
AUC_{0-t} (ng•hr/mL)	1481.95	2036.82	3519.53	4073.62	6418.04	5981.44

AUC calculation: BQL concentrations (<1 ng/mL) were not used in calculating AUC values; t = last time point collected which was quantifiable.

In conclusion, treatment with olanzapine pamoate monohydrate resulted in measurable olanzapine plasma concentrations for at least 13 days. Peak plasma concentrations occurred between 45 and 126 hours after dosing. Treatment-related findings were limited to an inflammatory response to the olanzapine pamoate monohydrate formulation in the injection site.

2.6.6.3 Repeat-dose toxicity

Note: The repeat-dose toxicity studies submitted in support to this application (Study R07100, A subchronic toxicity study in Fischer 344 rats given olanzapine pamoate monohydrate by intramuscular injection once a month for 3 months and Study D00200, A chronic toxicity study in beagle dogs given multiple doses of olanzapine pamoate monohydrate for 6 months followed by a 2-month reversibility phase) were previously reviewed by Lois Freed, Ph.D. on 12/13/2002 under IND 60701 N 032, submitted on 7/30/2002. As no new repeat-dose toxicity studies have been submitted since, and the present reviewer has no reason to disagree with Dr. Freed's assessments, the review data for these studies are directly reproduced from Dr. Freed's review. The "Key study Findings" are summarized by this reviewer.

1. Study title: A subchronic toxicity study in Fischer 344 rats given olanzapine pamoate monohydrate (Compound 426906) by intramuscular injection once a month for 3 months.

Key study findings:

Fischer 344 rats (10/sex/treatment group) received IM doses of either 0 mg/kg (vehicle control); 125 mg pamoic acid/kg; or 20, 50, or 100 mg olanzapine/kg body weight of OP Depot once every 4 weeks for 3 months. The rationale for dose selection was based on obtaining a graded response up to a maximally deliverable dose. Mean body weight was reduced in MD and HD males (5 and 7%). In females, body weight was reduced at the HD (3%). Pamoic acid had no effect on body wt in either males or females. No effects on food consumption were observed in either OPM or pamoic acid-treated groups. There were no drug-related effects on hematology or clinical chemistry parameters except for an increase in white blood cell count (due primarily to increases in segmented neutrophils and monocytes) in HD females. There were no drug-related effects on organ weights. Treatment-related gross and histopathologic changes in rats treated with OP Depot and pamoic acid were limited to injection site reactions in all groups consistent with a chronic inflammatory response to a foreign body and increasing in severity in a dose-related manner. The primary finding was inflammation associated with accumulation of injected material. In the drug-treated groups, the severity was dose-related, with moderate-to-marked inflammation observed at the HD. Injection site reactions from the pamoic acid-treated rats were infrequent and less severe than those from rats treated with OP Depot.

The TK data indicated that peak plasma concentrations for both olanzapine and pamoic acid were generally achieved within the first day after dosing. Plasma levels of olanzapine tended to remain fairly stable over the 3-mo dosing period. In general, plasma exposure (Cmax and AUC_{0-t} values) for both olanzapine and pamoic acid increased with increasing dose. For olanzapine, there was a less-than dose-proportionate increase in Cmax , but the AUC increased in a greater-than dose-proportionate manner in males (at all doses) and in females (between MD and HD). Peak plasma levels of pamoic acid

(Cmax) were markedly higher in both males (5-14 fold) and females (9-14 fold) when pamoic acid was administered alone as compared to when given in combination with olanzapine but the AUC was generally similar to that seen in the HD OPM group.

Study no: R07100
Conducting laboratory and location: Eli Lilly and Co, Greenfield, IN
Date of study initiation: 6/13/00
GLP compliance: Y
QA report: Y
Drug, lot #, radiolabel, and % purity: olanzapine pamoate monohydrate (Compound 426906), PPD04663 [also listed as lot 325SB8 and ADD7275-135; olanzapine pamoate monohydrate], PPD04660 [also listed as lot 686W02, (b) (4) (b) (4) pamoic acid], olanzapine potency = 43.4% "as is"; pamoic acid potency = 99.78%.
Formulation/vehicle: suspension/5% mannitol, 0.1% polysorbate 80 in water. OPM suspensions were prepared just prior to dosing.
Methods
Dosing:

Species/strain: Fischer 344 rat (b) (4) (b) (4)

#/sex/group or time point (main study): 10/sex/grp
Satellite groups used for toxicokinetics or recovery: [cf. sponsor's table below]
Age: 15-16 wks
Weight: 248.6-327.7 gm for males, 165.0-193.2 gm for females
Doses in administered units: 0 [VC], 0 [PC], 20, 50, and 100 mg/kg olanzapine.
The experimental design was summarized in the following sponsor's table:

Group[a]	Dose of Olanzapine/Injection	Dose of OPM/Injection	Number of Animals/Sex
01	0 mg vehicle/kg[b]	0 mg vehicle/kg[b]	10
02	125 mg pamoic acid /kg	125 mg pamoic acid /kg	10
03	20 mg/kg	46 mg/kg	10
04	50 mg/kg	115 mg/kg	10
05	100 mg/kg	230 mg/kg	10
06	125 mg pamoic acid /kg	125 mg pamoic acid /kg	12
07	20 mg/kg	46 mg/kg	22[c]
08	50 mg/kg	115 mg/kg	22[c]
09	100 mg/kg	230 mg/kg	22[c]

[a] Rats assigned to Groups 01 through 05 were included in the toxicity component of the study. Rats assigned to Groups 06 through 09 were included in the toxicokinetic component of the study.
[b] Vehicle control.
[c] Includes 4 rats/sex to be used as replacement animals.

Route, form, volume, and infusion rate: i.m. depot [biceps femoris muscle], 1 mL/kg, 1 injection per month [right biceps muscle on Day 28, left biceps muscle on Days 0 and 56]. Drug substance [olanzapine pamoate] was stated to be stable in suspension for 8 hrs at rm. temperature, and to be homogeneously distributed in suspension. Stability of OPM in suspension was documented at 5 and 25° C for 15 days. The stability of pamoic

acid [25 mg/mL] was documented at 25° C for 8 days; however, the concentration of pamoic acid [at 188 mg/mL] decreased from 102.9% to 92.9% of intended after 8 days at rm. temperature [i.e., 25° C]. Data documenting homogeneity was not provided; however, the various drug suspensions [20, 50, 100, and 150 mg/ml OPM; 25 mg/ml pamoic acid] were found to provide accurate doses.

Observations and times

Clinical signs: animals were observed daily. In addition, detailed physical examinations were performed on main-study animals on a weekly basis.

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

Food consumption: food consumption was recorded prior to start of dosing and weekly during the dosing period.

Ophthalmoscopy: ophthalmology examinations were performed on all main-study animals prior to start of dosing and "near the end of the live phase". "The adnexa, conjunctiva, cornea, sclera, anterior chamber, iris, and lens of the eyes were examined with focal illumination and examination lens following dilation of the pupil. The fundus was examined using indirect ophthalmoscopy.

ECG: n/a

Clinical pathology: blood samples were collected from all main-study animals just prior to necropsy for analysis of the following parameters:

Hematology: rbc ct, heg, hct, MCV, MCH, MCHC, reticulocyte ct, wbc ct [total, differential], platelet ct, blood cell morphology, APTT, PT.

Clinical chemistry: glucose, BUN, creatinine, total bilirubin, alkaline phosphatase, ALT, AST, GGT, CK, CA, Pi, Na, K, Cl, cholesterol, TG, total protein, albumin, globulin, A/G ratio.

Urinalysis: urine samples were collected overnight from 5/sex/grp [main-study animals only] "Near the end of the live phase" for analysis of the following parameters: color, clarity, specific gravity, pH, protein, glucose, occult blood, ketones, bilirubin, urobilinogen, volume, Na, K, Cl, creatinine, and total excretion of Na, K, Cl, and creatinine.

Gross pathology: a complete necropsy was performed on all main-study animals on Days 84-86.

Organ wts: the following organ wts were recorded in all main-study animals: kidneys, liver, heart, spleen, ovaries, testes, prostate, adrenals, thyroid/parathyroid, pituitary, brain.

Histopathology: the following tissues were examined microscopically in VC, PC, and HD grps: kidney, urinary bladder, liver, heart, aorta, trachea, lung, spleen, lymph node, thymus, salivary gland, duodenum, jejunum, ileum, cecum, colon, rectum, ovary, uterus, cervix, vagina, testis, mammary gland, Harderian gland, skeletal muscle, bone, bone marrow, adrenal thyroid, parathyroid, pituitary, brain [cerebrum, cerebellum], pancreas, tongue, esophagus, stomach, epididymes, prostate, seminal vesicle, skin, brainstem, spinal cord, sciatic nerve, eye, injection site. In addition, the injection site was examined at the lower doses. Tissue section were preserved in 10% neutral buffered formalin, processed, embedded in paraffin, and stained with H & E for examination. [Testes and eyes should have been preserved in different fixatives, e.g., Bouin's, Davidson's.]

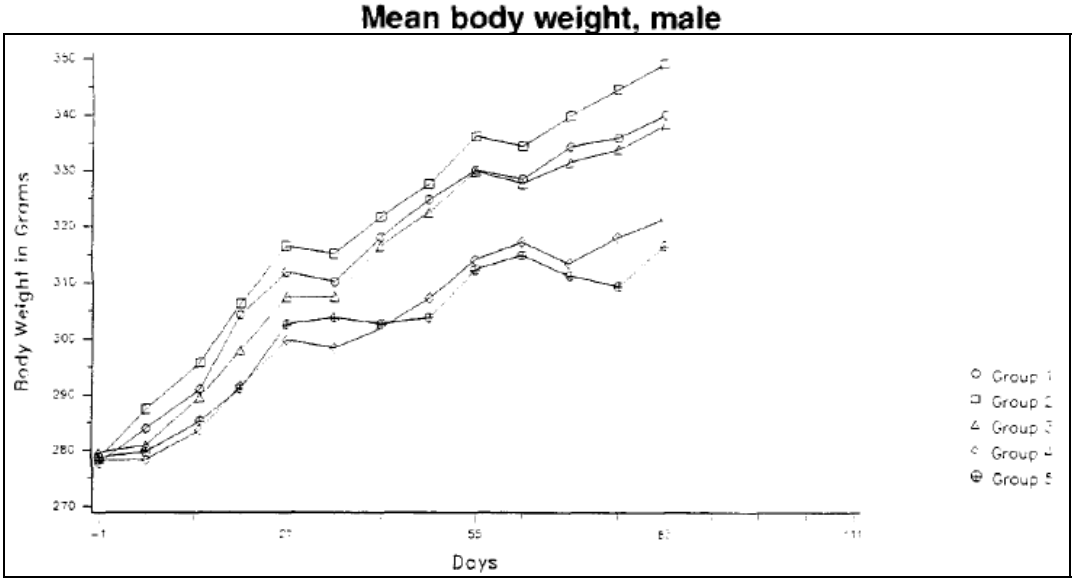
Toxicokinetics: blood samples were collected at 0, 1, 4, 8, 24, 120, 216, 336, 504, and 672 hrs following the 1st and 3rd doses of OPM [corresponds to Days 0.04, 0.17, 0.33, 1, 5, 9, 14, 21, and 28 postdosing] from 1/sex/grp/time point. In addition, blood samples were collected from "separate groups of rats" after the 1st and 3rd doses; sampling times in these animals were 0, 4, 8, 24, 120, 336, 504, and 672 hr postdosing [corresponding to 0, 0.17, 0.33, 1, 5, 14, 21, and 28 days postdosing]. Day 0 predose samples were not analyzed. Olanzapine and pamoic acid were quantitated in plasma using validated HPLC methods; LLOQ was 1 and 10 ng/mL, respectively.

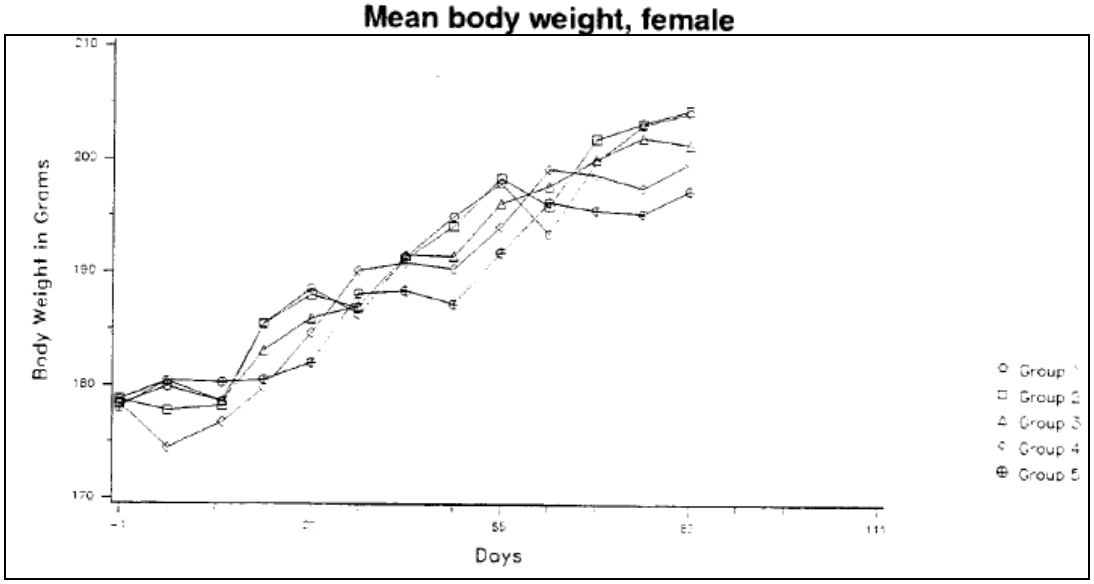
Results:

Mortality: there were no apparent drug-related deaths. Two satellite-TK animals died on Day 1 after blood collection; no cause of death was determined. [The sponsor did not identify what dose grp(s) these animals were in.]

Clinical signs: drug-related clinical signs [i.e., soiled hair coat, red nasal and/or eye discharge, and lacrimation] were evident at all doses of OPM in males and/or females. There were no clear effects of pamoic acid in either males or females.

Body weights: in males, final mean body wt was reduced at the MD and HD [5 and 7%, respectively, compared to CM]; overall mean body wt gain was reduced by 31 and 40% at the MD and HD. In females, mean body wt and overall body wt gain were significantly affected at the HD [3 and 26%, respectively]. Pamoic acid had no effect on body wt in either males or females. Body wt data were illustrated in the following sponsor's figures:





Food consumption: food consumption was not affected by pamoic acid or OPM in either males or females.

Ophthalmoscopy: no drug-related effects were reported.

Hematology: pamoic acid had no effect on the parameters assessed in either males or females. In males, a small [but significant, 3%] decrease was noted in rbc ct at the HD and 2-3% decreases in MCV and MCH were observed at all doses. In females, wbc ct was increased [38%] at the HD; this increase was due primarily to increases in segmented neutrophils and monocytes [120 and 73%, respectively], although lymphocytes, eosinophils, and basophil cts were also increased [16-30%]. The sponsor didn't consider any of these findings drug-related.

Clinical chemistry: pamoic acid had no effect on the parameters assessed in either males or females. There were also no drug-related effects in males. In females, the following were noted: (a) 18% decrease in cholesterol [HD], (b) 9-10% decrease in total protein and albumin [HD], (c) 69 and 160% increase in CK at the MD and HD [high values were observed in 4 MDF (658-1333 IU/L) and 1 HDF (5059 IU/L); high CF value: 532 IU/L], (d) 85% increase in total bilirubin [HD]; the individual data did not appear to be consistent with the mean values for this parameter. The range of individual values was 0.09-0.11 [with a note that "range exceeded result < 0.10 mg/dl"], whereas the mean value was 0.172±0.256 mg/ml [mean±SD]. None of these findings was considered drug-related by the sponsor.

Urinalysis: there were no clear pamoic acid- or drug-related effects. Urinary pH was significantly lower in PCF [6.9 vs 7.5 for CF], and urinary volume was increased 100% in PCM. In males, total K and creatinine excretion were slightly lower at the HD [26 and 16%, respectively].

Organ weights: there were no clear organ wt effects. In males, there were decreases in the absolute wt of organs [e.g., liver, thyroid, pituitary], probably due to lowered body wt. Relative wts were unaffected.

Gross pathology: the only finding of note was at the injection site. These findings are summarized in the following table:

TISSUE	FINDING	CM	PCM	LDM	MDM	HDM	CF	PCF	LDF	MDF	HDF
IJ site,L	lesion	0/10	2/10	10/10	11/10	11/10	0/10	0/10	10/10	12/10	11/10
IJ site,R	lesion	0/10	0/10	10/10	10/10	11/10	0/10	2/10	10/10	9/10	10/10
subcut	lesion	0/10	0/10	0/10	0/10	1/10	0/10	0/10	0/10	0/10	2/10

The "lesion" was characterized by the sponsor as follows: "…accumulations of yellow foreign material in the fascia, deep and sometimes distal to the biceps femoris muscle. Rarely, the subcutaneous tissue overlying the injection site had a similar lesion….Grossly, the injection sites from pamoic acid-treated rats were characterized by white or dark lesions in the fascia, deep and sometimes distal to the biceps femoris muscle."

Histopathology: pamoic acid- or OPM-related microscopic findings were observed only at the injection site. The findings are summarized in the following table:

TISSUE	FINDING	CM	PCM	LDM	MDM	HDM	CF	PCF	LDF	MDF	HDF
	muscular degeneration										
	slight	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10	1/10
	granulomatous inflam										
	minimal	0/10	3/10	0/10	0/10	0/10	0/10	1/10	1/10	0/10	0/10
	slight	0/10	1/10	2/10	2/10	0/10	0/10	0/10	8/10	2/10	0/10
	moderate	0/10	0/10	8/10	5/10	3/10	0/10	0/10	1/10	7/10	3/10
	marked	0/10	0/10	0/10	3/10	6/10	0/10	0/10	0/10	1/10	7/10
	muscular gran inflam										
	minimal	0/10	0/10	0/10	0/10	0/10	0/10	0/10	1/10	1/10	0/10
	slight	0/10	0/10	1/10	1/10	1/10	0/10	0/10	0/10	2/10	0/10
	moderate	0/10	0/10	1/10	1/10	4/10	0/10	0/10	0/10	0/10	2/10
	marked	0/10	0/10	0/10	1/10	0/10	0/10	0/10	0/10	0/10	0/10
	muscular brown pig [muscle]										
	slight	0/10	0/10	0/10	0/10	2/10	0/10	0/10	0/10	0/10	0/10
	granulomatous inflam										
	minimal	0/10	0/10	1/10	1/10	0/10	0/10	0/10	2/10	0/10	0/10
	slight	0/10	0/10	6/10	4/10	3/10	0/10	1/10	8/10	8/10	0/10
	moderate	0/10	0/10	1/10	3/10	2/10	0/10	0/10	0/10	1/10	8/10
IJ site, R*	marked	0/10	0/10	0/10	0/10	3/10	0/10	0/10	0/10	0/10	1/10
	muscular gran inflam										
	minimal	0/10	0/10	0/10	1/10	0/10	0/10	0/10	0/10	0/10	0/10
	slight	0/10	0/10	0/10	2/10	0/10	0/10	0/10	0/10	4/10	2/10
	moderate	0/10	0/10	0/10	2/10	2/10	0/10	0/10	0/10	1/10	3/10
	marked	0/10	0/10	0/10	0/10	1/10	0/10	0/10	0/10	0/10	1/10
subcut	minimal chronic inflam	0/10	0/10	0/10	0/10	1/10	0/10	0/10	0/10	0/10	1/10
	slight brown pig [macrophage]	0/10	0/10	0/10	0/10	1/10	0/10	0/10	0/10	0/10	1/10

*note: "Right leg was injected on Day 28 (8 weeks before necropsy)"

[The data in the table above were taken from the sponsor's summary histopathology table. There appears to be some discrepancies between that table and the sponsor's summary of the incidences of "granulomatous inflammation" by grade.]

Drug-related injection site changes were characterized "…by an inflammatory cell infiltrate resulting in a marked expansion of the fascia surrounding the muscle and, to a lesser degree, expansion of the connective tissue spaces (perimysium) between muscle fiber bundles. The expanded connective tissue spaces contained sheets of large polygonal to slightly spindle-shaped macrophages within apparent cell margins. Some of the macrophages appeared to form multinucleated syncytial cells, but distinct foreign-body macrophages were rare. Macrophages contained abundant vacuolated pale cytoplasm, and many of the vacuolar spaces had a regular shape and pattern consistent with intracellular crystalline material which had been extracted during tissue processing.

Some inflammatory foci, primarily in the left injection sites, contained variable numbers of small crystals of green-gold refractile foreign material (injected material) which appeared to be primarily intracellular. Admixed with the macrophages were variable numbers of lymphocytes and plasma cells and scattered granulocytes, most of which looked to be eosinophils. In some inflammatory foci, lymphocytes were the predominant cell type. The inflammatory cells infiltrated slightly between muscle fibers adjacent to the inflammatory reaction, but atrophy, degeneration, or necrosis of myocytes was typically minimal. Only minimal fibroplasia and minimal increased collagen deposition were apparent in either the right or left injection sites. Within the adjacent connective tissue were occasional perivascular and vascular accumulations of lymphocytes admixed with other inflammatory cells and scattered brown-gold pigment-laden macrophages".

The injection site changes resulting from pamoic acid were stated to "…have a different appearance…" than OPM-related changes. They were described as follows: "The granulomatous inflammation primarily surrounded adipocytes adjacent to the skeletal muscle. Macrophages were admixed with scattered lymphocytes, plasma cells, mast cells, and granulocytes. Rarely, the macrophages were multinucleated or formed foreign-body giant cells. Some inflammatory reactions were associated with aggregates of yellow foreign material (probably pamoic acid)."

Toxicokinetics: the data were summarized in the following sponsor's table:

	Administered Dose (mg/kg every 28 days)							
Parameter	20 mg OLZ/kg		50 mg OLZ/kg		100 mg OLZ/kg		125 mg PA/kg	
Sex	M	F	M	F	M	F	M	F
Olanzapine								
Day 0								
C_{max} (ng/mL)	115	91	121	155	200	456	NA	NA
AUC_{0-t} (ng•hr/mL)	6002	6919	16537	20569	57590	35073	NA	NA
Day 56								
C_{max} (ng/mL)	133	99	171	189	209	169	NA	NA
AUC_{0-t} (ng•hr/mL)	5387	6477	22993	15307	59992	48010	NA	NA
Pamoic Acid								
Day 0								
C_{max} (ng/mL)	1817	2928	3111	2979	4906	4165	24060	38567
AUC_{0-t} (ng•hr/mL)	141060	233436	341367	267694	589832	817656	576149	1606801
Day 56								
C_{max} (ng/mL)	2230	1720	2472	5012	2236	2755	31383	38994
AUC_{0-t} (ng•hr/mL)	247664	168404	394919	443327	537234	806213	463539	576236

Abbreviations: M = male; F = female; OLZ = olanzapine administered as olanzapine pamoate monohydrate; PA = pamoic acid; C_{max} = maximum plasma concentration; NA = not applicable.

BQL concentrations (< 1.00 ng/mL for olanzapine; < 10.00 ng/mL for pamoic acid) were not used in calculations.

A value of 0 ng/mL was assumed on Day 0 at time zero for AUC_{0-t} calculations; t = last quantifiable time point within each 28-day interval.

2. Study title: A chronic toxicity study in Beagle dogs given multiple doses of olanzapine pamoate monohydrate (LY170053 pamoate monohydrate) for 6 months followed by a 2-month reversibility phase.

Key study findings:
Beagle dogs (4 to 6/sex/treatment group) were administered 5, 10, or 20 mg/kg of OP Depot intramuscularly once every 2 weeks for 6 months. Doses were selected based on results of the single-dose study at equivalent doses where the high dose of 20 mg/kg used "nearly maximal concentrations that could be suspended and given in a single 2-ml dose in a 10-kg dog". Additional dogs (4 to 6/sex) received either vehicle or 25 mg/kg pamoic acid, a dose equivalent to that given in the 20-mg/kg OP Depot group. Four dogs/sex/ group were terminated at the end of the 6-month period, while 2 dogs/sex from the HD and vehicle control groups were followed for an additional 2-month reversibility phase.
Treatment-related toxicity was limited to injection site reactions seen in all OP Depot and pamoic acid groups. In dogs treated with OP Depot, clinical signs of injection site reactions consisted of swelling, firmness, redness, ulceration, and scabs ("scab" was defined as "an open area of skin with drainage"). The injection site reactions were dose-related in incidence, severity, and duration of reaction and generally resolved within 1 to 2 weeks. The injection site reactions in dogs given pamoic acid were mild and occurred in only a few dogs at a low incidence.
Grossly, the injection sites from dogs treated with OP Depot were characterized primarily by "accumulations of yellow foreign material within the biceps femoris muscle and adjacent muscles and connective tissues". Histologically, the injection site reactions in dogs given OP Depot were characterized by "focally extensive areas of myocyte loss with replacement by fibroblasts, collagen, and variable numbers of inflammatory cells consistent with chronic inflammation in response to tissue injury and injection of foreign material". The injection site reactions from pamoic acid-treated dogs were histologically similar. Observations in recovery animals [VC, HD] suggested reversibility of drug effects. No findings were detected in HDM at the end of the recovery period; minimal/slight chronic inflammation at the injection site was still evident in HDF.
TK data were provided for both olanzapine and pamoic acid. There were no consistent changes in plasma exposure [Cmax, AUC] to olanzapine with duration of dosing, or in dose-corrected plasma olanzapine levels. Plasma levels of olanzapine were >LLOQ by 56-63 days after the last dose, whereas pamoic acid was still detectable at the end of the recovery period.
Pamoic acid was absorbed more rapidly when given alone (Tmax = 1-3 hr) vs when given as the OPM formulation (Tmax = 53-104 hr, at HD), resulting in peak plasma levels of pamoic acid markedly higher in both males (20-18 fold) and females (18-13 fold) when pamoic acid was administered alone. Plasma AUCs for pamoic acid were also higher when pamoic acid was administered alone, but the effect was much smaller (1.2-2 fold). Plasma AUC for pamoic acid tended to be lower (23-76%) on Day 56 compared to Day 0.

Study no: D00200
Conducting laboratory and location: sponsor, IN
Date of study initiation: 5/17/00

GLP: Y
QA report: Y
Drug, lot #, radiolabel, and % purity: olanzapine pamoate monohydrate (Compound 426906), lot
CT15746/325SB8 [olanzapine], PPD04660/686W02 [pamoic acid]; "potency" = 99.78%
Formulation/vehicle: suspension/5% mannitol and 0.1% Tween 80 in sterile water.
Methods
Dosing:

Species/strain: Beagle dog (b) (4)
#/sex/group or time point (main study): 4/sex/grp
Satellite groups used for toxicokinetics or recovery: 2/sex for C and HD recovery
Age: 8-10 mo
Initial body weight: 7.5-10.4 kg for males, 8.0-10.1 kg for females
Doses in administered units: 0 [vehicle; VC], 0 [pamoic acid C (25 mg/kg); PC], 5, 10, 20 mg/kg

Dosing regimen: biweekly doses for 6 months. Injections were alternated between left and right hindlimbs. Dosing was withheld from 1 HDM on Day 28 due to "adverse clinical signs". During the first 6 injections, doses were administered as a single injection [21-gauge needle]. However, it was noted that results of clinical trials indicated that "…the rate of delivery of the injection may affect the degree of the reaction". Therefore, the dosing method was changed for the remaining doses, as follows:

7th injection: "all doses were administered over a period of >10 seconds using a 1-ml tuberculin syringe with those greater than 1 ml divided into two doses that were administered into the same muscle. However, in pamoic acid-or OPM-treated dogs, the smaller syringe and slower delivery rate caused obstruction of the needle on numerous occasions which subsequently resulted in the following: 1) the replacement of needles (from 21 to 20 gauge) during individual injections; 2) multiple injection attempts; and 3) for 3 dogs, incomplete dose administration due to the detachment of the needle from the syringe".

8th-13th injections: for C and LD grps, each animal received a single injection using a 21-gauge needle on a 1- or 3-mL syringe. For MD and HD animals, doses were administered in divided doses into the same muscle using 2 1-mL syringes. The duration of the dosing was not controlled.

Route, form, volume, and infusion rate: i.m. depot [biceps femoris muscle], 0.0333-0.133 ml/kg.

Stability and homogeneity [for 8 hrs in suspension at rm temperature] were stated to have been demonstrated prior to conduct of study. Data were provided documenting the stability of olanzapine pamoate in suspension at 5 and 25ºC for up to 15 days.
Suspensions were prepared on day of dosing.

Observations and times

Clinical signs: clinical signs, including injection site changes, recorded daily.

Physical examination: physical and neurological examinations were conducted in addition to daily observations. Physical examinations [general appearance, mental status, skin/pelage, eyes, external ears, oral cavity, palpation of abdomen and lymph nodes] were conducted prior to the start of dosing, "near the end" of the dosing period, and "near the

end" of the recovery period [Day 236]. Neurological examinations [behavior, posture, gait, muscle tone, cranial nerve reflexes ("menace reflex, resting pupillary size, symmetry pupillary light response, eye position, vestibular eye movements, facial sensation, symmetry of face and ears, swallowing reflex, and jaw muscle tone"), "spinal reflexes (patellar and flexor)"] were conducted prior to the start of dosing, on Day 2 ["soon after administration of the first dose"], before and after the last dose [Days 166 and 170], and on Day 236 [recovery animals].

Body weights: body wts were recorded prior to start of dosing, weekly during the dosing period, and on Day 181. In animals followed during the recovery period, body wts were also recorded on the 1st day of recovery [Day 182], weekly during the recovery period, and "near the end" of the recovery period [Day 237].

Food consumption: food intake was qualitatively assessed daily [in increments of 25%].

Ophthalmoscopy: examinations were performed prior to the start of dosing, at the end of the dosing period, and during the recovery period [nos]. The following structures were examined: "…eyelids, membrana nictitans, conjunctiva, cornea, sclera, anterior chamber, lens, iris." "The adnexa, cornea, sclera, anterior chamber, and lens were examined by biomicroscopy following dilatation of the pupil" and the fundus was examined using binocular indirect ophthalmoscopy.

ECG: ECGs were recorded from all dogs prior to start of dosing, prior to and at 120 hrs after the 1st, 2nd, 7th, and 13th doses. In addition, ECGs were recorded "near the end" of the recovery period in recovery animals. The following parameters were recorded from Lead II: abnormal rhythm disturbances, RR, PR, QRS, QT intervals.

Hematology: blood samples were collected prior to start of dosing and on Days 23, 51, 79, 107, 135, 162, and 181 of the dosing period, and on Day 237 [recovery period]. The following parameters were assessed: rbc ct, hgb, hct, MCV, MCH, MCHC, reticulocyte ct, blood cell morphology, wbc ct [total, differential], platelet ct, APTT, PT.

Clinical chemistry: blood samples were collected according to the same scheduled used for assessment of hematology. The following parameters were assessed: glucose BUN, creatinine, total bilirubin, alkaline phosphatase, ALT, AST, GGT, CK, Ca, Pi, Na, K, Cl, cholesterol, TG, total protein, albumin, globulin, A/G.

Urinalysis: urine samples were collected by cystocentesis at time of sacrifice for analysis of the following parameters: color, clarity, specific gravity, pH, protein, glucose, occult blood, ketones, bilirubin, urobilinogen. Microscopic analysis of sediment was performed on samples that were outside the normal range for "color, clarity, protein, occult blood, or bilirubin".

Gross pathology: a complete necropsy was conducted on all dogs. Main study animals were sacrificed on Days 182-183; recovery animals were sacrificed on Day 238.

Organ wts: wts of the following organs were recorded: kidneys, liver, heart, ovaries, testes, prostate, adrenals, thyroid/parathyroids, pituitary, brain.

Histopathology: the following tissues were examined microscopically in all animals: kidney, urinary bladder, liver, gallbladder, heart, aorta, trachea, lung, spleen, lymph node, thymus, salivary gland, pancreas, tongue, esophagus, stomach, duodenum, jejunum, ileum, cecum, colon, rectum, ovary, uterus, cervix, vagina, testis, epididymis, prostate, skin, injection sites, mammary gland [females only], lacrimal gland, skeletal

muscle, bone, bone marrow, adrenal, thyroid, parathyroid, pituitary, cerebrum, cerebellum, brain stem, spinal cord, sciatic nerve, eye.

All tissues were preserved in 10% neutral buffered formalin, except for eyes [Davidson's fixative], embedded in paraffin, and stained with H & E for examination. An independent peer review was conducted, which consisted of examination of sections of tissues from VC, PC, and HD grps [3/sex/grp] and all recovery animals, and of injection site and subcutaneous lesions of all dogs.

Toxicokinetics: blood samples were collected before each dose, and at 1, 4, 8, 24, 120, and 216 hrs following the 1st, 7th, and last dose. Olanzapine and/or pamoic acid were quantitated in plasma using HPLC [EC for olanzapine, fluorescence for pamoic acid]. LLOQ was 1 and 2 ng/mL for olanzapine and pamoic acid, respectively. It was noted that C samples were discarded without analysis, and that "…not all samples collected were analyzed for pamoic acid".

Results

Mortality: no unscheduled deaths occurred.

Clinical signs: no drug-related clinical signs were observed. Injection site changes were evident in all treated animals. The injection site data are summarized in the following table:

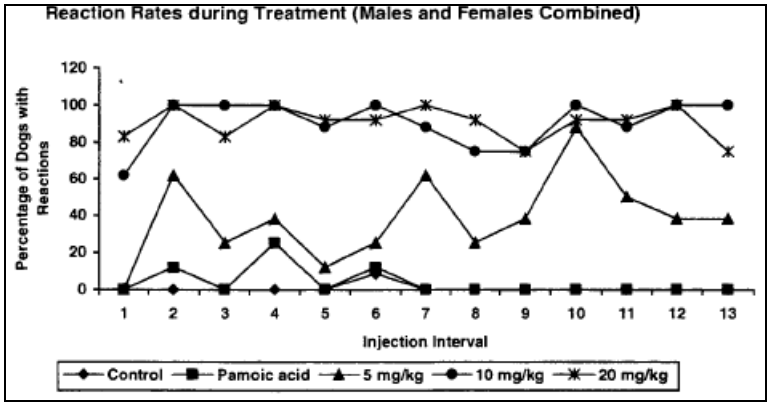
FINDINGS	MALES					FEMALES				
	VC	PC	LD	MD	HD	VC	PC	LD	MD	HD
MAIN-STUDY										
scab*	0/6	0/4	2/4	1/4	6/6	0/6	1/4	0/4	4/4	4/6
ulceration	0/6	0/4	2/4	2/4	5/6	0/6	0/4	0/4	4/4	4/6
swollen	0/6	1/4	4/4	4/4	6/6	0/6	2/4	4/4	4/4	6/6
red	0/6	0/4	2/4	3/4	6/6	1/6	1/4	1/4	4/4	5/6
firm	0/6	1/4	4/4	4/4	6/6	0/6	1/4	3/4	4/4	6/6
discharge	0/6	0/4	2/4	1/4	4/6	0/6	0/4	0/4	4/4	3/4
scar	0/6	0/4	2/4	1/4	5/6	0/6	0/4	0/4	4/4	4/6
RECOVERY										
scab	0/2				1/2	0/2				0/2
scar	0/2				2/2	0/2				1/2

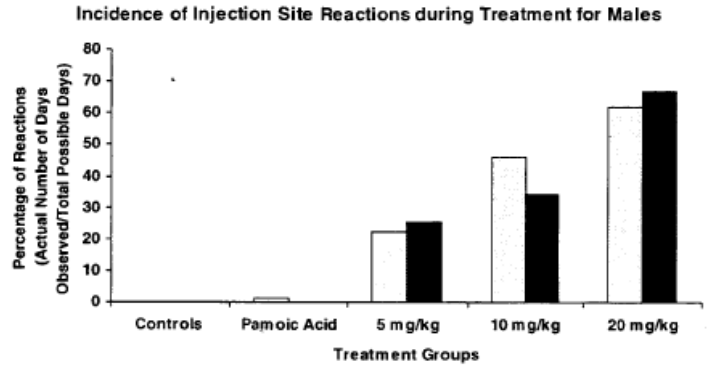
*defined as "an open area of skin with drainage"

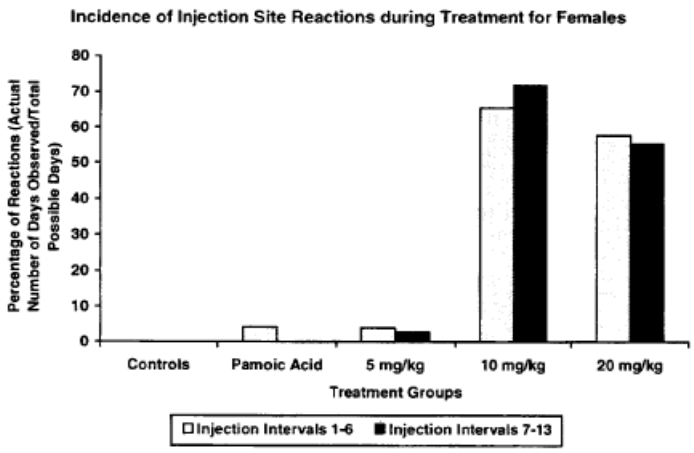
The sponsor summarized the severity and duration of injection site changes in the following table and figures:

Clinical Observation	Administered Dose (mg/kg/injection) 0[a]	0[b]	5	10	20
	Total Number of Dogs With Reactions During Treatment Average Number of Days Observed (Mean Range of Severity by Area)[c]				
			Males		
Swelling, firmness, ulceration, redness, and/or scabs	0/6	1/4 4	4/4 44	4/4 72	6/6[d] 116
Swelling	0/6	1/4 3 (2.0)	4/4 29 (1.7-3.3)	4/4 35 (1.9-3.7)	6/6 76 (2.1-3.5)
Firmness	0/6	1/4 2 (1.5)	4/4 29 (2.0-4.0)	4/4 61 (2.0-3.4)	6/6 71 (2.0-3.8)
			Females		
Swelling, firmness, ulceration, redness, and/or scabs	1/6 1.0	2/4 7	4/4 6	4/4[e] 126	6/6[d] 103
Swelling	0/6	2/4 7 (2.0-3.0)	4/4 4 (1.6-4.0)	4/4 73 (2.6-3.7)	6/6 71 (2.0-4.0)
Firmness	0/6	1/4 10 (2.0)	3/4 3 (1.0)	4/4 77 (2.5-3.8)	6/6 70 (2.2-4.1)

[a] Vehicle control.
[b] Pamoic acid control.
[c] Severity scale (area affected) for swelling and firmness: 1 = ≤0.5 cm, 2 = 0.6 to 1.0 cm, 3 = 1.1 to 3.0 cm, 4 = 3.1 to 5.0 cm, 5 = ≥5.1 cm.
[d] Swelling extended to hind limb area in 3 males and 2 females.
[e] Swelling extended to hind limb area in 1 female.







Surgical treatment [lancing] of lesions was required in a number of animals. During the lancing process, "…a thick, opaque, yellowish or pink liquid was removed". The sponsor noted that the incidence of lesions requiring surgical intervention was dose- and gender related. The data were summarized in the following sponsor's table:

Incidence of Surgically Incised Reactions

Animal Number	Dose Group (mg/kg/injection)	Days of Surgical Incision
		Males
312393	5	44, 61, 74, 102, 130, 157, 172
313084	10	159
312475	20	22, 143, 171
312495	20	19, 31, 61, 89, 118, 144, 171
313155	20	58, 74, 102, 115, 116, 129, 144, 157, 171
312365	20	44, 74, 102, 142, 171
312465	20	129, 145
		Females
290624	10	9, 61, 74, 89, 104, 118, 129, 159, 180
289914	10	74, 83, 89, 117, 144, 157, 171
289894	10	145, 157
289924	10	19, 88, 101, 115, 129, 143, 157, 171
289995	20	31, 117, 143, 171
289965	20	74, 101, 118, 129, 143, 158, 172
290565	20	22, 74, 105, 131
290605	20	31, 72, 88, 100, 114, 128, 129, 142, 143, 156, 170, 171

Physical examination: the only drug-related effect observed was local reaction at the injection site.

Body weights: there were no drug-related effects on body wt.

Food consumption: there were no apparent drug-related effects on body wt.

Ophthalmoscopy: no drug-related effects were reported.

ECG: data were provided only for heart rate. No drug-related effects on hr/ECG parameters were observed.

Hematology: there were no clear drug-related findings.

Clinical chemistry: there were no apparent drug-related findings.

Urinalysis: there were no apparent drug-related findings.

Organ weights: there were no significant drug-related findings. However, ovary wt tended to be increased in HDF [32-20%, absolute-relative] and in HFD during the recovery period [56-52%, A-R], compared to PC. [Ovary wts were fairly similar between VC and PC.]

Gross pathology: the only drug-related finding was "lesion" at injection sites. The lesion was characterized as an accumulation of "yellow foreign material within the biceps femoris muscle, adjacent muscles, and connective tissue deep to the muscle". The severity of the finding was based on the size of the affected area. In pamoic acid-treated animals, the lesion appeared "pale". The incidence of this finding is summarized in the following table:

SITE	MALES					FEMALES				
	VC	PC	LD	MD	HD	VC	PC	LD	MD	HD
MAIN-STUDY										
L1	0/4	2/4	4/4	5/4*	4/4	0/4	3/4	4/4	4/4	4/4
R1	0/4	1/4	3/4	4/4	4/4	0/4	0/4	4/4	4/4	4/4
RECOVERY										
L1	0/2				0/2	0/2				2/2
R1	0/2				0/2	0/2				2/2

*this is correct

Histopathology: the only drug-related findings were observed at the injection site. The data are summarized in the following table:

SITE	FINDING	MALES					FEMALES				
		VC	PC	LD	MD	HD	VC	PC	LD	MD	HD
MAIN-STUDY											
L1	chronic inflammation										
	minimal	0/4	2/4	0/4	0/4	0/4	1/4	1/4	0/4	0/4	0/4
	slight	0/4	1/4	0/4	1/4	0/4	0/4	1/4	0/4	0/4	0/4
	moderate	0/4	0/4	1/4	1/4	1/4	0/4	0/4	3/4	3/4	2/4
	marked	0/4	0/4	3/4	2/4	3/4	0/4	1/4	1/4	1/4	2/4
	necrosis										
	slight	0/4	0/4	0/4	0/4	1/4	0/4	0/4	0/4	0/4	0/4
	moderate	0/4	0/4	0/4	0/4	0/4	0/4	1/4	0/4	0/4	0/4
	marked	0/4	0/4	0/4	0/4	0/4	0/4	0/4	1/4	0/4	0/4
R1	chronic inflammation										
	minimal	0/4	1/4	0/4	1/4	0/4	0/4	1/4	0/4	1/4	0/4
	slight	0/4	1/4	1/4	1/4	1/4	0/4	1/4	1/4	1/4	3/4
	moderate	0/4	0/4	3/4	0/4	2/4	0/4	0/4	3/4	2/4	1/4
	marked	0/4	0/4	0/4	2/4	1/4	0/4	0/4	0/4	0/4	0/4
RECOVERY											
L1	chronic inflammation										
	minimal	0/2				0/2	0/2				1/2
	slight	0/2				0/2	0/2				1/2
R1	chronic inflammation										
	slight	0/2				0/2	0/2				1/2

The injection site changes in drug-treated animals were further described in the study report as follows:

"Injection site reactions were characterized by focally extensive areas of myocyte loss with replacement by fibroblasts, collagen, and variable numbers of inflammatory cells. Within and immediately adjacent to the foci of inflammation were scattered

atrophic, degenerative, necrotic, and/or regenerative myocytes. Two injection site reactions had prominent central areas of myocyte necrosis, which were given a separate diagnosis. A similar inflammatory response was often within adjacent connective tissue and/or expanded fascial spaces between muscle fiber bundles. The types and relative percentages of inflammatory cells within the inflammatory foci were variable, although the overall pattern was that of chronic inflammation in response to tissue injury and foreign material. For some injection sites, the inflammatory response was primarily granulomatous with sheets of large foamy macrophages and rare multinucleated giant cells. Macrophages often contained negative-staining clefts (consistent with loss of material, presumably compound, during tissue processing). Other inflammatory responses had a prominent lymphocytic response with numerous plasma cells. A few inflammatory responses had scattered small pockets of neutrophils."

Changes at the injection site in pamoic acid-treated animals were further described in the study report as follows:

"Injection site reactions from pamoic acid-treated dogs typically had more prominent fibroplasia, but less inflammatory cell infiltrate than injections [sic] site reactions from OPM-treated dogs. Therefore, a separate diagnosis of fibroplasia was often made in addition to a diagnosis of inflammation. Like injection site reactions from OPM-treated dogs, the injection site reactions from pamoic acid-treated dogs had focally extensive areas of myocyte loss with replacement by fibroblasts, collagen, and scattered mononuclear inflammatory cells (primarily macrophages and lymphocytes). The injection site from 1 pamoic acid-treated dog had a prominent central area of myocyte necrosis which was given a separate diagnosis."

It was noted that the injection site reactions in recovery animals involved a smaller area of tissue, but were otherwise similar to those observed in main-study animals.

Toxicokinetics: the data were summarized in the following table [units: Cmax in ng/mL, AUC(0-t) in ng▪hr/mL; means ±SEM]:

PARAMETER	DAY	MALES				FEMALES			
		PC	LD	MD	HD	PC	LD	MD	HD
OLANZAPINE									
C_{max} [ng/mL]	0		6.86 ± 0.65	13.04 ± 1.00	26.89 ± 3.77		9.27 ± 1.32	16.39 ± 0.85	37.35 ± 4.97
	84		14.45 ± 2.52	21.78 ± 3.55	48.08 ± 6.18		7.94 ± 1.90	41.96 ± 14.50	39.14 ± 5.92
	168		10.18 ± 2.16	26.80 ± 3.10	29.14 ± 6.84		9.01 ± 1.09	9.76 ± 0.78	53.53 ± 22.72
T_{max} [hr]	0		120 ± 0	144 ± 24	136 ± 16		120 ± 39	144 ± 24	136 ± 16
	84		12 ± 0	120 ± 0	120 ± 0		139 ± 50	120 ± 0	120 ± 0
	168		92 ± 28	120 ± 0	45 ± 24		72 ± 28	8 ± 0	99 ± 32

AUC [ng•hr/mL]	0		1327 ± 248	2719 ± 298	4703 ± 686		1275 ± 237	2736 ± 238	6476 ± 678
	84		2167 ± 318		7236 ± 931		1679 ± 512	4934 ± 1221	7410 ± 1416
	168		1392 ± 387	4069 ± 480	3204 ± 1045		1416 ± 393	1016 ± 142	7655 ± 2876
PAMOIC ACID									
C_{max} [ng/mL]	0	5100.30 ± 1136.42	96.14 ± 3.64	162.45 ± 20.34	256.48 ± 27.01	5585.98 ± 873.80	144.33 ± 10.53	217.23 ± 15.17	309.51 ± 25.65
	168	4273.48 ± 1545.20	91.50 ± 10.88	212.94 ± 36.18	241.59 ± 61.07	3948.17 ± 625.12	97.11 ± 15.10	133.34 ± 18.83	309.77 ± 115.97
T_{max} [hr]	0	1 ± 0	12 ± 0	144 ± 24	104 ± 16	1 ± 0	145 ± 69	144 ± 24	104 ± 16
	168	3 ± 2	72 ± 28	120 ± 0	53 ± 21	2 ± 1	72 ± 28	15 ± 5	67 ± 35
AUC [ng•hr/mL]	0	99110 ± 7901	18109 ± 1531	31686 ± 1868	46275 ± 4540	73791 ± 15089	27396 ± 1079	45148 ± 4281	59415 ± 4414
	168	43722 ± 10079	12150 ± 2280	32944 ± 3417	27752 ± 9905	93050 ± 14694	15444 ± 4035	10906 ± 2170	45843 ± 17110

Evaluation of TK in animals with and without local irritation indicated, according to the sponsor, that the presence of local tissue reaction did not appear to affect the PK of olanzapine pamoate. However, it was noted that there was a "high degree of interanimal variability". Pamoic acid, when administered alone, was absorbed and eliminated more rapidly than when administered in combination with olanzapine.
In recovery animals, olanzapine was <LLOQ by 56-63 days after the last dose, whereas pamoic acid was still quantifiable at the end of the recovery period. It was noted that plasma levels of pamoic acid were "nearing the limits of quantification" by the end of the recovery period.

Toxicology summary: I.M. depot toxicity studies of OPM were conducted in Fischer 344 rat [3-mo] and Beagle dog [6-mo + 2-mo recovery].

Rat: in the rat study, OPM was administered monthly for 3 months at doses of 20, 50, and 100 mg/kg i.m. Two control grps were included; the VC grp received vehicle and the PC grp received 125 mg/kg of pamoic acid. [The dose of pamoic acid administered to the PC grp was similar to that received by the HD grp.] Observations consisted of the following: clinical signs/physical examination, body wt, food consumption, ophthalmoscopy, hematology, clinical chemistry, urinalysis, TK, and terminal studies [gross pathology, organ wts, histopathology].
There were no apparent drug-related deaths. Two satellite animals died on Day 1 following blood collection; what grp(s) these animals belonged to was not stated. Drug-related clinical signs were evident in treated grps; however, no signs were clearly associated with pamoic acid. Mean body wt was reduced in MD and HD males [5 and 7%]. In females, body wt was reduced at the HD [3%]. Pamoic acid had no effect on body wt in either males or females. No effects on food consumption were observed in either OPM or pamoic acid-treated grps. There were also no effects of drug or pamoic acid on ophthalmology parameters. No changes related to pamoic acid were observed on hematology or clinical chemistry parameters. In drug-treated males, there were no clear effects on hematology or clinical chemistry parameters. In females, an increase in wbc ct [due primarily to increases in segmented neutrophil and monocyte cts], small decreases in cholesterol, total protein, and albumin, and substantial increases in CK [also increased at

the MD] and total bilirubin were observed at the HD. [The individual data did not appear to be consistent with the mean for total bilirubin.] The sponsor didn't consider any of these findings related to drug. Differences between VC and PC grps were observed on selected urinalysis parameters; however, neither parameter [pH, volume] was affected in both males and females. No drug related effects were observed on urinalysis parameters or on organ wts. There were no effects of pamoic acid on organ wts. The only finding detected upon gross examination was "lesions" at the injection site [i.e., in fascia]. All drug-treated animals were affected at L and R injection sites. In PC grps, only 2/sex were affected [1 site only]. In addition, s.c. lesions were detected in 1/10 HDM and 2/10 HDF. Lesions in drug-treated animals appeared to be the result of accumulation of injected material at the site and, rarely, in s.c. tissue. "Yellow foreign material" was noted to accumulate at the site, reflecting the presence of pamoic acid (according to the report). However, lesions in pamoic acid-treated animals were described as appearing "white or dark". Microscopic findings were also detected only at the injection site. The primary finding was inflammation associated with accumulation of injected material. The injection site reaction was much greater [incidence and severity] in drug-treated grps. In the drug-treated grps, the severity was dose-related, with moderate-to-marked inflammation observed at the HD. Inflammatory cells infiltrates [macrophages, lymphocytes, plasma cells, and/or granulocytes] were detected in fascia surrounding muscle, and "to a lesser extent" in the perimysium. The injection site changes in the PC grps were noted to "have a different appearance" than those in the OPM-treated grps. Muscle degeneration [characterized as "slight"] was only detected in 1 HDF.

The TK data indicated, for olanzapine, a less-than dose-proportionate increase in Cmax with dose in both males and females. However, the AUC for olanzapine increased in a greater-than dose-proportionate manner in males [at all doses] and in females [between 50 and 100 mg/kg]. Plasma levels of olanzapine tended to remain fairly stable over the 3-mo dosing period, except for a >2-fold decrease in Cmax between Day 0 and Day 56 in HDF.

Peak plasma levels of pamoic acid [Cmax] were markedly higher in both males [5-14 fold] and females [9-14 fold] when pamoic acid was administered alone as compared to when given in combination with olanzapine; the effect was greater on Day 56 than on Day 0. Overall, the AUC was not similarly affected. On Day 0, the AUC for pamoic acid was similar between the PC and HD gps in males; in females, the pamoic acid AUC was 2-fold higher in the PC grp. On Day 56, the AUC for pamoic acid in the PC grp was 0.9 and 0.7 times that in the HD grp in males and females, respectively. Across dose grps, Cmax increased in a less-than dose-proportionate manner, except at the MD in females on Day 56. The AUC for pamoic acid increased in a dose-proportionate manner in females; in males, the increase was less-than dose-proportionate.

Dog: in the dog study, OPM was administered biweekly [alternating left and right hindlimbs] for 6 months at doses of 5, 10, and 20 mg/kg i.m. Two control grps were included; the VC grp received vehicle and the PC grp received 25 mg/kg of pamoic acid. For the first 6 injections, doses were administered as a single injection using a 21-gauge needle; however, the method of administration was modified following the 6th injection due to observations made in ongoing clinical trials. [The rate of drug administration

appeared to "affect the degree of the reaction".] For the 7th injection, all doses were to be administered over a >10-sec period using a 1-mL syringe, with doses of >1 mL being administered as two separate injections into the same muscle. However, administration of pamoic acid and OPM using this regimen resulted in obstruction of the needle "on numerous occasions", resulting in the following: (a) replacement of the 20-gauge needle with a 21-gauge needle, (b) "multiple injection attempts", and (c) "for 3 dogs, incomplete dose administration due to the detachment of the needle from the syringe". For the 8th-13th injections, C and LD animals received a single injection using a 21-gauge needle using a 1- or 3-mL syringe. MD and HD animals received divided doses into the same muscle using two 1-mL syringes. The duration of dosing was not controlled.

Observations consisted of the following: clinical signs/physical examination, body wt, food consumption, ophthalmology, ECG, hematology, clinical chemistry, urinalysis, TK, and terminal studies [gross pathology, organ wts, histopathology]. Two per sex per grp for VC and HD grps only were followed for a 2-mo recovery period.

There were no unscheduled deaths or drug-related clinical signs. Injection site changes, i.e., ulceration, swelling, redness, firmness, discharge, and scar/scab were detected in all drug-treated grps. ["Scab" was defined as "an open area of skin with drainage".] Swelling and firmness were noted in all drug-treated animals. The severity of all injection site changes was not clearly dose-related; however, overall the injection site tended to be affected to a greater extent at the higher doses. Injection site changes were also greater [i.e., more animals affected, increased severity] in the PC as compared to the VC grp. The sponsor noted that lancing of lesions ["…a thick, opaque, yellowish or pink liquid…"] was required in a number of animals, with the incidence being dose- and gender related. For the most part, injection site effects were reversible; findings in recovery animals were limited to scab or scar in HD grps; PC animals were not followed during the recovery period. There were no apparent pamoic acid- or drug-related changes on ophthalmology or clinical pathology parameters. According to the sponsor, ECG parameters were not affected by either pamoic acid or drug; however, data were provided only for heart rate. Organ wts were not affected by pamoic acid or drug; however, ovary wt tended to be higher in HDF, both in main-study and recovery animals. Gross and microscopic findings were limited to the injection site. Gross findings consisted of an accumulation of yellow foreign material at the injection site and surrounding areas. A "pale" injection site was noted in PC and in all drug-treated animals. In recovery animals, paleness was still detected in both HDF, but not in VC grps or in HDM. The primary microscopic finding at the injection site was chronic inflammation. Chronic inflammation, characterized as focal areas of myocyte loss with replacement by fibroblasts, collagen, and inflammatory cells [macrophages, lymphocytes, neutrophils], was detected in all drug-treated animals. The severity was not clearly dose-related; however, injection sites in drug-treated animals were affected to a greater extent [incidence and severity] than in PC animals. Injection site changes were noted to be somewhat different in PC than in OPM-treated animals. In PC animals, the reaction was described as involving more "prominent fibroplasia" and less inflammatory cell infiltration than detected in OPM-treated animals; however, in both PC and OPM grps there were focal, extensive areas of myocyte loss with replacement by inflammatory cell infiltration [macrophages and lymphocytes]. Necrosis

was detected only sporadically. Observations in recovery animals [VC, HD] suggested some reversibility of drug effects. No findings were detected in HDM at the end of the recovery period; minimal/slight chronic inflammation at the injection site was still evident in HDF.
The TK data were provided for both olanzapine and pamoic acid. Interanimal variability was high, although the sponsor noted that local site irritation did not appear to affect the PK of OPM. There were no consistent changes in plasma exposure [Cmax, AUC] to olanzapine with duration of dosing, or in dose-corrected plasma olanzapine levels.

As noted in the rat study, pamoic acid was absorbed more rapidly when given alone [Tmax = 1-3 hr] vs when given as the OPM formulation [Tmax = 53-104 hr, at HD], resulting in peak plasma levels of pamoic acid markedly higher in both males [20-18 fold] and females [18-13 fold] when pamoic acid was administered alone; the effect was greater on Day 0 than on Day 56. Plasma AUCs for pamoic acid were also higher when pamoic acid was administered alone, but the effect was much smaller [1.2-2 fold]. Plasma AUC for pamoic acid tended to be lower [23-76%] on Day 56 compared to Day 0, except in MDM. Trends in dose-corrected plasma exposure were not consistent.

Plasma levels of olanzapine were >LLOQ by 56-63 days after the last dose, whereas pamoic acid was still detectable at the end of the recovery period.
(End citation)

2.6.6.4 Genetic toxicology

Note: The genetic toxicology studies on pamoic acid conducted in support of this application (i.e., Ames assay, *In vitro* chromosomal aberration assay in CHO cells, *In vitro* chromosomal aberration assay in human lymphocytes, *In vitro* mouse lymphoma, *In vivo* micronucleus assay in mice, *In vivo* chromosomal aberration assay in mice) were previously reviewed by Lois Freed, Ph.D. on 12/13/2002 under IND 60701 N 032, submitted on 7/30/2002. As no new genetic toxicology studies have been submitted since, and the present reviewer has no reason to disagree with Dr. Freed's assessments, the genetic toxicology review data are directly reproduced from Dr. Freed's review, as follows.

1. Study title: The effect of pamoic acid given intramuscularly for 2 consecutive days on the induction of micronuclei in bone marrow of ICR mice.
Study no: 020115MNT5058
Conducting laboratory and location: sponsor, Greenfield, IN
Date of study initiation: 1/11/02
GLP: Y
QA reports: Y
Drug, lot #, % purity: 015784 [pamoic acid], lot no. PPD04730, potency = 100.7%
Formulation/vehicle: suspensions/0.75% CMC, 5% mannitol, 0.1% polysorbate 80.
Methods:
Strains/species/cell line: ICR mice (b) (4) 5/sex/grp

Doses: 0, 290, 374, and 586 mg/kg i.m. for CM, LDM, MDM, and HDM, respectively; 0, 355, 467, and 684 mg/kg i.m. for CF, LDF, MDF, and HDF, respectively. [Doses varied because they were not corrected for body wt.] Basis of dose selection: dose-range finding study. Doses were administered on Days 0 and 1. Cyclophosphamide was administered [by gavage] only on Day 1.
Test agent stability: stated to be stable in suspension for 8 hrs at 25□C.
Controls
Negative controls: vehicle
Positive controls: cyclophosphamide
Observations: clinical signs [daily], animals were sacrificed 24 hrs after the 2nd dose of pamoic acid. Bone marrow was collected from both femurs [one slide from each femur]. One slide was fixed per animal, stained with Wright's-Giemsa mixture. 2000 PCEs were counted per animal. Bone marrow toxicity was calculated [PCE/NCE]; data from animals with PCE/NCE ratio <0.3 were not included in the grp analyses. MN-PCEs were determined based on examination of 2000 PCEs/animal.
Criteria for positive results: not stated
Results: Clinical signs consisted of leg weakness [all doses] and rough hair coat [HDM]. There was no evidence of bone marrow cytotoxicity [i.e., no decrease in PCE/NCE ratio] or increase in MN-PCEs at any dose of pamoic acid. Cyclophosphamide produced increase in MN-PCEs of ≈5-8 fold.

2. Study title: The effect of pamoic acid (Compound 015784) on the induction of reverse mutations in *Salmonella typhimurium* and *Escherichia coli* using the Ames test.
Study no: 020109AMT5058, 020116AMS5058, 020123AMS5058
Conducting laboratory and location: sponsor, Greenfield, IN
Date of study initiation: Study 020109AMT5058: 1/7/02, Study 020116AMS5058: 1/14/02, Study 020123AMS5058: 1/21/02
GLP compliance: Study No's: 020116AMS5058, 020123AMS5058
QA reports: Y
Drug, lot #, % purity: Pamoic acid, lot 686W02, purity = 99.78%
Formulation/vehicle: DMSO
Methods:
Strains/species/cell line: *S .typhimurium* tester strains, TA1535, TA1537, TA98, and TA100; *E. coli* strain, WP2uvrA.
Dose selection criteria: doses were selected on the basis of data from a preliminary range-finding study [Study 020109AMT5058]. In this study, concentrations of 312.5-5000 µg/plate were not associated with cytotoxicity or increases in revertants when tested using *S. typhimurium* strains TA1535, TA1537, TA98, TA100, and *E coli* WP2uvrA.
Test agent stability: not determined
Metabolic activation system: Arochlor-induced rat liver S9 [strain not specified]
Controls:
Negative controls: DMSO
Positive controls: 2-NF, 9AmAc, 2AA, MNNG
Exposure conditions:
Incubation and sampling times: 48 hrs at 37^{o}C

Concentrations used in definitive study: Study 020116AMS5058: 312.5, 625, 1250, 2500, and 5000 µg /plate [±S9]; Study 020123AMS5058 [TA98 only]: 312.5, 625, 1250, 2500, and 5000 µg /plate [±S9].
Analysis:
No. of replicates: concentrations were tested in triplicate plates.
Counting method: Artek 880 Automated Colony Counter. It was noted that "Automated counting permitted measuring approximately 86% of the plate. Therefore, the area counted and the total area of the plate were measured with the instrument in order to determine a correction factor." The summary table provided corrected counts.
Criteria for positive results: increase in revertants that exceed the C value by ≥2-fold [TA98, TA100, WP2uvrA] or 3-fold [TA1535, TA1537] "for two successive concentrations of drug". "If the above criteria were met for only one concentration level, the determination of a positive response was made on the basis of scientific judgment".

Results:
Study 020116AMS5058
There were no increases in revertants at any concentration, with or without S9, for any tester strain. There was also no evidence of cytotoxicity.
Study 020123AMS5058
This study was conducted in order to confirm the results obtained in Study 020116AMS5058 with TA98. In the previous study, the C values with TA98 exceed the historical control range. No increases in revertants and no cytotoxicity were observed with TA98 in the repeat study; the C values were within the HC range.

3. Study title: The effect of pamoic acid (Compounds 015784 and 062998) on the *in vitro* induction of chromosome aberrations in Chinese Hamster ovary cells.
Study no: 020109CAB5058, 020116CAB5058, 020116CAB5058B, 020130CAB5058B, 020213CAB5058, 020213CAB5058A, 020220CAB5084, 020313CAB5058, 020313CAB5058A, 020313CAB5058B, 020313CAB5058C.
Conducting laboratory and location: sponsor, Greenfield, IN
GLP: all studies, except that drug concentrations and stability in vehicle were not determined.
QA reports: Y
Drug lot information
686W02: original lot tested in animals and humans. Obtained from (b) (4).
19310: obtained from the same supplier as lot 686W02; contained (b) (4) impurities".
X35-7717-188B: this lot was prepared (b) (4) from lot no. 686W02.
H82-L7H-04A, H82-L7H-04B: these lots were prepared from lot no. 19320. Lot H82-L7H-04A was "prepared by controlled crystallization from a solution of pamoic acid in DMSO".
Lot H82-L7H-04B "was crystallized from the residual slurry left from the preparation of lot H82-L7H-04A".
USP G-4: USP reference standard ["highly purified"]; obtained from the USP.

D11A: disodium salt of pamoic acid [Compound (b) (4) obtained from (b) (4) (b) (4) Described as having "high purity".
According to the study report, all drug lots were analyzed by HPLC [Method B05607]; two (b) (4) analogues were present "to various degrees in the lots tested". Structures of these analogues were illustrated in the following sponsor's figure:

(b) (4)

Preliminary cytotoxicity testing

Study 011219CTX5058 [lot 686W02]: in the absence and presence of S9, % cell survival was <50% of C only at the highest concentration tested [i.e., 12% at 4000 µg/ml, compared to VC; the next highest concentration was 1000 µg /ml].

Study 011219CTX5058 [lot 686W02, extended exposure, -S9 only]: % cell survival was <50%of C only at the highest concentration tested [i.e., 2% at 4000 µg /ml].

Study 020206CTX5058 [lot USP G-4]: % cell survival was <50% of C only at the highest concentration tested [i.e., 14 and 41% at 2000 µg/ml in absence and presence of S9, respectively].

Study 020206CTX5058 [lot USP G-4, extended exposure, -S9 only]: % cell survival was <50% of C at the 2 highest concentrations tested [i.e., 38 and 0% at 1000 and 2000 µg/ml respectively].

Study 020206CTX5058A [lot 19310]: % cell survival was >50% of C at all concentrations tested [10-2000 µg/ml] in the absence of S9. In the presence of S9, % cell survival was <50% of C at the 2 highest concentrations tested [41 and 0% at 1000 and 2000 µg /ml, respectively].

Study 020206CTX5058A [lot 19310, extended exposure]: cytotoxicity was not concentration-related in this study. % cell survival was as follows: 63, 46, 57, 31, 53, 37, 51, 59, 48, 48, 45, 42, 92, and 0% of C at 10, 50, 100, 150, 200, 250, 300, 350, 400, 450, 500, 750, 1000, and 2000 µg /ml, respectively.

Study 020206CTX5084 [lot D11A]: % cell survival was >50% at all concentrations tested [10- 2000 µg/ml] in the absence and presence of metabolic activation.

Study 020109CAB5058

Drug, lot #, % purity: pamoic acid [Compound 015784], 686W02 (b) (4) used for clinical trials], purity = 99.78%.

Methods:

Strains/species/cell line: CHO cells
Dose selection criteria: concentrations were selected on the basis of results from a preliminary range-finding study.
Metabolic activation system: Arochlor-induced rat liver S9 [strain not specified]
Controls:

Vehicle: DMSO

Negative controls: DMSO

Positive controls: cyclophosphamide, Mitomycin C

Exposure conditions:

Incubation and sampling times: 4 hrs with drug

Doses used in definitive study: 250, 500, 750, 1000, 1250, 1500, 1750, and 2000 µg/ml [-S9]; 400, 600, 800, 900, 1000, 1100, 1200, 1300, and 1400 µg/ml [+S9]. [In an amendment, it was noted that "due to unexpected toxicity", cells tested in the presence of S9 were not scored.]

Analysis:

No. of replicates: 1 culture/concentration for cytotoxicity. According to the protocol, chromosomal aberrations were evaluated in triplicate per concentration and C, duplicate for PC; however, according to the data tables, concentrations were tested in duplicate [100 cells/culture].

Counting method: n/a

Criteria for positive results: concentration-related, statistically significant increase in chromosomal aberration [trend test].

Results

The results of this study were previously submitted to the IND [Amendment N-028].

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

Table 1: Chromosome Aberrations in Chinese Hamster Ovary (CHO) Cells Treated With Compound 015784 (Lot 686W02) in the Absence of Metabolic Activation, Study 020109CAB5058 (Combined Results From 2 Cultures)

		Number and Type of Aberration															% Cells With Aberrations			
		Gap			Chromatid Exchange					Chromosome Exchange			Other							
Treatment (µg/mL)	Cells Scored	TG	SG	TB	TR	QR	CR	ID	SB	D	R	CI	DM	PU	GT	Number Aberrations Per Cell	Excluding Gaps (TA)	Including Gaps (TAG)	% Cells With >1 Aberration	% Cells With Diplochromosomes
Vehicle Control: Dimethyl sulfoxide																				
	200	2														0	0	1	0	0
Positive Control: Mitomycin C																				
1	25	7	3	5	6	3	2	2				1				0.76	48***	60	20	5
Test Compound: 015784																				
1250	200	1														0	0	0.5	0	1.5
1500	200	2														0	0	1	0	5.5***
1750	200	3	1	3	5	5		1				2				0.08	3.5***	4.5	2.5	13.5***

Abbreviations: TG = chromatid gap, SG = chromosome gap, TB = chromatid break, TR = triradial, QR = quadriradial, CR = complex rearrangement, ID = interstitial deletion, SB = chromosome break (includes acentric fragment), D = dicentric, R = ring chromosome, CI = chromosome intrachange, DM = "double minute" fragment, PU = pulverized chromosome, GT = >10 aberrations.

***Significantly greater than vehicle control (p≤.001).

% cell survival was ≤50% of C only at the HC evaluated [i.e., 1750 µg/ml]. In addition, ppt was detected at the 2 highest concentrations tested [1750, 2000 µg/ml]; the 2000-µg/mL concentration was not analyzed due to excessive ppt.

Study 020116CAB5058

Drug, lot #, % purity: pamoic acid [Compound 015784], 686W02 (b) (4) used for clinical trials], purity = 99.78%.

Methods:

Strains/species/cell line: CHO cells

Dose selection criteria: concentrations were selected based on the results of previous studies

[Study 011219CTX5058 (range-finding), 020109CAB5058 (definitive)]
Metabolic activation system: Arochlor-induced rat liver S9 [strain not specified]
Controls:

Vehicle: DMSO

Negative controls: DMSO

Positive controls: cyclophosphamide

Comments: pamoic acid was not tested in the absence of metabolic activation

Exposure conditions:

Incubation and sampling times: 4 hrs with drug

Doses used in definitive study: 650, 700, 750, 800, 850, 900, 950, 1000, 1050, 1100, 1150, 1200, and 1250 µg/mL [+S9].

Analysis:

No. of replicates: 1 culture/concentration for cytotoxicity. According to the protocol, concentrations were tested in triplicate; PCs were tested in duplicate cultures. However, according to the data tables, concentrations were tested in duplicate, with 200 cells scored per concentration.

Counting method: n/a

Criteria for positive results: concentration-related, statistically significant increase in chromosomal aberration [trend test].

Results

The results of this study were previously submitted to the IND [Amendment N-028].
The data were summarized in the following sponsor's table:

Table 5: Chromosome Aberrations in Chinese Hamster Ovary (CHO) Cells Treated With Compound 015784 (Lot 686W02) in the Presence of Metabolic Activation, Study 020116CAB5058 (Combined Results From 2 Cultures)

		Number and Type of Aberration															% Cells With Aberrations			
		Gap			Chromatid Exchange					Chromosome Exchange			Other							
Treatment (µg/mL)	Cells Scored	TG	SG	TB	TR	QR	CR	ID	SB	D	R	CI	DM	PU	GT	Number Aberrations Per Cell	Excluding Gaps (TA)	Including Gaps (TAG)	% Cells With >1 Aberration	% Cells With Diplochromosomes
Vehicle Control: Dimethyl sulfoxide																				
	200		1	1	1											0.01	1	1.5	0	0
Positive Control: Cyclophosphamide																				
10	25			2	6	2	1		1							0.48	44***	44	4	0
Test Compound: 015784																				
900	200	9	6	24	20	26	13	3	3			6				0.475	15.5***	21	12.5	13.5***
950	200	7	9	15	13	15	8	1				7				0.295	13***	17	9	19.5***
1000	200	18	11	31	18	21	7	1	2		1	7			1	0.49	19***	27	12.5	16.5***

% cell survival was <50% of C at concentrations >1000 µg/mL; the greatest cytotoxicity was detected at the HC [26% of C].
There was no explanation as to why all concentrations were not examined for chromosomal aberrations.

Study 020116CAB5058B

The results of this study were previously submitted to the IND 60701 [Amendment N-028].

Drug, lot #, % purity: pamoic acid [Compound 015784], 686W02 (b) (4) used for clinical trials], purity = 99.78%.

Methods:

Strains/species/cell line: CHO cells

Dose selection criteria: concentrations were selected on the basis of data from previous studies [Study 011219CTX5058 (range-finding), Study 020109CAB5058 (definitive)]
Metabolic activation system: n/a
Controls:
Vehicle: DMSO
Negative controls: DMSO
Positive controls: Mitomycin C
Comments: pamoic acid was not tested in the presence of metabolic activation.
Exposure conditions:
Incubation and sampling times: 4 hrs with drug.
Doses used in definitive study: 1000, 1250, 1500, 1750 µg/ml
Analysis:
No. of replicates: same as for other CHO assays
Counting method: n/a
Criteria for positive results: concentration-related, statistically significant increase in chromosomal aberration [trend test].

Results

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

Table 3: Chromosome Aberrations in Chinese Hamster Ovary (CHO) Cells Treated With Compound 015784 (Lot 686W02) in the Absence of Metabolic Activation, Study 020116CAB5058B (Combined Results From 2 Cultures)

		Number and Type of Aberration																	
		Gap			Chromatid Exchange					Chromosome Exchange			Other				% Cells With Aberrations		
Treatment (µg/mL)	Cells Scored	TG	SG	TB	TR	QR	CR	ID	SB	D	R	CI	DM	PU	GT	Number Aberrations Per Cell	Excluding Gaps (TA)	Including Gaps (TAG)	% Cells With >1 Aberration	% Cells With Diplochromosomes
Vehicle Control: Dimethyl sulfoxide																				
	200	4	1													0	0	2.5	0	0
Positive Control: Mitomycin C																				
1	25	7	1	16	12	6	6	4	1			3				1.92	72***	76	56	0
Test Compound: 015784																				
1250	200	2	1		1	1										0.01	0.5	2	0.5	1
1500	200	6										1				0.005	0.5	2.5	0	2.5
1750	200	5	3	6	1	3			1			3				0.07	2.5	5	2	10***

Abbreviations: TG = chromatid gap, SG = chromosome gap, TB = chromatid break, TR = triradial, QR = quadriradial, CR = complex rearrangement, ID = interstitial deletion, SB = chromosome break (includes acentric fragment), D = dicentric, R = ring chromosome, CI = chromosome intrachange, DM = "double minute" fragment, PU = pulverized chromosome, GT = >10 aberrations.
***Significantly greater than vehicle control (p≤.001).

% cell survival was <50% of C only at the HC tested [45% at 1750 µg/mL].

Study 020130CAB5058B

Drug, lot #, % purity: pamoic acid [Compound 015784], 686W02 (b) (4) used for clinical trials], purity = 99.78%.
Formulation/vehicle: DMSO
Methods:
Strains/species/cell line: CHO cells
Dose selection criteria: concentrations were selected in the basis of previous studies [011219CTX5058B, 020109CAB5058B, 020116CAB5058BB, 02123CAB5058].
Metabolic activation system: n/a

Controls:

Vehicle: DMSO

Negative controls: DMSO

Positive controls: Mitomycin C

Comments: pamoic acid was not tested in the presence of metabolic activation

Exposure conditions:

Incubation and sampling times: 19 hrs with drug

Doses used in definitive study: 1200, 1250, 1300, 1325, 1350, 1375, 1400, 1425, 1450, 1475, and 1500 µg/ml.

Analysis:

No. of replicates: same as for the other CHO assays

Counting method: n/a

Criteria for positive results: concentration-related, statistically significant increase in chromosomal aberration [trend test].

Results

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

Table 7: Chromosome Aberrations in Chinese Hamster Ovary (CHO) Cells Treated With Compound 015784 (Lot 686W02) Utilizing an Extended Exposure Period in the Absence of Metabolic Activation, Study 020130CAB5058B (Combined Results From 2 Cultures)

		Number and Type of Aberration															% Cells With Aberrations			
		Gap			Chromatid Exchange					Chromosome Exchange			Other							
Treatment (µg/mL)	Cells Scored	TG	SG	TB	TR	QR	CR	ID	SB	D	R	CI	DM	PU	GT	Number Aberrations Per Cell	Excluding Gaps (TA)	Including Gaps (TAG)	% Cells With >1 Aberration	% Cells With Diplochromosomes
Vehicle Control: Dimethyl sulfoxide																				
	200	3														0	0	1.5	0	0
Positive Control: Mitomycin C																				
0.5	25	1		18	6	3	6		1	1					4	3	72***	72	64	0
Test Compound: 015784																				
1250	200	12		8	3											0.055	4***	8.5	1	0
1325	200	7	1	25	4		1									0.15	9.5***	11.5	3	0
1400	200	10		25	3	1	1									0.15	8.5***	12	3	0

Abbreviations: TG = chromatid gap, SG = chromosome gap, TB = chromatid break, TR = triradial, QR = quadriradial, CR = complex rearrangement, ID = interstitial deletion, SB = chromosome break (includes acentric fragment), D = dicentric, R = ring chromosome, CI = chromosome intrachange, DM = "double minute" fragment, PU = pulverized chromosome, GT=>10 aberrations.

***Significantly greater than vehicle control (p≤.001).

No cytotoxicity was observed in this study. In fact, % cell survival [compared to C] was >100% at all concentrations tested [range: 118-272% of C].

Study 020213CAB5058

Drug, lot #, % purity: pamoic acid [Compound 015784], USP-G4, "preliminary potency" = 100%

Formulation/vehicle: DMSO

Methods:

Strains/species/cell line: CHO cells

Dose selection criteria:

Metabolic activation system: Arochlor-induced rat liver S9 [strain not specified]

Controls:

Vehicle: DMSO

Negative controls: DMSO
Positive controls: Mitomycin C, cyclophosphamide [+S9 not evaluated]
Exposure conditions:
Incubation and sampling times: 4 hrs with drug
Doses used in definitive study: 300, 400, 500, 750, 1000, 1100, 1200, 1300, 1400, 1500, 1600, and 1750 µg/mL [-S9]; 300, 400, 500, 750, 1000, 1100, 1200, 1300, 1400, 1500, 1600, and 1750 µg/mL [+S9]. [Note: cultures tested in the presence of S9 were not evaluated.]
Analysis:
No. of replicates: same as for the other CHO assays
Counting method: n/a
Criteria for positive results: same as for the other CHO assays

Results

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

Table 9: Chromosome Aberrations in Chinese Hamster Ovary (CHO) Cells Treated With Compound 015784 (Lot USP G-4) in the Absence of Metabolic Activation, Study 020213CAB5058 (Combined Results From 2 Cultures)

		Number and Type of Aberration															% Cells With Aberrations			
		Gap			Chromatid Exchange					Chromosome Exchange			Other							
Treatment (µg/mL)	Cells Scored	TG	SG	TB	TR	QR	CR	ID	SB	D	R	CI	DM	PU	GT	Number Aberrations Per Cell	Excluding Gaps (TA)	Including Gaps (TAG)	% Cells With >1 Aberration	% Cells With Diplochromosomes
Vehicle Control: Dimethyl sulfoxide																				
	200		2	1												0.005	0.5	1.5	0	0
Positive Control: Mitomycin C																				
0.5	25		2	8	1	6	2								1	1.08	32***	40	24	0
Test Compound: 015784																				
750	200	2														0	0	1	0	0
1000	200	1		1												0.005	0.5	1	0	0
1100	200	1														0	0	0.5	0	0

Abbreviations: TG = chromatid gap, SG = chromosome gap, TB = chromatid break, TR = triradial, QR = quadriradial, CR = complex rearrangement, ID = interstitial deletion, SB = chromosome break (includes acentric fragment), D = dicentric, R = ring chromosome, CI = chromosome intrachange, DM = "double minute" fragment, PU = pulverized chromosome, GT = >10 aberrations.
***Significantly greater than vehicle control (p≤.001).

Study 020213CAB5058A

Drug, lot #, % purity: pamoic acid [Compound 015784], 19310, "preliminary potency" = 100%
Formulation/vehicle: DMSO
Methods:
Strains/species/cell line: CHO cells
Dose selection criteria: concentrations used were selected on the basis of a preliminary range-finding study [Study 020206CTX5058A].
Metabolic activation system: Arochlor-induced rat liver S9 [strain not specified]
Controls:
Vehicle: DMSO
Negative controls: DMSO
Positive controls: Mitomycin C, cyclophosphamide
Exposure conditions:
Incubation and sampling times: 4 hrs with drug
Doses used in definitive study: 600, 750, 900, 1000, 1100, 1250, 1500, and 1750 µg/mL [-S9]; 300, 400, 500, 600, 700, 750, 800, 900, 1000, 1050, 1100, 1250, 1500, and 1750 µg/mL [+S9]
Analysis:

No. of replicates: same as for other CHO cell assays
Counting method: n/a
Criteria for positive results: same as for the other CHO cell assays

Results

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

Table 13: Chromosome Aberrations in Chinese Hamster Ovary (CHO) Cells Treated With 015784 (Lot 19310) i the Absence of Metabolic Activation, Study 020213CAB5058A (Combined Results From 2 Cultures)

		Number and Type of Aberration															% Cells With Aberrations			
		Gap			Chromatid Exchange					Chromosome Exchange			Other							
Treatment (µg/mL)	Cells Scored	TG	SG	TB	TR	QR	CR	ID	SB	D	R	CI	DM	PU	GT	Number Aberrations Per Cell	Excluding Gaps (TA)	Including Gaps (TAG)	% Cells With >1 Aberration	% Cells With Diplochromosomes
Vehicle Control: Dimethyl sulfoxide																				
	200	3		1												0.005	0.5	2	0	0
Positive Control: Mitomycin C																				
0.5	25	1	1	6	3	9	4	1				5				1.12	60***	60	28	0
Test Compound: 015784																				
1250	200	1	1	2												0.01	1	2	0	0
1500	200			1	1											0.01	1	1	0	0.5
1750	200	2	1	1		1	1		2							0.025	1	1.5	0.5	0.5

Table 15: Chromosome Aberrations in Chinese Hamster Ovary (CHO) Cells Treated With Compound 015784 (Lot 19310) in the Presence of Metabolic Activation, Study 020213CAB5058A (Combined Results From 2 Cultures)

		Number and Type of Aberration															% Cells With Aberrations			
		Gap			Chromatid Exchange					Chromosome Exchange			Other							
Treatment (µg/mL)	Cells Scored	TG	SG	TB	TR	QR	CR	ID	SB	D	R	CI	DM	PU	GT	Number Aberrations Per Cell	Excluding Gaps (TA)	Including Gaps (TAG)	% Cells With >1 Aberration	% Cells With Diplochromosomes
Vehicle Control: Dimethyl sulfoxide																				
	200	5	1	3	1	3		1								0.04	2	3.5	1.5	2
Positive Control: Cyclophosphamide																				
10	25	5		5	6	3		2	1			2				0.76	44***	60	28	2
Test Compound: 015784																				
750	200	5		2	3	3						1				0.045	3	4.5	1	9.5***
1000	200	40	2	24	17	28	1	10	7			20				0.535	23.5***	33	15.5	16***
1100	32	9	1	25	6	3	2	5	4			3				1.5	46.8***	56.2	40.6	13***

% cell survival was >50% of C at all concentrations tested in the absence of metabolic activation. In the presence of metabolic activation, the degree of cytotoxicity was not concentration-related; at the HC, % cell survival was 68% of C [range: 33-142% of C].

Study 020220CAB5084

Drug, lot #, % purity: disodium salt of pamoic acid [Compound 062998], D11A, "preliminary potency"= 98%

Methods:

Strains/species/cell line: CHO cells

Dose selection criteria: concentrations were selected on the basis of a preliminary range-finding study [Study 020206CTX5084].
Metabolic activation system: Arochlor-induced rat liver S9 [strain not specified]
Controls:
Vehicle: DMSO
Negative controls: DMSO
Positive controls: Mitomycin C, cyclophosphamide
Exposure conditions:
Incubation and sampling times: 4 hrs with drug
Doses used in definitive study: 250, 500, 750, 1000, 1250, 1500, 1750, and 2000 µg/mL
Analysis:
No. of replicates: same as for the other CHO cell assays
Counting method: n/a
Criteria for positive results: same as for the other CHO cell assays

Results

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

Table 23: Chromosome Aberrations in Chinese Hamster Ovary (CHO) Cells Treated With Compound 062998 (Lot D11A) in the Absence of Metabolic Activation, Study 020220CAB5084 (Combined Results From 2 Cultures)

		Number and Type of Aberration															% Cells With Aberrations			
		Gap			Chromatid Exchange					Chromosome Exchange			Other							
Treatment (µg/mL)	Cells Scored	TG	SG	TB	TR	QR	CR	ID	SB	D	R	CI	DM	PU	GT	Number Aberrations Per Cell	Excluding Gaps (TA)	Including Gaps (TAG)	% Cells With >1 Aberration	% Cells With Diplochromosomes
Vehicle Control: Dimethyl sulfoxide																				
	200															0	0	0	0	0.5
Positive Control: Mitomycin C																				
0.5	25	1	1	4	4	3	1	1				3				0.64	44***	44	16	0
Test Compound: 062998																				
1500	200		1													0	0	0.5	0	0
1750	200	3		1		1					1					0.015	1	2.5	0.5	0.5
2000	200	4	1	7	2	2	2									0.065	2.5**	4	2	4.5***

Table 25: Chromosome Aberrations in Chinese Hamster Ovary (CHO) Cells Treated With Compound 062998 (Lot D11A) in the Presence of Metabolic Activation, Study 020220CAB5084 (Combined Results From 2 Cultures)

		Number and Type of Aberration															% Cells With Aberrations			
		Gap			Chromatid Exchange					Chromosome Exchange			Other							
Treatment (µg/mL)	Cells Scored	TG	SG	TB	TR	QR	CR	ID	SB	D	R	CI	DM	PU	GT	Number Aberrations Per Cell	Excluding Gaps (TA)	Including Gaps (TAG)	% Cells With >1 Aberration	% Cells With Diplochromosomes
Vehicle Control: Dimethyl sulfoxide																				
	200	4	1													0	0	2.5	0	0
Positive Control: Cyclophosphamide																				
10	25	8	1	11	7	8	5	4	3	1		5				1.76	76***	80	48	1
Test Compound: 062998																				
500	200	6	1		3			1								0.02	1	3	0.5	0.5
750	200	4				2	1				1					0.02	1.5	3.5	0.5	1.5
1000	200	4	2	1	5			1								0.035	3.5**	5	0	3**

In the absence of metabolic activation, % cell survival was >50% of C at all concentrations tested. In the presence of metabolic activation, % cell survival was <50% at the 2 highest concentrations [26 and 29% of C at 1750 and 2000 µg/mL, respectively.

Study 020313CAB5058

Drug, lot #, % purity: pamoic acid [Compound 015784], USP G-4, "preliminary potency" = 100%

Methods:

Strains/species/cell line: CHO cells

Dose selection criteria: concentrations were selected on the basis of data from Study 020213CAB5058.

Metabolic activation system: Arochlor-induced rat liver S9 [strain not specified]

Controls:

Vehicle: DMSO

Negative controls: DMSO

Positive controls: cyclophosphamide

Comments: pamoic acid was not tested in the absence of metabolic activation

Exposure conditions:

Incubation and sampling times: 4 hrs with drug

Doses used in definitive study: 200, 400, 500, 550, 600, 650, 700, 800, 1000, 1250, 1500, and 2000 µg/mL.

Analysis:

No. of replicates: same as for the other CHO cell assays

Counting method: n/a

Criteria for positive results: same as for the other CHO cell assays

Results

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

Table 11: Chromosome Aberrations in Chinese Hamster Ovary (CHO) Cells Treated With Compound 015784 (Lot USP G-4) in the Presence of Metabolic Activation, Study 020313CAB5058 (Combined Results From 2 Cultures)

Treatment (µg/mL)	Cells Scored	Number and Type of Aberration														Number Aberrations Per Cell	% Cells With Aberrations		% Cells With >1 Aberration	% Cells With Diplochromosomes
		Gap			Chromatid Exchange					Chromosome Exchange			Other				Excluding Gaps (TA)	Including Gaps (TAG)		
		TG	SG	TB	TR	QR	CR	ID	SB	D	R	CI	DM	PU	GT					
Vehicle Control: Dimethyl sulfoxide																				
	200															0	0	0	0	0
Positive Control: Cyclophosphamide																				
15	25	2		2	4	1		2								0.36	28***	36	8	8
Test Compound: 015784																				
400	200															0	0	0	0	1.5
600	200	7		2	15	11	1	8				1				0.19	11.5***	13.5	5	21***
1000	200	5		12	21	12	1	8	2			4				0.30	18***	19	7.5	29.5***

Cytotoxicity was not concentration-related between concentrations of 500 and 1250 µg/ml. % cell survival was <50% of C at concentrations of 550, 700, 1500, and 2000 µg/mL [48, 48, 2, and 0%, respectively].

Study 020313CAB5058A

Drug, lot #, % purity: pamoic acid [Compound 015784], X35-7717-188B, "preliminary potency" = 100%

Methods:

Strains/species/cell line: CHO cells
Dose selection criteria:
Metabolic activation system: Arochlor-induced rat liver S9 [strain not specified]
Controls:
Vehicle: DMSO
Negative controls: DMSO
Positive controls: cyclophosphamide
Comments: pamoic acid was not tested in the absence of metabolic activation
Exposure conditions:
Incubation and sampling times: 4 hrs with drug
Doses used in definitive study: 600, 700, 800, 900, 950, 1000, 1100, 1200, and 1300 µg/mL
Analysis:
No. of replicates: same as for the other CHO cell assays
Counting method: n/a
Criteria for positive results: same as for the other CHO cell assays

Results

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

Table 21: **Chromosome Aberrations in Chinese Hamster Ovary (CHO) Cells Treated With Compound 015784 (Lot X35-7717-188B) in the Presence of Metabolic Activation, Study 020313CAB5058A (Combined Results From 2 Cultures)**

		Number and Type of Aberration																	
		Gap			Chromatid Exchange					Chromosome Exchange			Other				% Cells With Aberrations		
Treatment (µg/mL)	Cells Scored	TG	SG	TB	TR	QR	CR	ID	SB	D	R	CI	DM	PU	GT	Number Aberrations Per Cell	Excluding Gaps (TA)	Including Gaps (TAG)	% Cells With >1 Aberration	% Cells With Diplochromosomes
Vehicle Control: Dimethyl sulfoxide																				
	200				1											0.005	0.5	0.5	0	0
Positive Control: Cyclophosphamide																				
15	25	3	1	15	8	2	2	6				5				1.52	64***	72	48	1
Test Compound: 015784																				
600	200	1			4	2						1				0.035	3	3.5	0.5	20***
700	200	3	1	6	5	6		2								0.095	5***	6.5	3.5	29.5***

Cytotoxicity was not concentration related [range: 18-61% of C]; maximum cytotoxicity was observed at 1000 µg/mL [i.e., % cell survival 18% of C].

Study 020313CAB5058B

Drug, lot #, % purity: pamoic acid [Compound 015784], H82-L7H-04-B, "preliminary potency" = 100%

Methods:

Strains/species/cell line: CHO cells
Dose selection criteria: concentrations selected on the basis of data from Study 020116CAB5058
Metabolic activation system: Arochlor 1254-induced rat liver S9 [strain not specified]
Controls:
Vehicle: DMSO
Negative controls: DMSO
Positive controls: cyclophosphamide
Comments: pamoic acid was not tested in the absence of metabolic activation
Exposure conditions:
Incubation and sampling times: 4 hrs with drug
Doses used in definitive study: 600, 700, 800, 900, 950, 1000, 1100, 1200, and 1300 µg/mL
Analysis:
No. of replicates: same as for the other CHO cell assays
Counting method: n/a
Criteria for positive results: same as for the other CHO cell assays

Results

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

Table 19: **Chromosome Aberrations in Chinese Hamster Ovary (CHO) Cells Treated With Compound 015784 (Lot H82-L7H-04-B) in the Presence of Metabolic Activation, Study 020313CAB5058B (Combined Results From 2 Cultures)**

		Number and Type of Aberration															% Cells With Aberrations			
		Gap			Chromatid Exchange					Chromosome Exchange			Other							
Treatment (µg/mL)	Cells Scored	TG	SG	TB	TR	QR	CR	ID	SB	D	R	CI	DM	PU	GT	Number Aberrations Per Cell	Excluding Gaps (TA)	Including Gaps (TAG)	% Cells With >1 Aberration	% Cells With Diplochromosomes
Vehicle Control: Dimethyl sulfoxide																				
	200	2	3		1											0.005	0.5	3	0	0
Positive Control: Cyclophosphamide																				
15	25	3	1	1	2	1			1				1			0.24	24***	36	0	2
Test Compound: 015784																				
600	200	2	3								1					0.005	0.5	3	0	1
700	200	3		1	1							1				0.015	1.5	3	0	0
800	200	9	1	2	1	1	1					1				0.03	2	6	1	2
900	200	10	2	5	3	1	1		3			1				0.07	4***	8.5	1	3***

Cytotoxicity was not concentration-related [range: 41-90% of C]; maximum cytotoxicity [i.e., % cell survival 41% of C] was observed at the LC and HC.

Study 020313CAB5058C

Drug, lot #, % purity: pamoic acid [Compound 015784], H82-L7H-04-A, "preliminary potency" = 100%

Methods:

Strains/species/cell line: CHO cells

Dose selection criteria: concentrations were selected on the basis of data from Study 020116CAB5058.

Metabolic activation system: Arochlor 1254-induced rat liver S9 [strain not specified]

Controls:

Vehicle: DMSO

Negative controls: DMSO

Positive controls: cyclophosphamide

Comments: pamoic acid was not tested in the absence of metabolic activation

Exposure conditions:

Incubation and sampling times: 4 hrs with drug

Doses used in definitive study: 600, 700, 800, 900, 950, 1000, 1100, 1200, and 1300 µg/mL

Analysis:

No. of replicates: same as for the other CHO cell assays

Counting method: n/a

Criteria for positive results: same as for the other CHO cell assays

Results

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

Table 17: **Chromosome Aberrations in Chinese Hamster Ovary (CHO) Cells Treated With Compound 015784 (Lot H82-L7H-04-A) in the Presence of Metabolic Activation, Study 020313CAB5058C (Combined Results From 2 Cultures)**

		Number and Type of Aberration															% Cells With Aberrations			
		Gap			Chromatid Exchange					Chromosome Exchange			Other							
Treatment (µg/mL)	Cells Scored	TG	SG	TB	TR	QR	CR	ID	SB	D	R	CI	DM	PU	GT	Number Aberrations Per Cell	Excluding Gaps (TA)	Including Gaps (TAG)	% Cells With >1 Aberration	% Cells With Diplochromosomes
Vehicle Control: Dimethyl sulfoxide																				
	200															0	0	0	0	0.5
Positive Control: Cyclophosphamide																				
10	25	1	2	7	3	8		1	1			1			2	1.64	52***	60	32	0
Test Compound: 015784																				
600	161	2	1		1											0.006	0.62	1.86	0	1
800	200	3		6	5	8	1				1	4				.125	8***	9	4	3
900	158	5	2	30	13	12	14	2	5			2			1	0.557	18.99***	22.78	13.29	3.5

Additional studies [data not provided]: the sponsor measured the pH [Compound 015784: 900-1000 µg/mL; +S9; 0, 1, 2, and 4 hrs] and osmolarity [Compound 015784: 900-1000 µg/mL (+S9), 1250-1750 µg/mL (-S9)] of the culture medium during treatment with pamoic acid in "2 small studies". Neither the pH nor osmolarity was affected by presence of drug in the incubation medium.

Conclusions: from this series of CHO cell assays, the sponsor concluded that pamoic acid induced statistically significant increases in the % of cells with chromosomal aberrations [- gaps] in the presence [4-hr exposure] and absence [19-hr exposure] of metabolic activation; the initial positive response in the absence of metabolic activation with 4-hr exposure was not reproducible. [Pamoic acid also produced increases in endoreduplication, both in the absence and presence of metabolic activation.]

4. Study title: Chromosomal aberrations *in vivo* in mouse bone marrow cells exposed to pamoic acid (Compound 015784).
Study no: 23539-0-451OECD
Conducting laboratory and location: (b) (4)
Date of study initiation: 3/8/02
GLP compliance: Y, except that animals were received prior to approval of the protocol.
QA reports: Y
Drug, lot #, % purity: 015784, lot PPD04958, potency = 100.7%
Methods:
Strains/species/cell line: female CD-1 (ICR) BR mice (b) (4) [n = 5/group/sampling time].
Dose selection criteria:
Basis of dose selection: maximum homogenous suspension of 188 mg/mL in vehicle. No toxicity was expected based on previous studies.
Range finding studies: none conducted
Test agent stability: olanzapine pamoate monohydrate demonstrated to be stable in suspension at 5 and 25◦ C for 15 days; pamoic acid demonstrated to be stable in suspension for 8 hrs at 25◦ C. [The data indicated that the concentration of pamoic acid in suspension at a concentration of 188 mg/mL fell from 102.9% of intended initially to 92.9% of intended after 8 hrs at 25◦ C].

Metabolic activation system: n/a
Controls:
Vehicle: sterile vehicle [0.05% mannitol in 0.75% CMC, 0.1% polysorbate 80, 0.05% Dow Corning Antifoam 1510-US]
Negative controls: vehicle
Positive controls: cyclophosphamide [80 mg/kg p.o.]
Exposure conditions:
Incubation and sampling times: animals were sacrificed at 18 [all doses] and 42 [C, HD] hrs post-dosing.
Doses used in definitive study: 0, 328.67, 457.88, and 641.64 mg/kg i.m.
Study design: females were used in order to maximize the mg/kg dose. Animals were observed at dosing, at 1 hr postdosing, and daily until sacrifice.
Analysis: at sacrifice, hind limbs [tibias] were removed and bone marrow was prepared [using 5% Giemsa stain] for analysis. 100 metaphases were examined per animal, except that >25 metaphases were examined in cases in which ε25% of metaphases had aberrations. MI was calculated by examining 1000 cells per animal.
Criteria for positive results: "…a statistically significant dose-related increase in the number of structural aberrations for at least one dose level."

Results

No compound-related clinical signs were observed. There was no evidence of bone marrow cytotoxicity or increase in % cells with structural or numerical aberrations at any dose of pamoic acid tested, at either time point.

5. Study title: Mutagenicity test on pamoic acid (Compound 15784) in the L5178Y TK +/- mouse lymphoma forward mutation assay.
Study no: 23539-0-431ICH1
Volume #: 6.1
Conducting laboratory and location: (b) (4)
Date of study initiation: 1/25/02
GLP compliance: Y, except that drug concentrations and stability were not determined
QA report: Y
Drug, lot #, radiolabel, and % purity: pamoic acid (Compound 015784), lot 686W02, "assigned potency" = 99.8%
Formulation/vehicle: DMSO
Methods:
Strains/species/cell line: L5178Y TK +/- mouse lymphoma
Dose selection criteria:
Basis of dose selection: dose-range finding study.
Range finding study: performed using 4- and 24-hr treatment [+/-S9] at concentrations up to 3000 µg/mL.
Test agent stability: not determined
Metabolic activation system: male Sprague-Dawley rat, Arochlor 1254-induced liver S9
Controls:
Vehicle: DMSO
Negative controls: DMSO
Positive controls: MMS [-S9], methylcholanthrene [MCA, +S9]
Exposure conditions:
Incubation and sampling times: 4- and 24-hr treatment [-S9], 4-hr treatment [+S9]
Doses used in definitive study:
-S9, 4-hr: 13 concentrations were tested; of these, 8 were selected for analysis.

-S9, 24-hr: 12 concentrations were tested; of these, 7 were selected for analysis.
+S9, 4-hr: 13 concentrations were tested; of these, 7 were selected for analysis.
In the initial assay [+S9, 4-hr], cytotoxicity was not concentration-related; therefore, this assay was repeated with 19 concentrations, of which 7 were selected for analysis.
Analysis:
No. of replicates: one culture/concentration; VC was tested in triplicate cultures; PC was tested in duplicate cultures.
Counting method: (b) (4) (b) (4) Colony sizing was performed on all cultures.
Criteria for positive results: (a) >2-fold increase in mutant frequency [MF], (b) "It is desirable to obtain this relationship for at least three concentrations, but this goal depends on the concentration steps chosen for the assay and cytotoxicity at which mutagenic activity appears". (c) The requirement for a concentration-related response is "waived if a large increase in mutant frequency (4-fold or higher) is obtained for a single concentration at or near the highest testable cytotoxicity".

Results

Pamoic acid solution: a ppt was noted when solution was added to culture medium at concentrations >1000 µg/mL, and the medium appeared viscous at concentrations of 750-1000 µg/mL. Stable ppt was detected at concentrations >2500 µg/mL.
Range-finding study: cytotoxicity was calculated as cell density as % of C. % cell density was "0%" at concentrations >1500 µg/mL, both in the absence and presence of S9 after 4 hrs of treatment, and at concentrations >750 µg/mL in the absence of S9 after 24 hrs of treatment.
Definitive study:
-S9, 4-hr treatment: concentrations of 200-1000 µg/mL were scored. There were no increases in MF at these concentrations. It was noted that "Culture described as viscous at dosing and at the termination of treatment" at the HC. RG at the two highest concentrations [900 and 1000 µg/mL] was 18.1 and 17.8%, respectively. The PC produced increases in both small and large colonies.
-S9, 24-hr treatment: concentrations of 6.25-150 µg/mL were scored. There were no significant increases in MF at these concentrations. RG at the HC was 16.4%. The PC produced increases in small and large colonies.
+S9, 4-hr treatment:
Initial assay: concentrations of 100-800 µg/mL were scored. Cytotoxicity was not concentration-related [lowest RG (20.2%) occurred at 600 µg/mL]. There were no increases in MF at any concentration. The PC produced increases in small and large colonies.
Repeat assay: concentrations of 700-840 µg/mL were scored. Cytotoxicity was concentration-related [RG at the HC was 15.8%]. There was no increase in MF at any concentration. The PC produced increases in small and large colonies.

6. Study title: Chromosomal aberrations in cultured human peripheral blood lymphocytes with pamoic acid (Compound 015784).
Study no: 23539-0-449OECD
Volume #: 6.1
Conducting laboratory and location: (b) (4)
Date of study initiation: 4/16/02
GLP compliance: Y, except that drug concentrations and stability were not determined.
QA report: Y
Drug, lot #, radiolabel, and % purity: pamoic acid, lot 686W02, "assigned potency" = 99.8%.
Formulation/vehicle: DMSO solution
Methods:

Strains/species/cell line: human peripheral blood lymphocytes
Dose selection criteria:
Basis of dose selection: solubility limit
Range finding studies: none
Test agent stability: not determined
Metabolic activation system: Arochlor 1254-induced rat liver S9 [strain not specified]
Controls:
Vehicle: DMSO
Negative controls: culture medium
Positive controls: Mitomycin C, cyclophosphamide
Exposure conditions:
Incubation and sampling times: 4- and H22-hr treatment [-S9], 4-hr treatment for [+S9]
Doses used in definitive studies:
-S9, 4-hr: cytotoxicity: 505, 758, 1010, 1210 ⌈g/mL; clastogenicity: 253, 505, 758, 1010 µg/mL.
-S9, 21.8-hr: cytotoxicity 127, 253, 505, 758, 1010, 1210, 1410, 1610, 1810, 2010 µg/mL; clastogenicity: 127, 253, 505 µg/mL.
+S9, 4-hr: cytotoxicity: 127, 253, 505, 758, 1010, 1210, 1410, 1610, 1810, 2010 µg/mL; clastogenicity: 127, 253, 505, 758 µg/mL.
Analysis:
No. of replicates: duplicate cultures for VC, treatment, and PC.
Counting method: "if possible", 100 cells were scored per culture, except for "At least 25 cells" were scored in those cultures in which >25% of cells had aberrations. MI was calculated on the basis of 1000 cells per culture. Cells were stained with 5% Giemsa for examination; the method of cell analysis was not stated.
Criteria for positive results: (a) significant increase [$p<0.01$] in number of cells with aberrations at one or more concentrations and (b) concentration-related increase.

Results

-S9, 4-hr treatment: concentrations of 253-1010 µg/mL were scored. Cytotoxicity was assessed at concentrations of 10, 505, 758, 1010, and 1210 ⌈g/mL. The MI was similar to VC at concentrations of 10 and 505 ⌈g/mL. The MI was decreased at concentrations of 758 and 1010 µg/mL, but not in a concentration-related manner [38 and 30% reduction compared to VC, respectively]. [At a concentration of 1210 µg/mL, the % decrease in MI was 44%; MF was not determined at this concentration.] It was noted that "Slight" ppt was detected at all concentrations. There was no increase in the number of cells with structural aberrations, or in endoreduplication or polyploidy. The PC produced increases in structural aberrations [but not in endoreduplication or polyploidy].
-S9, 22-hr treatment: concentrations of 127, 253, and 505 µg/mL were scored. MI was reduced by 51, 65, and 65%, respectively, at these concentrations. [MI was decreased by 82, 92, 99, and 100% at concentrations of 758-1410 µg/mL; these concentrations were not scored for aberrations.] Ppt was detected at > 505 µg/mL. Ppt was characterized as "slight" at 505 µg/mL, and as preventing analysis at >1410 µg/mL. Pamoic acid did not significantly increase the number of cells with aberrations [structural or numerical]; however, the % cells with structural aberrations [-gaps] tended to be higher at the HC scored; the greatest increase at the HC was in simple breaks [i.e., chromatid/chromosome breaks and acentric fragments]. The PC produced significant increases in structural aberrations, but not in endoreduplication or polyploidy. The data were summarized in the following sponsor's table:

Table 4: Chromosome Aberrations in Human Lymphocytes - Without Metabolic Activation - 21.8 Hour Treatment, 21.8 Hour Harvest

Assay No.: 23539 Trial No.: B2 Date: 06/05/02 Lab No.: CY6182 Test Article: Pamoic Acid

				CELLS SCORED	% MITOTIC INDEX REDUCTION [a]	# ENDOREDUPLICATED CELLS	# POLYPLOID CELLS	JUDGEMENT (+/-) [b]	NUMBERS AND PERCENTAGES (%) OF CELLS SHOWING STRUCTURAL CHROMOSOME ABERRATIONS: Gaps	Simple Breaks	chte	chre	mab	TOTALS [c] -g	TOTALS [c] +g	JUDGEMENT (+/-) [d]
CONTROLS NEGATIVE:	RPMI 1640		A	100		0	0		3					0	3	
			B	100		0	0		1					0	1	
			TOTAL	200					4					0	4	
			AVERAGE	%	-	**0.0**	**0.0**		**2.0**					**0.0**	**2.0**	
VEHICLE:	DMSO	10.0µL/mL	A	100		0	0							0	0	
			B	100		0	0							0	0	
			TOTAL	200										0	0	
			AVERAGE	%	0	**0.0**	**0.0**							**0.0**	**0.0**	
POSITIVE:	MMC	0.300µg/mL	A	50		0	0		7	14	9			22	26	
			B	50		0	0		2	13	7			17	19	-
			TOTAL	100					9	27	16			39	45	
			AVERAGE	%	-	**0.0**	**0.0**	-	**9.0**	**27.0**	**16.0**			**39.0**	**45.0**	+

Table 4 (continued): Chromosome Aberrations in Human Lymphocytes - Without Metabolic Activation - 21.8 Hour Treatment, 21.8 Hour Harvest

Assay No.: 23539 Trial No.: B2 Date: 06/05/02 Lab No.: CY6182 Test Article: Pamoic Acid

			CELLS SCORED	% MITOTIC INDEX REDUCTION [a]	# ENDOREDUPLICATED CELLS	# POLYPLOID CELLS	JUDGEMENT (+/-) [b]	NUMBERS AND PERCENTAGES (%) OF CELLS SHOWING STRUCTURAL CHROMOSOME ABERRATIONS: Gaps	Simple Breaks	chte	chre	mab	TOTALS [c] -g	TOTALS [c] +g	JUDGEMENT (+/-) [d]
CONTROLS TEST ARTICLE	127µg/mL	A	100		0	0		3	1				1	4	
		B	100		0	0		5	1				1	6	
		TOTAL	200					8	2				2	10	
		AVERAGE	%	51	**0.0**	**0.0**	-	**4.0**	**1.0**				**1.0**	**5.0**	-
	253µg/mL	A	200		0	0		2					0	2	
		AVERAGE	%	65	**0.0**	**0.0**	-	**1.0**					**0.0**	**1.0**	-
	505µg/mL	A	193		0	1		6	5			1	6	11	
		AVERAGE	%	65	**0.0**	**0.5**	-	**3.1**	**2.6**			**0.5**	**3.1**	**5.7**	-

chte: chromatid exchange chre: chromosome exchange mab: multiple aberrations, greater than 4 aberrations

[a] % Mitotic index reduction as compared to the vehicle control.

[b] Significantly greater in % polyploidy and % endoreduplication than the vehicle control, $p \leq 0.01$.

[c] -g = # or % of cells with chromosome aberrations; +g = # or % of cells with chromosome aberrations + # or % of cells with gaps.

[d] Significantly greater in -g than the vehicle control, $p \leq 0.01$. RPMI 1640 = culture medium DMSO = Dimethylsulfoxide MMC = Mitomycin C

+S9, 4-hr treatment: concentrations of 127, 253, 505, and 758µg/mL were scored. MI was reduced by 11, 49, 43, and 47%, respectively, at these concentrations. [MI was reduced by 61, 56, and 100% at concentrations of 1010, 1210, and 1410-1810 µg/mL, respectively; these concentrations were not scored for aberrations.] Ppt was detected at concentrations >253 µg/mL; the ppt was characterized as "slight" at 505 µg/mL and was too severe to allow scoring at 2010 µg/mL. Pamoic acid did not produce significant increases in the number of cells with structural aberrations or in endoreduplication or polyploidy; however, the % cells with structural aberrations [-gaps] tended to be higher at the HC scored. The PC produced significant increases in % cells with structural aberrations, but not in endoreduplication or polyploidy. The data were summarized in the following sponsor's table:

Table 6: Chromosome Aberrations in Human Lymphocytes - With Metabolic Activation - 4.0 Hour Treatment, 22.2 Hour Harvest

Assay No.: 23539 Trial No.: B1 Date: 05/02/02 Lab No.: CY5162 Test Article: Pamoic Acid

				CELLS SCORED	% MITOTIC INDEX REDUCTION [a]	# ENDOREDUPLICATED CELLS	# POLYPLOID CELLS	JUDGEMENT (+/-) [b]	NUMBERS AND PERCENTAGES (%) OF CELLS SHOWING STRUCTURAL CHROMOSOME ABERRATIONS: Gaps	Simple Breaks	chte	chre	mab	TOTALS [c] -g	TOTALS [c] +g	JUDGEMENT (+/-) [d]
CONTROLS NEGATIVE:	RPMI 1640		A	100		0	0		2					0	2	
			B	100		0	0		2					0	1	
			TOTAL	200					4					0	3	
			AVERAGE	**%**	-	**0.0**	**0.0**		**2.0**					**0.0**	**1.5**	
VEHICLE:	DMSO	10.0 µL/mL	A	100		0	0		2	1	1			2	4	
			B	100		0	0		2					0	2	
			TOTAL	200					4	1	1			2	6	
			AVERAGE	**%**	**0**	**0.0**	**0.0**		**2.0**	**0.5**	**0.5**			**1.0**	**3.0**	
POSITIVE:	CP	25.0 µg/mL	A	53		0	0		4	10	4			14	17	
			B	50		0	0		3	13	3			15	18	
			TOTAL	103					7	23	7			29	35	
			AVERAGE	**%**	-	**0.0**	**0.0**	-	**6.8**	**22.3**	**6.8**			**28.2**	**34.0**	+

Table 6 (continued): Chromosome Aberrations in Human Lymphocytes - With Metabolic Activation - 4.0 Hour Treatment, 22.2 Hour Harvest

Assay No.: 23539 Trial No.: B1 Date: 05/02/02 Lab No.: CY5162 Test Article: Pamoic Acid

			CELLS SCORED	% MITOTIC INDEX REDUCTION [a]	# ENDOREDUPLICATED CELLS	# POLYPLOID CELLS	JUDGEMENT (+/-) [b]	NUMBERS AND PERCENTAGES (%) OF CELLS SHOWING STRUCTURAL CHROMOSOME ABERRATIONS: Gaps	Simple Breaks	chte	chre	mab	TOTALS [c] -g	TOTALS [c] +g	JUDGEMENT (+/-) [d]
TEST ARTICLE	127 µg/mL	A	100		0	0		4	2				2	6	
		B	100		0	0		4					0	4	
		TOTAL	200					8	2				2	10	
		AVERAGE	**%**	**11**	**0.0**	**0.0**	-	**4.0**	**1.0**				**1.0**	**5.0**	-
	253 µg/mL	A	100		0	0		1					0	1	
		B	100		0	0		1					0	1	
		TOTAL	200					2					0	2	
		AVERAGE	**%**	**49**	**0.0**	**0.0**	-	**1.0**					**0.0**	**1.0**	-
	505 µg/mL	A	100		0	1		1	1				1	2	
		B	100		0	0		4					0	4	
		TOTAL	200					5	1				1	6	
		AVERAGE	**%**	**43**	**0.0**	**0.5**	-	**2.5**	**0.5**				**0.5**	**3.0**	-
	758 µg/mL	A	100		0	1		2	3	1			4	6	
		B	100		0	0		4	2				2	6	
		TOTAL	200					6	5	1			6	12	
		AVERAGE	**%**	**47**	**0.0**	**0.5**	-	**3.0**	**2.5**	**0.5**			**3.0**	**6.0**	-

chte: chromatid exchange chre: chromosome exchange mab: multiple aberrations, greater than 4 aberrations

[a] % Mitotic index reduction as compared to the vehicle control.

[b] Significantly greater in % polyploidy and % endoreduplication than the vehicle control, $p \leq 0.01$.

[c] -g = # or % of cells with chromosome aberrations; +g = # or % of cells with chromosome aberrations + # or % of cells with gaps.

[d] Significantly greater in -g than the vehicle control, $p \leq 0.01$. RPMI 1640 = culture medium DMSO = Dimethylsulfoxide CP = Cyclophosphamide

HC data were provided in the following sponsor's table:

Historical Control Data

Control Data of Chromosome Aberrations in Human Lymphocytes 1/2000 Through 12/2000, Approximately 20 - 24 Hour Harvest

	Activation		# of Aberrations per Cell	% of Cells With Aberrations	% of Cells With >1 Aberrations	% Poly-ploid Cells	% Endore-duplicated Cells
Negative Control	Without	MIN	0.0	0.0	0.0	0.0	0.0
3 Hour Treatment		MAX	0.01	1.0	0.5	1.0	0.0
		AVG	0.00	0.24	0.02	0.12	0.00
		SD (±)	0.00	0.34	0.11	0.27	0.00
		N	21	21	21	21	21
Solvent Control (Pooled)	Without	MIN	0.0	0.0	0.0	0.0	0.0
3 Hour Treatment		MAX	0.01	1.0	000	0.5	0.0
		AVG	0.00	0.24	0.00	0.17	0.00
		SD (±)	0.00	0.34	0.00	0.24	0.00
		N	21	21	21	21	21
Positive Control –	Without	MIN	0.29	23.2	4.0	0.0	0.0
Mitomycin C		MAX	0.98	47.0	26.0	1.0	0.0
3 Hour Treatment		AVG	0.55	35.11	12.67	0.12	0.00
		SD (±)	0.19	7.57	6.30	0.31	0.00
		N	21	21	21	21	21
Negative Control	Without	MIN	0.0	0.0	0.0	0.0	0.0
Continuous Treatment		MAX	0.01	0.5	0.5	0.5	0.0
		AVG	0.00	0.18	0.03	0.11	0.00
		SD (±)	0.00	0.25	0.11	0.21	0.00
		N	19	19	19	19	19
Vehicle Control (Pooled)	Without	MIN	0.0	0.0	0.0	0.0	0.0
Continuous Treatment		MAX	0.02	1.5	0.5	1.0	0.0
		AVG	0.00	0.29	0.02	0.12	0.00
		SD (±)	0.00	0.43	0.09	0.28	0.00
		N	29	29	29	21	21
Positive Control –	Without	MIN	0.24	16.7	4.7	0.0	0.0
Mitomycin C		MAX	0.92	48.0	26.0	1.5	0.0
Continuous Treatment		AVG	0.53	34.10	12.66	0.13	0.00
		SD (±)	0.17	7.04	5.90	0.39	0.00
		N	28	28	28	20	20

N = Number of trials

	Activation		# of Aberrations per Cell	% of Cells With Aberrations	% of Cells With >1 Aberrations	% Poly-ploid Cells	% Endore-duplicated Cells
Negative Control	With	MIN	0.0	0.0	0.0	0.0	0.0
3 Hour Treatment		MAX	0.02	2.0	0.5	1.5	0.5
		AVG	0.00	0.36	0.01	0.14	0.03
		SD (±)	0.01	0.52	0.08	0.36	0.12
		N	36	36	36	35	35
Solvent Control (Pooled)	With	MIN	0.0	0.0	0.0	0.0	0.0
3 Hour Treatment		MAX	0.06	4.0	2.0	0.5	0.5
		AVG	0.01	0.54	0.08	0.12	0.02
		SD (±)	0.01	0.89	0.34	0.22	0.09
		N	45	45	45	37	37
Positive Control	With	MIN	0.12	10.0	1.5	0.0	0.0
CP = Cyclophosphamide		MAX	0.95	56.3	24.0	1.0	0.0
3 Hour Treatment		AVG	0.50	32.72	11.51	0.04	0.00
		SD (±)	0.20	10.13	6.36	0.18	0.00
		N	45	45	45	37	37

Genetic toxicology summary and conclusions

The sponsor conducted the following genetic toxicology studies of pamoic acid: Ames test, *in vitro* chromosomal aberration assay in CHO cells, *in vitro* chromosomal aberration assay in human lymphocytes, *in vitro* mouse lymphoma assay, *in vivo* micronucleus assay in mice, *in vivo* chromosomal aberration assay in mice.

Pamoic acid [concentrations up to 5000 μg/plate] was negative in the Ames test and the *in vitro* mouse lymphoma assay [with or without metabolic activation (Arochlor-induced rat liver S9)], and in the *in vivo* assays in mice.

The results of the *in vitro* chromosomal aberration assays conducted using CHO cells are summarized in the attached table [SA = structural aberrations excluding gaps, DIPLO = endoreduplication, RCS = relative cell survival]. [In the sponsor's briefing document, it was noted that additional studies (data not provided) demonstrated that pH and osmolarity were not affected by the presence of pamoic acid in the medium. Also, as in the *in vitro* human lymphocyte assay, cytotoxicity was frequently assessed at concentrations not scored for aberrations even at concentrations were not associated with excessive cytotoxicity.] Pamoic acid produced increases in % of cells with structural and numerical [endoreduplication] aberrations, with and without metabolic activation, in one or more of the assays conducted. The sponsor concurred that pamoic acid was positive in this assay, with and without metabolic activation. In the absence of a structural alert for genotoxicity for pamoic acid, it was hypothesized that either an impurity or a metabolite was responsible for the positive findings. However, the sponsor noted that (a) no metabolite was identified in either *in vitro* or *in vivo* PK/ADME studies of pamoic acid and (b) "…testing of highly purified lots of pamoic acid prepared by different methods or obtained from different vendors confirmed the initial findings and eliminated the possibility that an impurity was responsible for the result". Therefore, it was concluded that pamoic acid itself was responsible for the positive findings. And, as noted by the sponsor, the positive findings were not artifacts resulting from changes in the pH or osmolarity of the culture medium.

The sponsor conducted additional genotoxicity assays in order assess (a) the "*in vivo* relevance of the response observed *in vitro* in the CHO cell…and (b) the effect of pamoic acid in an *in vitro* chromosomal aberration assay using a different cell line [i.e., human lymphocytes]. As previously noted, pamoic acid was negative in two *in vivo* assays in mice [i.e., *in vivo* micronucleus assay, *in vivo* chromosomal aberration assay].

In the *in vitro* chromosomal aberration assay in human lymphocytes, pamoic acid did not produce significant increases in % of cells with structural or numerical aberrations. However, with the 4-hr treatment [both with and without S9], higher concentrations should have been tested for aberrations since the MI was not reduced >50% at the HCs tested. At the high-concentrations tested both in the absence [continuous treatment] and presence [4-hr] of metabolic activation, the % of cells with structural aberrations was increased compared to VC. The % of affected cells [absence of S9-presence of S9] at the HC was 3.1-3.0% compared to 0-1% for the VC. According to the HC data provided for VC [covering the period 1/2000-12/2000], the range and average of % of cells with structural aberrations [it was not specified if these data represented + or – gaps] were as follows: -S9 [n = 29]: 0-1.5 [range], 0.29 [average]; +S9 [n = 45]: 0-4 [range], 0.54 [average]. [The vehicle(s) used were not specified, other than that VC or SC (solvent) data are "pooled".] Therefore, the % of affected cells obtained with pamoic acid at the HC in the absence of S9 exceeds the HC range, whereas the % of affected cells at the HC in the presence of S9 was within the HC range. [It should be noted that the HC data were collected using a 3-hr treatment period, as opposed to the 4-hr treatment period used with pamoic acid.]

I*n vitro* chromosomal aberration in CHO cells
[INCUB = incubation time, SA = structural aberrations
excluding gaps, DIPLO = endoreduplication, RCS = relative cell survival]

STUDY	INCUB [hr]	BATCH	S9	[PA]	SA [% cells]	ENDO [% cells]	RCS [%]
020109CAB5058	4	686W02 (clinical)	-	0	0	0	>50 at all but 1750 µg/mL
				1250	0	1.5	
				1500	0	5.5***	
				1750	3.5***	13.5***	
020116CAB5058	4	686W02 (clinical)	+	0	1	0	≥50
				900	15.5***	13.5***	
				950	13***	19.5***	
				1000	19***	16.5***	
020116CAB5058B	4	686W02 (clinical)	-	0	0	0	45 at HC
				1250	0.5	1	
				1500	0.5	2.5	
				1750	2.5	10***	
020130CAB5058B	19	686W02	-	0	0	0	>100 at all conc
				1250	4***	0	
				1325	9.5***	0	
				1400	8.5***	0	
020213CAB5058	4	USP-4	-	0	0.5	0	≥50 at all conc
				750	0	0	
				1000	0.5	0	
				1100	0	0	
020213CAB5058A	4	19310	-	0	0.5	0	>50 at all conc
				1250	1	0	
				1500	1	0.5	
				1750	1	0.5	
			+	0	2	2	not conc-related; 68 at 1750 µg/mL
				750	3	9.5***	
				1000	23.5***	16***	
				1100	46.8***	13***	
020220CAB5084	4	D11A	-	0	0	0.5	>50 at all conc
				1500	0	0	
				1750	1	0.5	
				2000	2.5**	4.5***	
			+	0	0	0	>50 at all conc
				500	1	0.5	
				750	1.5	1.5	
				1000	3.5**	3**	
020313CAB5058	4	USP G-4	+	0	0	0	48 [550,700 µg/mL] 2-0 [1500-2000 µg/mL]
				400	0	1.5	
				600	11.5***	21***	
				1000	18***	29.5***	
020313CAB5058A	4	X35-7717-188B	+	0	0.5	0	not conc-related; max of 18 at 1000 µg/mL
				600	3	20***	
				700	5***	29.5***	
020313CAB5058B	4	H82-L7H-04-B	+	0	0.5	0	not conc-related, max of 41 at 600 and 1300 µg/mL
				600	0.5	1	
				700	1.5	0	
				800	2	2	
				900	4***	3***	
020313CAB5058C	4	H82-L-7H-04-A	+	0	0	0.5	<50 at >800 µg/mL, but ≥30 at all conc
				600	0.62	1	
				800	8***	3	
				900	18.99***	3.5	

*p<0.05, **p<0.01, ***p<0.001400

It is the sponsor's conclusion that "no further genotoxicity or carcinogenicity assessments are warranted for the planned IM olanzapine depot NDA" based on the following: (a) "…the results of all of the *in vitro* and the *in vivo* genotoxicity tests on pamoic acid have been negative except for the single positive result in the *in vitro* chromosome aberration test in CHO cells", (b) the lack of "….evidence of a continuing tissue response or angiogenesis that might be considered early indicators of a neoplastic potential", and (c) "…humans appear to be much less sensitive to foreign body tumorigenesis than rodents…" The fact that pamoic acid was positive only in the *in vitro* chromosomal aberration assay in CHO cells [a "cell-line specific" effect, as described by the sponsor] is not a basis for dismissing the relevance of these findings in assessing human risk. [According to Anita Bigger, Ph.D., (Chair, Genetic Toxicology Subcommittee), compounds not infrequently test positive in only one cell line.] Concern regarding the positive results obtained in CHO cells is also not diminished by the negative responses in other *in vitro* and *in vivo* assays, or by the lack of preneoplastic findings in the 3-mo i.m. toxicity of pamoic acid in rat.
Furthermore, pamoic acid was reproducibly positive in the *in vitro* assay in CHO; it was not a "single positive result" as described by the sponsor. Therefore, additional evaluation of the carcinogenic potential of pamoic acid is needed. The sponsor may elect to conduct an alternative assay [e.g., heterozygous p53$^{+/-}$ knockout model] in lieu of a 2-yr bioassay, with justification provided for the assay selected.

Conclusions

Additional assessment of carcinogenic potential is necessary based on the reproducibly positive findings [in the presence and absence of metabolic activation] induced by pamoic acid in the *in vitro* chromosomal aberrations assays conducted in CHO cells. Negative findings in other genotoxicity assays or the lack of preneoplastic findings in a 3-month general toxicity study in rat do not reduce the concern regarding the carcinogenic potential of the olanzapine pamoate i.m. depot formulation. The Division recommended that the sponsor needs to "conduct either a 2-yr bioassay (in one species) or an alternative assay [e.g., heterozygous p53$^{+/-}$ knockout model]. Justification should be provided for the approach selected".
(End citation)

2.6.6.5 Carcinogenicity

Study title: **An Oncogenicity Study in Fischer 344 Rats Given Intramuscular Injections of Olanzapine Pamoate Monohydrate Once Monthly for 2 Years**

Background: The positive result of pamoic acid testing in the in vitro chromosome aberration assay in CHO cells (see 2.6.6.4. Genetic Toxicity) prompted some concern that pamoic acid might be carcinogenic, although all other genetic toxicology studies were negative. Therefore, a 2-year IM study in rats was conducted using once/4 weeks injections of OPM; pamoic acid alone was also evaluated in this study. The carcinogenicity protocol of OPM i.m. depot formulation was reviewed under IND 60701 (N 038 and 041) by Dr. Lois Freed (3/26/2003). As stated in the review, "Based on the positive responses in the in vitro chromosomal aberration assay, the Division suggested that the sponsor consider conducting a p53+/- assay in lieu of a 2-year bioassay. The sponsor chose not to do so for the following reasons: (a) the limited muscle mass of the mouse would further restrict the dose; (b) the sensitivity of the p53+/- mouse to"…solid state carcinogenicity as evidenced by the high rate of sarcomas that developed when implanted with Biomedic transponders". As a result the sponsor believes that the p53+/- transgenic model would likely develop neoplasms in response to any parenterally administered foreign material with prolonged absorption

characteristics and would be a poor choice in which to evaluate the true carcinogenic potential of the OPM drug product" (end citation)

In consultation with the Division, it was agreed that in view of the limitations of repeated i.m. injections to rodents by the volume that can be injected into the relatively small muscle mass available and the attainable concentration of drug substance in the formulation, a once/4 weeks dosing was acceptable for use in the OPM rodent carcinogenicity study even though the clinical development program was intended to support labeling for IM administration in humans using either once/4 weeks or once/2 weeks injections.

The protocol of the 2-year carcinogenicity study of OPM in the rat was approved by the Executive CAC and the doses were selected in accordance with the Executive CAC recommendations (IND 60701, Executive CAC Meeting Minutes of 3/11/2003). As the active substance of OPM is olanzapine that had been previously tested for carcinogenicity in two species (mice and rats), it was agreed that a carcinogenicity study in one species would be sufficient for the assessment of the olanzapine pamoate depot formulation.

Key study findings: In the 2-year study to evaluate the carcinogenic potential of a sustained-release formulation of olanzapine administered by once per 4 weeks intramuscular injections of olanzapine pamoate monohydrate (OPM) to Fischer 344 rats (60/sex/dose) at doses of 0 (vehicle), 0 (pamoic acid), 5, 10, and 20 mg /kg for males and 0, 0, 10, 25, and 50 mg /kg for females (dose range equivalent to 0.1-1.2x the MRHD of 405 mg/ 4 weeks on a mg/m2 basis), there was no carcinogenic effect attributable to OPM or pamoic acid since there was no dose-related effect on incidence and distribution of neoplastic lesions and they were similar among groups. Survival at the end of 2 years (45%, 48%, 48%, 45%, or 35% for males and 65%, 55%, 72%, 70%, or 72% for females in vehicle control, pamoic acid alone, LD, MD, or HD groups, respectively) showed that enough animals of both sexes were exposed for sufficient amount of time. In the high dose group, the mortality rate was 12% higher than the vehicle control in the males, and 6% lower than control in the females (statistically non-significant). There were no drug-related clinical signs. There were small but significant decreases in body weight gain of both genders in OPM dosed groups relative to control throughout treatment (by about 2% and 4% in HD males and females, respectively), while food consumption was slightly increased (by about 6%) in both genders. Pamoic acid alone did not affect body weight or food consumption. The only non-neoplastic pathologic finding attributable to OPM was the presence of residual test article formulation in injection sites and an associated chronic inflammatory response and muscle degeneration. The incidence and severity of residual test article accumulation and associated changes in the injection sites was clearly dose-proportional (out of 60 rats/sex/group, the incidence of this finding was 0, 1, 14, 34 and 52 for males and 0, 0, 22, 48 and 50 for females in vehicle control, pamoic acid alone, LD, MD, and HD groups, respectively).

Plasma concentrations of olanzapine and pamoic acid determined over 14 days (336 h) following 3, 12 and 18 months of dosing showed a dose-dependent increase in olanzapine exposure; the $AUC_{0\text{-}336h}$ values achieved at HD were lower or equal to those in humans at MRHD. Exposures to pamoic acid ($AUC_{0\text{-}336h}$) achieved at HD were equal to or higher than those in humans at MRHD. Dose-limiting factors were the amount of test article

feasible to be injected i.m. in the rat and the local injection site reaction. In animals receiving pamoic acid alone, Cmax values of pamoic acid were higher compared to the high-dose OPM groups.

Adequacy of the carcinogenicity study and appropriateness of the test model:
The study was conducted according to standard procedures to assess the carcinogenic potential of the test article.
The Fischer 344 rat was selected because this species and strain is commonly used as the test system for pharmaceuticals and because this was the same species and strain used in the existing oral carcinogenicity studies with olanzapine.
The intramuscular route was selected because it is the intended route of exposure in humans with this formulation. The justification of injection volume of 0.1 ml/100 g of body weight with a total volume not to exceed 0.2 ml was based on "the maximum that should be injected intramuscularly in rats as accepted by most Institutional Animal Care and Use Committees, supported by US and European humane societies" and on "practical limitations of injectability through a 21 or 23 gauge needle".
Pamoic acid was also evaluated in this study since it "represents that part of the molecule that would be released upon ionization and since no published carcinogenicity data could be cited supporting its long term safety by this or any other route of administration".
The selection of doses for the 2-year study was based on the 3-month study in the same species and strain at doses of 0, 20, 50, and 100 mg/kg of OPM administered i.m. once a month for 3 months, that resulted in 26% to 39% reduction in body weight gains in HDM, HDF and MDM and in absolute body weight reductions of < 10%, as compared with the controls. The MD and HD in the 3-month study induced significant, dose-limiting, chronic inflammatory reactions in the injection site characterized by atrophy, degeneration, or necrosis of myocytes, fibroplasias, and increased collagen deposition observable for at least 2 months after injection. Injection-site reactions induced by administration of pamoic acid alone were infrequent and less severe than those for the OPM-treated rats.
The doses for the 2-year carcinogenicity study (0, 5, 10, 20 mg/kg of OPM for males and 0, 10, 25, 50 mg/kg of OPM for females, i.m.) were selected in accordance with the Executive CAC recommendations (IND 60701, Executive CAC Meeting Minutes of 3/11/2003). The employed dose range was equivalent to 0.1-1.2 times the MRHD of intramuscular OPM in humans (405 mg/4 weeks) on mg/m2 basis. Effect of pamoic acid alone at i.m. doses similar to those administered in the high-dose OPM group (37 mg/kg in males and 92.5 mg/kg in females) was assessed in parallel in additional groups of rats.
A MTD was achieved in this study based on dose-related injection site adverse effects in both genders (chronic inflammatory reactions and residual test substance accumulation in the injection site affecting nearly all animals of both genders at HD); additionally, a non-significant (12%) increase in mortality occurred in HD males by the end of the study, and a small decrease in body weight gain in females (4% lower than the control by the end of the study), as determined by the statistical reviewer, Dr. Rahman. Plasma exposures to olanzapine and pamoic acid increased with the increase in OPM dose. Olanzapine AUC_{0-336h} values achieved at HD were lower or equal than those in humans at MRHD (300 mg every 2 weeks or 405 mg every 4 weeks). Exposures to pamoic acid (AUC_{0-336h}) achieved at HD were equal to or higher than those in humans at MRHD. Dose-limiting factors

were the amount of test article feasible to be injected i.m. in the rat and local injection site reaction.
It is concluded that this is a valid carcinogenicity study.

Note: Executive CAC meeting minutes for rat protocol dose selections and final study reviews are appended in the Attachment Section of this review

Evaluation of tumor findings:

Sponsor's analysis: The sponsor's analyses did not show statistically significant dose-response relationship among vehicle control, LD, MD, and HD groups or between vehicle control and pamoic acid alone in any of the tested tumor types.

Statistical reviewer analysis: Statistical review and evaluation of the results of this study was independently conducted by the statistical reviewer Dr. Mohammad Atiar Rahman. Adjustment for the multiple dose-response relationship testing was done using the results of Lin and Rahman (1998). Adjustment for multiple pairwise comparisons was done using the results of Haseman (1983 (see Statistical Review for statistical methods and references).
According to Dr. Rahman's review, "tests did not show statistically significant dose-response relationship or pairwise difference in tumor incidence between the untreated (i.e., vehicle) control and any of the treated groups in any observed tumor types. From the mortality and body weight data it can be concluded that the used high dose might have reached MTD in both sexes. For a final determination of the adequacy of the doses used, other clinical signs and histopathological toxic effects must be considered".
This reviewer agrees with Dr Rahman's conclusion. The adequacy of the doses used is mainly supported by the amount of test article feasible to be injected i.m. in the rat and the local injection site reaction (presence of residual test article formulation in injection sites and an associated chronic inflammatory response and muscle degeneration). The incidence and severity of residual test article accumulation and associated chronic inflammatory changes in the injection sites was clearly dose-proportional and affected the majority of the treated animals at MD and HD.
In conclusion, there was no significant dose-response relationship or pairwise difference in tumor incidence between the untreated control and any of the treated groups in any observed tumor types in any group of rats administered OPM for 104 weeks.

CAC concurrence: Yes

Study no.: R03603 and R03703 (oncogenic studies); R04103 and R04203 (companion TK studies)
Volume and page: electronic submission
Conducting laboratory and location: Eli Lilly and Company; (b) (4) (b) (4)
Date of study initiation: R03603: 5/23/2003; R03703: 6/6/2003; R04103: 7/25/2003; R04203:7/30/2003
GLP compliance: yes (OECD)
QA report: yes
Drug, lot #, and % purity:
Test article: Olanzapine pamoate monohydrate (Compound 426906)

Lot number: ML114 (=CTM0081)
Purity: 99.8%
Vehicle control: 0.75% CMC sodium, 5% mannitol, and 0.1% polysorbate 80 in Water for Injection, USP
Pamoic Acid control: Compound 015784
PPD-B0837-11, 265 mg pamoic acid/vial
PPD-B0837-12, 530 mg pamoic acid/vial

Doses: 0, 0, 5, 10, and 20 mg/kg/day (males); 0, 0, 10, 25 and 50 mg/kg/day (females).
Treatment groups: The animals were randomized by body weight stratification to the different dose groups as indicated in the sponsor's table below:

Group	Dose of Olanzapine for males[a] (mg/kg)	Dose of Olanzapine for females[b] (mg/kg)
01	0[c]	0[c]
02	0[d]	0[d]
03	5	10
04	10	25
05	20	50

[a] Studies R03603 and R04103.
[b] Studies R03703 and R04203.
[c] Vehicle control; Studies R03603 and R03703 only.
[d] Pamoic acid control; 37 mg/kg (M), 92.5 mg/kg (F)

Basis of dose selection: The selection of doses for the 2-year study was based on the 3-month study in the same species and strain at doses of 0, 20, 50, and 100 mg/kg of OPM administered i.m. once a month for 3 months, that resulted in 26% to 39% reduction in body weight gains in HDM, HDF and MDM and in absolute body weight reductions of < 10%, as compared with the controls. The MD and HD in the 3-month study induced significant, dose-limiting, chronic inflammatory reactions in the injection site characterized by atrophy, degeneration, or necrosis of myocytes, fibroplasias, and increased collagen deposition observable for at least 2 months after injection. Injection-site reactions induced by administration of pamoic acid alone were infrequent and less severe than those for the OPM-treated rats. The doses were selected in accordance with the Executive CAC recommendations (IND 60701, Executive CAC Meeting Minutes of 3/11/2003)
Species/strain: Rat, Fischer 344

Number/sex/group (main study): 60/sex/group
TK study: 57-66 sex/group
Route, formulation, volume: Intramuscular injection, 21 or 23 gauge needles; Suspension of OPM in vehicle (0.75% CMC sodium, 5% mannitol, and 0.1% polysorbate 80 in Water for Injection, USP); Injection volume of 0.1 ml/100 g of body weight with a total volume not to exceed 0.2 ml.
Frequency of dosing: Once every 28 days (+/- 1 day)
Treatment duration: 2 years for oncogenicity studies; 18 doses at 4-week intervals for toxicokinetic studies

Satellite groups used for toxicokinetics: 57-66 animals/sex/group
Age: 5 to 7 weeks
Animal housing: The rats were housed in groups of 5 animals per sex per cage in stainless steel suspended cages with wire mesh floors in a controlled environment.
Restriction paradigm for dietary restriction studies: non applicable
Drug stability/homogeneity: Dose form: suspension; Storage conditions: room temperature; Stable under the storage conditions used in these studies; Homogeneous under the conditions used in these studies
Dual controls employed: Two control groups (a vehicle control and a pamoic acid alone control) of 60 rats/sex each
Interim sacrifices: none
Deviations from original study protocol: Evaluation of protocol deviations indicates that these changes did not adversely affect the study integrity.

Observation times:
Live Phase
Observations: Daily for mortality and morbidity; detailed examinations performed weekly for the first 14 weeks, then every 2 weeks through the end of live phase (main study only).
Body weights: Weekly for the first 14 weeks, then every 2 weeks through Day 726 (main study) or Day 489 (TK study), beginning on Day -2; body weights from TK study were used for dose calculations and are not included in the submitted report.
Food consumption: Weekly for the first 14 weeks, then every 2 weeks through Day 726, beginning on Day -2 (main study only).
Clinical Pathology
Hematology (main study only).: Blood films prepared at terminal necropsy from all surviving animals and animals euthanized at unscheduled intervals, when possible. These samples were examined "only as necessary for diagnostic purposes".
Morphologic Pathology (main study only)
Scheduled necropsies: Days 732 through 736 (M); Days 730 through 739 (F)
Histopathology: Conducted on a full list of tissues from all main study animals from control and treated groups and all unscheduled deaths from the satellite allocation, as well as all gross lesions from all rats. Tissues were evaluated by light microscopy. Pathology peer review was conducted by Peter Mann, DVM.

Tissues collected for histopathology evaluation

Adrenal(s)	Heart	Sciatic Nerve
Aorta	Ileum	Seminal Vesicle(s)
Bone	Injection Site(s)[a]	Skeletal Muscle
Bone Marrow	Jejunum	Skin
Brain Stem	Kidney(s)	Spinal Cord
Cecum	Liver	Spleen
Cerebellum	Lung	Stomach
Cerebrum	Mammary Gland	Submandibular Lymph Node
Colon	Mesenteric Lymph Node	Testis(es)
Duodenum	Pancreas	Thymus
Epididymis(ides)	Parathyroid(s)	Thyroid(s)
Esophagus	Pituitary	Tongue
Eye(s)	Prostate	Trachea
Harderian Gland	Salivary Gland(s)	Urinary Bladder

[a] Collected from both legs and labeled L1 for left leg and R1 for right leg
[b] Paired organs were collected and when possible, both were evaluated

Toxicokinetics: Collection: After 3, 12, and 18 doses
Collection time points: Group 02 (pamoic acid only): 0, 0.5, 4, 8, 24, 48, and 120 hours postdose
Groups 03, 04, and 05 (test compound LD, MD and HD): 0, 4, 8, 24, 120, and 336 hours postdose

Results

Mortality: By the end of the study, survival rates in males given 0, 5, 10, or 20 mg olanzapine/kg were 45%, 48%, 45%, or 35%, respectively. Females given 0, 10, 25, or 50 mg olanzapine/kg had 65%, 72%, 70%, or 72% survival at termination, respectively. Rats given pamoic acid alone had a survival rate of 48% and 55% in the males and females, respectively. Sponsor's analysis showed no significant difference among vehicle control, pamoic acid control, and OPM low, medium, and high dose groups.
Details of unscheduled deaths by cause are presented in the following sponsor's tables:

Mortality by cause

Males

Cause of Death	Group >	01	02	03	04	05
	Dose (mg/kg) >	0	0	5	10	20
	Number >	60	60	60	60	60
Large granular lymphocytic leukemia, systemic		8	19	4	11	15
Pituitary neoplasm		9	3	9	5	9
Chronic progressive nephropathy, kidney		2	3	6	2	6
Thrombosis, heart		1	1	0	2	0
Mesothelioma, systemic		1	0	0	1	1
Undetermined		3	4	6	1	2
Islet cell neoplasms, pancreas		3	0	0	0	0
All other neoplasms		4	0	5	5	4
All other non-neoplastic causes		2	1	1	6	2
Total Unscheduled Deaths		33	31	31[a]	33	39
Total Died		11	8	7	10	14
Total Killed Clinical		17	18	18	20	20
Total Killed Moribund		5	5	5	3	5

[a] This total includes animal number 3031 that was an accidental death

Females

Cause of Death	Group >	01	02	03	04	05
	Dose (mg/kg) >	0	0	10	25	50
	Number >	60	60	60	60	60
Large granular lymphocytic leukemia, systemic		7	10	5	6	6
Pituitary neoplasm		5	10	1	6	4
Undetermined		5	3	5	3	5
All other neoplasms		3	3	2	1	2
All other non-neoplastic causes		1	1	4	2	0
Total Unscheduled Deaths		21	27	17	18	17
Total Died		5	13	6	4	3
Total Killed Clinical		14	13	10	13	11
Total Killed Moribund		2	1	1	1	3

Statistical reviewer's analysis: Percentage of survival in the high dose group at the end of Weeks 52, 78, and 91 is shown in the table excerpted from Dr. Rahman's review:

Percentage of survival in the high dose group at the end of Weeks 52, 78, and 91

	Percentage of survival		
	End of 52 weeks	End of 78 weeks	End of 91 weeks
Male	100%	90%	80%
Female	98%	94%	88%

It was concluded that enough rats in both sexes were exposed to the high dose of OPM for a sufficient amount of time.
The mortality rate at the end of the experiment in the high dose group males was 12% higher than the control, while in the HD females it was about 6% lower than control; these changes were not statistically significant (table excerpted from Dr. Rahman's review):

Percentage of Mortality at the End of the Experiment

Male	**Cont.**	**Low**	**Medium**	**High**
	53%	52%	55%	65%
Female	**Cont.**	**Low**	**Medium**	**High**
	33%	28%	30%	27%

Clinical signs: No drug-related clinical observations were noted in OPM-treated groups.
Body weights and food consumption:
Sponsor's analysis:
Body weights of males given OPM were significantly decreased throughout the treatment period. The change in mean body weight for males given OPM was ≤ 6% of the control values. Body weights of females given OPM were significantly decreased during the first 90-day period and sporadically thereafter. The decreases in mean body weight for females given OPM were ≤ 5% of the control values throughout the study.
No changes in body weight or food consumption parameters were found for males and females given pamoic acid alone.
Food consumption was increased throughout the study for males and females given OPM. The magnitude of the increase was ≤ 6% in both genders, statistically significant in females.
Mean body weights (in grams) are shown in the sponsor's tables on the next page; the per cent change relative to control on study days 96 and 362 is shown in the sponsor's table below:

Body Weight, % Change from Control

	Pamoic Acid		Olanzapine					
Monthly Dose (mg/kg):	37	94	5	10	10	25	20	50
Parameter Sex:	M	F	M	F	M	F	M	F
Body Weight, Day 96	–	–	↓3*	↓3*	↓4*	↓4*	↓5*	↓3*
Body Weight, Day 362	–	–	↓3*	–	↓4*	–	↓5*	–

Abbreviations: F = female, M = male, ↑ = increase, ↓ = decrease, – = no important findings.
*p≤.05; statistical significance based on actual data, not on percent change from control.

Statistical reviewer's analysis:
The following table excerpted from Dr. Rahman's review shows the percent difference in mean body weight gain from the concurrent control, defined as,

$$\text{Percent difference} = \frac{(\text{Final BW} - \text{Baseline BW})_{\text{Treated}} - (\text{Final BW} - \text{Baseline BW})_{\text{Control}}}{(\text{Final BW} - \text{Baseline BW})_{\text{Control}}}$$

Percent Difference in Mean body Weight Gain from Combined Controls

Male			Female		
Low	Medium	High	Low	Medium	High
1.24	1.10	-1.88	3.76	- 2.50	-4.03

Source: Table 5 of sponsor's submission

Dr Rahman determined that relative to the control, there had been about 2% decrement in body weight gain in male high dose group and about 4% decrement in body weight gain in female high dose group.

Gross pathology: The most consistent gross pathology findings were observed in the spleen, pituitary, brain stem, testes, kidneys, and injection sites. The incidence of noteworthy gross pathology findings is presented with histopathology correlates in the following sponsor's table.

Incidence of noteworthy gross and histopathology findings

Males

Organ	Group >	01	02	03	04	05
Gross Finding	Dose (mg/kg) >	0	0	5	10	20
Histopathologic Finding	Number >	60	60	60	60	60
Spleen						
Enlarged		26	22	18	21	25
Large granular lymphocytic leukemia		24	33	21	28	25
Pituitary						
Enlarged		25	14	23	16	18
Adenoma or carcinoma, adenohypophysis		31	26	30	21	24
Brain Stem						
Depression		18	8	17	12	12
Pituitary neoplasm (adenoma or carcinoma)		31	26	30	21	24
Testes						
Discoloration		39	34	30	35	32
Adenoma, interstitial cell		41	44	42	41	40
Kidney						
Discoloration, enlarged, granular		41	47	46	39	47
Chronic progressive nephropathy		59	58	55	57	57
Injection Site (Left)						
Discoloration, yellow		1	2	32	50	60
Accumulation, yellow crystals		0	1	2	27	48
Injection Site (Right)						
Discoloration, yellow		1	2	30	55	60
Accumulation, yellow crystals		0	1	14	34	53

Incidence of noteworthy gross and histopathology findings

Females

Organ / Gross Finding / Histopathologic Finding	Group >	01	02	03	04	05
	Dose (mg/kg) >	0	0	10	25	50
	Number >	60	60	60	60	60
Spleen						
Enlarged		9	16	10	9	12
Large granular lymphocytic leukemia		15	22	21	10	20
Mammary Gland						
Nodule(s)		17	19	21	18	21
Adenoma, adenocarcinoma, fibroadenoma		16	11	14	18	16
Pituitary						
Enlarged		19	26	22	19	21
Adenoma or carcinoma, pars distalis		28	37	28	20	26
Brain stem						
Depression		11	21	9	11	16
Pituitary neoplasm (adenoma or carcinoma)		28	37	28	20	26
Uterus						
Nodule(s)		8	9	6	4	5
Endometrial stromal polyp		7	9	8	4	9
Kidneys						
Discoloration, enlarged, granular		21	26	23	16	17
Chronic progressive nephropathy		55	54	52	54	54
Injection Site (Left)						
Discoloration, yellow		0	2	44	56	53
Accumulation, yellow crystals		0	0	12	35	50
Injection Site (Right)						
Discoloration, yellow		0	3	49	59	55
Accumulation, yellow crystals		0	0	22	48	50

Histopathology:

Non-neoplastic findings:

Single to multiple accumulations of yellow crystals at the injection sites present in a dose-dependent manner in all OPM-treated groups was the only test article-related non-neoplastic histopathology finding in both males and females. The crystals were assumed to be test article formulation, and were intermixed with mononuclear cells (macrophages) and fibrous connective tissue; in some lesions, lymphocytes and plasma cells were present at the periphery. Muscle degeneration and chronic inflammation were observed at some injection sites. The incidence and severity of these local injection site effects was clearly dose-proportional.

Neoplastic findings: The incidence of tumors among all groups is summarized in the sponsor's table on the next page.

Tumor Summary

Males

Group >	01	02	03	04	05
Dose (mg/kg) >	0	0	5	10	20
Animals Examined >	(60)	(60)	(60)	(60)	(60)
Tumor Bearing Animals	58	59	56	57	59
Animals with Malignant Tumors	36	40	29	36	33
Animals with Benign Tumors	56	55	53	55	54
Animals with Multiple Tumors	42	52	37	45	43
Animals with Single Tumors	16	7	19	12	16
Animals with Multiple Malignant Tumors	28	37	24	31	28
Animals with Multiple Benign Tumors	25	28	25	19	22
Animals with Metastasising Tumors	0	0	1	0	1
Total Tumors	241	329	236	275	299
Total Malignant Tumors	143	236	151	194	218
Total Benign Tumors	98	93	85	81	81
Total Metastasizing Tumors	0	0	1	0	1

Females

Group >	01	02	03	04	05
Dose (mg/kg) >	0	0	10	25	50
Animals Examined >	(60)	(60)	(60)	(60)	(60)
Tumor Bearing Animals	53	54	48	44	50
Animals with Malignant Tumors	24	29	26	18	25
Animals with Benign Tumors	41	43	40	33	39
Animals with Multiple Tumors	33	34	38	26	33
Animals with Single Tumors	20	20	10	18	17
Animals with Multiple Malignant Tumors	18	23	23	15	21
Animals with Multiple Benign Tumors	15	18	15	13	18
Animals with Metastasising Tumors	1	0	1	0	1
Total Tumors	180	191	186	144	181
Total Malignant Tumors	119	123	129	95	118
Total Benign Tumors	61	68	57	49	63
Total Metastasizing Tumors	1	0	1	0	1

The most common neoplasms were tumors of the mammary gland (females), uterus, testes, pituitary and adrenals, and leukemia. Sponsor's neoplastic incidence tables by tumor type are appended in Attachment 3 to this review.

Leukemia: In this study, out of 60 rats/sex/group, the incidence of large granular lymphocytic leukemia (most frequently seen in liver, spleen, bone marrow, and lymph nodes) was 25, 34, 21, 30, and 25 in males and 16, 22, 21, 13, and 21 in females for vehicle control, pamoic acid control, LD, MD and HD, respectively. It is recognized as a common spontaneous systemic neoplastic disease of F344 rats older than a year of age.

Pituitary adenoma (pars distalis and pars intermedia) incidence was 31, 25, 29, 21, and 23 in males and 28, 36, 28, 20, and 24 in females for vehicle control, pamoic acid control, LD, MD and HD, respectively. It was a common, spontaneous neoplasm. Additionally, in males, one case of pituitary carcinoma was diagnosed in each of pamoic

acid control, LD and HD groups; in females, one pituitary carcinoma was found in pamoic acid control, and two in HD group.
Pheochromocytoma (benign and malignant) in the adrenal gland was found in 7, 9, 3, 3, and 10 males, and in 3, 4, 1, 1, and 2 females out of 60 animals/sex/group from vehicle control, pamoic acid control, LD, MD and HD groups, respectively.
Mammary fibroadenoma, adenoma, and adenocarcinoma were common tumors in all female groups, but not in the males. In females, the incidence of fibroadenoma was 12, 11, 13, 14, and 15 ; the incidence of adenocarcinoma was 3, 0, 1, 3, and 1, and the incidence of adenoma was 1, 0, 0, 1, 0 for vehicle control, pamoic acid control, LD, MD and HD groups, respectively.
Interstitial (Leydig) cell adenoma in the testis was found in 41, 44, 42, 41, and 40 out of 60 animals from vehicle control, pamoic acid control, LD, MD and HD groups, respectively. It is a common spontaneous tumor for F344 rats.
Endometrial stromal polyp in the uterus is a common proliferative lesion that occurred in 7, 9, 8, 4, and 9 out of 60 females from vehicle control, pamoic acid control, LD, MD and HD groups, respectively.
According to the pathology report, all other neoplasms were considered to be incidental, spontaneous, and unrelated to test article administration. There were no rare tumors (less than 5/group) that were considered to be test article-related.

Sponsor's analysis: Sponsor's analyses (methods specified in Dr. Rahman's statistical review) did not show statistically significant dose-response relationship among vehicle control, and the low, medium, and high dose OPM groups in any of the tested tumor types, neither a statistically significant difference between untreated (vehicle) control and pamoic acid-only group in any of the tested tumor types. Based on the incidence and distribution of neoplasms among all groups, there was no carcinogenic effect attributable to the test article.

Statistical reviewer's analysis: According to Dr. Rahman's independent analysis, tests did not show statistically significant dose-response relationship or pairwise difference in tumor incidence between the untreated control and any of the treated groups in any observed tumor types.

Toxicokinetics:

Plasma exposures to olanzapine and pamoic acid in the groups treated with the test article (OP depot) and exposure to pamoic acid in the groups treated with pamoic acid alone are shown in the following sponsor's table:

TK data: OPM 2-Year Carcinogenicity Study in Rats

		Vehicle Control		Pamoic Acid		OP Depot[a]		OP Depot[a]		OP Depot[a]	
	Dose (mg/kg)/4 weeks	0	0	37	92.5	5	10	10	25	20	50
Toxicokinetics	Sex/number of animals	M:0	F:0	M:57	F:57	M:66	F:66	M:66	F:66	M:66	F:66
Week 8	Olanzapine										
	C_{max} (ng/mL)	NA	NA	NA	NA	56	90	67	123	130	176
	$AUC_{0-336\,h}$ (ng•h/mL)	NA	NA	NA	NA	2933	3560	5467	8338	8754	16322
	Pamoate[b]										
	C_{max} (ng/mL)	NA	NA	16674	28684	3137	1795	1873	2637	2709	2591
	$AUC_{0-336\,h}$ (ng•h/mL)	NA	NA	242759	427823	99474	141467	170845	246425	200833	343559
Week 44	Olanzapine										
	C_{max} (ng/mL)	NA	NA	NA	NA	23	65	48	139	92	238
	$AUC_{0-336\,h}$ (ng•h/mL)	NA	NA	NA	NA	1437	3779	3789	8449	4241	25218
	Pamoate[b]										
	C_{max} (ng/mL)	NA	NA	18645	36079	887	2122	1337	4561	1947	3518
	$AUC_{0-336\,h}$ (ng•h/mL)	NA	NA	333163	675436	66143	193737	123075	395584	130801	517485
Week 68	Olanzapine										
	C_{max} (ng/mL)	NA	NA	NA	NA	85	99	41	106	73	187
	$AUC_{0-336\,h}$ (ng•h/mL)	NA	NA	NA	NA	2113	4934	3783	6738	6647	9597
	Pamoate[b]										
	C_{max} (ng/mL)	NA	NA	17704	51085	1664	1742	1239	2667	1760	4512
	$AUC_{0-336\,h}$ (ng•h/mL)	NA	NA	319104	719735	69916	116634	129323	185339	195107	266701

[a] Dose expressed in mg olanzapine/kg.

[b] AUC values in the group that received pamoic acid alone were calculated from 0 - 120 hours. The corresponding pamoic acid doses following OP Depot administration were 6.2, 12.4, and 24.9 mg/kg in males and 12.4, 31.1, and 62.2 mg/kg in females.

Plasma concentrations of olanzapine and pamoic acid were determined over 14 days (336 h) following 3, 12 and 18 months of dosing with the test article (OPM) in the 2-year carcinogenicity study; the TK of pamoic acid were also determined following the administration of pamoic acid alone at doses of 37 mg/kg and 92.5 mg/kg for males and females respectively injected i.m. at 4-week intervals. In both males and females, exposure to olanzapine and pamoic acid (AUC_{0-336h}) increased with increasing OPM dosage. No sex-related differences were observed in the toxicokinetics at a common dose of 10 mg/kg. Peak plasma concentrations of both olanzapine and/or pamoic acid occurred within the first day of dosing in all dose groups on each day evaluated followed by a general decline for up to 336 hours postdose

In separate groups of animals that received pamoic acid alone, peak plasma concentrations were approximately 10-fold higher than those observed in the high-dose OPM groups, which received similar, but slightly lower, pamoic acid doses. Exposure to pamoic acid, as assessed by AUC values, was higher in the pamoic acid alone groups compared to the high-dose OPM groups. Additionally, there was a faster decline in the plasma concentrations of pamoic acid in the pamoic acid alone groups versus the groups treated with OPM. Animals receiving pamoic acid alone rarely had quantifiable concentrations of pamoic acid at 120 hours postdose, but animals receiving OPM almost always had quantifiable concentrations of pamoic acid at 336 hours postdose.

Olanzapine exposure in humans upon administration of OPM in therapeutic doses is shown in the following sponsor's table:

OPM Steady-State PK parameters in humans, Multiple dose
Geometric mean (%CV)

2-Week Injection Interval

Dose (mg)	N	$C_{max,ss}$ (ng/mL)	$T_{max,ss}$ (hr)	$AUC_{\tau,ss}$ (ng×hr/mL)	$t_{1/2}$ (hr)	CL_{ss}/F (L/hr)	V_{ss}/F (L)	$C_{av,ss}$ (ng/mL)
100	3	13.5 (56.2)	96.0 (95.68 - 167.83)	3530 (46.7)	322 (28.7)	28.4 (46.7)	13700 (82.9)	10.5 (46.7)
150	12	29.7 (29.0)	48.1 (19.28 - 334.23)	7540 (26.2)	422 (75.8)	19.9 (26.2)	12800 (86.8)	22.4 (26.2)
160	7	26.1 (63.6)	95.9 (48 - 312)	6870 (51)	352 (189)	23.3 (51.0)	13200 (383)	20.4 (51.0)
210	17	39.3 (37.7)	48.7 (0 - 190.92)	10400 (46.2)	561 (90.4)	19.2 (46.2)	16300 (73.5)	31.0 (46.2)
300	19	46.1 (41.7)	82.8 (19.87 - 335.72)	12400 (46.5)	751 (192)	24.1 (46.5)	28300 (168)	37.0 (46.5)

4-Week Injection Interval

Dose (mg)	N	$C_{max,ss}$ (ng/mL)	$T_{max,ss}$ (hr)	$AUC_{\tau,ss}$ (ng×hr/mL)	$t_{1/2}$ (hr)	CL_{ss}/F (L/hr)	V_{ss}/F (L)	$C_{av,ss}$ (ng/mL)
210	21	22.8 (57.2)	48 (0 – 503)	9150 (44.7)	553 (87.5)	21.9 (44.7)	18300 (128)	13.6 (44.7)
255	6	25.4 (44.8)	336 (0 - 503)	12400 (51.5)	718 (102)	20.6 (51.5)	20800 (101)	18.4 (51.5)
300	14	39.6 (45.2)	167 (44 – 507)	18900 (44.0)	590 (93.3)	15.9 (44.0)	16700 (113)	28.1 (44.0)
405	29	47.6 (52.5)	96.0 (24 - 669)	23600 (50.0)	995 (110)	17.1 (50.0)	24600 (120)	35.2 (50.0)

CV = coefficient of variation

Exposure to pamoic acid in humans upon 4 consecutive i.m. administrations of OPM depot at a dose of 300 mg given every 2 weeks (Clinical study F1D-EW-LOBO) is shown in the following sponsor's table.

Geometric Mean (Geometric CV) Pamoic Acid PK Parameters after the Fourth Injection of IM Olanzapine Depot 300 mg given every 2 weeks

CT Lot	N_{PK}	$C_{max,ss}$ (ng/mL)	$t_{max,ss}$[a] (h)	$AUC_{\tau,ss}$ (ng•h/mL)	$t_{1/2}$ (h)	$C_{av,ss}$ (ng/mL)	PTF (%)
Overall	7	500 (70.2)	24.00 (6 – 264)	108000 (57.9)	423 (79.8)	323 (57.9)	85.3 (30.7)
CT21907	3	639 (30.1)	24.00 (6 - 264)	129000 (15.0)	278 (67.1)	385 (15.0)	98.1 (36.9)
CT501950	4	415 (94.4)	26.50 (6 - 48)	95030 (81.5)	580 (73.3)	283 (81.5)	76.8 (25.0)

Abbreviations: $AUC_{\tau,ss}$ = area under the time-versus-time curve during one injection interval at steady state; $C_{av,ss}$ = average drug concentration under steady-state conditions during multiple dosing; $C_{max,ss}$ = maximum observed drug concentration during one injection interval at steady state; CT = clinical trial; h = hour; N_{PK} = minimum observed drug concentration during a dosing interval at steady state; PTF = peak-to-trough fluctuation; $t_{max,ss}$ = observed sampling time of $C_{max,ss}$; $t_{½}$ = apparent terminal elimination half-life.

[a] Median and range reported.

Olanzapine exposure (AUC_{0-336h}) values achieved at HD of OPM depot formulation in rat carcinogenicity study were equal to or lower than those in humans at MRHD (300 mg/2 weeks or 405 mg/4 weeks). Exposures to pamoic acid (AUC_{0-336h}) achieved at HD of OPM depot formulation in rat carcinogenicity study were equal to or higher than those in humans at MRHD (300 mg/ 2 weeks). Dose-limiting factors were the amount of test article feasible to be injected i.m. in the rat and the local injection site reaction.

Conclusions:

In conclusion, based on the lack of a dose-response relationship or difference in tumor incidence between control and any of the treated groups in any of the observed tumor types in a valid carcinogenicity study, there was no carcinogenic effect attributable to the test article (OPM) or pamoic acid alone.

2.6.6.6 Reproductive and developmental toxicology

Fertility and early embryonic development

No fertility and early embryonic development studies were conducted with OP Depot.

Embryofetal development

Study title: An Embryo-Fetal Development (Segment II) and a Companion Toxicokinetic Study of Olanzapine Pamoate Monohydrate Administered by Intramuscular Injection to CD Rats

Key study findings: A single intramuscular injection of olanzapine pamoate monohydrate on Gestation Day 6 in the rat at doses of 0 (vehicle control), 10, 25, and 75 mg/kg or pamoic acid (the formulating agent) alone at a dose of 93 mg/kg did not induce signs of systemic maternal toxicity or adverse embryo/fetal developmental effects in any dose group. Injection site treatment-related histopathologic findings were observed in all compound-treated groups and were indicative of an inflammatory response to pamoic acid or OPM. Measurable olanzapine and pamoic acid plasma concentrations were registered throughout gestation until the termination on Gestation Day 20. Based on these findings, the no-observed-adverse-effect level (NOAEL) for both systemic maternal effects and developmental effects was 75 mg/kg, corresponding to olanzapine $AUC_{0\text{-}t}$ value of 8448 ng•hr/mL and C_{max} of 153 ng/mL. This dose represents the highest dose that could be administered based on the maximum achievable concentration of the test article in suspension and the maximum tolerated intramuscular dose volume for the test animal used in this study.

Study no.: R07403 (embryo-fetal development)
R07503 (satellite toxicokinetic study)
Volume # and page #: N.A.
Conducting laboratory and location:
Eli Lilly and Company
2001 West Main Street
Greenfield, IN 46140
Date of study initiation: Study R07403: 22 Nov. 2003; Study R07503: 30 Nov. 2003
GLP compliance: yes
QA reports: yes
Lot numbers and potency: Olanzapine Pamoate Monohydrate (OPM) Lot CT23905/210 mg olanzapine/vial and Lot PPD-B0837-9/60 mg olanzapine/vial; pamoic acid (Compound 015784), Lot PPD-B0837-11/265 mg pamoic acid/vial.
Vehicle: 0.75% carboxymethylcellulose sodium, 5% mannitol, and 0.1% polysorbate 80 in water for injection, USP; Lot CT24129

Methods

Doses: Single i.m. dose of OPM (10, 25, 75 mg/kg) or pamoic acid (93 mg/kg) on Gestation Day 6. The dosage per kg was based on the maternal GD 6 body weight. The dose selection was based on the results of a 3-month study at 0, 20, 50, or 100 mg

OPM/kg i.m. once/month. Based on the incidence and severity of the injection site reactions, 100 mg/kg was considered to have exceeded the MTD. The high dose in the present study was selected based on the maximum achievable concentration of the test article in suspension, the maximum tolerated intramuscular dose volume (0.15 ml/animal), and an approximate body weight of 275 g. The LD and MD were consistent with those used in the 2-year rodent study with OPM (Study R03703). The pamoic acid group was included to evaluate the potential effects of the formulating agent alone. The dose level of 93 mg pamoic acid/kg approximated the concentration of pamoic acid in the 75 mg OPM/kg group.

Species/strain: Rat, Crl: CD (SD) IGS BR (Sprague-Dawley)

Number/sex/group: 24-25

Route, formulation, volume, and infusion rate**:** i.m., suspension in vehicle (vehicle composition: 0.75% carboxymethylcellulose sodium, 5% mannitol, and 0.1% polysorbate 80 in water for injection, USP). Both dose volume and concentration was adjusted to achieve a dose ≤ 0.15 ml/animal.

Satellite groups used for toxicokinetics: Groups of mated females were administered the same doses with the same dosing regimen as described above.

Study design: Mated female Sprague-Dawley rats (24-25/group) were given single i.m. doses of OPM (0, 10, 25, or 75 mg/kg) or pamoic acid (the depot formulating agent) (93 mg/kg) on Gestation Day 6. The OPM dose levels were 0 (vehicle control), 10, 25, or 75 mg/kg; the pamoic acid dose was 93 mg/kg.The high OPM dose was selected based on the maximum tolerated intramuscular dose volume and the maximum achievable concentration of the test article in suspension. Administration of a single dose was intended to mimic the proposed clinical delivery of the sustained-release formulation.

The rats were euthanized on Gestation Day 20 for evaluation of maternal reproductive parameters and the fetuses were assessed for viability, weight, gender, and morphology. Toxicokinetic evaluations were conducted in satellite groups of mated females administered the same doses with the same dosing regimen as described above. Plasma concentrations of olanzapine and pamoic acid were determined over 14 days following test article administration, at the following time points: 1, 4, 8, 24, 120, 216, and 336 hours post-dose (corresponding to 0.04, 0.17, 0.33, 1, 5, 9, and 14 days post-dose). Exposure to both olanzapine and pamoic acid ($AUC_{0\text{-}t}$ and C_{max}) were assessed.

Parameters and endpoints evaluated:

Maternal: survival and clinical signs (daily), body weight and food consumption (on Gestation Days 4, 6, 10, 14, 18, and 20), reproductive parameters (number of corpora lutea, number of implantations, live and dead fetuses, and resorptions).

Fetal: viability, individual weight, gender, external, visceral and skeletal anomalies.

For maternal reproductive and fetal parameters, the litter was selected as the independent sampling unit.

Histopathology: Preserved tissue specimens (injection site) from control and compound-treated rats were stained with hematoxylin and eosin and examined by light microscopy

Results

Mortality (dams):

All treated female rats survived until study termination.

Clinical signs (dams): There were no clinical signs considered to be compound related. Incidental findings observed infrequently and independently of dose included alopecia, haircoat soiling, nose and eye discharge, and decreased feces.

Body weight (dams): There were no differences in gestational body weight or total corrected body weight gain between the OPM treated groups and control, as well as between the pamoic acid-treated group and control.

Food consumption (dams): Food consumption on Gestation Days 6 through 10 was increased by approximately 6% to 7% in all OPM- treated groups when compared to control. This effect was of no toxicological significance since it did not correlate with any body weight changes. There were no significant effects on food consumption in the pamoic acid treated group.

Toxicokinetics: Maternal plasma exposure parameters for olanzapine and pamoic acid (AUC_{0-t}, C_{max} and T_{max}) as determined over 14 days after administration of the single dose of test article are shown in the sponsor's table below. Following the administration of OPM, plasma concentrations of olanzapine and pamoic acid were quantifiable in the MD and HD groups through 336 hours postdose (GD 20) and in the LD group through 120 hours postdose (GD 11) and 336 hours postdose for olanzapine and pamoic acid, respectively.

	Treatment Group			
Parameter	10 mg/kg OLZ	25 mg/kg OLZ	75 mg/kg OLZ	93 mg/kg PA
Olanzapine				
C_{max} (ng/mL)	127	132	153	NA
AUC_{0-t} (ng•hr/mL)	3778	4980	8448	NA
T_{max} (hr)	8	1	4	NA
Pamoic acid				
C_{max} (ng/mL)	19,115	19,479	17,586	101,154
AUC_{0-t} (ng•hr/mL)	556,240	827,770	1,804,874	1,538,998
T_{max} (hr)	8	8	24	4

Abbreviations: OLZ = olanzapine, PA = pamoic acid, C_{max} = maximal plasma concentration, AUC_{0-t} = area under the plasma concentration-time curve from time zero to time t, t = last quantifiable time point, T_{max} = time to maximal concentration, NA = not applicable.

AUC_{0-t} values for both olanzapine and pamoic acid increased with increasing dose. C_{max} remained relatively constant independent of dose. In animals receiving pamoic acid alone, C_{max} values of pamoic acid were much higher compared to the high-dose OPM group; however, AUC_{0-t} values were similar.

Olanzapine AUC_{0-t} value achieved at the HD in the rat developmental toxicity study was 8448 ng•hr/mL; the corresponding C_{max} was 153ng/mL. The peak pamoic acid concentrations (C_{max}) were much higher in the pamoic acid group than those observed in the high-dose OPM group which received a similar amount of pamoic acid. The corresponding C_{max} values were 101, 154 ng/ml and 17, 586 ng/ml, respectively.

Terminal and necroscopic evaluations: C-section data (implantation sites, pre- and post-implantation loss, etc.): There were no compound-related effects on maternal reproductive endpoints in either the OPM- or pamoic acid- treated groups in comparison to control (see sponsor's table below).

Treatment Group:	Vehicle	93 mg/kg PA	10 mg/kg OLZ	25 mg/kg OLZ	75 mg/kg OLZ
Pregnant females at cesarean section	25	25	24	25	25
Corpora lutea/dam	14.0	14.4	14.6	14.1	13.6
Implantations/dam	12.9	13.1	13.7	13.2	13.0
Preimplantation loss/dam (%)	8.2	9.3	5.6	6.4	4.9
Total resorptions/litter (early and late)	0.5	0.7	0.6	0.6	0.7
Live fetuses/litter	12.4	12.4	13.1	12.6	12.3
Dead fetuses/litter	0.0	0.0	0.0	0.0	0.0
Postimplantation loss/litter (%)	4.3	5.6	4.3	5.4	5.5

Abbreviations: OLZ = olanzapine, PA = pamoic acid.

Offspring (malformations, variations, etc.): There were no compound-related effects on weight, morphology or gender ratio.

Treatment Group:	Vehicle	93 mg/kg PA	10 mg/kg OLZ	25 mg/kg OLZ	75 mg/kg OLZ
Fetal weight (gender combined; g)	3.9	3.9	3.9	4.0	3.9
Male fetuses/litter (%)	54.8	51.2	53.3	55.9	46.9
Fetuses with malformations/litter (%)	1.0	0.0	0.6	0.3	0.3
Fetuses with deviations/litter (%)	15.5	8.1	13.0	14.8	17.0
Fetuses with variations/litter (%)	3.0	2.4	2.7	1.4	1.0
Affected implants/litter (%)	5.3	5.6	4.6	5.7	5.8
Malformations - fetal (litter) incidences					
Innominate artery-absent	1(1)	0(0)	0(0)	0(0)	1(1)
Cardiovascular system-transposition	0(0)	0(0)	1(1)[a]	0(0)	0(0)
Brain-lateral ventricle-dilated	0(0)	0(0)	0(0)	1(1)	0(0)
Digestive system-transposition	0(0)	0(0)	1(1)[a]	0(0)	0(0)
Spleen-transposition	0(0)	0(0)	1(1)[a]	0(0)	0(0)
Cervical vertebra-arch-fused	0(0)	0(0)	1(1)	0(0)	0(0)

[a] Observations from Fetus 14 from Litter 3058.

Abbreviations: OLZ = olanzapine, PA = pamoic acid.

Gross and Histopathology

Treatment-related changes attributable to OPM and pamoic acid were observed at injection sites (granulomatous inflammation with a dose-related severity). Injection site reactions in pamoic acid-treated rats had a less severe inflammatory response, but more evidence of tissue damage.

The injection site reactions from OPM-treated rats were described as "granulomatous inflammation characterized by infiltrates of macrophages admixed with lymphocytes, plasma cells, and eosinophils, involving muscle and/or the perimysium and tissues between muscle bundles. Greenish-yellow crystals (foreign material) within the center of granulomas and within macrophages were frequently observed at the HD OPM and less often at MD and LD (see sponsor's table below). Although fibroplasia was evident, there was little or no significant atrophy, degeneration, or necrosis of myocytes in OPM-treated rats. Overall, these histologic lesions were consistent with a foreign body response."
Injections site reactions in pamoic acid-treated rats had a different microscopic appearance compared to OPM-treated rats. The inflammatory infiltrate of macrophages, lymphocytes, and multinucleated giant cells was "less intense; contained no foreign material; and was associated with degenerated, atrophic and regenerating muscle fibers, fibrosis, and in some rats, degeneration or necrosis of nerves and arteries". The associated changes were given a diagnosis of degenerative alteration. In contrast to OPM-treated rats, the perimysium and associated structures were infrequently affected.

Injection site histopathology findings in pregnant rats treated with OPM or pamoic acid

	Treatment Group				
Injection site reaction[a]	Vehicle	93 mg/kg PA	10 mg/kg OLZ	25 mg/kg OLZ	75 mg/kg OLZ
Number of female rats	25	25	25	25	25
Granulomatous inflammation					
Minimal	0	6	10	0	0
Slight	0	6	14	19	6
Moderate	0	1	0	6	18
Marked	0	0	0	0	1
Degenerative alteration					
Minimal	0	4	0	0	0
Slight	0	13	0	0	0

[a] Incidence.

Abbreviations: OLZ = olanzapine, PA = pamoic acid.

Conclusion:

Following a single intramuscular injection of the sustained-release injectable formulation of olanzapine pamoate monohydrate (OPM) at doses of 10, 25 and 75 mg/kg in the pregnant rat on Gestation Day 6, there were no compound-related signs of maternal toxicity or adverse effects on the growth, viability, and structural development of the embryo and fetus. Maternal effects were limited to local reactions at the intramuscular injection site consistent with the results of previous studies with OPM in the rat (Hoffman 2000, as cited by the sponsor). Microscopically, the principal change in OPM-treated groups was a granulomatous inflammation reaction consistent with a foreign body response with a dose-related severity and associated with mild or no irritation in surrounding tissues. The injection site reactions from the formulating agent (pamoic acid)-treated rats had less cellular infiltrate and less foreign material than those from OPM-treated rats although there was greater residual irritation of surrounding tissues. No effects on maternal reproductive or embryo-fetal developmental parameters were observed in any dose group. The NOAEL for both systemic maternal effects and developmental effects was 75 mg of OPM/kg, corresponding to olanzapine AUC_{0-t} value

of 8448 ng•hr/mL and C_{max} of 153 ng/mL. This dose represents the highest OPM dose that could be administered based on the maximum achievable concentration of the test article in suspension and the maximum tolerated intramuscular dose volume for the test animal used in this study.

Study title: An Embryo-Fetal Development and Companion Toxicokinetic Study in Female New Zealand White Rabbits Given a Single Intramuscular Injection of Olanzapine Pamoate Monohydrate

Key study findings: A single intramuscular injection of olanzapine pamoate monohydrate in pregnant New Zealand White rabbits (20/dose/group) on Gestation Day 7 at doses of 10, 25, or 75 mg/kg or pamoic acid (the formulating agent) alone at a dose of 100 mg/kg did not induce treatment-related adverse effects on the maternal reproductive or embryo/fetal developmental parameters. There were also no adverse treatment-related signs of systemic maternal toxicity (i.e., effects on survival, clinical signs, body weights, or food consumption) at any dose. Compound-related effects were limited to injection site observations mainly in the pamoic acid group.
Olanzapine and pamoic acid plasma exposure (AUC) assessed in satellite groups of pregnant females (4 animals/dose group) increased with increasing dose and was present throughout organogenesis in the 25- and 75-mg OPM/kg dose groups. The NOEL for systemic maternal and developmental toxicity was 75 mg/kg, corresponding to an olanzapine $AUC_{0\text{-}t}$ value of 9703 ng·hr/mL and olanzapine C_{max} of 51 ng/mL. This dose represents the highest dose that could be administered based on the maximum achievable concentration of the test article in suspension and the maximum tolerated intramuscular dose volume for the test animal used in this study.

Study no.: Study B00006 and Study B00007 (satellite TK study).
Volume # and page #: N.A.
Conducting laboratory and location:
Eli Lilly and Company
2001 West Main Street
Greenfield, IN 46140

Date of study initiation: Study B00006: 22 June 2004 through 12 August 2004
Study B00007: 24 June 2004 through 24 September 2004
GLP compliance: Yes
QA reports: yes
Drug, lot #, and % purity: Olanzapine pamoate monohydrate Lot CT23905, with an assigned potency of 210 mg olanzapine /vial, and Lot PPD-B0837-9, with an assigned potency of 60 mg olanzapine / vial. Pamoic acid Lot PPD-B0837-11, assigned potency of 265 mg pamoic acid/vial. (The assayed concentrations were from 100% to 110% of theoretical values for olanzapine and 99% of theoretical values for pamoic acid).
Vehicle (Lot CT24129) was composed of 0.75% carboxymethylcellulose sodium, 5% mannitol, and 0.1% polysorbate 80 in water for injection, USP.

Methods

Doses: 0, 10, 25, or 75 mg OPM/kg

Species/strain: Rabbit, New Zealand White

Number/sex/group: 20 (main study); 4 (satellite TK study)

Route, formulation, volume, and infusion rate: i.m.; suspension; dose volume: 0.5 ml/kg body weight in all groups. The dose administered was adjusted based on the animals Gestation Day 7 body weight.

Satellite groups used for toxicokinetics: yes, at the same OPM dose levels

Study design: Mated female New Zealand White rabbits (20/group) were given intramuscular injection of OPM at doses of 0, 10, 25, or 75 mg/kg, or pamoic acid alone (the depot formulating agent), at a dose of 100 mg/kg on Gestation Day 7. The high dose was selected based on the maximum tolerated intramuscular dose volume (0.5 ml/kg body weight) and the maximum achievable concentration of the test article in suspension. The rabbits were euthanized on Gestation Day 28 for evaluation of maternal reproductive parameters and the fetuses were assessed for viability, weight, gender, and morphology. Additional mated rabbits (4/ group) received by the same route 10, 25, or 75 mg OPM/kg or 100 mg pamoic acid/kg on Gestation Day 7 and were sampled for TK evaluation over a 21-day period. A control of the vehicle alone was provided for the main study (Study B00006).

Treatment groups:

Group	Dose of Olanzapine (mg/kg)	Dose of Olanzapine Pamoate Monohydrate (mg/kg)[a]	Dose of Pamoic Acid (mg/kg)
01	0 (Study B00006 only)[b]	0 (Study B00006 only)[b]	0 (Study B00006 only)[b]
02	0	0	100
03	10	23	0
04	25	57.5	0
05	75	172.6	0

[a] Based on 43.5% olanzapine per mole of OPM.

[b] Vehicle.

TK: Blood samples were collected for the measurement of plasma olanzapine and pamoic acid concentrations from 4 animals/OPM treatment group/time point at 1, 4, 8, 24, 120, 240, 360, and 504 hours postdose (time points correspond to 0.04, 0.2, 0.3, 1, 5, 10, 15, and 21 days post dose). In the pamoic acid group, blood samples were collected for the measurement of plasma pamoic acid concentration from 4 animals/time point at 1, 4, 8, and 24 hours postdose. Note: Only 2 animals/time point were available in the pamoic acid group for TK beyond the 1st day evaluation since 2 rabbits in the pamoic acid group died after their 24-hour sample collection.

Parameters and endpoints evaluated:

- Maternal survival and clinical signs (examined daily for survival and beginning on Gestation Day 4 for general physical condition and clinical signs); body weight (on GD 4, 7, 10, 14, 17, 20, 24, and 28) and food consumption (daily from GD 4); gross examinations at necropsy on GD28. No tissues were collected.
- Maternal reproductive parameters (numbers of corpora lutea, implantations, live and dead fetuses, and resorptions). Conceptuses were categorized as live fetuses, dead fetuses, late resorptions, early resorptions, or implantation scars.

- Fetal parameters: Viability, weight (individual) and external, visceral, and skeletal anomalies (late resorptions were examined for external anomalies whenever possible). Fetuses were categorized as normal, variant, deviant, or malformed based on the most severe anomaly present.

Results

Mortality (dams): No mortality occurred in the OPM-treated groups; in the pamoic acid group, 1 animal was euthanized on GD21 due to anorexia and cachexia (during Gestation Days 15 through 21, food consumption ranged from 0 to 10 g/day and the body weight was decreased by approximately 580 g). This was an isolated event and not likely to be compound-related. In the satellite TK study, 2 rabbits from the pamoic acid group died approximately 5 minutes after the 24-hour blood collection from the jugular vein, due to trauma in the cervical region secondary to the venipuncture, as confirmed by gross internal examination.

Clinical signs (dams): Compound-related clinical signs involved a limited number of injection site observations mainly in the pamoic acid group (2/20 animals); in the OPM-treated groups there was only one 1 case of injection site reaction at the LD (see sponsor's table below).

Treatment Group:	Vehicle	100 mg/kg PA	10 mg/kg OLZ	25 mg/kg OLZ	75 mg/kg OLZ
Deaths (Study B00006)	0	1	0	0	0
Deaths (Study B00007)	0	2	0	0	0
Clinical signs[a]					
Scab	0(0)	1(4)	1(12)	0(0)	0(0)
Ulceration	0(0)	2(25)	0(0)	0(0)	0(0)
Skin red	1(6)	0(0)	0(0)	0(0)	1(1)
Injection site red	0(0)	1(4)	0(0)	0(0)	0(0)
Injection site scab	0(0)	1(3)	0(0)	0(0)	0(0)
Injection site swollen	0(0)	1(5)	0(0)	0(0)	0(0)

Abbreviations: OLZ = olanzapine, PA = pamoic acid.

[a] Rabbits affected (total occurrences).

Body weight (dams): No statistically significant effects on maternal body weight were observed at the LD and MD of the OPM groups. At the HD, the mean maternal body weight was increased approximately 5% vs. control, statistically significant on both gestation days 17 and 20 (see sponsor's table on the next page). In the pamoic acid group, there was a decrease in body weights vs. control (approximately 3%, statistically significant). We agree with the sponsor's opinion that these minimal statistically significant effects on body weights are not toxicologically important or adverse.

Treatment Group:	Vehicle	100 mg/kg PA	10 mg/kg OLZ	25 mg/kg OLZ	75 mg/kg OLZ
Body weight (g)					
Gestation Day 17	3527	3468	3544	3648	3686*
Gestation Day 20	3571	3507	3588	3674	3735*

*p<.05.

Food consumption (dams): Maternal food consumption was increased in OPM MD and HD groups during the intervals of GD 7 through 17 (17% to 38%) and GD7 through 20

(10% to 38%), respectively. In the LD OPM group, food consumption values were increased by 39%, relative to controls on GD 7 through 10 and decreased by 17% on GD 14 through 17. These differences in relative food consumption were not likely to be toxicologically important or adverse. There were no effects on food consumption in the pamoic acid group (see sponsor's table below).

Treatment Group:	Vehicle	100 mg/kg PA	10 mg/kg OLZ	25 mg/kg OLZ	75 mg/k OLZ
Relative food consumption (g/day/1000 g)					
Gestation Days 7-10	52	50	71*	71*	71*
Gestation Days 10-14	50	48	55	62*	61*
Gestation Days 14-17	49	45	41*	58*	61*
Gestation Days 17-20	57	50	50	55	62*

Abbreviations: OLZ = olanzapine, PA = pamoic acid.
*p<.05.

Toxicokinetics: Following the administration of OPM, plasma concentrations of olanzapine and pamoic acid were quantifiable through Gestation Day 28, the last day of the study, in the 25- and 75-mg/kg dose groups. In the 10-mg/kg dose group, plasma concentrations of olanzapine and pamoic acid were quantifiable through GD 17 and 28, respectively. AUC_{0-t} values for both olanzapine and pamoic acid increased with increasing dose. C_{max} values were similar independent of dose. In animals receiving pamoic acid alone, C_{max} values of pamoic acid were much higher compared to the high-dose OPM group; however, AUC_{0-t} values were similar. TK data are presented in the sponsor's table below.

Treatment Group:	100 mg/kg PA	10 mg/kg OLZ	25 mg/kg OLZ	75 mg/kg OLZ
Olanzapine				
C_{max} (ng/mL)	NA	40	36	51
$AUC_{0-t\ hr}$ (ng•hr/mL)	NA	2437	4804	9703
T_{max} (hr)	NA	4	10	5
Pamoic acid				
C_{max} (ng/mL)	12437	891	1025	1643
$AUC_{0-t\ hr}$ (ng•hr/mL)	555101	52015	152451	438491
T_{max} (hr)	5	4	4	249

Abbreviations: OLZ = olanzapine, PA = pamoic acid, C_{max} = maximum plasma concentration, AUC_{0-t} = area under the plasma concentration-time curve from time zero to time t, t = last quantifiable time point, T_{max} = time to maximal concentration, NA = not applicable.

Terminal and necroscopic evaluations:
Maternal necropsy findings: On gross internal examination, treatment-related findings were limited to effects due to trauma in the cervical region secondary to the venipuncture (extensive hematoma in the cervical region) registered in 2 animals from the pamoic acid group of the satellite TK study. There were no other treatment-related observations. Histopathology examination was not performed in this study.
C-section data (implantation sites, pre- and post-implantation loss, etc.):

There were no compound-related effects on maternal reproductive endpoints (number of corpora lutea, implantations, pre- and post-implantation loss, number of fetuses/litter) in either the OPM- or pamoic acid-treated groups vs. control group (see sponsor's table below).

Treatment Group:	Vehicle	100 mg/kg PA	10 mg/kg OLZ	25 mg/kg OLZ	75 mg/kg OLZ
Pregnant females at cesarean section	18	19	19	18	19
Corpora lutea/dam	9.8	10.2	10.2	9.5	9.9
Implantations/dam	9.4	9.4	9.7	9.1	9.8
Preimplantation loss/dam (%)	5.2	6.8	4.4	6.6	1.0
Total resorptions/litter (early and late)	0.7	0.1	0.6	0.3	0.3
Live fetuses/litter	8.7	9.3	9.1	8.6	9.5
Dead fetuses/litter	0.1	0.0	0.0	0.1	0.0
Postimplantation loss/litter (%)	7.9	1.2	5.9	7.0	3.3

Abbreviations: OLZ = olanzapine, PA = pamoic acid.

Offspring (malformations, variations, etc.):
There were no compound-related effects on fetal growth and development as assessed by fetal weight and morphology (see sponsor's table below).

Treatment Group:	Vehicle	100 mg/kg PA	10 mg/kg OLZ	25 mg/kg OLZ	75 mg/kg OLZ
Fetal weight (gender combined; g)	34.9	35.0	35.6	36.8	35.7
Male fetuses/litter (%)	48.9	54.6	48.6	51.7	54.1
Fetuses with malformations/litter (%)	2.6	1.4	2.2	1.4	1.6
Fetuses with deviations/litter (%)	33.5	25.5	35.3	23.5	31.6
Fetuses with variations/litter (%)	38.5	52.5	43.1	40.9	39.7
Affected implants/litter (%)	9.8	2.6	7.9	8.3	4.9

Abbreviations: OLZ = olanzapine, PA = pamoic acid.

Conclusion: A single intramuscular injection of olanzapine pamoate monohydrate at doses of 10, 25 and 75 mg/kg gestation day 7 in the rabbit resulted in measurable olanzapine plasma concentrations in the MD and HD groups through gestation day 28, the termination of the study.
Maternal effects were limited to local injection site reactions observed mainly in the group treated with the formulating agent (pamoic acid) alone. No developmental effects were observed in any dose group. Based on these findings, the NOEL for maternal and developmental toxicity was 75 mg/kg, corresponding to an olanzapine $AUC_{0\text{-}t}$ value of 9703 ng·hr/mL and olanzapine C_{max} of 51 ng/ml. This dose represents the highest dose that could be administered based on the maximum achievable concentration of the test article in suspension and the maximum tolerated intramuscular dose volume for the test animal used in this study.

Prenatal and postnatal development

Study title: A Prenatal and Postnatal Development Study, Including Maternal Function of Olanzapine Pamoate Monohydrate Administered by Intramuscular Injection to Female CD Rats

Key study findings: Administration of OPM at i.m. doses of 10, 25 and 75 mg/kg, or pamoic acid at an i.m. dose of 93 mg/kg on gestation days 6, 16 and post-partum day 4 resulted in a slight increase in gestation length (+0.4 days) at HD, but did not cause maternal toxicity. The mean number of pups born, live litter size, live born index (%), sex ratio per litter at birth and postnatal survival from birth to PND 4 (culling) and PND 21 (weaning) were unaffected by the maternal OPM administration at all dose levels.
Mean F_1 progeny body weights in the HD group were significantly increased by up to 12.9% and 12.5%, for males and females, respectively, during the pre-culling period (PND 1-4) and by up to 8.5% and 9.8%, respectively, during the post-culling period (PND 7-21). Mean body weights were also increased at the MD and HD OPM males during most of the maturation phase (up to 6.9% and 9.0%, respectively). The mean body weights exceeded the mean values in the historical control data. The effects on pre- and post-weaning body weights were considered to be a result of the slightly increased gestation length observed in the HD OPM group. The mean age of attainment of sexual maturation in F1 male offspring was slightly, but significantly, decreased in the HD OPM group that was attributed to the increased group mean body weights noted throughout the postnatal period. Because this change was within historical control values and associated with increased growth of the pups, it was not likely to be adverse.
Behavioral changes were noted at the HD of 75 mg/kg: delayed negative geotaxis performance in F_1 males and females, decreased motor activity of F_1 females at the age of weaning, lack of habituation to startle response in the F_1 males as well as decreased learning capacity demonstrated by a statistically significant trend to prolongation of the escape time and increased (n.s.) number of errors during memory trials in a water maze. F_1 reproductive capacity (as assessed by fertility and gestation indices, sperm analysis, F_2 live litter size, live born index (%), sex ratio per litter at birth and postnatal survival from birth to PND 1) was unaffected in either OPM or pamoic acid groups.
Conclusion: The NOAEL for maternal systemic and reproductive toxicity of olanzapine pamoate monohydrate (OPM) when administered to pregnant rats via intramuscular injection on GD 6, GD 16 and PN day 4 is 75 mg/kg based on the lack of adverse drug-related findings in the F_0 females. The NOAEL for F_1 developmental toxicity is 25 mg/kg, based on the findings of drug-related neurobehavioral developmental effects at 75 mg/kg

Study no.: WIL-353058
Volume #, and page #: N.A.
Conducting laboratory and location: (b) (4) (b) (4)

Date of study initiation: July 28, 2004
GLP compliance: Yes
QA reports: yes
Drug, lot #, and % purity: olanzapine pamoate monohydrate; pamoic acid
Lot numbers and potency:
CT23905 (210 mg olanzapine/vial)
PPD-B0837-9 (60 mg olanzapine/vial)
PPD-B0837-11 (265 mg pamoic acid/vial)

Vehicle: 0.75% carboxymethylcellulose sodium, 5% mannitol, 0.1% polysorbate 80 in water for injection, USP; Lot CT 509162

Methods

Doses: OPM by intramuscular injection at doses of 10, 25 and 75 mg/kg, pamoic acid at an i.m. dose of 93 mg/kg body weight, or vehicle control (0.75% carboxymethylcellulose sodium, 5% mannitol, 0.1% polysorbate 80 in water for injection), each administered on gestation day (GD) 6, GD 16 and Lactation Day (LD) 4. This timing of administration was chosen because the test article depot formulation "was shown in previous studies to yield a sustained exposure for at least 2 weeks".

Species/strain: Rat, Crl:CD®(SD)

Number/sex/group: $F_{0\ dams}$: 25/group; $F_{1\ generation}$: one pup/sex/litter

Route, formulation, volume, and infusion rate: Intramuscular injection, Suspension, Dose volume: 0.11 to 0.20 ml/animal, Frequency of administration: Once daily on GD 6, GD 16 and lactation day (LD) 4.

Satellite groups used for toxicokinetics: None

Study design and methods: Virgin female rats (12 weeks old) and untreated resident male rats (19 weeks old) of the same strain and source were cohabited 1:1 in a cage for mating. After confirmation of mating (by an intravaginal copulatory plug or by the presence of sperm in a vaginal lavage), the female was placed in an individual maternity cage and the day was designated as GD 0. The bred females were assigned to groups using computer program that randomized the animals based on stratification of the GD 0 body weights. The control and test article were administered to the F_0 maternal animals by i.m. injection on GD 6, GD 16, and postnatal day 4 using 23-gauge sterile needles. The F_0 maternal animals were assigned to study groups as follows:

Group Number	Test Article	Dosage Level (mg/kg)	Dosage Concentration (mg/mL)	Number of Animals
1	Vehicle Control	0	0	25
2	Pamoic Acid	93	200	25
3	OPM	10	20	25
4	OPM	25	50	25
5	OPM	75	100	25

The progeny F_1 and F_2 were not directly administered the test article during the study; the F_1 pups were potentially exposed to the test article *in utero* and during lactation, through maternal milk.

All F_0 females were allowed to deliver naturally and rear their pups to weaning (PND 21). During the gestation and lactation periods and the period of expected parturition the F_0 female rats were observed twice daily for mortality, clinical signs and for initiation and completion of parturition. On the day of parturition (PND 0), pups were sexed and examined for gross malformations, and the numbers of stillborn and live pups were recorded. Individual gestation length was calculated using the date delivery started.

All surviving F_0 females with viable pups on postnatal day 21, females with total litter loss and that did not deliver (up to post-mating day 25) were euthanized. A gross necropsy was performed for each of these females. For females that delivered, the numbers of former implantation sites were recorded. For females that failed to deliver, pregnancy status was determined, and if evidence of macroscopic implantations were present, the number of implantation sites and corpora lutea were recorded. Tissues were preserved in 10% neutral-buffered formalin for possible future histopathologic examination only as deemed necessary by the gross findings. Uteri with no macroscopic evidence of implantation were opened and subsequently placed in a 10% ammonium sulfide solution for detection of early implantation loss.

F1 Litters were examined daily for survival and pups were individually weighed on PN days 1, 4, 7, 10, 14, 17, and 21. Standardization of litter size to 8 pups per litter (4/ sex where possible) was performed on PN day 4 by a random selection.

Litter parameters were defined as follows:

Mean Live Litter Size	=	Total No. of Viable Pups on PND 0 / No. of Litters with Viable Pups PND 0	
Live Born Index	=	Σ [Viable Pups Per Litter on PND 0/No. of Pups Born Per Litter] / No. of Litters Per Group	x 100
Postnatal Survival Between Birth and PND 4 (Pre-Selection) (% Per Litter)	=	Σ (Viable Pups Per Litter on PND 4/No. of Pups Born Per Litter) / No. of Litters Per Group	x 100
Postnatal Survival for All Other Intervals (% Per Litter)	=	Σ [Viable Pups Per Litter at End of Interval N/Viable Pups Per Litter on PND 4 (Post-Selection)] / No. of Litters Per Group	x 100
Where N	=	PND 4 (Post-Selection)-7, 4 (Post-Selection)-14 or 4 (Post-Selection)-21	

Prior to weaning (PN Day 21), one male and one female pup per litter were randomly selected for evaluation of attainment of developmental landmarks, evaluation of sensory function and behavioral testing. All of the selected F1 pups were allowed to attain sexual maturity. The remaining F_1 pups were euthanized and necropsied on PND 21. Tissues were saved in 10% neutral-buffered formalin for possible histopathologic examination; however, histopathological evaluation was not conducted on any of these tissues.

Indicators of physical and functional development of the progeny were evaluated as follows. Growth (body weight) was registered individually on PND 1, 4, 7, 10, 14, 17, and 21. Negative geotaxis assessment was performed on all pups/litters beginning on PND 6. In addition, one pup/sex/litter was selected for functional observational battery testing on PND 11, 21 and 60, locomotor activity assessment on PND 21 and 61, acoustic startle response on PND 20 and 60 and learning and memory assessment beginning on PND 62. Attainment of landmarks of sexual maturity (vaginal opening and balano-preputial separation) was evaluated in selected pups (1/sex/litter). The F_1 pups selected for sexual developmental landmarks assessment were assigned to an F_1 maturational

phase, including reproductive functional assessment. Upon reaching sexual maturity, F_1 animals (one/sex/litter) were mated within each group, avoiding sibling pairings. All F_1 females were allowed to deliver and were housed with their offspring until PN Day 1, when the F_2 pups were euthanized. The F_1 females were necropsied on post-partum day 14; F_1 males were necropsied following the last female necropsy. Sperm assessment was conducted for selected F_1 males. The brain and reproductive organs were weighed from all F_1 parental animals at the scheduled termination. Histological examination of reproductive tissues was conducted for F_1 parental animals in the vehicle control, pamoic acid control and HD OPM group. In the LD and MD OPM groups, histopathology was performed on the uterus, vagina and ovaries.

Parameters and endpoints evaluated: F_0 maternal animals: general toxicity (weight, food consumption, clinical signs) as well as pregnancy maintenance, parturition and lactation; F_1 generation: growth, viability and development, including reproductive and behavioral performance (as described above).

Results

F_0 in-life: There were no maternal (F_0) OPM- or pamoic acid-related deaths. All females survived to the scheduled necropsies. No OPM- or pamoic acid-related clinical findings were noted at the daily examinations at any dose level. During gestation, in all OPM-treated groups, decreased (44%-56%) mean maternal body weight gains per day were noted during GD 6-9, and increased (8%-26%) mean body weight gains per day were noted during GD 16-18 (see sponsor's table below). These body weight changes coincided with dose administration on GD 6 and 16, but had no corresponding effects on mean body weights during gestation; therefore, the effects were considered OPM-related, but not adverse.

Maternal (F_0) body weight gain during gestation (g/day)

Dose (mg/kg):	0[a]	93[b]	10	25	75
Body weight gain (g/day)					
GD 6-9	3.2	2.9	1.8*	1.7*	1.4*
GD 16-18	13.7	15.0	15.8*	17.3*	14.8*

[a] Vehicle.
[b] Pamoic Acid.
*p<0.05.

During lactation, a significant overall decrease (-2.9%) in mean maternal body weight was noted at the HD OPM group compared to the vehicle control group. However, the difference was slight, and there were no corresponding effects on mean body weight gains per day or mean total body weight change (LD 1-21). There were no pamoic acid-related effects on mean body weight gains per day, mean total body weight gain or on mean body weights during either gestation or lactation.

There was an overall trend of increased food consumption in all OPM-treated groups vs. control during GD 6-9, 16-18 and 18-20 (see sponsor's table below). The increases were OPM-related, but were not adverse. There were no effects on body weight parameters or food consumption in the OPM-treated groups during lactation or in the pamoic acid group during gestation and lactation.

The fertility indices in the vehicle control, 10, 25, and 75 mg/kg OPM groups, and pamoic acid group were 92.0%, 96.0%, 88.0%, 92.0% and 100%; none of the differences from the vehicle control group were significant.

Gestation length was slightly increased (mean +0.4 days) for F_0 dams in the HD group (22 days vs. 21.6 days in vehicle control). The change was statistically significant but was within the conducting laboratory historical control data range. The mean gestation length in the pamoic acid group (21.6 days) was equal to the vehicle control group value. The slight increase in gestation length was not considered adverse because there were no untoward effects on reproductive performance and parturition.

PRE/POSTNATAL DEV STUDY IN RATS: F0 SUMMARY OF FOOD CONSUMPTION DURING GESTATION [G/KG/DAY]

Group	Statistic	Days 0-6	Days 6-9	Days 9-12	Days 12-16	Days 16-18	Days 18-20	Overall
1	Mean	73.9	70.7	73.5	69.8	69.2	61.0	69.7
	SD	5.33	5.70	5.94	5.96	5.65	8.01	NA
	N	23	23	23	23	23	23	23
	LSM	73.9	70.7	73.5	69.8	69.2	61.0	69.7
	LSM s.e.	1.21	1.21	1.21	1.21	1.21	1.21	0.83
3	Mean	75.7	78.1	77.4	71.3	76.9	63.4	73.8
	SD	7.32	4.79	5.61	3.71	5.75	6.21	NA
	N	23	23	23	22	23	23	23
	LSM	75.7	78.1	77.4	71.4	76.9	63.4	73.8
	LSM s.e.	1.21	1.21	1.21	1.23	1.21	1.21	0.83
	Bonferroni p-value#	NT	NT	NT	NT	NT	NT	NT
	Trend p-value#	NT	<.001*	NT	NT	<.001*	0.150	<.001*
4	Mean	75.2	78.2	74.0	69.5	77.2	65.6	73.3
	SD	6.24	6.86	5.63	5.70	6.56	5.63	NA
	N	22	22	22	22	22	22	22
	LSM	75.2	78.2	74.0	69.5	77.2	65.6	73.3
	LSM s.e.	1.24	1.24	1.24	1.24	1.24	1.24	0.85
	Bonferroni p-value#	NT	NT	NT	NT	NT	NT	NT
	Trend p-value#	NT	<.001*	NT	NT	<.001*	0.007*	0.003*
5	Mean	72.8	76.3	77.8	71.4	78.7	66.8	74.0
	SD	4.52	5.20	6.15	3.71	5.50	6.18	NA
	N	23	23	23	23	23	23	23
	LSM	72.8	76.3	77.8	71.4	78.7	66.8	74.0
	LSM s.e.	1.21	1.21	1.21	1.21	1.21	1.21	0.83
	Bonferroni p-value#	NT	NT	NT	NT	NT	NT	NT
	Trend p-value#	0.483	0.002*	0.079	0.581	<.001*	<.001*	0.001*
ALL	Trt F-test p-value++							<.001*
INTN	Trt*Time p-value++							<.001*
	LinTrt*Time p-value++							<.001*
	LinTrt*LinTime p-value#							<.001*
	LinTrt*QuadTime p-value#							0.691

: Level of significance tested = .05.
++ : Level of significance tested = .01.
ǂ : Sequential trend test nonmonotonic.
NA : Not available.
NT : Not tested.
* : Statistically significant.

F_0 necropsy: No OPM- or pamoic acid-related macroscopic findings or effects on organ weights, and no important microscopic findings were observed in the F_0 parental females at the scheduled necropsies (PN day 21). No OPM- or pamoic acid-related effects were observed on the number of former implantation sites and the implantation loss.

F_1 physical development: The mean number of pups born, live litter size, live born index (%), pre-culling live litter size, percentage of males per litter at birth and postnatal survival from birth to PND 4 (pre-selection) and PND 4 (post-selection) to 7, PND 4 to 14, and PND 4 to 21 were unaffected by the F_0 maternal OPM administration at all dose levels. Differences from the vehicle control group were slight, not significant and did not occur in a dose-related manner.

No OPM- or pamoic acid-related effects were observed on F_1 pup survival, general physical condition or the functional observational battery assessments.

Survival: The number of pups (litters with pups) that were found dead or euthanized *in extremis* was 10(6), 17(8), 6(3), 7(5), and 14(9) in the control, pamoic acid and 10, 25, and 75 mg/kg OPM groups, respectively. An F_1 male in the LD OPM group, as well as one F_1 male and one F_1 female in the pamoic acid control group were found dead during the week following weaning (PN Day 24-26). There was no evidence that these deaths were related to the respective compounds.

Body weights: Mean F_1 male and female pup body weights in the HD group were significantly increased by up to 12.9% and 12.5%, respectively, during the pre-culling period (PND 1-4) and by up to 8.5% and 9.8%, respectively, during the post-culling period (PND 7-21). The mean body weights also exceeded the mean values in the historical control data. Mean body weight gains in the HD OPM group pups were similar to the vehicle control group during the pre- and post-culling periods.

Mean F1 offspring body weights (g)

Dose (mg/kg):	0[a]		93[b]		10		25		75	
Gender:	M	F	M	F	M	F	M	F	M	F
Mean Body Weight (g)										
PND 1	6.7	6.2	6.8	6.4	7.1	6.7	6.9	6.5	7.4*	6.9*
PND 4	9.3	8.8	9.1	8.6	9.7	9.2	9.7	9.2	10.5*	9.9*
Overall pre-culling	8.0	7.5	7.9	7.5	8.4	8.0	8.3	7.9	8.9*	8.4*
PND 7	15.0	14.5	14.2	13.2	14.5	14.2	15.3	14.6	16.1	15.5
PND 10	21.7	20.9	20.3	18.8	20.8	20.3	21.9	21.1	23.3	22.5
PND 14	31.1	29.9	29.7	28.0	30.1	29.3	31.5	30.5	33.6*	32.6*
PND 17	38.5	36.9	37.1	35.0	36.7	35.6	38.1	36.7	40.8*	39.5*
PND 21	50.3	48.1	48.0	45.1	47.9	46.4	50.1	48.0	54.6*	52.8*
Overall post-culling	31.3	30.0	29.9	28.0	30.0	29.2	31.4	30.2	33.7*	32.6*

[a] Vehicle.
[b] Pamoic Acid.
*p≤0.05.

OPM-related effects on F_1 body weights were also noted prior to behavioral testing and during the maturation phase. Mean body weights were increased in the males at 25 and 75 mg/kg OPM during most of the maturation phase (up to 6.9% and 9.0%, respectively), including during the period of behavioral testing. Mean body weights in the 75 mg/kg OPM group females were also increased, but only prior to behavioral testing on PND 20 and 21 and on PND 28, 35 and 42 during the maturation phase. The effects on pre- and post-weaning body weights were considered to be a result of the slightly increased gestation length observed in the 75 mg/kg OPM group. The body weight increases were therefore considered to be test article-related but not adverse due to the lack of effects on related parameters.

No test article-related macroscopic findings were noted for F_1 pups. No drug-related microscopic findings were observed in the F_1 parental males and females at the scheduled necropsies.

F_1 landmarks of sexual maturation:

Males: Compared to the vehicle control group, the mean age of attainment of balanopreputial separation was slightly, but significantly, decreased in the HD OPM group. The earlier acquisition of balanopreputial separation noted in the 75 mg/kg group F_1 males (see sponsor's table below) were attributed to the increased group mean body

weights noted previously throughout the postnatal period. Because the change in mean age at balanopreputial separation was slight, associated with increased growth of the pups, and within historical control values (41.6 to 49.0 days of age), it was not considered adverse. There were no effects of F_0 maternal pamoic acid administration on the mean age of acquisition.

Sexual maturation landmarks: Attainment of Balanopreputial separation in males

Dose (mg/kg):	0[a]	93[b]	10	25	75
Mean Day of Acquisition (PND)	46.1	46.0	46.2	44.9	44.8*
Body Weight at Day of Acquisition	237.9	239.1	240.2	240.0	243.7

[a] Vehicle.
[b] Pamoic Acid.
*p<0.05

Females: The mean ages of attainment of vaginal patency in the 10, 25 and 75 mg/kg OPM groups, as well as pamoic acid control were not significantly different from the vehicle control group. The mean body weight at the age of attainment of vaginal patency in the 75 mg/kg group was significantly higher than the value in the vehicle control group, but was within the historical control data range.

Sexual maturation landmarks: Attainment of vaginal patency in females

Dose (mg/kg):	0[a]	93[b]	10	25	75
Mean Day of Acquisition (PND)	31.6	32.3	32.0	31.5	31.5
Body Weight at Day of Acquisition	102.4	103.7	104.4	103.5	109.6*

[a] Vehicle.
[b] Pamoic Acid.
*p≤0.05.

F_1 Functional Observational Battery - PND 11-21 and PND 60: There were no compound-related effects in the FOB assessment (i.e., forelimb and hindlimb grip strength, muscle tone, gait, mobility, stereotypic behavior, clinical signs) on PND 11, 21, and 60.

F_1 behavioral evaluation: A delayed negative geotaxis performance in the F_1 males and females, a lack of habituation to startle response in the F_1 males and reduced performance on memory trials in the Biel Maze in the F_1 males were found at the HD of 75 mg/kg OPM.

- Negative geotaxis: A significant overall decrease in the percentage of pups exhibiting successful negative geotaxis during PN days 6-8 was found at 75 mg/kg OPM (see sponsor's table on the next page). This effect was compensated later in development, as all F_1 pups successfully completed the test on PN day 11.

F1 Negative geotaxis

Dose (mg/kg):	0[a]		93[b]		10		25		75[c]	
Gender	M	F	M	F	M	F	M	F	M	F
Mean % Per Litter Expressing a Positive Response										
PND 6	26.9	24.6	15.7	15.0	17.8	19.3	23.9	17.0	9.6	7.6
PND 7	57.6	51.1	56.0	43.3	53.3	47.2	59.1	51.1	50.9	34.4
PND 8	91.3	80.4	80.0	72.0	85.1	72.8	80.7	75.0	77.4	64.9
PND 9	93	94	94	95	98	90	94	91	95	91
PND 10	98	98	100	99	100	98	100	99	99	100
PND 11	99	99		100		100		100	100	
PND 12	99	100								
PND 13	100									

[a] Vehicle.
[b] Pamoic Acid.
[c] A significant overall trend was noted for both sexes ($p<0.05$)
Note: Values subjected to statistical analysis (PND 6-8) are presented to one decimal as shown on table 26. Values not analyzed statistically (PND 9-13) are presented as whole numbers as shown on table 102.

- F1 Acoustic Startle Response

The acoustic startle response habituation was evaluated longitudinally in selected pups on PND 20 and again as offspring approached sexual maturity (PND 60).

A significant increase in maximum startle response (VMAX) was noted in HDM on PND 60 compared to the vehicle control group (see sponsor's table below). These males failed to habituate over the 50-block test session. In HDF, there were increases in VMAX and VAVE on PND 20, but by PND 60, HD females displayed a normal pattern of peak startle response and there were no effects on the pattern of the habituation response over the entire 50-block test session.

There were no effects on startle responding in either males or females at LD and MD, or in pamoic acid control group.

F1 Acoustic Startle Response (PN Days 20 and 60)

Dose (mg/kg):	0[a]		93[b]		10		25		75	
Gender:	M	F	M	F	M	F	M	F	M	F
PND 20 V_{MAX}										
Trials 1-10	163.7	165.2	170.5	147.3	151.1	155.6	154.6	196.6	197.4	211.5*
PND 20 V_{AVE}										
Trials 1-10	30.5	30.6	31.9	27.5	29.9	29.8	29.8	36.6	38.8	39.0*
PND 60 V_{MAX}										
Trials 1-10	139.8	108.3	117.1	103.6	136.4	70.2	108.6	67.6	171.1	110.3
Trials 11-20	95.2	70.9	71.6	69.9	90.0	50.9	77.1	48.2	99.3	70.0
Trials 21-30	86.7	74.3	79.4	67.4	85.0	52.1	78.4	38.8	109.8	56.5
Trials 31-40	59.2	81.4	76.8	58.9	75.7	47.6	69.9	36.4	122.2*	63.8
Trials 41-50	66.2	65.8	88.9	53.4	72.3	36.9	53.5	40.7	131.2*	70.9

[a] Vehicle.
[b] Pamoic Acid.
*$p<0.05$.

VMAX - maximum startle response (millivolts)
VAVE – average startle response (millivolts)

- F1 Locomotor activity

Locomotor activity patterns (total activity as well as ambulatory activity counts) in F1 generation were evaluated on PND 21 and 61 (as offspring approached sexual maturity).

The only significant difference from the vehicle control group was an overall trend of decreased total and ambulatory counts in the HD OPM group females on PND 21 (see sponsor's table below). However, there were no effects in these females on PND 61.

F1 Locomotor activity (PN Day 21)

Dose (mg/kg):	0[a]		93[b]		10		25		75	
Gender:	M	F	M	F	M	F	M	F	M	F
PND 21 Ambulatory Counts										
Overall	113.4	127.6	116.8	109.8	132.8	116.1	105.7	111.6	120.2	98.4*
PND 21 Total Counts										
Overall	327.2	376.6	340.8	320.6	375.1	344.3	329.2	329.4	370.1	304.5*

[a] Vehicle.
[b] Pamoic Acid.
*p<0.05.

- F1 Learning and memory (Biel Maze Swimming Trials)

Latencies to locate a submerged platform during the learning and memory trials (PN Day 61) were significantly altered by OPM treatment. There was an overall trend towards an increased time to escape for the HD OPM group males during the memory Path A trials (see sponsor's table below). A transient trend to increased time to escape with a corresponding increase in the mean number of errors was noted for the HD females for the learning Path A trials (Trials 1-4), but the opposite effects in these same females was noted in the subsequent, learning Path B trials (Trials 5-10), i.e., an overall trend of decreased time to escape with a corresponding decrease in the mean number of errors vs. control. For this reason, the effects in the females were not considered to be biologically relevant or related to the prenatal exposure to OPM.

F1 Learning and memory (Biel Maze Swimming Trials)

Dose (mg/kg):	0[a]		93[b]		10		25		75	
Gender:	M	F	M	F	M	F	M	F	M	F
Escape Time/Learning/Path A (Trials 1-4)										
Overall (seconds)	46.5	42.2	45.2	42.5	48.6	42.8	50.9	44.4	53.9	53.2*
Errors/Learning/Path A (Trials 1-4)										
Overall	11.2	9.5	10.9	9.8	11.8	10.9	12.1	10.7	12.9	13.3*
Escape Time/Memory/Path A (Trials 11 and 12)										
Overall (seconds)	60.4	51.4	59.4	61.1	42.6	54.0	59.3	57.8	80.9*	48.3
Errors/Memory/Path A (Trials 11 and 12)										
Overall	15.5	11.7	14.9	15.6	10.3	13.4	15.9	14.5	19.2	11.8
Escape Time/Learning Path B (Trials 5-10)										
Overall	68.4	65.5	66.6	56.1	72.1	60.9	63.0	57.9	62.9	48.8*
Errors/Learning Path B (Trials 5-10)										
Overall	14.2	13.8	13.6	12.4	15.0	13.8	13.6	12.0	12.7	10.7*

[a] Vehicle.
[b] Pamoic Acid.
*p<0.05.

- F1 Reproductive Performance

No effects were noted on F1 reproductive performance in either OPM or pamoic acid groups. The mean number of normal estrous cycles and length of estrous cycles were similar to the vehicle control group values. Female mating indices were 100% all groups, and female fertility indices were 100%, 100%, 100%, 100%, and 95.7% in the vehicle

control, pamoic acid, LD, MD and HD OPM groups. A single HD female had evidence of mating but failed to deliver; this female was non-gravid.

There were no significant overall differences in mean body weight gains per day among groups during the maturation and mating phases (females PND 28-84 and males PND 28-133). Mean F_1 maternal body weights, body weight gains per day and total body weight gain during gestation (GD 0-20) were unaffected. Mean F_1 maternal food consumption (g/kg/day) was decreased in the HD OPM group, but since there were no corresponding effects on mean body weights or body weight gains, this slight decrease was not considered a sign of toxicity.

Mean F_1 maternal food consumption

Dose (mg/kg):	0[a]	93[b]	10	25	75
Food consumption (g/kg/day)					
Overall	74.6	75.5	75.4	73.7	71.9*

[a] Vehicle.
[b] Pamoic acid.
*p≤0.05.

There were no effects on mean gestation lengths or parturition in the F_1 females. Mean F_1 gestation lengths in the LD, MD and HD OPM groups were 21.6, 21.6 and 21.7 days, respectively, compared to mean gestation lengths of 21.6 days in the vehicle control group and 21.8 days in the lab historical control data.

- F_1Sperm Evaluation

Cauda Epididymal sperm was evaluated for sperm motility, sperm morphology and sperm concentration.

- Sperm motility (sperm motility, progressive motility and path velocity): The only significant difference from the vehicle control group was an increased path velocity in the HD OPM group and in the pamoic acid control group (as shown in sponsor's table below). This increase was not a manifestation of adverse effect in view of the absence of changes in sperm motility or the ability of the animals to sire a litter.

F_1Sperm Motility

Dose (mg/kg):	0[a]	93[b]	10	25	75
Path velocity (μm/s)	183.5	200.6*	197.5	191.5	200.1*

[a] Vehicle.
[b] Pamoic acid.
*p≤0.05.

- Sperm morphology: There were no effects on sperm morphology in the OPM groups or in the pamoic acid group.
- Sperm concentration: Mean testicular and epididymal sperm concentrations and sperm production rates in the OPM groups or in the pamoic acid group were not different from the vehicle control group.

Necropsy findings

- Organ Weights

No effects were observed on mean organ weights (absolute and relative to final body and brain weights) in the OPM groups or pamoic acid group F_1 males and females. The only significant difference from the vehicle control group was an increased left testis mean weight relative to brain weight in the HD OPM group and in the pamoic acid control vs. vehicle control (see sponsor's table on the next page). This increase was not likely to be

caused by the maternal OPM administration since the vehicle control group left testis weights were low, and there were no effects on the right testis weight relative to brain weight or on absolute and relative to final body weight testis weights in either HD OPM or pamoic acid group. The left testis/brain wt values in these groups were also within the conducting laboratory historical control data range.

Organ weights F_1 males

				Testis		Epididymis		Cauda Epid	
Group	Statistic	Prostate	Sem Ves Cg/Fluid	Left	Right	Left	Right	Left	Right
1	Mean	49.98	104.35	87.90	92.40	34.41	35.81	16.0167	16.5645
	SD	11.641	15.384	10.427	15.170	5.574	3.258	2.98254	1.70014
	N	22	22	22	22	21	22	22	22
2	Mean	51.08	105.45	94.53	96.17	36.24	36.60	16.8866	17.0560
	SD	10.170	17.513	7.949	11.577	3.872	3.335	2.02623	1.91824
	N	24	24	24	24	24	24	24	24
3	Mean	45.90	102.29	93.42	94.49	35.93	36.52	16.2154	16.3724
	SD	10.985	14.217	7.715	7.287	3.558	3.909	2.40434	1.89737
	N	22	22	22	22	22	22	22	22
	Dunnett p-value#	NT	NT	NT	NT	NT	NT	NT	NT
	Trend p-value#	NT	NT	NT	NT	NT	NT	NT	NT
4	Mean	50.44	99.19	93.62	98.26	36.01	37.33	16.6150	17.3258
	SD	10.704	13.220	11.095	14.510	6.509	3.579	3.61316	2.25642
	N	22	22	22	22	22	22	22	22
	Dunnett p-value#	NT	NT	NT	NT	NT	NT	NT	NT
	Trend p-value#	NT	NT	0.051	NT	NT	NT	NT	NT
5	Mean	46.81	100.97	93.90	91.50	35.72	34.61	16.4865	16.0553
	SD	11.104	19.933	8.677	24.670	2.957	6.130	2.03443	3.73382
	N	23	23	23	23	23	23	23	23
	Dunnett p-value#	NT	NT	NT	NT	NT	NT	NT	NT
	Trend p-value#	0.636	0.382	0.047*	0.946	0.386	0.503	0.498	0.812

Groups: 1-Vehicle control; 2- Pamoic acid; 3 – LD OPM; 4- MD OPM; 5- HD OPM

F_1 Histopathology

- Unscheduled Deaths

The only microscopic finding in the single male unscheduled deaths that occurred in the LD OPM group and in the pamoic acid control group between PN Day 24-26 was severe hypoplasia of the right testis and epididymis, which was attributed to dying prior to sexual maturity. In the single female from the pamoic acid group that was found dead on PND 25, the only microscopic finding was hypoplasia of the reproductive organs, which was attributed to this female dying prior to reaching sexual maturity.

- Scheduled Necropsy

There were no microscopic findings that could be attributed to F_1 prenatal exposure to OPM or pamoic acid in the F_1 males at the scheduled necropsy. In single males in the MD and HD OPM groups that failed to sire a litter, the only findings were hydronephrosis and lymphocyte infiltrate of the prostate, respectively. In the F_1 females, at the scheduled necropsy on Post-Partum Day 14, the only finding in the OPM-treated-groups with potential relationship to treatment was minimal to mild increases in vaginal mucification observed in 0/23, 1/24, 3/23, 0/22, and 6/22 post-partum F_1 females derived from F_0 dams given vehicle control, pamoic acid, or 10, 25, and 75 mg/kg OPM, respectively. According to the sponsor, mucin production in these F_1 females was slightly increased over amounts occurring during a normal estrous. The change was minimal to mild in severity, sporadic in nature, and was not dose-dependent. Test article related changes were not observed in other male or female reproductive organs collected for microscopic examination.

F_2 Litter Data and Postnatal Survival

The mean number of F2 pups born, live litter size, live born index (%), percentage of males per litter at birth and postnatal survival from birth to PND 1 in the OPM-treated groups were not significantly different from the vehicle control group. These parameters were also unaffected in the pamoic acid group. The only statistically significant difference from the vehicle control group was a decreased mean litter proportion of male pups in the pamoic acid group (47.0% per litter) which was within the conducting laboratory reproductive historical control data range.

In summary, i.m. administration of OPM at doses of 10, 25 and 75 mg/kg, or pamoic acid at an i.m. dose of 93 mg/kg on gestation days 6, 16 and post-partum day 4 did not cause maternal toxicity. Gestation length was slightly increased (mean +0.4 days) at the HD. The slight increase in gestation length was not adverse because there were no untoward effects on reproductive performance and parturition. The mean number of pups born, live litter size, live born index (%), pre-culling live litter size, percentage of males per litter at birth and postnatal survival from birth to PND 4 (pre-selection) and from PND 4 to 21 (post-selection to weaning) were unaffected by F_0 maternal OPM administration at all dose levels.

Mean F_1 progeny body weights in the HD group were significantly increased by up to 12.9% and 12.5%, for males and females, respectively, during the pre-culling period (PND 1-4) and by up to 8.5% and 9.8%, respectively, during the post-culling period (PND 7-21). Mean body weights were also increased at the MD and HD OPM group males during most of the maturation phase (up to 6.9% and 9.0%, respectively). The mean body weights exceeded the mean values in the historical control data. The effects on pre- and post-weaning body weights were considered to be a result of the slightly increased gestation length observed in the HD OPM group. The body weight increases were therefore considered to be test article-related but not adverse due to the lack of effects on related parameters. No OPM- or pamoic acid-related effects were observed on F_1 pup survival, general physical condition or the functional observational battery assessments. The mean age of attainment of sexual maturation in F1 male offspring was slightly, but significantly, decreased in the HD OPM group that was attributed to the increased group mean body weights noted throughout the postnatal period. Because the change was slight, associated with increased growth of the pups, and within historical control values, it was not considered adverse

Behavioral changes were induced at the HD of 75 mg/kg: delayed negative geotaxis performance in F_1 males and females, decreased motor activity of F_1 females at the age of weaning, lack of habituation to startle response in F_1 males and reduced performance on memory trials in the water maze in F_1 males during assessment on PND 60-62 (i.e., near sexual maturation). A significant increase in the maximum startle response was observed for these males as well as a decreased learning capacity demonstrated by a statistically significant trend to prolongation of the escape time and increased (n.s.) number of errors during memory trials in water maze.

No effects were noted on F_1 reproductive capacity in either OPM or pamoic acid groups. Spermatogenic endpoints (i.e., motility, morphology) in the F_1 males were unaffected by OPM or pamoic acid. The mean number of F_2 pups born, live litter size, live born index (%), percentage of males per litter at birth and postnatal survival from birth to PND 1 in

the OPM-treated groups were not significantly different from the vehicle control group. These parameters were also unaffected in the pamoic acid group.

Conclusion: The NOAEL for maternal general and reproductive toxicity of olanzapine pamoate monohydrate (OPM) when administered to pregnant rats via intramuscular injection on GD 6, GD 16 and LD 4 is 75 mg/kg based on the lack of adverse test article-related findings in the F_0 females. The NOAEL for F_1 developmental toxicity is 25 mg/kg, based on the findings of drug-related neurobehavioral developmental effects at 75 mg/kg (delayed negative geotaxis performance in F_1 pups of both genders, lack of habituation to startle response and reduced performance on memory trials in the males).
There were no observed adverse effects on maternal or F_1 neonatal parameters in the pamoic acid group. Thus, the NOAEL for intramuscular injection of pamoic acid was 93 mg/kg.

Plasma exposure and safety margins in developmental toxicology studies:
- Olanzapine exposure
When based on dose adjusted for body weight or for body surface area, multiples above 1 were seen at HD in both animal species tested vs. humans. However, when comparing exposures using plasma AUC values, exposure multiples in both rat and rabbit vs. human exposure at MRHD are all less than 1 (as shown in sponsor's tables below and on the next page). The ratio of Cmax values obtained in animals versus those achieved in humans was generally greater than 1.
- Pamoic acid exposure
Systemic exposure to the pamoate ion assayed as pamoic acid was monitored in OP Depot developmental toxicity studies in rats and rabbits. The pamoate exposures observed at the HD in these studies were about 4x (rabbits) to 40x (rats) the human exposures at MRHD (see sponsor's table on the next page).

Olanzapine Exposure Multiples Based on Dose

				Exposure Multiple Based on Dose		Exposure Multiple Based on Dose Corrected for Surface Area	
		mg/kg/day	mg/m^2/day	300 mg/ 2 wk	405 mg/ 4 wk	300 mg/ 2 wk	405 mg/ 4 wk
Human	300 mg/2 wk	0.31[a]	11.3				
	405 mg/4 wk	0.19[a]	7.6				
Rat	Embryo-fetal 75 mg/kg once	2.7	16.1	8.8	13.0	1.4	2.1
Rabbit	Embryo-fetal 75 mg/kg once	2.7	32.1	8.8	13.0	2.8	4.2

Olanzapine Exposure Multiples Based on Cmax or AUC for 300 mg/2 wk

		C_{max} (ng/mL)	AUC (ng•h/mL)	Exposure Multiple Based on C_{max} M	Based on C_{max} F	Based on AUC M	Based on AUC F
Human	300 mg/2 wk[a]	49.7	13600				
Rat	Embryo-fetal 75 mg/kg on GD 6	153	8448	–	3.1	–	0.6
Rabbit	Embryo-fetal 75 mg/kg on GD 7	51	9703	–	1.0	–	0.7

Olanzapine Exposure Multiples Based on Cmax or AUC for 405 mg/4 wk

		C_{max} (ng/mL)	AUC (ng•h/mL)	Exposure Multiple Based on C_{max} M	Based on C_{max} F	Based on AUC M	Based on AUC F
Human	405 mg/4 wk[a]	54.0	26100				
Rat	Embryo-fetal 75 mg/kg on GD 6	153	8448	–	2.8	–	0.32
Rabbit	Embryo-fetal 75 mg/kg on GD 7	51	9703	–	0.9	–	0.37

Exposure Multiples for Pamoic Acid Based on $C_{max,ss}$

		$C_{max,ss}$	Exposure Multiple for Pamoic Acid
Human	300 mg/2 wk	445.9[a]	
Rat	Embryo-fetal 75 mg/kg on GD 6	17586	39.4
Rabbit	Embryo-fetal 75 mg/kg on GD 7	1643	3.7

[a] Human exposure values obtained from Study F1D-EW-LOBE

Thus, exposure multiples in the evaluation of OP Depot developmental toxicity relative to the MRHD were small due to limitations in dose volumes that could be administered. Maximal suspension concentrations were used in all animal studies. The highest OPM i.m. dose (75 mg/kg) used in the embryofetal (Segment II) studies in rats and rabbits, as well as in the prenatal/postnatal (Segment III) study in rats is the highest dose that could be administered based on the maximum achievable concentration of the test article in suspension and the maximum tolerated intramuscular dose volume for the test animals used.

2.6.6.7 Local tolerance

The primary toxicity of OP Depot in laboratory animals was injection site irritation (reviewed under single- and repeat-dose OP Depot general toxicology studies).

2.6.6.8 Special toxicology studies

Antigenicity, immunotoxicity, mechanistic, or dependence studies were not conducted with OP Depot. Dependency studies were conducted with oral olanzapine and reviewed under NDA 20-592 (in these studies, no evidence of olanzapine drug dependence was demonstrated in rats or rhesus monkeys). No preclinical studies have been conducted using direct administration of olanzapine metabolites.

Studies on Impurities

Two lots of drug substance (325SB8 and ML114 relabeled as CTM00881) were used in all toxicology studies and also used in clinical trials. Thus, impurity exposures tested and qualified in toxicology were also qualified by their use in clinical studies.

Olanzapine-Related Impurities

(b) (4) is a (b) (4) (b) (4) product of olanzapine that may form during the olanzapine pamoate drug substance manufacturing process and may be isolated along with the drug substance. A set of qualification studies by the oral route were conducted to support the oral olanzapine drug product specification (NDA 20-592); these studies were also used to support the RAIM olanzapine product. Oral qualification data can be applied in light of the greater exposures that would be expected by the oral versus the IM route.

The oral qualification studies are summarized by the sponsor as follows:

> "Olanzapine containing elevated levels of (b) (4) as well as 2 other impurities found as degradation products in the olanzapine oral drug product (b) (4) was not mutagenic in an Ames assay and in an in vivo mouse micronucleus test (NDA 20-592).
>
> To assess the safety of impurities observed during stability testing of olanzapine tablets, Fischer 344 rats were given oral doses of either vehicle or a suspension of olanzapine with impurities once daily for 14 days (Tox 63; NDA 20-592). The olanzapine-treated group was given approximately 16 mg olanzapine/kg plus approximately 167 µg/kg of each of the 3 impurities: (b) (4) (b) (4), also known as the (b) (4) and formed by the (b) (4) olanzapine (b) (4) and (b) (4) (b) (4) The dose of olanzapine, 16 mg/kg, was equivalent to the top dose used in the 6-month study in rats and was expected to produce well-characterized pharmacologic and toxicologic effects.
>
> The dose of each impurity was chosen to provide a substantial safety margin (about 100-fold) relative to the maximum human daily dose of a 20-mg tablet containing the respective impurity at a 0.5% concentration (1.67 µg of impurity/60 kg). All of the effects in this study were consistent with those observed in earlier rodent studies with olanzapine and suggest that olanzapine with elevated levels of impurities does not exhibit toxicities different from those reported for olanzapine alone." (End citation)

Olanzapine Pamoate Monohydrate-Related Impurities

Concentrations of two impurities - (b) (4), the (b) (4) (b) (4) of pamoic acid in Lot 325SB8 (used in the single- and repeat-dose toxicology studies) and Lot CTM00881 (used in the reproduction and carcinogenicity studies) were at or above their proposed acceptance criteria and lots used in clinical studies. In the animal studies, no systemic toxicity findings were observed with OP Depot or pamoic acid; the observed effects were limited to injection site reactions. Based on dose (mg/m2)

the highest doses tested in rat 3-month study and in dog 6-month study provided multiples about 2-fold and 3-fold, respectively, for these 2 impurities when compared to the maximum recommended human dose. Based on AUC values, multiples of 4.8 x and 1.5-2x are achieved at the highest doses tested in rat 3-month study and dog 6-month study, respectively (see sponsor's tables on the next page).

Since all specified impurities were present at similar concentrations in drug product lots used in both nonclinical and clinical studies and since no new impurities were identified in long-term stability studies, the specified impurities (b) (4) and (b) (4) are qualified.

Exposure Multiples for Olanzapine Pamoate Monohydrate Degradants or Process Impurities Based on Dose in mg/kg

				Exposure Multiple			
	Dose/Frequency	mg/kg	mg/kg/day	Total Impurities (b) (4)			(b) (4)
Human	300 mg/2 weeks	4.29	0.31				
	405 mg/4 weeks	5.79	0.21				
Rat	3-Month repeat-dose	100	3.57	35.5[a,c,e]	11.7	9.7	7.4[d]
	100 mg/kg/4 weeks			52.5[b,c,e]	17.3	14.4	10.9
Rat	Embryo-fetal	75	2.68	15.8	8.8	4.7	7.0
	75 mg/kg, once			11.7	6.5	3.5	5.2
Rabbit	Embryo-fetal	75	2.68	15.8	8.8	4.7	7.0
	75 mg/kg, once			11.7	6.5	3.5	5.2
Rat	2-Year carcinogenicity	20	0.71	2.1	1.2	0.6	0.9
	20 (M) mg/kg/4 weeks			3.1	1.7	0.9	1.4
	50 (F) mg/kg/4 weeks	50	1.79	5.3	2.9	1.6	2.3
				7.8	4.3	2.3	3.5
Dog	6-Month repeat-dose	20	1.43	14.2	4.7	3.9	3.0
	20 mg/kg/2 weeks			21.0	6.9	5.8	4.4

Abbreviations: F = female, LSN = Lilly Serial Number, M = male.

[a] First row indicates multiples compared to 300 mg/2 weeks.

[b] Second row indicates multiples compared to 405 mg/4 weeks.

[c] Values for impurity concentrations in drug substance Lot 325SB8 were adjusted to report values on an olanzapine equivalent basis rather than a weight percent basis.

[d] Original values for (b) (4) were corrected for detector response against an authentic sample of the impurity.

[e] Impurity concentrations at or above the (b) (4) reporting threshold were summed to determine the results for total impurities.

Exposure Multiples for Olanzapine Pamoate Monohydrate Degradants or Process Impurities Based on Dose in mg/m2

				Exposure Multiple			
				Total Impurities			(b) (4)
		mg/m^2	mg/m^2/day	(b) (4)			
Human	300 mg/2 weeks	158.6	11.3				
	405 mg/4 weeks	214.1	7.6				
Rat	3-Month repeat-dose	600	21.4	5.8[a,c,e]	1.9	1.6	1.2[d]
	100 mg/kg/4 weeks			8.5[b,c,e]	2.8	2.3	1.8
Rat	Embryo-fetal	450	16.1	2.6	1.4	0.8	1.1
	75 mg/kg once			1.9	1.1	0.6	0.8
Rabbit	Embryo-fetal	900	32.1	5.1	2.8	1.5	2.3
	75 mg/kg dosed once			3.8	2.1	1.1	1.7
Rat	2-Year carcinogenicity	120 (M)	4.29	0.3	0.2	0.1	0.2
	20 (M) mg/kg/4 weeks			0.5	0.3	0.1	0.2
	50 (F) mg/kg/4 weeks	300 (F)	10.7	0.9	0.5	0.3	0.4
				1.3	0.7	0.4	0.6
Dog	6-Month repeat-dose	400	28.6	7.7	2.5	2.1	1.6
	20 mg/kg/2 weeks			11.4	3.7	3.1	2.4

Abbreviations: F = female, LSN = Lilly Serial Number, M = male.

[a] First row indicates multiples compared to 300 mg/2 weeks.

[b] Second row indicates multiples compared to 405 mg/4 weeks.

[c] Values for impurity concentrations in drug substance Lot 325SB8 were adjusted to report values on an olanzapine equivalent basis rather than a weight percent basis.

[d] Original values for (b) (4) were corrected for detector response against an authentic sample of the impurity.

[e] Impurity concentrations at or above the (b) (4) reporting threshold were summed to determine the results for total impurities.

Exposure multiples for Olanzapine Pamoate Monohydrate Degradants or Process Impurities Based on AUC

	AUC,ss (ng/mL)	Exposure Multiple	Exposure Multiple	Exposure Multiple	Margin of Safety
		Total Impurities			(b) (4)
Specification limits (%)		(b) (4)			
Study and effect level:					
Human study					
Max. planned dose = 300 mg/2 weeks	13600				
3-Month rat study					
100 mg/kg/4 weeks	54001	1.31	3.97	4.76	6.27
6-Month dog study					
20 mg/kg/2 weeks	19126	0.46	1.41	1.69	2.22
2-Year rat carcinogenicity study					
Average of 20 mg/kg in males and 50 mg/kg in females on Week 68	8122	0.66	1.19	2.24	1.49

Abbreviations: LSN = Lilly Serial Number, Max = maximum.

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2.6.6.9 Discussion and Conclusions

Due to dose-limiting factors (i.e., the amount of test article feasible to be injected i.m., maximal concentration achievable in the suspension formulation and local injection site reaction), relatively low olanzapine exposures could be achieved with OP Depot in the nonclinical toxicology studies, which resulted in low nonclinical-to-clinical exposure multiples. In the OP Depot nonclinical studies, lower doses of olanzapine were administered as compared to the oral dosage form. However, this does not constitute a safety issue since the safety of systemic olanzapine was adequately evaluated by previous nonclinical studies with the approved oral and rapid-acting IM olanzapine formulations. The sustained long-term systemic plasma exposure to olanzapine upon OP Depot administration was not associated with systemic toxicity, and monitorable clinical signs were observed in the tested nonclinical species.

Systemic exposure to the pamoate ion (assayed as pamoic acid) at the highest doses administered in the animal studies (resulting primarily in injection site reactions only), approximated or exceeded human levels of pamoic acid. Exposure multiples (C_{max}) for pamoic acid ranged from 0.5 to 0.7 (dogs) and from 5 to 6 (rats) in repeat-dose studies; 4 and 39 in rabbit and rat embryo-fetal studies, respectively; and 4 to 10 in the 2-year rat carcinogenicity study.

General toxicity: Pivotal studies are the repeat-dose toxicity studies in rats and dogs. OP Depot was given intramuscularly to rats once every 4 weeks for 3 months and to dogs once every 2 weeks for 6 months. Due to limitations in dose volume and suspendability, systemic toxicity was not elicited with OP Depot. Key findings include:

- Injection site reactions indicative of chronic inflammation were the major finding in all toxicology studies with OP Depot, in both rats and dogs. The reaction in dogs was more pronounced, appearing within a few days after administration and diminishing in a week or 2 thereafter. Histologic evidence of chronic inflammation and fibrosis was present at necropsy; the inflammation persisted, though significantly reduced, after a 2-month recovery period.
- Pamoic acid (the formulation agent) did not exert systemic toxicity. Injection site reactions from the pamoic acid-treated animals were less frequent and less severe than those from animals treated with OP Depot.

Genetic toxicity: Olanzapine was previously tested for genetic toxicity (under NDA 20-592) and was negative in a full range of standard tests that included bacterial mutation tests and in vitro and in vivo mammalian tests.

Pamoic acid was negative in the Ames test, the mouse lymphoma assay, and the chromosome aberration assay in human lymphocytes and was also negative in 2 in vivo assays (the mouse micronucleus test and the mouse bone marrow chromosome aberration assay). Although reproducibly positive results were obtained in an in vitro chromosome aberration assay in Chinese hamster ovary (CHO) cells (at concentrations >500 µg/ml, about 1000-fold greater than the peak plasma concentrations in humans), the absence of oncogenicity in the 2-year carcinogenicity study in rats supports a lack of genotoxic hazard to humans.

Carcinogenicity: In the 2-year study to evaluate the carcinogenic potential of a sustained-release formulation of olanzapine administered by once per 4 weeks intramuscular injections of olanzapine pamoate monohydrate (OPM) to Fischer 344 rats (60/sex/dose) at doses of 0 (vehicle), 0 (pamoic acid), 5, 10, and 20 mg /kg for males and 0, 0, 10, 25,

and 50 mg /kg for females, there was no carcinogenic effect attributable to OPM or pamoic acid since there was no dose-related effect on incidence and distribution of neoplastic lesions and they were similar among groups. Effect of pamoic acid alone at i.m. doses similar to those administered in the high-dose OPM group (37 mg/kg in males and 92.5 mg/kg in females) was assessed in parallel in additional groups of rats. The doses were selected in accordance with the Executive CAC recommendations (IND 60701, Executive CAC Meeting Minutes of 3/11/2003). A MTD was achieved in this study based on dose-related injection site adverse effects in both genders (chronic inflammatory reactions and residual test substance accumulation in the injection site affecting nearly all animals of both genders at HD). Olanzapine plasma exposure (AUC) values achieved at HD were lower or equal than those in humans at MRHD (300 mg every 2 weeks or 405 mg every 4 weeks), while exposures to pamoic acid (AUC) achieved at HD were equal to or higher than those in humans at MRHD. Dose-limiting factors were suspendability, amount of test article feasible to be injected i.m. in the rat and local injection site reaction.

Reproductive and developmental toxicology:

- Fertility and Early Embryonic Development

No fertility studies were conducted with OP Depot, as agreed by the Division. In oral olanzapine rat fertility studies impaired fertility due to reduced estrous cycling secondary to hyperprolactinemia was described (NDA 20-592). We agree with the sponsor's rationale for not repeating these studies with OP Depot as it "would have no impact on the risk to patients on OP Depot since similar olanzapine exposures were observed clinically upon oral olanzapine administration and OPM i.m. administration".

- Embryo-Fetal Development

Embryo/fetal studies in rats and rabbits from dams treated with OPM Depot formulation during gestation [i.m. doses of 10, 25 and 75 mg/kg on gestation day 6 (rat) or 7 (rabbit) with plasma exposures maintained throughout the period of organogenesis] showed no OPM- or pamoic acid-related maternal systemic toxicity, embryo/fetotoxicity (as indicated by the lack of effect on embryo/fetal intrauterine growth and survival) or increased incidence of structural malformations up to the maximum feasible dose tested (75 mg/kg).

- Pre- and Postnatal Toxicity

A prenatal/postnatal study with OPM Depot was conducted in rats at i.m. doses of 0, 10, 25, and 75 mg/kg given to dams on gestations days 6 and 16 and again on post-partum day 4. Changes in behavioral development of offspring were observed at the highest dose (a delay in negative geotaxis early in the development of the F1 pups, a lack of habituation to the startle response in F1 males and a reduced performance on memory trials in water maze). These effects are qualitatively similar to the transient decrease in Figure-8 maze activity observed in the 2-generation study conducted with oral olanzapine (NDA 20-592, as cited by the sponsor), and therefore, do not represent new findings of concern. Although there are differences in behavioral assessment methods compared to the earlier 2-generation study with oral olanzapine, the results are qualitatively similar and suggest no new risk due to olanzapine pamoate monohydrate use.

Impurities and degradation products: (b) (4) ((b) (4) product of olanzapine that may form during OPM drug substance manufacturing process) has oral qualification data that can be applied in the light of the similar or greater

exposures that would be expected by the oral vs. the i.m. route. Olanzapine containing elevated levels of Compound (b) (4) as well as 2 other impurities found as degradation products in oral olanzapine drug (Compounds (b) (4) were tested and found to be negative for genotoxicity in the Ames test and in vivo mouse micronucleus test, as well as for general toxicity in rats at doses about 100x higher than the MRHD of oral olanzapine in humans (NDA 20-252).

Compounds (b) (4) (the (b) (4) (b) (4) of pamoic acid) were at or above their proposed acceptance criteria in Lot 325SB8 (used in the single-and repeat-dose toxicology studies) and Lot CTM00881 (used in the reproduction and carcinogenicity studies). In all these studies, no systemic adverse findings were observed with OP Depot or pamoic acid. The highest tested dose levels used in rat 3-month and dog 6-month toxicity studies provided multiples from about 2- to 3-fold above the acceptance criteria for these 2 impurities when compared to MRHD (based on dose in mg/m2) and multiples from 1.5 to 4.8x the MRHD (based on plasma exposure, AUC).

Since "drug products derived from the 2 lots of drug substance mentioned above (Lot 325SB8 and ML114 which was re-labeled as CTM00881) were used in all toxicology studies and also in clinical trials", the impurity exposures tested and qualified in toxicology studies were also qualified by their use in clinical studies.

2.6.6.10 Tables and Figures

Incorporated in the text

2.6.7 TOXICOLOGY TABULATED SUMMARY

Repeat-Dose Toxicity: 3-Month Study in Rats

Report title: A Subchronic Toxicity Study in Fischer 344 Rats Given Olanzapine Pamoate Monohydrate (Compound 426906) by Intramuscular Injection Once a Month for 3 Months

Species/strain: Rat/Fischer 344 | Duration of dosing: 3 months

Initial age: 15 to 16 weeks | Duration of reversibility: None | Document ID: Tox87

Date of first dose: 21 June 2000 | Route: Intramuscular | GLP compliance: Yes

NOAEL: 100 mg/kg for systemic effects; <20 mg/kg for local reactions | Vehicle: 5% mannitol and 0.1% polysorbate 80 in Water for Injection

Special features: Dosing was once/4 weeks due to the extended exposure to test article. A pamoic acid comparator group was included.

Dose (mg/kg)/4 weeks		0[a]		PA - 125[b]		OP Depot - 20[c]		OP Depot - 50[c]		OP Depot - 100[c]	
Sex		M	F	M	F	M	F	M	F	M	F
Toxicokinetics	Number of animals	0	0	12	12	22	22	22	22	22	22
Number of deaths		NA	NA	0	0	0	0	0	1	0	1
Day 0											
Olanzapine											
C_{max} (ng/mL)		NA	NA	NA	NA	115	91	121	155	200	456
AUC_{0-t} (ng*h/mL)		NA	NA	NA	NA	6002	6919	16537	20569	57590	35073
T_{max} (h)		NA	NA	NA	NA	4	8	24	8	216	8
Pamoate											
C_{max} (ng/mL)		NA	NA	24060	38567	1817	2928	3111	2979	4906	4165
AUC_{0-t} (ng*h/mL)		NA	NA	576149	1606801	141060	233436	341367	267694	589832	817656
T_{max} (h)		NA	NA	8	8	8	4	8	8	8	8
Day 56											
Olanzapine											
C_{max} (ng/mL)		NA	NA	NA	NA	133	99	171	189	209	169
AUC_{0-t} (ng*h/mL)		NA	NA	NA	NA	5387	6477	22993	15307	59992	48010
T_{max} (h)		NA	NA	NA	NA	4	4	8	4	8	120
Pamoate											
C_{max} (ng/mL)		NA	NA	31383	38994	2230	1720	2472	5012	2236	2755
AUC_{0-t} (ng*h/mL)		NA	NA	463539	576236	247664	168404	394919	443327	537234	806213
T_{max} (h)		NA	NA	4	8	8	4	8	4	24	4

Dose (mg/kg)/4 weeks	0[a]		PA - 125[b]		OP Depot - 20[c]		OP Depot - 50[c]		OP Depot - 100[c]	
Sex:number of animals	M:10	F:10	M:10	F:10	M:10	F:10	M:10	F:10	M:10	F:10
Noteworthy Findings										
Number of deaths	0	0	0	0	0	0	0	0	0	0
Body weight (g), Day 83 (% change)[d]	340	204	–	–	0	↓1	↓5*	↓2	↓7*	↓3*
Body weight gain (g), Day 83 (% change)[d]	62.4	25.8	–	–	↓6	↓13	↓31*	↓18	↓39*	↓26*
FC/body weight, (g/kg) Day 83 (% change)[d]	51.6	63.6	–	–	↑2	–	↑5*	–	↑4*	–
EFU (g), Day 83 (% change)[d]	4.55	2.54	–	–	↓7	↓13	↓31*	↓19	↓39*	↓25*
Clinical signs (n[e])										
Lacrimation	0	0	0	0	0	5	6	10	5	9
Red ocular discharge	1	1	0	1	3	9	5	9	5	9
Red nasal discharge	0	0	0	0	3	0	7	5	7	8
Soiling around chin	0	0	0	0	1	2	3	6	4	5
Soiling: perineal, urogenital, or anal	0	1	0	0	0	0	0	2	0	2
Ophthalmology	–	–	–	–	–	–	–	–	–	–
Enzyme induction	NE	NE	NE	NE	NE	NE	NE	NE	NE	NE
Hematology	–	–	–	–	–	–	–	–	–	–
Clinical chemistry	–	–	–	–	–	–	–	–	–	–
Urinalysis	–	–	–	–	–	–	–	–	–	–
Gross pathology (n[e])										
Injection site lesion, left leg	0	0	2	0	10	10	11	12	11	11
Injection site lesion, right leg	0	0	0	2	10	10	10	9	11	10
Organ weight/body weight	–	–	–	–	–	–	–	–	–	–

(Continued)

Repeat-Dose Toxicity: 3-Month Study in Rats (concluded)

Dose (mg/kg)/4 weeks	0[a]		PA - 125[b]		OP Depot - 20[c]		OP Depot - 50[c]		OP Depot - 100[c]	
Sex:number of animals	M:10	F:10	M:10	F:10	M:10	F:10	M:10	F:10	M:10	F:10
Histopathology (n[e])										
Injection site, left[f]										
Granulomatous inflammation										
Minimal	0	0	3	1	0	1	0	0	0	0
Slight	0	0	1	0	1	8	0	2	0	0
Moderate	0	0	0	0	9	1	6	7	4	3
Marked	0	0	0	0	0	0	4	1	6	7
Injection site, right[f]										
Granulomatous inflammation										
Minimal	0	0	0	0	1	2	1	0	0	0
Slight	0	0	0	1	6	8	4	7	2	0
Moderate	0	0	0	0	1	0	4	2	4	8
Marked	0	0	0	0	0	0	0	0	4	2

Abbreviations: ↑ = increase, ↓ = decrease, – = no important findings, AUC_{0-t} = area under the concentration versus time curve where t is the last quantifiable time point within each 28-day period, C_{max} = maximum observed plasma concentration, EFU = efficiency of food utilization, F = female, FC = food consumption, M = male, NA = not applicable, NE = not evaluated, NOAEL = no-observed-adverse-effect level, OP = olanzapine pamoate, PA = pamoic acid, T_{max} = time of maximum plasma concentration.

[a] Vehicle control.
[b] Pamoic acid control.
[c] Dose expressed in mg olanzapine/kg.
[d] For controls, group means are shown. For treated groups, percent differences from controls are shown. Statistical significance is based on actual data (not on the percent differences).
[e] Number of animals or tissues affected.
[f] Left leg injected on Days 0 and 56 (12 and 4 weeks before necropsy); right leg injection on Day 28 (8 weeks before necropsy).
[g] p≤.05 (Trend test).

Repeat-Dose Toxicity: 6-Month Study with 2-Month Reversibility Period in Dogs

Report title: A Chronic Toxicity Study in Beagle Dogs Given Multiple Doses of Olanzapine Pamoate Monohydrate (LY170053 Pamoate Monohydrate) for 6 Months Followed by a 2-Month Reversibility Phase

Species/strain: Dog/beagle
Initial age: 8 to 10 months
Date of first dose: 17 May 2000
NOAEL: 20 mg/kg for systemic effects; <5 mg/kg for local reactions
Duration of dosing: 6 months
Duration of reversibility: 2 months
Route: Intramuscular
Vehicle: 5% mannitol, 0.1% polysorbate 80 in Sterile Water for Injection, USP
Document ID: Tox88
GLP compliance: Yes
Special features: Dosing was once every 2 weeks due to the extended exposure to test article. A pamoic acid comparator group was included.

Dose (mg/kg)/2 weeks	0[a]		PA - 25[b]		OP Depot - 5[c]		OP Depot - 10[c]		OP Depot - 20[c]	
Sex:number of animals	M:6[d]	F:6[d]	M:4	F:4	M:4	F:4	M:4	F:4	M:6[d]	F:6[d]
Toxicokinetics, Day 0										
Olanzapine										
C_{max} (ng/mL)	NE	NE	NE	NE	6.86	9.27	13.04	16.39	26.89	37.35
AUC_{0-t} (ng•h/mL)	NE	NE	NE	NE	1327	1275	2719	2736	4703	6476
T_{max} (h)	NE	NE	NE	NE	120	120	144	144	136	136
Pamoate										
C_{max} (ng/mL)	NE	NE	5100.3	5585.98	96.14	144.33	162.45	217.23	256.48	309.51
AUC_{0-t} (ng•h/mL)	NE	NE	99110	73791	18109	27396	31686	45148	46275	59415
T_{max} (h)	NE	NE	1	1	120	145	144	144	104	104
Toxicokinetics, Day 84										
Olanzapine										
C_{max} (ng/mL)	NE	NE	NE	NE	14.45	7.94	21.78	41.96	48.08	39.14
AUC_{0-t} (ng•h/mL)	NE	NE	NE	NE	2167	1679	3759	4934	7236	7410
T_{max} (h)	NE	NE	NE	NE	120	139	120	120	120	120
Toxicokinetics, Day 168										
Olanzapine										
C_{max} (ng/mL)	NE	NE	NE	NE	10.18	9.01	26.80	9.76	29.14	53.53
AUC_{0-t} (ng•h/mL)	NE	NE	NE	NE	1392	1416	4069	1016	3204	7655
T_{max} (h)	NE	NE	NE	NE	92	72	120	8	45	99

(Continued)

Repeat-Dose Toxicity: 6-Month Study with 2-Month Reversibility Period in Dogs (continued)

Dose (mg/kg)/2 weeks	0[a]		PA - 25[b]		OP Depot - 5[c]		OP Depot - 10[c]		OP Depot - 20[c]	
Sex:number of animals	M:6[d]	F:6[d]	M:4	F:4	M:4	F:4	M:4	F:4	M:6[d]	F:6[d]
Toxicokinetics, Day 168 (concluded)										
Pamoate										
C_{max} (ng/mL)	NE	NE	4273.48	3948.17	91.50	97.11	212.94	133.34	241.59	309.77
AUC_{0-t} (ng•h/mL)	NE	NE	43722	93050	12150	15444	32944	10906	27752	45843
T_{max} (h)	NE	NE	3	2	72	72	120	15	53	67
Noteworthy Findings										
Died or sacrificed moribund	0	0	0	0	0	0	0	0	0	0
Body weight	–	–	–	–	–	–	–	–	–	–
Food consumption	–	–	–	–	–	–	–	–	–	–
Clinical observations (n[e])										
Injection site reaction	0	1	1	2	4	4	4	4	6	6
Neurological examinations	–	–	–	–	–	–	–	–	–	–
Ophthalmology	–	–	–	–	–	–	–	–	–	–
Heart rate	–	–	–	–	–	–	–	–	–	–
Electrocardiography	–	–	–	–	–	–	–	–	–	–
Enzyme induction	NE	NE	NE	NE	NE	NE	NE	NE	NE	NE
Hematology	–	–	–	–	–	–	–	–	–	–
Clinical chemistry	–	–	–	–	–	–	–	–	–	–
Urinalysis	–	–	–	–	–	–	–	–	–	–
Gross pathology (n[e])										
Injection site lesion										
Left leg	0	0	2	3	4	4	5	4	4	4
Right leg	0	0	1	0	3	4	4	4	4	4
Organ weight	–	–	–	–	–	–	–	–	–	–

Dose (mg/kg)/2 weeks	0[a]		PA - 25[b]		OP Depot - 5[c]		OP Depot - 10[c]		OP Depot - 20[c]	
Sex:number of animals	M:6[d]	F:6[d]	M:4	F:4	M:4	F:4	M:4	F:4	M:6[d]	F:6[d]
Histopathology (n[e])										
Injection site, chronic inflammation										
Left										
Minimal	0	1	2	1	0	0	0	0	0	0
Slight	0	0	1	1	0	0	1	0	0	0
Moderate	0	0	0	0	1	3	1	3	1	2
Marked	0	0	0	1	3	1	2	1	3	2
Right										
Minimal	0	0	1	1	0	0	1	1	0	0
Slight	0	0	1	1	1	1	1	1	1	3
Moderate	0	0	0	0	3	3	0	2	2	1
Marked	0	0	0	0	0	0	2	0	1	0
Reversibility Evaluations										
Number evaluated	2	2							2	2
Histopathology (n[e])										
Injection site, chronic inflammation										
Left										
Minimal	0	0							0	1
Slight	0	0							0	1
Right										
Slight	0	0							0	1

Abbreviations: – = no important findings, AUC_{0-t} = area under the concentration versus time curve where t is the last quantifiable time point within each 2-week period, C_{max} = maximum observed plasma concentration, F = female, M = male, NE = not evaluated, NOAEL = no-observed-adverse-effect level, OP = olanzapine pamoate, PA = pamoic acid, T_{max} = time of maximum plasma concentration.

[a] Vehicle control.
[b] Pamoic acid control.
[c] Dose expressed in mg olanzapine/kg.
[d] Two dogs were assigned to the reversibility phase of the study.
[e] Number of animals or tissues affected.

Reproductive and Developmental Toxicity: Effects on Pre- and Postnatal Development Including Maternal Function in Rats

Report title: A Prenatal and Postnatal Development Study, Including Maternal Function, of Olanzapine Pamoate Monohydrate Administered by Intramuscular Injection to Female CD Rats
Design similar to ICH4.1.2? Yes
Duration of dosing: Dosed on GD 6, GD 16, and Lactation Day 4
Species/strain: Rat/CD
Day of mating: Gestation Day 0
Document ID: WIL-353058
Initial age: approximately 12 weeks
Litters culled/not culled: culled to 8 pups/litter on PND 4
GLP compliance: Yes
Date of first dose: 20 July 2004
Route: Intramuscular
Vehicle: 0.75% carboxymethylcellulose sodium, 5% mannitol, and 0.1% polysorbate 80 in water for injection, USP
Special features: Doses were given on Gestation Day 6, Gestation Day 16, and Lactation Day 4 due to the extended exposure to test article. A pamoic acid comparator group was included.
No-observed-adverse-effect level:
Maternal toxicity 75 mg/kg
Developmental toxicity 25 mg/kg
Reproductive toxicity 75 mg/kg

	Dose (mg/kg)	0[a]	PA - 93[b]	OP Depot - 10[c]	OP Depot - 25[c]	OP Depot - 75[c]
F_0 females	# pregnant	23	25	23	22	23
	# died or sacrificed moribund	0	0	0	0	0
	# aborted or with total resorption of litter	0	0	0	0	0
	Clinical observations	–	–	–	–	–
	Necropsy observations	–	–	–	–	–
	Body weight gain (g/day)					
	Gestation Days 6 - 9	3.2	2.9	1.8*	1.7*	1.4*
	Gestation Days 16 - 18	13.7	15.0	15.8*	17.3*	14.8*
	Lactation	–	–	–	–	–
	Food consumption (g/kg/day)					
	Gestation Days 6 - 9	70.7	70.7	78.1*	78.2*	76.3*
	Gestation Days 16 - 18	69.2	70.9	76.9*	77.2*	78.7*
	Gestation Days 18 - 20	61.0	63.1	63.4	65.6*	66.8*
	Lactation	–	–	–	–	–

	Dose (mg/kg)	0[a]	PA - 93[b]	OP Depot - 10[c]	OP Depot - 25[c]	OP Depot - 75[c]
F_0 females	Mean duration of gestation (days)	21.6	21.6	21.7	21.7	22.0*
	Abnormal parturition	0	0	0	0	0
F_1 litters	# of litters evaluated	23	25	23	22	23
Preweaning	Implantation loss (%)	4.6	4.3	3.7	4.2	5.2
	Mean # pups/litter	15.9	15.8	15.3	16.0	15.0
	Live born index (%)	98.0	98.5	99.8	98.8	98.3
	# of litters with stillborn pups	5	5	1	3	4
	Postnatal survival to PND 4 (%)	96.3	96.0	98.3	97.2	95.8
	Postnatal survival from PND 4 - 21 (%)	99.5	97.4	100	100	99.1
	# of total litter losses	0	0	0	0	0
	Pup body weight, g (% change)[d]					
	Preculling PND 1 - 4 (M/F)	8.0/7.5	↓1/0	↑5/↑7	↑4/↑5	↑11*/↑12*
	Postculling PND 4 - 21 (M/F)	31.3/30.0	↓4/↓7	↓4/↓3	0/↑1	↑7*/↑9*
	Pup sex ratio (% male)	53.5	50.6	49.1	48.7	52.1
	Pup clinical observations	–	–	–	–	–
	Negative geotaxis, % positive (% change)[d]					
	PND 8 (M/F)	91.3/80.4	↓12/↓10	↓7/↓6	↓12/↓7	↓15*/↓19*
	PND 10 (M/F)	–/–	–/–	–/–	–/–	–/–
	Pup necropsy observations	–	–	–	–	–
F_1 males	# evaluated	23	24	23	22	23
Postweaning	# died or sacrificed moribund	0	1	1	0	0
	Clinical observations	–	–	–	–	–
	Necropsy observations	–	–	–	–	–
	Body weight, PND 28 (% change)[d]	87.7	↓1	↓1	↑4	↑8*
	Body weight, PND 28 - 133 (% change)[d]	403.6	↑5*	↑4	↑5	↑5
	Food consumption	–	–	–	–	–

(Continued)

Reproductive and Developmental Toxicity: Effects on Pre- and Postnatal Development Including Maternal Function in Rats (continued)

	Dose (mg/kg)	0[a]	PA - 93[b]	OP Depot - 10[c]	OP Depot - 25[c]	OP Depot - 75[c]
F_1 males Postweaning	Balanopreputial separation (days)	46.1	46.0	46.2	44.9	44.8*
	Auditory startle PND 60 V_{max} (% change)[d]					
	Trials 31-40	59.2	↑30	↑28	↑18	↓106
	Trials 41-50	66.2	↑34	↑9	↓19	↓98
	Biel maze (% change)[d]					
	Escape time, memory trial (seconds)	60.4	↓2	↓29	↓2	↑34*
	Memory trial, errors	15.5	↓4	↓34	↑3	↑24
	Locomotor activity (total counts)	327.2	340.8	375.1	329.2	370.1
	Histopathology of reproductive organs	–	–	NE	NE	–
	Spermatogenic endpoints	–	–	–	–	–
F_1 females Postweaning	# evaluated	23	24	23	22	23
	# died or sacrificed moribund	0	1	0	0	0
	Clinical observations	–	–	–	–	–
	Necropsy observations	–	–	–	–	–
	Body weight (g) PND 28 (% change)[d]	81.3	↓6	↓3	0	↑8*
	Body weight during maturation	–	–	–	–	–
	Body weight during gestation	–	–	–	–	–
	Food consumption	–	–	–	–	–
	Mean age of vaginal patency (days)	31.6	32.3	32.0	31.5	31.5
	Auditory startle PND 60 V_{max} (% change)[d]					
	Trials 31-40	81.4	↓28	↓42	↓55	↓22
	Trials 41-50	65.8	↓19	↓44	↓38	↑8
	Biel maze (% change)[d]					
	Escape time, memory trial (seconds)	51.4	↑19	↑5	↑12	↓6
	Memory trial, errors	11.7	↑33	↑15	↑24	↑1
	Locomotor activity (total counts)	376.6	320.6	344.3	329.4	304.5*
	Mean # days prior to mating	2.8	3.1	2.1	3.3	2.9

	Dose (mg/kg)	0[a]	PA - 93[b]	OP Depot - 10[c]	OP Depot - 25[c]	OP Depot - 75[c]
F_1 females Postweaning	Mating index (%)	100	100	100	100	100
	Fertility index (%)	100	100	100	100	95.7
	Estrous cycling index (%)	100	100	95.7	100	100
	Mean duration of gestation (days)	21.6	21.9*	21.6	21.6	21.7
	Abnormal parturition	0	0	0	0	0
	Histopathology of reproductive organs	–	–	–	–	–
F_2 litters	# of litters evaluated	23	24	23	22	22
	Implantation loss (%)	3.9	5.5	5.8	3.8	6.4
	Mean # pups/litter	14.0	14.0	14.1	14.3	14.8
	Live born index (%)	99.2	98.8	97.8	99.1	98.1
	PND 1 body weight	–	–	–	–	–
	Postnatal survival, birth to PND 1 (%)	98.6	97.8	92.7	98.4	96.3
	Pup body weight	–	–	–	–	–
	Pup sex ratio (% male)	54.7	47.0	46.5	52.1	50.9
	Pup clinical observations	–	–	–	–	–
	Pup necropsy observations	–	–	–	–	–

Abbreviations: # = number, ↑ = increase, ↓ = decrease, – = no important findings, F = female, GD = gestation day, M = male, NE = not evaluated, OP = olanzapine pamoate, PA = pamoic acid, PND = postnatal day.

[a] Vehicle control.

[b] Pamoic acid.

[c] Dose expressed in mg olanzapine/kg.

[d] For controls, group means are shown. For treated groups, percent differences from controls are shown. Statistical significance based on actual data (not on the percent differences).

* $p \leq .05$ (Trend test for OP Depot groups versus vehicle; t-test for PA group versus vehicle).

OVERALL CONCLUSIONS AND RECOMMENDATIONS

Conclusions:

- Single-dose pharmacokinetics of OP Depot indicated rapid absorption and sustained exposure to olanzapine and pamoic acid in rats, with slower absorption but sustained exposure to both olanzapine and pamoic acid in dogs. Exposure to olanzapine peaked within the first few days and was sustained for weeks.
- Repeat-dose toxicokinetics for both olanzapine and pamoic acid indicate no consistent sex difference and no accumulation of either olanzapine or pamoic acid. In rats, rabbits, and dogs, plasma concentrations of olanzapine following IM administration of OP Depot increased with increasing dose. OP Depot produced initial peak plasma concentrations of olanzapine followed by a gradual decline in concentration for up to 28 days postdose, demonstrating a sustained release profile.
- Aspects of olanzapine disposition other than absorption (i.e., distribution, metabolism, excretion, etc) were not studied under this application since they had been previously evaluated for the oral and the rapid acting IM form and were expected "to remain unchanged once the compound is absorbed"
- The plasma profile of pamoic acid following administration of OP Depot was similar to that of olanzapine. When administered IM as a single agent, pamoic acid was also readily absorbed, underwent very little metabolism, and was excreted largely unchanged via bile into the feces.
- The general toxicity of OP Depot was evaluated in rats and beagle dogs. In the pivotal repeat-dose toxicity studies, OP Depot was given intramuscularly to rats once every 4 weeks for 3 months and to dogs once every 2 weeks for 6 months. Due to limitations in dose volume and suspendability, systemic toxicity was not elicited with OP Depot. In the OP Depot studies, lower exposures to olanzapine were achieved as compared to the oral dosage form.
- Injection site reactions indicative of chronic inflammation were the major finding in all toxicology studies with OP Depot, in both rats and dogs. The reaction in dogs was more pronounced, appearing within a few days after administration and diminishing in a week or 2 thereafter. Histologic evidence of chronic inflammation and fibrosis was present at necropsy; the inflammation persisted, though significantly reduced, after a 2-month recovery period.
- Pamoic acid (the formulation agent) did not exert systemic toxicity. Injection site reactions from the pamoic acid-treated animals were less frequent and less severe than those from animals treated with OP Depot.
- Pamoic acid genetic toxicity testing showed reproducibly positive results in the in vitro chromosome aberration assay in Chinese hamster ovary (CHO) cells (at concentrations >500 μg/ml, about 1000-fold greater than the peak plasma concentrations in humans). However, the absence of oncogenicity in the 2-year carcinogenicity study in rats supports a lack of genotoxic hazard to humans.
- There was no carcinogenic effect attributable to either OPM or pamoic acid in the 2-year rat carcinogenicity study. Olanzapine pamoate monohydrate was administered by once per 4 weeks intramuscular injections at doses of 0 (vehicle), 0 (pamoic acid), 5, 10, and 20 mg /kg for males and 0, 0, 10, 25, and 50 mg /kg for

females (on a mg/m2 basis, HD was equivalent to 0.5 and 1.2x the MRHD of 405 mg/ 4 weeks, or to 0.3 and 0.8x the MRHD of 300 mg/ 2 weeks, for males and females, respectively). Pamoic acid alone was assessed in parallel at i.m. doses similar to those administered in the high-dose OPM group (37 mg/kg in males and 92.5 mg/kg in females).

- Embryo/fetal studies in rats and rabbits from dams treated with OPM Depot formulation during gestation [i.m. doses of 10, 25 and 75 mg/kg on gestation day 6 (rat) or 7 (rabbit) with plasma exposures maintained throughout the period of organogenesis] showed no OPM- or pamoic acid-related maternal systemic toxicity, embryo/fetotoxicity (as indicated by the lack of effect on embryo/fetal intrauterine growth and survival) or increased incidence of structural malformations up to the maximum feasible dose tested (75 mg/kg).
- Changes in behavioral development of rat offspring (a delay in negative geotaxis in the early postnatal period, a lack of habituation to the startle response in F1 males and a reduced performance on memory trials in water maze) were seen at the highest dose (75 mg/kg) employed in a prenatal/postnatal study with OPM Depot given to dams on gestations days 6 and 16 and again on post-partum day 4. These effects are qualitatively similar to the transient decrease in Figure-8 maze activity observed in the 2-generation study conducted with oral olanzapine (NDA 20-592, as cited by the sponsor), and therefore suggest no new risk due to olanzapine pamoate monohydrate use.
- Relatively low olanzapine exposures could be achieved with OP Depot in the nonclinical studies, which resulted in low nonclinical-to-clinical exposure multiples. However, this should not be considered a safety issue since the safety of systemic olanzapine was adequately evaluated by previous nonclinical studies with the approved oral and rapid-acting IM olanzapine formulations. The sustained long-term systemic plasma exposure to olanzapine upon OP Depot administration was not associated with systemic toxicity, and monitorable clinical signs were observed in the tested nonclinical species.
- Systemic exposure to the pamoate ion (assayed as pamoic acid) at the highest doses administered in the animal studies (resulting primarily in injection site reactions only), approximated or exceeded human levels of pamoic acid. Exposure multiples (C_{max}) for pamoic acid ranged from 0.5 to 0.7 (dogs) and from 5 to 6 (rats) in repeat-dose toxicology studies; 4 and 39 in rabbit and rat embryo-fetal studies, respectively; and 4 to 10 in the 2-year rat carcinogenicity study.

Unresolved toxicology issues (if any): None

Recommendations: Approvable

Suggested labeling:
We generally agree with the proposed labeling. The safety factor under 13.1 "Carcinogenesis, Mutagenesis, Impairment of Fertility" (1st paragraph, line 12) needs to be changed from (b) (4) (b) (4) to "…equivalent to 0.3 (males) and 0.8 (females) times the maximum recommended human dose of 300 mg every 2 weeks on a mg/m2 basis".

APPENDIX/ATTACHMENTS

Attachment 1

Executive CAC meeting minutes for rat protocol dose selections

Executive CAC
Meeting date: 3/11/03

Committee: Joseph Contrera, Ph.D., HFD-901, Acting Chair
Al DeFelice, Ph.D., HFD-110, Alternate Member
Karen Davis Bruno, Ph.D., HFD-510, Alternate Member
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.

The committee did not address the sponsor's proposed statistical evaluation for the 2-yr carcinogenicity bioassay, as this does not affect the sponsor's ability to initiate the bioassay. The sponsor may seek guidance on the statistical evaluation of bioassay results from agency staff separately. Data files should be submitted electronically following section E of the "Guidance for Industry, Providing Regulatory Submission in Electronic Format, New Drug Application."

IND #60,701
Drug Name: olanzapine pamoate i.m. depot
Sponsor: Eli Lilly and Co

Rat Carcinogenicity Protocol: the sponsor has proposed a 2-yr bioassay to be conducted in Fischer 344 rats. Olanzapine pamoate is to be administered monthly at doses of 0, 5, 10, and 20 mg/kg to males and females. The sponsor based selection of the HD on microscopic findings at the injection site in a 3-mo i.m. depot study in Fischer 344 rats, taking into account limitations in dosing volume.

Executive CAC Recommendations and Conclusions: the ExeCAC concurred with the sponsor's dose-selection for males (i.e., 0, 5, 10, and 20 mg/kg), but recommended higher doses in females (0, 10, 25, and 50 mg/kg). This recommendation was based on decreases in body weight gain observed in mid-dose and high-dose males and high-dose females in the 3-month i.m. depot study. The ExeCAC also recommended that a pamoic-acid only control group (for males and females) be added to the protocol in order to be able to assess the tumorigenic potential of pamoic acid alone.

The sponsor should be informed that if histological evaluation of tissues from only control and high dose treatment groups is planned, histopathologic examination of other dose groups will be needed under any of the following circumstances: (a) for any macroscopic findings in the low and mid dose groups for a given tissue, that tissue will need to be examined microscopically in all dose groups, (b) for an increase (even if not statistically significant) in the incidence of tumors (rare or common) in the high dose group for a tissue, that tissue would need to be examined microscopically at the next lower dose group, (c) for an increase in tumors in an organ for a tumor type that should be analyzed across tissue sites as well as by tissue site (e.g., hemangiosarcoma, lymphoma etc. [cf. McConnell et al, JNCI 76:283, 1986]), all relevant tissues

should be examined for that dose level and the next lower dose level, (d) for an excessive decrease in body weight (relative to controls) or survival in the examined dose group, lower dose groups should be examined.

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Joseph Contrera, Ph.D.
Acting Chair, Executive CAC

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/Division File, HFD-120
/LMFreed, HFD-120
/SHardemanPM, 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/

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PHARMACOLOGIST

Barry Rosloff
2/7/2008 05:02:42 PM
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