CENTER FOR DRUG EVALUATION AND RESEARCH

APPLICATION NUMBER:

209321Orig1s000

NON-CLINICAL REVIEW(S)

MEMORANDUM

DEPARTMENT OF HEALTH & HUMAN SERVICES
Public Health Service
Food and Drug Administration

Division of Neurology Products (HFD-120)
Center for Drug Evaluation and Research

Date: April 30, 2019
From: Lois M. Freed, Ph.D.
Supervisory Pharmacologist

Subject: NDA 209-321 (Ruzurgi, amifampridine, 3,4-diaminopyridine)

NDA 209-321 was originally received on August 11, 2017, for amifampridine (3,4-diaminopyridine, 3,4-DAP) (b) (4) The CMC and clinical (except for the final TQT study report) modules were provided in the August 11, 2017, submission. The remainder of the application (including the nonclinical modules) was received on December 7, 2017. Based on a preliminary review of the NDA, a Refuse-to-File (RTF) letter was issued (January 31, 2018), based on CMC and nonclinical deficiencies. The nonclinical deficiency was the lack of adequate plasma exposure data for the 6-month dietary toxicity study. The resubmission after RTF was received on June 15, 2018, and the NDA was filed as a Priority application (Filing Communication, August 14, 2018), with no filing review issues identified. Clinical development was conducted by the NDA sponsor (Jacobus Pharmaceuticals) under IND 54313.

The nonclinical studies conducted to support clinical development and the NDA for 3,4-DAP consist of primary and secondary pharmacology, PK/ADME, and subchronic and chronic oral toxicity (up to 6 and 9 months in duration in rat and dog, respectively) studies and a standard genetic toxicology battery. If the NDA is approved, reproductive and developmental toxicology and carcinogenicity studies are to be conducted post-approval, as agreed to by the Division (see Pre-NDA Meeting Minutes, March 17, 2016).

The nonclinical data have been reviewed by Dr. Banks-Muckenfuss (Pharmacology/Toxicology NDA Review and Evaluation, NDA 209321, Melissa Banks-Muckenfuss, Ph.D., April 18, 2019). Based on that review, Dr. Banks-Muckenfuss recommends (b) (4)

The sponsor's nonclinical pharmacology studies confirm published studies identifying 3,4-DAP as a nonselective blocker of voltage-gated potassium channels (IC_{50} values of 187.6-1575.1 µM after 15 min of exposure in mammalian cells expressing K*v* 1-3). In an in vitro phrenic nerve-diaphragm mouse (female C57BL/6J) preparation, 3,4-DAP, but not the major human circulating

metabolite, N-(4-amino-pyridin-3-yl)acetamide (3-Ac-DAP), facilitated neuromuscular transmission consistent with potassium channel blockade. In an in vitro receptor binding/enzyme inhibition panel, neither 3,4-DAP nor 3-Ac-DAP demonstrated ≥50% binding or inhibition at 10 µM.

The PK/ADME evaluation of 3,4-DAP was minimal; only in vitro studies assessing effects on transporters and CYP-450 enzymes, serum protein binding, and metabolic stability were conducted. Serum protein binding was low for both 3,4-DAP and 3-Ac-DAP (25.3 and 43.3%, respectively). When 3,4-DAP (10 µM) was incubated with hepatocytes from mouse, rat, monkey, and human, metabolic stability was highest in mouse, with 80.9, 19.7, 12.5, and 62.6% 3,4-DAP remaining after 120 min. (No other information, e.g., strain or sex, was provided for the hepatocytes used.) 3,4-DAP was not metabolized in vitro by dog hepatocytes.

Dose-ranging and pivotal toxicity studies of 3,4-DAP were conducted in C57BL mouse, Sprague-Dawley rat, and Beagle dog. Studies in mouse consisted of two 14-day dietary palatability and toxicity (non-GLP) studies and a 28-day dietary toxicity (GLP) study. Studies in rat consisted of dose-ranging (7- and 14-day) studies, 14- and 28-day studies, and a 6-month (+ 1-month recovery) study; 3,4-DAP was administered in the diet in all studies. Studies in dog consisted of a 10-day dose-ranging study and a 9-month (+1-month recovery) study; 3,4-DAP was administered orally as a capsule in both studies. The studies in mouse were conducted to support dose selection for a future carcinogenicity study and will not be discussed further.

In rat, the primary drug-related findings were reduced body weight (relative to control) and food consumption in all studies. In the first 14-day study (non-GLP), one male at 150 mg/kg and all animals (5/sex/group) at 450 and 1500 mg/kg were terminated on Day 4 because of excessive body weight and food consumption effects; the actual doses were estimated to be 127-110, 41-81, and 114-40.9 mg/kg, respectively. In the second 14-day study (non-GLP) and the 28-day and 6-month (GLP) studies (intended doses of 0, 15,45, and 135 mg/kg), excessive body weight effects (≥10% decrease in body weight relative to control) were consistently observed at the highest dose tested (135 mg/kg; actual dose ranged from ~100 to 170 mg/kg), except for high-dose males (8%) in the 6-month study, possibly due to the lower dose actually administered (~100 mg/kg). The 7-day (GLP) study (0, 15, 45, and 135 mg/kg) was conducted to address the lack of adequate toxicokinetic (TK) data in the pivotal studies. Similar to the previous studies, reduced body weight (relative to control) and food consumption were the primary findings. The TK data (Day 7) are summarized in the following table:

INTENDED (ACHIEVED) DOSE	**MALES**			**FEMALES**		
	T_{max} (hr)	**C_{max} (ng/mL)**	**$AUC_{(0-t)}$ (ng*hr/mL)**	**T_{max} (hr)**	**C_{max} (ng/mL)**	**$AUC_{(0-t)}$ (ng*hr/mL)**
3,4-DAP						
15 (18.80)	7	11.9	176	5	13.6	200
45 (57.26)	5	83.2	1110	5	152	1650
135 (121.04)	5	780	10600	7	757	14100
3-Ac-DAP						
15 (18.83)	5	683	10700	5	580	9540
45 (55.24)	5	2160	33100	5	2010	28500
135 (118.80)	7	3540	62300	7	2700	54300

In dog, the primary drug-related findings were convulsions and reduced body weight (relative to control). In the 10-day dose-ranging (non-GLP) study, 3,4-DAP was orally (capsule) administered to two dogs (M, F) at rising doses of 0.065 to 2.1 mg/kg BID, with a 4-day washout between doses. At 2.1 mg/kg BID, both animals were sacrificed because of severe clinical signs, including sustained convulsions. Two additional animals, administered a single morning dose of 1.3 mg/kg, exhibited severe clinical signs (no convulsions) and the rising-dose phase was terminated. In the multiple-dose phase, animals (2/sex/group) were administered 3,4-DAP at doses of 0, 0.26, and 1.05 mg/kg BID. The only findings were transient clinical signs (Day 1 only) at the high dose (HD) and increased liver weight and hepatocellular vacuolation at both doses in males and in HD females. In the 9-month (GLP) study, 3,4-DAP was administered orally (capsule) at doses of 0, 0.065, 0.26, 0.52, and 1.05 mg/kg BID. Following the second daily dose on Day 1, 4 HD animals (2M, 2F) were sacrificed after exhibiting severe clinical signs, including sustained convulsions; clinical signs in survivors including abnormal gait, convulsions, tremors, and prostration. Dosing was stopped at the HD on Day 2, and the group was terminated on Day 100. The only other finding of note was a decrease in body weight, relative to control, of 12% in males at 0.52 mg/kg BID. TK data (mean ± SD) for 3,4-DAP (Day 273) are summarized in the following table (levels of 3-Ac-DAP were <LOQ):

DOSE (mg/kg BID)	**MALES**				**FEMALES**			
	T_{max} (hr)	**C_{max} (ng/mL)**	**$AUC_{(0-t)}$ (ng*hr/mL)**	**$t_{1/2}$ (hr)**	**T_{max} (hr)**	**C_{max} (ng/mL)**	**$AUC_{(0-t)}$ (ng*hr/mL)**	**$t_{1/2}$ (hr)**
0.13	7	35.9±8.55	190±51.0	3.65	1	34.9±10.0	187±66.1	2.20±0.555
0.52	4.5	108±19.0	740±131	2.88±0.340	7	112±13.1	758±101	2.73±0.205
1.04	4	197±36.5	1440±272	2.72±0.291	4	215±36.2	1280±169	2.69±0.320

(b) (4)

the information provided in the sponsor's Clinical Pharmacology Summary, the C_{max} and $AUC_{(0\text{-}last)}$ for 3,4-DAP at 30 mg in adults (fed state) are 63.1±39.2 ng/mL and 210.9±121.8 ng*hr/mL, respectively; for metabolite, 3-Ac-DAP, the values are 265.1±104.5 and 1740.3±5904 ng/mL, respectively. No plasma exposure data were provided for children. The most concerning toxicity (convulsions) in animals was observed only in dog, indicating that the parent compound is responsible. Plasma C_{max} in dog at the highest dose not associated with convulsions is ~3 times that in adults at the maximum recommended single dose of 3,4-DAP. This is a known risk for 3,4-DAP (e.g., Lindquist S, Stangel M. Neuropsychiatr Dis Treat 7:341-349, 2011). According to the sponsor, seizures have been reported in the compassionate use program for 3,4-DAP (summarized in the sponsor's Summary of Clinical Safety).

A battery of genetic toxicology studies was conducted for 3,4-DAP. 3,4-DAP was negative in the Ames assay and in in vivo mouse micronucleus and chromosomal aberration assays. 3,4-DAP was positive for clastogenicity (increased small colonies) in the in vitro mouse lymphoma *tk* assay, in the absence of metabolic activation (24-hr incubation). Metabolite, 3-Ac-DAP was negative in in vitro (Ames and mouse lymphoma *tk*) assays.

Reproductive and developmental toxicology studies and carcinogenicity studies were not conducted for 3,4-DAP. The Division agreed that, based on the seriousness of the indication, these studies may be conducted post-approval if the NDA is approved.

Impurities: During the review period, two potentially genotoxic impurities (b) (4) were identified as substantive review issues (Late Cycle Meeting Background Package, November 30, 2018), based on internal (Q)SAR evaluations. The sponsor demonstrated both impurities were negative in adequately conducted Ames assays.

Conclusions and Recommendations

An incomplete battery of nonclinical studies was submitted to support the NDA for 3,4-DAP for treatment of Lambert-Eaton myasthenic syndrome. However, the Division previously agreed that the reproductive and developmental toxicology and carcinogenicity studies may be conducted post-approval. Dr. Banks-Muckenfuss has concluded that the nonclinical data support approval for the treatment of LEMS (b) (4) according to the meeting minutes, discussion of the nonclinical response at the meeting included the following:

> "Considering the intended patient population(s), it is possible that some portions of the nonclinical program may be submitted post-approval. It would be expected, however, that the genotoxicity studies will be completed as soon as possible and that the planned chronic toxicity studies in rodent and non-rodent will be completed prior to NDA submission…"

Therefore, the sponsor was informed that only the genotoxicity and chronic toxicity studies would need to be included in the NDA. In the more recent pre-NDA meeting (minutes dated March 17, 2016), there were no questions on or discussion of juvenile animal toxicology studies; the Division confirmed that reproductive and developmental toxicity and carcinogenicity studies could be conducted post-approval. It is my understanding that the clinical team has concluded the clinical data are adequate to support the safety and effectiveness of 3,4-DAP for the treatment of LEMS in pediatric patients.

A juvenile animal study should be conducted post-approval because some of the developmental endpoints assessed (particularly long-term effects on learning and memory) cannot be fully evaluated in humans during clinical development.

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/s/

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

PHARMACOLOGY/TOXICOLOGY NDA REVIEW AND EVALUATION

Application number:	209321
Supporting documents:	1, 5, 9, 14, 25
Applicant's letter date:	8/11/17; 6/15/18; 10/17/18; 11/13/18; 3/6/19
CDER stamp date:	8/11/17; 6/15/18; 10/17/18; 11/13/18; 3/6/19
Product:	3,4-DAP (3,4-diaminopyridine, amifampridine)
Indication:	Lambert Eaton Myasthenia
Applicant:	Jacobus Pharmaceutical Co. Inc.
Review Division:	DNP
Reviewer:	Melissa Banks-Muckenfuss, PhD
Supervisor:	Lois Freed, PhD
Division Director:	Billy Dunn, MD
Project Manager:	Michelle Mathers, MS MBA

Disclaimer

Except as specifically identified, all data and information discussed below and necessary for approval of NDA 209321 are owned by Jacobus Pharmaceutical Company or are data for which Jacobus Pharmaceutical Company has obtained a written right of reference. Any data or information described or referenced below from reviews or publicly available summaries of a previously approved application are for descriptive purposes only and are not relied upon for approval of NDA 209321.

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1 Executive Summary

1.1 Introduction

Jacobus Pharmaceuticals, Inc. has developed 3-4-DAP (3,4-diaminopyridine, amifampridine), a potassium channel blocker, for (b) (4) treatment of Lambert-Eaton Myasthenia.

1.2 Brief Discussion of Nonclinical Findings

3,4-DAP has generally been accepted as a potassium channel blocker for decades (Kirsch & Narahashi, 1978; Judge & Bever, 2006), although there has been some debate in recent literature (Wu et al., 2009). In the in vitro assays conducted by the sponsor, 3,4-DAP was shown to block a variety of voltage-gated potassium channels (with IC_{50}s ranging from 188-1575 mcM). 3,4-DAP was also shown to weakly inhibit the norepinephrine transporter (IC_{50}= 230 mcM), and major human metabolite 3-Ac-DAP showed weak effects on muscarinic receptors, as an antagonist of M_3 (IC_{50}= 2500 mcM) and an agonist of M_4 and M_5 (EC_{50}= 150 and 200 mcM, respectively).

In vitro, 3,4-DAP is metabolized to 3-Ac-DAP in mouse, rat, monkey, and human hepatocyte preparations; dog hepatocytes did not form 3-Ac-DAP. In vivo, metabolite 3-Ac-DAP was shown to be a major human metabolite; rodents, but not dogs, also showed in vivo exposure to 3-Ac-DAP. The sponsor conducted a 7-day bridging TK study in adult rats to estimate systemic exposures to 3,4-DAP and 3-Ac-DAP in the 6-month dietary administration toxicity study in rat. The estimated exposures achieved in the 6-month study in rats suggested that 3,4-DAP and 3-Ac-DAP were adequately assessed compared to the estimated AUC exposures at the MRHD in humans. Doses in the 9-month study in adult dogs were limited by a steep dose-response for CNS toxicity; C_{max} exposures at the HD, which showed severe CNS toxicity, were approximately 3- to 4-fold those after a 30 mg dose in humans. At the maximum dose tested for the full 9-month duration (i.e., the HMD of 1.04 mg/kg/day given BID), the C_{max} was approximately 1.5-fold that after a 30 mg dose in humans. The AUC margin at the HMD in dogs compared to the estimated AUC at the MRHD was approximately 1.5- to 1.7-fold.

Doses in the subchronic and chronic toxicity studies were primarily limited by body weight reductions in mice and rats and adverse CNS effects (e.g., convulsions) in dogs. In the 6-month dietary toxicity study in rats, drug-related reductions in body weight gain were observed at 45 mg/kg (calculated averages of 32 and 42 mg/kg in males and females, respectively) and 135 mg/kg (calculated averages of 101 and 135 mg/kg in males and females, respectively). The NOAELs were the HD in males and the MD in females, based on mean body weight reduction. In the 9-month dog toxicity study, the HD (2.1 mg/kg/day, given BID) resulted in adverse CNS effects (e.g., sustained convulsions) and early sacrifice of 4 HD animals; therefore, administration of the HD was discontinued. In HMDM (i.e., 1.04 mg/kg/day BID), mean body weight was reduced approximately 11% compared to controls at the end of the dosing period, and showed

partial recovery. The NOAELs were 0.52 mg/kg/day in males (based on mean body weight reductions exceeding 10%), and 1.04 mg/kg/day in females (based on severe CNS signs at 2.1 mg/kg/day, in females and males).

Reproductive and developmental toxicology studies and the carcinogenicity assessment have not been conducted; it was previously agreed upon that these may be submitted post-approval (EoP2 Meeting Minutes, dated 6/17/14). A juvenile animal toxicology study has not been conducted to support the use of 3,4-DAP in children; this should be conducted Phase 4.

3,4-DAP was negative in an Ames assay and in the in vivo mouse micronucleus and chromosomal aberration assays. 3,4-DAP was positive in an in vitro mouse lymphoma assay, showing increases in both small and large colonies (with a proportionally larger increase in small colonies). Major metabolite 3-Ac-DAP was negative in the in vitro Ames and mouse lymphoma assays.

The sponsor identified several potential impurities, including: (b) (4) [redacted] were considered positive for potential mutagenicity in an internal (Q)SAR assessment; however, Ames assays were conducted for these impurities and both were negative for mutagenicity.

1.3 Recommendations

1.3.1 Approvability

From a nonclinical perspective, the application is recommended for approval in the treatment of Lambert-Eaton Myasthenia (b) (4) [redacted]

1.3.2 Additional Nonclinical Recommendations

PMRs

1) A full reproductive toxicology battery (i.e., fertility, embryofetal development, and pre- and postnatal development studies) should be conducted in Phase 4.
2) A carcinogenicity assessment (i.e., a 2-year rat bioassay and a mouse carcinogenicity study) should be conducted in Phase 4; these studies should include TK.
3) A juvenile animal toxicology study should be conducted in Phase 4.

1.3.3 Labeling

The following suggestions are provided for labeling.

———INDICATIONS AND USAGE———

RUZURGI is a potassium channel blocker …

8 USE IN SPECIFIC POPULATIONS
8.1 Pregnancy
Risk Summary

…

(b) (4)

…

12 CLINICAL PHARMACOLOGY
12.1 Mechanism of Action

The mechanism by which amifampridine exerts its therapeutic effect in LEM patients has not been fully elucidated. Amifampridine is a broad spectrum potassium channel blocker.

13 NONCLINICAL TOXICOLOGY
13.1 Carcinogenesis, Mutagenesis, and Impairment of Fertility

Carcinogenesis

Carcinogenicity studies of amifampridine have not been conducted.

Mutagenesis

Amifampridine was negative in vitro in a bacterial reverse mutation assay and in vivo in mouse micronucleus and chromosomal aberration assays. Amifampridine was positive in an in vitro mouse lymphoma tk assay in the absence of metabolic activation.

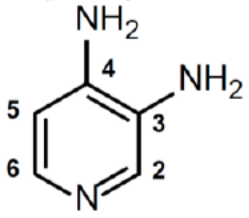
Impairment of Fertility

(b) (4)

2 Drug Information

2.1 Drug

CAS Registry Number	54-96-6
Generic Name	amifampridine
Chemical Name	3,4-diaminopyridine, 3,4-DAP 3,4-pyridinediamine
Molecular Formula/Molecular Weight	$C_5H_7N_3$; 109.13 g/mol
Structure or Biochemical Description	(from the sponsor's submission)



Pharmacologic Class	potassium channel blocker

2.2 Relevant INDs, NDAs, BLAs and DMFs

IND 54,313

2.3 Drug Formulation

3,4-DAP is formulated as 10 mg tablets, which are comprised of the following (below from the sponsor's submission):

Table 1 Composition of 3,4-Diaminopyridine, 10 mg Tablets

Component and Quality Standard (and Grade, if Applicable)	Function	(b) (4) Tablet Batch		10 mg Tablet	
		Quantity Per Unit (g)	%	Quantity Per Unit (mg)	%
3,4-DAP (free base)	Active pharmaceutical ingredient	1000	4.05	10.0	4.05
Microcrystalline cellulose, NF (b) (4)					
Dibasic calcium phosphate, USP					
Sodium starch glycolate, USP					
Colloidal silicon dioxide, NF					
Magnesium stearate, NF (b) (4)					
Total unit dose					

3,4-DAP = 3,4-Diaminopyridine; NF = National Formulary; USP = United States Pharmacopeia; (b) (4)

(b) (4)

2.4 Comments on Novel Excipients

None.

2.5 Comments on Impurities/Degradants of Concern

The sponsor identified several potential process and degradant impurities, including: (b) (4)

(b) (4)

(b) (4)

in the sponsor's (Q)SAR assessment but were positive in the Agency's internal (Q)SAR assessment. Ames assays were conducted on these two impurities; both demonstrated a lack of mutagenic potential.

2.6 Proposed Clinical Population and Dosing Regimen

3,4-DAP is proposed for (b) (4) Lambert-Eaton Myasthenia (LEM, also referred to as Lambert-Eaton myasthenic syndrome or LEMS), in patients (b) (4)

The proposed recommended starting (b) (4) administered 2 to 3 times per day, titrated in 5 to 10 mg increments until the optimal dose is reached. The proposed maximum recommended total daily dose is (b) (4) mg in divided doses over 24 hours (b) (4) with no single dose to exceed 30 mg.

(b) (4)

2.7 Regulatory Background

Jacobus initially submitted NDA 209321 for 3,4-diamopyridine for the treatment of Lambert Eaton myasthenic syndrome on December 5, 2017, but the NDA was not filed (letter dated January 31, 2018). After discussion with the Agency, the sponsor addressed the issues, and the NDA was resubmitted (6/15/18).

3 Studies Submitted

3.1 Studies Reviewed

Pharmacology and PK studies

Dose-ranging and acute/subacute studies in rats, mice, and dogs

Study 20149900: A 7-day Study of 3,4-DAP by Dietary Administration in Rats

Study 20049262: A 6-month Study of 3,4-DAP by Dietary Administration in Rats with a 1-month Recovery Period

Study 20055756: A 9-month Study of 3,4-DAP by Oral Capsule Administration in Dogs with a 1-month Recovery Period

4 Pharmacology

4.1 Primary Pharmacology

The binding of 3,4-DAP (1 to 3000 micromolar (mcM)) at Kv1.1, Kv1.2, Kv1.3, Kv1.4, Kv1.5, Kv2.1, Kv3.2, Kv3.4, Kv4.3/KChIP2.2, and hERG channels expressed in HEK293 or CHO cells was evaluated and compared to that for 4-aminopyridine (Study 160428.KBD; non-GLP). For the voltage-gated potassium channels, the IC_{50} ranged from 188 to 1575 mcM; the IC_{50}s for comparator 4-AP ranged from 41 to 2129 mcM. The IC_{50} at the hERG channel was >3000 mcM for 3,4-DAP and 1866 to 2401 mcM for 4-AP. Metabolite 3-Ac-DAP was not tested in these binding assays; however, a lack of clear binding at the hERG and Kv (assessed by inhibition of α-dendrotoxin) channels was observed in the secondary pharmacology assay.

Supporting pharmacology information was also submitted in an in vitro murine neuromuscular junction study report. A study By R. Maselli (date not provided) studied the effects of 3,4-DAP and 3-Ac-DAP (0.1, 1, 10, or 100 mcM) on left hemi-diaphragm muscle with attached phrenic nerve isolated from C57BL/6J mice. The results of the study showed that 3,4-DAP, but not metabolite 3-Ac-DAP, enhanced action potential-evoked neurotransmitter release, particularly in conditions in which the likelihood of release was low.

4.2 Secondary Pharmacology

Standard receptor binding assays were conducted for 3,4-DAP and 3-Ac-DAP. A binding assay of 3,4-DAP and 3-Ac-DAP (each at 10 mcM) at a total of 44 receptor, ion channel, transporter, and/or enzyme sites demonstrated no secondary activity. A second assay of 44 sites focused on sites related to abuse potential, showed inhibition of binding activity at adrenergic receptors (α1A, α2A, and α2B), muscarinic receptors (M1, M2, M3, M4, and M5), and the NE transporter for 1 mM 3,4-DAP, as well as inhibition of binding at muscarinic receptors (M3, M4, and M5) for 1 mM 3-Ac-DAP. In subsequent assays, 3,4-DAP and 3-Ac-DAP were shown to have relatively weak affinities and functional (antagonist and/or agonist) activities at these receptors (see the sponsor's summary Tables 4, 5, and 6, below).

Table 4 Cerep Drug Abuse Potential Profile: Binding Affinity of 3,4-DAP and 3-Ac-DAP at Receptors and Transporters Relevant to Abuse Potential

Receptor	Assay	Ligand Type	3,4-DAP[a] K_i (μM)	3-Ac-DAP[b] K_i (μM)
Adrenergic (NE)	α_{1A}	Antagonist	330	ND
	α_{2A}	Antagonist	84	ND
	α_{2B}	Antagonist	500	ND
Muscarinic (ACh)	M_1	Antagonist	410	ND
	M_2	Antagonist	760	ND
	M_3	Antagonist	140	450
	M_4	Antagonist	100	550
	M_5	Antagonist	69	180
NE transporter	NE transporter	Antagonist	470	ND

3-Ac-DAP = *N*-(4-amino-pyridin-3-yl)acetamide; 3,4-DAP = 3,4-diaminopyridine; ACh = acetylcholine; K_i = inhibition constant; NE = norepinephrine; ND = not determined.
[a] 3,4-DAP is listed as RS-30-13 in the study report.
[b] 3-Ac-DAP is listed as RS-85-5 in the study report.

Table 5 Cellular Functional Activity of 3,4-DAP

Receptor	Assay	Antagonist IC_{50} (μM)	Agonist EC_{50} (μM)	% Agonist Response at 3 mM
Adrenergic (NE)	α_{1A}	>1000	2500	60%
	α_{2A}	>1000	Inactive	-2%
	α_{2B}	>10000	Inactive	10%
Muscarinic (ACh)	M_1	>1000	2800	55%
	M_2	6400	Inactive	16%
	M_3	>1000	2700	56%
	M_4	Inactive	NC	52%
	M_5	Inactive	Inactive	0%
NE transporter	NE transporter	230	-	-

3,4-DAP = 3,4-diaminopyridine; ACh = acetylcholine; EC_{50} = half maximal effective concentration; IC_{50} = half maximal inhibitory concentration; NC = not calculated; NE = norepinephrine.
Note: 3,4-DAP is listed as RS-30-13 in the study report

Table 6 Cellular Functional Activity of 3-Ac-DAP

Receptor	Assay	Antagonist IC_{50} (μM)	Agonist EC_{50} (μM)	% Agonist Response at 3 mM
Muscarinic (ACh)	M_3	2500	Inactive	15%
	M_4	Inactive	150	46%
	M_5	Inactive	200	42%

3-Ac-DAP = *N*-(4-amino-pyridin-3-yl)acetamide.
Note: 3-Ac-DAP is listed as RS-85-5 in the study report.

4.3 Safety Pharmacology

Dedicated safety pharmacology studies were not conducted. Binding at the hERG channel was evaluated in the pharmacology studies for both 3,4-DAP and 3-Ac-DAP, but the studies were not standard GLP hERG assays. However, a human TQT study was conducted.

5 Pharmacokinetics/ADME/Toxicokinetics

5.1 PK/ADME

Several in vitro ADME studies were provided.

In vitro incubation of radiolabeled 3,4-DAP in hepatocyte preparations from mice, rats, dogs, monkeys, and humans showed that the major human metabolite (3-Ac-DAP) was formed in mouse (7-15% of total radioactivity (TRA); $t_{1/2}$ >120 minutes), rat (33-77% of TRA; $t_{1/2=}$ 52 minutes), and monkey hepatocytes (30-80% of TRA; $t_{1/2=}$ 50 minutes), as well as human hepatocytes (24-55% of TRA; $t_{1/2}$ >120 minutes). 3,4-DAP was metabolically stable in dog hepatocyte preparations (91-92% of TRA remained as 3,4-DAP). An in vitro human hepatocyte cytotoxicity assay did not clearly show cell loss in incubations with 3,4-DAP or 3-Ac-DAP (up to 200 mcM).

Cytochrome P450 enzymes were not shown to play a role in the metabolism of 3,4-DAP. 3-Ac-DAP was not metabolized in human liver microsomes. 3,4-DAP and 3-Ac-DAP (0, 0.1, 1, 4, 10, 40, 100, and 200 mcM) did not show inhibition of cytochrome P450 isoforms CYP1A2, CYP2B6, CYP2C8, CYP2C9, CYP2C19, CYP2D6, or CYP3A4 in human liver microsomes. 3,4-DAP (up to 10 mcM) did not clearly demonstrate induction of CYP1A2, CYP2B6, or CYP3A4. An in vitro assay of 3,4-DAP (1 mcM) in the presence of N-acetyl-transferase enzymes, NAT-1 and NAT-2, showed that 3,4-DAP was rapidly metabolized in the presence of NAT-2 and was metabolized to a lesser extent, and less rapidly, in the presence of NAT-1.

In vitro assays to assess the permeability of 3,4-DAP and 3-Ac-DAP (up to 200 mcM) in human Caco-2 cells showed mixed results; overall, the studies suggested that 3,4-DAP and 3-Ac-DAP were unlikely substrates or inhibitors at P-gp transporters. Neither 3,4-DAP nor 3-Ac-DAP inhibited the BCRP transporter. In vitro, neither 3,4-DAP and 3-Ac-DAP (1, 10, and 50 mcM) inhibited OAT1, OAT3, OCT2, OATP1B1 or OATP1B3, with the exception of 3,4-DAP at OCT2 (31% inhibition at 200 mcM). 3,4-DAP and 3-Ac-DAP were not shown to be substrates at OAT1, OAT3, or OCT2 transporters.

In vitro, the half-lives of 3,4-DAP and 3,4-Ac-DAP in human plasma were 335 and 262 hr, respectively, and human plasma protein binding was approximately 25% and 43%, respectively.

In vivo ADME was studied in a dedicated dietary administration TK study in rats (see **Toxicokinetics**, below) and in the toxicity studies conducted in animals.

5.2 Toxicokinetics

Study 20149900: A 7-day Study of 3,4-DAP by Dietary Administration in Rats
Conducted by (b) (4)

Drug: 3,4-DAP (3,4-diaminopyridine), batch 1250 (4800J), 99.9% pure

The sponsor stated that stability analyses were previously performed (Study 20049259), demonstrating that the drug is stable in the vehicle (i.e., diet mixture) when prepared and stored under the same conditions at concentrations bracketing those used in the present study.

Animals: Sprague Dawley (b) (4) CD(SD) rats (b) (4)
8 weeks old
M: 246-279 g; F: 169- 217 g
Animals were individually housed.

Diet: PMI Nutrition International Certified Rodent Chow® Meal No. 5CR4 (14% protein)

Summary design (see Text Table 1 from the sponsor, below)

Text Table 1
Experimental Design

Group No.	Test Material	Target Dose Level (mg/kg/day)[a]	Dose Concentration (PPM)	Number of Animals Main Study	
				Males	Females
1	Control Article[b]	0	0	8	8
2	3,4-DAP	15	195	8	8
3	3,4-DAP	45	585	8	8
4	3,4-DAP	135	1755	8	8

PPM = parts per million.
[a] Approximate value based on a 260-gram rat consuming 20 grams of test diet/day.
[b] PMI Nutrition International Certified Rodent Chow® Meal No. 5CR4 (14% protein).

Dose formulation analysis indicated that all formulations used for dosing were within 20% of the nominal concentrations. The average achieved doses (based on food consumption and body weight) are provided in sponsor's Text Table 2, below.

Text Table 2
Average Mean Test Article Consumption (mg/kg/day)

Group No.	Target Dose Level (mg/kg/day)	Calculated Group Mean Dose Level (mg/kg/day)	
		Main Study	
		Males	Females
2	15	18.80	18.83
3	45	57.26	55.24
4	135	121.04	118.80

No mortality occurred in the study. Brown staining and/or wet fur in the urogenital region and red skin on the tail occurred in two to five HD animals on D8. Drug-related body weight loss and/or reduced body weight gain were observed in HDM, HDF, and MDF; on D8, average body weights were 14%, 9%, and 4.5%, respectively, lower than those of controls. Drug-related reductions in food consumption (up to 33%) were observed at the HD.

On Day 7, blood samples were collected from the jugular vein for plasma analysis of 3,4-DAP and 3-Ac-DAP (N=4/sex/group/time point) at 0500 (approximately 1 hr prior to lights on), 0700, 0900, 1300, 1700 (approximately 1 hr prior to lights out); a final sample was obtained on Day 8 at 0100. TK parameters calculated included C_{max}, T_{max}, and AUC.

The sponsor reported that plasma concentrations of 3,4-DAP were LLOQ in the control samples, but 23 of 48 control samples showed low concentrations of metabolite 3-Ac-DAP (0.698 to 7.19 ng/mL, compared to 54.5 to 657 ng/mL in drug-treated animals). The reason for the contamination was not identified. In drug-treated animals, C_{max} was observed between 0500 and 0700. Systemic exposures to 3,4-DAP and 3-Ac-DAP increased with increasing dose, in a greater-than-dose-proportional (3,4-DAP) and dose-proportional (3-Ac-DAP, between 15 and 45 mg/kg) or less-then-dose-proportional (3-Ac-DAP, between 45 and135 mg/kg) fashion. No clear sex difference was observed. See the sponsor's summary TK data, below.

Table 2.1: Summary 3,4-DAP Toxicokinetic Parameters in Sprague Dawley Rat Plasma Following Dietary Administration (ad libitum) of 3,4-DAP on Day 7

Analyte	Gender	Dose (mg/kg/day)	T_{max} (hr)	C_{max} (ng/mL)	SE C_{max} (ng/mL)	C_{max}/D (ng/mL/(mg/kg))	$AUC_{(0-t)}$ (hr*ng/mL)	SE $AUC_{(0-t)}$ (hr*ng/mL)	$AUC_{(0-t)}$/D (hr*ng/mL/(mg/kg))
3,4-DAP	Male	15	7	11.9	0.461	0.794	176	10.2	11.7
		45	5	83.2	13.0	1.85	1110	92.0	24.6
		135	5	780	109	5.78	10600	754	78.8
	Female	15	5	13.6	2.92	0.908	200	9.60	13.3
		45	5	152	38.1	3.37	1650	204	36.7
		135	7	757	226	5.61	14100	2540	105

Note: Clock times 0500, 0700, 0900, 1300, 1700, and 0100 (Day 8) were assumed as 5, 7, 9, 13, 17, and 24 hours on Day 7, respectively.
SE = Standard error.

Table 2.2: Summary 3-Ac Toxicokinetic Parameters in Sprague Dawley Rat Plasma Following Dietary Administration (ad libitum) of 3,4-DAP on Day 7

Analyte	Gender	Dose (mg/kg/day)	T_{max} (hr)	C_{max} (ng/mL)	SE C_{max} (ng/mL)	C_{max}/D (ng/mL/(mg/kg))	$AUC_{(0-t)}$ (hr*ng/mL)	SE $AUC_{(0-t)}$ (hr*ng/mL)	$AUC_{(0-t)}$/D (hr*ng/mL/(mg/kg))
3-Ac	Male	15	5	683	27.7	45.5	10700	478	710
		45	5	2160	55.0	48.1	33100	1020	735
		135	7	3540	419	26.3	62300	5190	461
	Female	15	5	580	49.6	38.7	9540	415	636
		45	5	2010	198	44.6	28500	1550	633
		135	7	2700	334	20.0	54300	2700	402

Note: Clock times 0500, 0700, 0900, 1300, 1700, and 0100 (Day 8) were assumed as 5, 7, 9, 13, 17, and 24 hours on Day 7, respectively.
SE = Standard error.

6 General Toxicology

6.2 Repeat-Dose Toxicity

MOUSE

Three dietary studies were conducted in mice.

Non-GLP 14-day dietary palatability and toxicity studies were conducted in CD-1 mouse, testing nominal doses up to 1500 mg/kg/day. In the palatability study, moribund euthanasia, body weight losses (13-25%; D2), and clinical signs (e.g., increased/decreased activity, suspected dehydration, reduced fecal output/size, and/or hunched posture) occurred at ≥ 450 mg/kg/day; at 150 mg/kg/day, one female was sacrificed early due to body weight loss (22%; D13). Body weight reductions were also observed at ≥ 150 mg/kg/day in surviving animals (up to 8% in males and 15% in females); these were associated with reduced food consumption. Calculated doses based on average food consumption exceeded the nominal doses of 150 mg/kg/day (actual 168 – 212 mg/kg/day), 450 mg/kg/day (actual 735 – 805 mg/kg/day), and 1500 mg/kg/day (actual 2255 – 2476 mg/kg/day). In the toxicity study, transient body weight losses and reduced body weight gains resulted in reduced mean body weights compared to control at ≥ 45 mg/kg/day in females (~7-10% on D8, ~4-1% on D15) and 135 mg/kg/day in males (~7% on D8, ~4% on D15); this primarily occurred in the first week of dosing. Increased serum potassium (~1.4x control [ss]) was observed in HDM on Day 16. Calculated doses based on average food consumption exceeded the nominal doses of 15 mg/kg/day (21 – 35 mg/kg/day), 45 mg/kg/day (71 – 92 mg/kg/day), and 135 mg/kg/day (223 – 309 mg/kg/day), with the dose in females

exceeding that in males. Mean plasma concentrations for 3,4-DAP and metabolite 3-Ac-DAP showed increased exposures with increased doses (F > M at HD); mean plasma exposure to metabolite 3-Ac-DAP exceeded that of 3,4-DAP.

A GLP 28-day toxicity study in CByB6F1 hybrid mouse was conducted by dietary administration (0, 15, 45, and 135 mg/kg/day). No mortality was observed, but drug-related transient body weight losses and reductions in mean body weight (as much as 9%) were seen at 135 mg/kg. Reduced reticulocyte counts (~25% [ss] in females; ~7% in males [nss]) and increased TBIL (~30% [ss] in females) were reported at 135 mg/kg/day on Day 30/31. Absolute liver and spleen weights were reduced at HD (approximately 10-20%), and uterus and thymus weights were also reduced in HDF (approximately 35%); no clearly drug-related histopathological alterations were observed. Calculated doses based on average food consumption exceeded the nominal doses (i.e., approximately 18-29 mg/kg/day, 45-79 mg/kg/day, and 161-270 mg/kg/day).

RAT

The sponsor conducted non-GLP 14-day dietary palatability and toxicity studies in rat.

The palatability study tested doses ranging from 15 to 1500 mg/kg by dietary administration in Sprague Dawley rats (SD rats; 5/sex/gp). One male at 150 mg/kg and all animals at 450 and 1500 mg/kg were sacrificed on Day 4 because of reduced body weight and food consumption. Mean body weights were 10%, 23%, and 25% (males) and 14%, 21%, and 28% (females) lower than controls at 150, 450, and 1500 mg/kg, respectively, on Day 15 (150) or Day 4 (450 and 1500). The NOAEL was 45 mg/kg (calculated dose based on average food consumption was approximately 40-41 mg/kg).

The toxicity study tested doses of 15, 45, and 135 mg/kg 3,4-DAP (calculated doses based on average food consumption were 16-17 mg/kg, 50 mg/kg, and 154-170 mg/kg) in SD rats (5/sex/gp). Reduced body weight gains were observed, particularly in the first week at 135 mg/kg (93% and 45% during W1 and W2 in males, and 86% and 56% during W1 and W2 in females); on Day 15, mean body weight compared to controls was reduced 21% in males and 13% in females at 135 mg/kg. The altered body weights correlated with reduced food consumption in the first week of the study. Reduced adipose tissue was observed in one HDF. Mean plasma concentrations of 3,4-DAP and 3-Ac-DAP increased with increasing dose, and no clear sex difference was observed. The NOAEL for the study was 45 mg/kg.

Study 20049261: A 28-day Dietary Toxicity Study of 3,4-DAP in Rats with a 14-day Recovery Period. Conducted by (b) (4) (FDA); initiated 5/30/14

Animals: Sprague Dawley (b) (4) CD(SD) rats (b) (4)
Drug: 3,4-DAP, lot 1250, 99.6% pure
Doses: 0, 15, 45, and 135 mg/kg
(calculated doses of approximately 15-17, 46-51, and 123-139 mg/kg)
Diet: PMI Nutrition International Certified Rodent Chow® Meal No. 5CR4 (14% protein)

Summary design table from the sponsor's submission, below.

Group No.	Test Material	Target Dose Level (mg/kg/day)[a]	Dose Concentration (PPM)	No. of Animals			
				Main Study		Recovery Study	
				Males	Females	Males	Females
1	Control Article	0	0	10	10	5	5
2	3,4-DAP	15	195	10	10	-	-
3	3,4-DAP	45	585	10	10	-	-
4	3,4-DAP	135	1755	10	10	5	5

PPM = parts per million; - = not applicable.
[a] Approximate value based on a 260 gram rat consuming 20 grams of test diet/day.

Satellite TK groups (4/sex/gp) were also used, but only mean plasma concentrations were obtained. Animals were individually housed. Dose formulations were approximately 93% to 114% of the nominal concentrations.

No mortality was observed. Clinical signs including yellow and/or brown staining of the urogenital area fur, ungroomed fur, and red fur staining around the areas (which were considered signs of "general malaise") were observed at the HD. Reduced body weights (i.e., losses and/or reduced gains) were observed at the MD in females and at the HD in both sexes, particularly in the first two weeks. Overall, reduced body weight gains were observed in MDF (29%), and HD animals (44%); the final mean body weights (compared to controls) were reduced approximately 9% in MDF, 12% in HDF (maximum observed 17%, at D15), and 17% in HDM (maximum observed 18% at D15) on Day 29. These reductions correlated with food consumption at the HD and showed recovery.

A few clinical and anatomical pathology alterations were observed. RBC parameters were slightly reduced (5% to <10%) in HD animals. Reticulocytes were increased in HDF (~80%) and HDM (34%). Serum glucose was reduced in HD animals (approximately 20%). Cholesterol (approximately 25-40%) and phosphorus (approximately 15-20%) were increased in MD and HD males. Liver weights were reduced (15%) in HDM; no histopathological correlates were observed. Minimal cardiomyopathy was observed in 1 of 10 HDF. Uterine dilatation was observed in 1 HDF (marked) and 1 recHDF (moderate), while mild dilatation was observed in 1 ConF. Minimal to mild mandibular salivary gland hypertrophy (characterized by the pathologist as "an increased amount of pale staining basophilic cytoplasm resulting in an increased size of the cells of the serous acini") was observed at the HD but showed recovery. The NOAEL was 45 mg/kg, based on reduced body weights.

Study title: A 6-month Study of 3,4-DAP by Dietary Administration in Rats with a 1-month Recovery Period

Study no.:	20049262 (Sponsor: (b) (4) 6Month_Rat)
Study report location:	EDR, SDN1
Conducting laboratory and location:	(b) (4)
Date of study initiation:	1/13/15
GLP compliance:	Yes (FDA), except Drug characterization (GMP)
QA statement:	Yes
Drug, lot #, and % purity:	3,4-DAP (3,4-diaminopyridine), Batch 1250 (3057J), 99.6% pure

Methods (See study design tables below, from the sponsor)

Route of administration:	Diet (PMI Nutrition International Certified Rodent Chow® Meal No. 5CR4 (14% protein))
Species/Strain:	Sprague Dawley (b) (4) CD(SD) rats (b) (4) 8 weeks old M: 226 – 278 g; F: 171-245 g
Age:	8 weeks
Weight:	M: 226- 278 g; F: 171-245 g
Satellite groups:	Recovery: as in sponsor's table below TK: 5/sex/group
Unique study design:	Animals were individually housed to accurately assess food consumption/ drug dose.
Deviation from study protocol:	Several deviations were noted, but did not affect the integrity of the study. On Day 93, plasma samples for bioanalysis were taken at the wrong time and were therefore not used for TK evaluation.

Text Table 1
Experimental Design

Group No.	Test Material	Target Dose Level (mg/kg/day)[a]	Dose Concentration (PPM)	No. of Animals			
				Main Study		Recovery Study	
				Males	Females	Males	Females
1	Control Article[b]	0	0	15	15	5	5
2	3,4-DAP	15	195	15	15	-	-
3	3,4-DAP	45	585	15	15	-	-
4	3,4-DAP	135	1755	15	15	5	5

PPM = parts per million; - = not applicable.
[a] Approximate value based on a 260 gram rat consuming 20 grams of test diet/day.
[b] PMI Nutrition International Certified Rodent Chow® Meal No. 5CR4 (14% protein).

- Approximate doses achieved in males were 11, 32, and 101 mg/kg/day. Approximate doses achieved in females were 14, 42, and 135 mg/kg/day.

Observations and Results

Mortality

There was no clearly drug-related mortality.

Clinical Signs

Dose-related clinical signs were observed, including: thin fur, signs of stress, and red fur-staining (muzzle, face), and yellow fur-staining (anal, urogenital; M) at ≥ LD (and some controls); ungroomed fur, and brown fur-staining (anal, urogenital) at ≥ MD; and wet fur and black fur-staining (abdominal) at HD. Brown fur-staining was observed in 2 HD recovery animals (1M, 1F).

Body Weights

Drug-related body weight losses and/or reduced gains were observed at ≥ MD, resulting in overall body weight gain reductions of approximately 15% in MD and HD males, and 10 to 35% in 3,4-DAP-treated females. On D182, mean body weights compared to controls were reduced 8% and 9% at MD and HD (respectively) in males, and 3%, 10%, and 15% at LD, MD, and HD, respectively, in females.

Increased body weight gains were observed during the recovery period in HDF.

Food Consumption

Transient, dose-related reductions in food consumption (up to 25% in W1, [ss] at HD) were observed primarily during the first month of treatment.

Ophthalmoscopy

Ocular examination was conducted by a board-certified veterinary ophthalmologist (W. Greentree, DVM, DACVO). No drug-related alterations were reported.

Hematology [parameters assessed, below, from the sponsor]

Red blood cell count Hemoglobin concentration Hematocrit Mean corpuscular volume Red blood cell distribution width Mean corpuscular hemoglobin concentration Mean corpuscular hemoglobin Reticulocyte count (absolute) Platelet count	White blood cell count Neutrophil count (absolute) Lymphocyte count (absolute) Monocyte count (absolute) Eosinophil count (absolute) Basophil count (absolute) Large unstained cells Other cells (as appropriate)
Activated partial thromboplastin time Fibrinogen	Prothrombin time

Slightly increased (≤10%) RBC parameters and slight reductions in reticulocytes (up to 20% in M) were observed in MDM and HDM. The sponsor reported no drug-related changes.

Clinical Chemistry [parameters assessed, below, from the sponsor]

Alanine aminotransferase	Total protein
Aspartate aminotransferase	Albumin
Alkaline phosphatase	Globulin (calculated)
Gamma-glutamyltransferase	Albumin/globulin ratio
Creatine kinase	Glucose
Total bilirubin	Cholesterol
Urea nitrogen	Triglycerides
Creatinine	Sodium
Calcium	Potassium
Phosphorus	Chloride

Slightly reduced serum triglycerides (25-40%) were observed in HDM and HDF. Slightly increased serum calcium (~3%) was observed in MDM and HDM.

Urinalysis [parameters assessed, below, from the sponsor]

Color	Protein
Appearance/clarity	Glucose
Specific gravity	Bilirubin
Volume	Ketones
pH	Blood

No drug-related changes were observed.

Gross Pathology

The incidence of thin hair coat was increased in HDF (7 vs. 2 Control).

Organ Weights

Drug-related increases in adrenal weight and decreases in liver weight were observed at ≥ MD; see the sponsor's Test Table 3, below. The pathologist stated that these changes were without microscopic correlate.

Text Table 3
Summary of Organ Weight Data – Terminal Euthanasia (Day 183/184)

	Males			Females		
Group	2	3	4	2	3	4
Dose (mg/kg/day)	15	45	135	15	45	135
No. Animals per Group	15	15	15	15	15	15
Adrenal gland (No. Weighed)[a]	13	15	15	15	14	15
Absolute value	5.194	13.858	15.429	0.509	-4.624	-8.716
% of body weight	6.266	**24.365**	**24.412**	2.145	3.996	5.014
% of brain weight	2.148	**15.875**	**14.936**	0.672	-4.077	-7.510
Liver (No. Weighed)	13	15	15	15	14	15
Absolute value	-4.697	**-12.303**	**-16.123**	-2.000	-8.350	**-13.148**
% of body weight	-4.097	-4.469	**-10.334**	0.492	1.198	1.062
% of brain weight	-7.717	-10.744	**-16.473**	-2.047	-8.064	**-12.231**

[a] All values expressed as percent difference of control group means.
Based upon statistical analysis of group means, values highlighted in bold are significantly different from control group – $p \leq 0.05$; refer to data tables for actual significance levels and tests used.

Histopathology

Adequate Battery	Yes; Con and HD only, plus target organs
Separate, Signed Report	Yes, R. Long, DVM, DACVP
Peer Review	No

Histological Findings
Drug-related minimal to mild salivary gland hypertrophy was reported at the HD. See the sponsor's Text Table 5, below. The pathologist described the alteration as, "… characterized by an increased amount of pale staining basophilic cytoplasm resulting in an increased size of the cells of the serous acini of the mandibular salivary gland…" and attributed the change to "… some alteration in the autonomic control of [secretion] release from the mandibular salivary gland." This finding showed recovery.

Text Table 5
Summary of Microscopic Findings – Terminal Euthanasia (Day 183/184)

	Males				Females			
Group	1	2	3	4	1	2	3	4
Dose (mg/kg/day)	0	15	45	135	0	15	45	135
No. Animals Examined	15	13	15	15	15	15	14	15
Gland, salivary (No. Examined)	15	13	15	15	15	15	14	15
Hypertrophy	(0)[a]	(0)	(0)	(11)	(0)	(0)	(0)	(9)
Minimal	-	-	-	6	-	-	-	5
Mild	-	-	-	5	-	-	-	4

[a] Numbers in parentheses represent the number of animals with the finding.
"-" indicates the severity is not applicable because there were no animals with the finding or the tissue was not examined in the group.

Additionally, minimal to marked uterine dilatation occurred with increased incidence and severity (see table, below). Minimal cardiomyopathy showed slightly increased incidence in HDM (7 vs. 4 ConM) at the end of the dosing period but showed higher incidence in control males at the end of the recovery period (3/5 recConM vs. 1/5 HDM).

	Terminal					Recovery	
	ConF	LDF	MDF	HDF		ConF	HDF
Uterus, dilatation	3	-	-	9		1	2
Minimal	1	-	-	5		1	1
Mild	2	-	-	2		0	0
Moderate	0	-	-	2		0	0
Marked	0	-	-	0		0	1

- = not examined

Toxicokinetics [table below, from the sponsor]

Group No.	Subgroup	No. of Males/ Females	Sample Collection Time Points[a]				
			Day 1[b]	Day 2[c]	Day 93[c]	Day 100[c]	Day 182
1 to 4	A	3/3	X	X	X	X	X
	B	2/2[d]	-	-	-	-	-

X = sample collected; - = not applicable.

[a] Sample was collected as soon as practical after the lights turned on for the day, estimated between the time of 0600 and 0700 hours.

[b] Sample was collected prior to dose initiation on Day 1.

[c] Sample was collected approximately 24 hours after food was offered, as soon as practical after the lights turned on for the day.

[d] Animals in Subgroup B were dosed; however, blood was not collected from these animals unless blood was unable to be collected from animals in Subgroup A.

Sampling was not adequate to determine toxicokinetics. Plasma samples were analyzed for 3,4-DAP and metabolite 3-Ac-DAP using a validated analytical procedure. Mean plasma concentrations of 3,4-DAP and metabolite 3-Ac-DAP increased with increasing dose of 3,4-DAP. Mean plasma concentrations were generally greater than dose proportional for 3,4-DAP.

	3,4-DAP (ng/mL)				Metabolite 3-Ac-DAP (ng/mL)			
	D1	D2	D100	D182	D1	D2	D100	D182
MALES								
0	BQL	BQL	BQL	BQL	BQL	BQL	BQL	BQL
15/ **11**	BQL	6.38	11.2	5.79	BQL	497	375	269
45/ **32**	BQL	35.0	57.6	51.8	BQL	1360	1330	1220
135/ **101**	BQL	267	346	475	BQL	2690	2130	2670
FEMALES								
0	BQL	BQL	BQL	BQL	BQL	BQL	BQL	BQL
15/ **14**	BQL	10.5	13.0	9.69	BQL	468	389	303
45/ **42**	BQL	42.2	58.9	21.5	BQL	1320	1140	686
135/ **135**	BQL	83.7	862	359	BQL	1460	2390	2350

(calculated average dose in bold)

Drug Consumption Analysis

Based on food consumption data, the actual doses approximated the nominal doses in females but were lower than expected in males. See the table from the sponsor, below.

Text Table 2
Average Mean Test Article Consumption (mg/kg/day)

Group No.	Target Dose Level (mg/kg/day)	Calculated Group Mean Dose Level (mg/kg/day)			
		Main Toxicity Study		Toxicokinetic	
		Males	Females	Males	Females
2	15	10.85	13.85	10.83	13.93
3	45	31.48	42.74	33.20	41.49
4	135	100.72	134.76	107.42	135.01

The sponsor's stated NOAELs, the HD in males and the MD in females (based on reduced body weights in HDF), are reasonable.

DOG

Study 20062977: A Rising-dose and Multiple-dose Tolerance Study of 3,4-DAP by Oral Capsule Administration in Dogs (non-GLP)

Conducted by [redacted] (b) (4)

Study design (tables from the sponsor)

Text Table 1
Experimental Design for the Rising-dose Study

Group No.	Test Material	Cycle	Dose Level (mg/kg/dose)	Dose Level (mg/kg/day)	No. of Animals Rising-dose Study	
					Males	Females
1	3,4-DAP	1	0.065	0.13	1	1
		2	0.13	0.26		
		3	0.26	0.52		
		4	0.52	1.04		
		5	1.05	2.1		
		6	2.1	4.2		
		7	1.3[b]	1.3	1[a]	1[a]

[a] Due to the unscheduled euthanasia of the male and female following Cycle 6, the alternate animals were assigned to the rising-dose phase of the study.
[b] During Cycle 7, animals received only the AM dose due to clinical signs resulting in a single dose of only 1.3 mg/kg/day.

Text Table 2
Experimental Design for the Multiple-dose Study

Group No.	Test Material	Dose Level (mg/kg/dose)	Dose Level (mg/kg/day)	No. of Animals Multiple-dose Study	
				Males	Females
1	Empty Capsule	0	0	2	2
2	3,4-DAP	0.26	0.52	2	2
3	3,4-DAP	1.05	2.1	2	2

In the rising-dose phase, both animals given 4.2 mg/kg/day were euthanized following adverse CNS signs (e.g., sustained and non-sustained convulsions, hyper-reactivity, slight to severe tremors, decreased activity, lying on side). A single dose of 1.3 mg/kg was also followed by clinical signs that were considered adverse, including: salivation,

labored breathing, coughing, and excessive licking and pawing at face. The remainder of the daily doses was not administered.

All animals survived the multiple-dose phase. Adverse clinical signs observed at 2.1 mg/kg/day included abnormal gait, decreased activity, retching, incoordination, coughing, sneezing, tremors, salivation, and labored breathing. Dose-related weight losses were observed through Day 8 and were associated with reduced food consumption. In LDM, HDM, and HDF, increased liver weights were observed, which correlated with mild to moderate hepatocellular vacuolation. Systemic exposure to 3,4-DAP increased with dose and was approximately dose proportional. Maximum plasma concentrations of 3,4-DAP generally occurred at the first collection time point following either the first or second daily dose (i.e., 1 or 7 hours post first daily dose). The half-life ranged from 2.48 to 3.93 hours. Exposure on Day 10 was similar to that on Day 1. No clear sex differences were observed.

Study title: A 9-month Study of 3,4-DAP by Oral Capsule Administration in Dogs with a 1-month Recovery Period

Study no.:	20055756
Study report location:	EDR
Conducting laboratory and location:	(b) (4)
Date of study initiation:	1/8/15
GLP compliance:	Yes (FDA), except: Drug characterization
QA statement:	Yes
Drug, lot #, and % purity:	3,4-DAP, lot 1250 (3057J), 99.6% pure

Methods

Doses:	0, 0.13, 0.52, 1.04, and 2.1 mg/kg/day
Frequency of dosing:	BID (6 hr ±30 min apart)
Route of administration:	Oral, capsule (size #12)
Dose volume:	n/a
Formulation/Vehicle:	n/a
Species/Strain:	Beagle dogs (b) (4)
Number/Sex/Group:	4/sex/gp
Age:	5-6 mo
Weight:	6.0-9.5 kg
Satellite groups:	Recovery: 2/sex/gp
Deviation from study protocol:	Several deviations were reported but did not affect the integrity and/or interpretation of the study.

Observations and Results

Mortality [twice daily]

Four HD animals (2M, 2F) were sacrificed on Day 1 following the second dose because they exhibited sustained convulsions; no histopathologic correlates were reported. Following the first dose at HD, sneezing, partly closed eyes, and repetitive behaviors (i.e., face pawing and head shaking) were observed. Approximately 1.25- 4.5 hr after receiving the second daily dose, nine of the twelve HD animals exhibited clinical signs including: abnormal gait, coughing, partly to completely closed eye(s), incoordination, hyperactivity, tremors, prostration, salivation, sustained or non-sustained convulsions, sneezing, lateral recumbency (i.e., lying on side), dilated pupil(s), and repetitive behaviors (i.e., face pawing and head shaking). Surviving animals recovered by the next day, but dosing of the entire HD group was discontinued on Day 2 and the animals were transferred off of the study (on Day 100). Tissues from the 4 HD animals sacrificed early were examined microscopically; autolysis of tissues involving the abdominal cavity, thoracic cavity, lung, thymus, eyes, brain and/or spinal cord was reported. One HDF had a pale focus in the cardiac portion of the glandular stomach, without correlating microscopic lesion.

Clinical Signs [daily]

Clinical signs were observed in the HD group, as discussed above (see **Mortality**). No other clinical signs were considered clearly drug-related.

Body Weights [at least weekly]

Mean body weight reduction (compared to control) of approximately 11% was observed in HMDM (1.04 mg/kg/day) at the end of the dosing period. Partial recovery was observed.

Food Consumption [daily, quantitative]

Food consumption was slightly (i.e., up to 20%) and variably reduced in LMD (0.52 mg/kg/day) and/or HMD animals, as well as in LDF early in the dosing period.

Ophthalmoscopy

Evaluations were conducted prestudy and on Day 270 by a board-certified veterinary ophthalmologist (W. F. Greentree, DVM, DACVO). Examinations were conducted using a hand-held slit lamp and indirect ophthalmoscope. No drug-related changes were reported.

ECG

Electrocardiogram measurements were obtained from all animals prestudy and on Day 269. Lead II was evaluated by a board-certified veterinary cardiologist (L.B. Lehmkuhl, DVM, MS, DACVIM). QTc was calculated using the Van de Water formula. Heart rate

and QTc interval were reduced in HMDF (see tables below from the sponsor). Note that although Text Table 17 is labeled "Summary of Mean HR Interval Data (msec)," the HR data presented in the summary are identified as heart rate in "bpm" (i.e., beats per minute) in the individual data sheets.

Text Table 17
Summary of Mean HR Interval Data (msec)

	Males				Females			
Dose (mg/kg/day) =	0	0.13	0.52	1.04	0	0.13	0.52	1.04
All dogs								
Before dosing								
Pretest	80	98	96	82	77	99	87	87
Vs. control	---	*+18*	*+16*	*+2*	---	*+22*	*+10*	*+10*
Last dosing week (Day 269)	86	83	85	78	87	96	75	68
Vs. pretest	*+6*	*-15*	*-11*	*-4*	*+10*	*-3*	*-12*	*-19*
Vs. control	---	*-3*	*-1*	*-8*	---	*+9*	*-12*	*-19*
Recovery dogs								
Before dosing								
Pretest	74	91	92	77	74	114	89	83
Last dosing week (Day 269)	80	77	85	86	82	97	77	56
Vs. pretest	*+6*	*-14*	*-7*	*-9*	*+8*	*-17*	*-12*	*-27*
Vs. control	---	*-3*	*+5*	*+6*	---	*+15*	*-5*	*-26*
End of recovery (Day 297)	91	81	92	103	85	98	102	69
Vs. pretest	*+17*	*-10*	*0*	*+26*	*+11*	*-16*	*+13*	*-14*
Vs. control	---	*-10*	*+1*	*+12*	---	*+13*	*+17*	*-16*

Text Table 18
Summary of Mean QTc Interval Data (msec)

	Males				Females			
Dose (mg/kg/day) =	0	0.13	0.52	1.04	0	0.13	0.52	1.04
All dogs								
Before dosing								
Pretest	224	232	237	234	237	236	237	233
Vs. control	---	*+8*	*+13*	*+10*	---	*-1*	*0*	*-4*
Last dosing week (Day 269)	216	217	224	218	231	222	218	207*
Vs. pretest	*-8*	*-15*	*-13*	*-16*	*-6*	*-14*	*-19*	*-26*
Vs. control	---	*+1*	*+8*	*+2*	---	*-9*	*-13*	*-24*
Recovery dogs								
Before dosing								
Pretest	236	232	225	227	225	237	244	233
Last dosing week (Day 269)	211	218	221	218	228	230	218	201
Vs. pretest	*-25*	*-14*	*-4*	*-9*	*+3*	*+3*	*-26*	*-32*
Vs. control	---	*+7*	*+10*	*+7*	---	*+2*	*-10*	*-27*
End of recovery (Day 297)	217	214	212	226	213	217	213	193
Vs. pretest	*−19*	*-18*	*-13*	*-1*	*-12*	*-20*	*-31*	*-40*
Vs. control	---	*+3*	*-5*	*-9*	---	*+4*	*0*	*-20*

* - Statistically different from control (Group 1) at $p < 0.05$.

The reduction in HR and shortened QTc in HMDF resulted primarily from two animals (HR: 47 and 46 bpm; QTC: 191 and 184 msec); data were available for one of these animals after the recovery period, and showed persistence of the effect (HR: 50 bpm and QTc 183 msec). It is noted that a recovery control animal showed a HR of 90 bpm

but a QTc of 199 msec. The contributing scientist indicated that the shortened mean QTc interval (10% or ~24 ms) observed in HMDF was not considered "biologically significant" and was not observed in males.

Clinical Pathology [schedule as below, from the sponsor]

Group Nos.	Time Point	Hematology	Coagulation	Clinical Chemistry	Urinalysis
All animals	Week -1	X	X	X	X
1 to 4	Week 20	X	X	X	X
1 to 4	Week 26	-	-	-	X
1 to 4	Day 274	X	X	X	-
1 to 4	Day 274/275	-	-	-	X[a]
1 to 4	Day 303	X	X	X	X[a]
Unscheduled euthanasia (when possible)	Before euthanasia	X	X	X	X[b]

X = sample collected; - = not applicable.
Note: Alternate Animal No. 23282 was not assigned to study; therefore, pretest data collected for this animal was not reported.
[a] If an adequate urine sample was not collected at scheduled euthanasia via the urine collection cage, then cystocentesis at gross necropsy was used.
[b] Collected by cystocentesis at gross necropsy.

Hematology [parameters measured below, from the sponsor]

Red blood cell count Hemoglobin concentration Hematocrit Mean corpuscular volume Red blood cell distribution width Mean corpuscular hemoglobin concentration Mean corpuscular hemoglobin Reticulocyte count (absolute) Platelet count	White blood cell count Neutrophil count (absolute) Lymphocyte count (absolute) Monocyte count (absolute) Eosinophil count (absolute) Basophil count (absolute) Large unstained cells Other cells (as appropriate)

Activated partial thromboplastin time Fibrinogen	Prothrombin time

Blood smear slides were prepared but not evaluated.

On Day 137, RBC parameters were reduced approximately 10% to 15% and reticulocytes were reduced 40% to 50% in LMDM and HMDM. On Day 274, RBC parameters remained slightly reduced (10% to 15%) in HMDM but showed recovery by Day 303.

Clinical Chemistry [parameters measured below, from the sponsor]

Alanine aminotransferase	Total protein
Aspartate aminotransferase	Albumin
Alkaline phosphatase	Globulin (calculated)
Gamma-glutamyltransferase	Albumin/globulin ratio
Creatine kinase	Glucose
Total bilirubin	Cholesterol
Urea nitrogen	Triglycerides
Creatinine	Sodium
Calcium	Potassium
Phosphorus	Chloride

Phosphorus was reduced 15% in HMDM on Day137. Triglycerides were reduced 30% to 40% in LMDM and HMDM on Day 274.

Urinalysis [parameters measured below, from the sponsor]

Color	Protein
Appearance/clarity	Glucose
Specific gravity	Bilirubin
Volume[a]	Ketones
pH	Blood

[a] If urine was collected by cystocentesis, total volume was not determined.

No drug-related differences were reported.

Gross Pathology

No clearly drug-related changes were observed.

Organ Weights

Thymus weight was reduced (41%) in HMDF and remained reduced (34%) in the recHMDF.

Histopathology

Adequate Battery	Yes
Separate, Signed Report	Yes, C.L. Johnson, DVM, DACVP
Peer Review	No

Histological Findings
One HMDM (#6852) showed focal, minimal degeneration of the white matter of the spinal cord; no related clinical signs were reported. Minimal mononuclear cell infiltration of the salivary gland was observed in a few 3,4-DAP-treated animals (2 LDF, 1 LMDF, 1 HMDF and 1 recHMDF, and 1 HMDM and 1 recHMDM). Mononuclear cell infiltration of the salivary gland has previously been reported as a common spontaneous lesion in

beagle dogs (cf., Morishima et al., 1990). The pathologist did not consider these alterations drug-related.

Toxicokinetics [schedule as below, from the sponsor]

Group No.	Sample Collection Time Points (Time Post First Daily Dose) on Day 1, Week 20, and Day 273							
	0[a] hr	1 hr	2 hr	6 hr[b]	7 hr	8 hr	12 hr	24 hr
1	X	X	X	X	X	X	X	X
2	X	X	X	X	X	X	X	X
3	X	X	X	X	X	X	X	X
4	X	X	X	X	X	X	X	X

x = sample collected.
[a] Sample was collected before the first daily dose.
[b] Sample was collected before the second daily dose.

Plasma samples were collected and analyzed for 3,4-DAP and 3-Ac-DAP using a validated analytical procedure. A number of estimated TK parameters (i.e., T_{max}, C_{max}, C_{max}/D, $AUC_{(0-t)}$, $AUC_{(0-t)}/D$, $AUC_{(0-6h)}$, and $t_{1/2}$) were for D1, W20, and D273 data. $AUC_{(0-t)}$ was calculated from time 0 to the time at which the last quantifiable concentration was observed.

3,4-DAP was not detected in control plasma samples. 3,4-DAP was generally quantifiable throughout the 24 hr sampling period, except in a few LD and LMD animals (3,4-DAP was quantifiable for 12 hr). Maximum 3,4-DAP plasma concentrations were usually observed 1 to 2 hr after the first or second dose. Systemic exposure increased with increasing dose and was approximately dose-proportional. No accumulation or sex differences were observed.

Text Table 19
Summary Mean (± SD) 3,4-DAP Toxicokinetic Parameters in Beagle Dog Plasma Following Oral Administration of 3,4-DAP on Day 1

Analyte	Day	Sex	Dose (mg/kg/day)	T_{max} (hr)	C_{max} (ng/mL)	$AUC_{(0-t)}$ (hr*ng/mL)	$AUC_{(0-6)}$ (hr*ng/mL)	$T_{1/2}$ (hr)
3,4-DAP	1	Male	0.13	5 (1 - 12)	29.8 ± 10.3	231 ± 92.2	83.0 ± 29.3	1.59 ± ID
		Male	0.52	1 (1 - 8)	112 ± 25.6	760 ± 193	298 ± 117	2.71 ± 0.242
		Male	1.04	2 (1 - 12)	170 ± 43.6	1440 ± 231	528 ± 179	2.50 ± 0.232
		Male	2.1	1.5 (1 - 8)	409 ± 255	2370 ± 819	891 ± 592	2.93 ± 0.596
		Female	0.13	1.5 (1 - 8)	32.9 ± 11.2	209 ± 37.7	91.3 ± 29.3	2.93 ± 1.19
		Female	0.52	1 (1 - 8)	100 ± 20.9	581 ± 50.8	286 ± 55.0	3.28 ± 1.67
		Female	1.04	4.5 (1 - 7)	185 ± 44.0	1200 ± 247	481 ± 124	2.58 ± 0.363
		Female	2.1	7 (2 - 7)	500 ± 111	2750 ± 287	1220 ± 186	2.57 ± 0.289

ID = Insufficient Data

Text Table 20
Summary Mean (± SD) 3,4-DAP Toxicokinetic Parameters in Beagle Dog Plasma Following Oral Administration of 3,4-DAP on Day 136

Analyte	Day	Sex	Dose (mg/kg/day)	T_{max} (hr)	C_{max} (ng/mL)	$AUC_{(0-t)}$ (hr*ng/mL))	$AUC_{(0-6)}$ (hr*ng/mL)	$T_{1/2}$ (hr)	R_{AUC}[a]
3,4-DAP	136	Male	0.13	4.5 (1 - 8)	25.2 ± 6.12	140 ± 27.5	57.4 ± 22.7	2.81 ± 1.09	0.696 ± 0.321
		Male	0.52	7 (1 - 8)	99.2 ± 13.2	701 ± 108	231 ± 69.8	2.90 ± 0.471	0.952 ± 0.204
		Male	1.04	7 (1 - 8)	182 ± 37.9	1330 ± 88.1	492 ± 43.8	2.70 ± 0.0837	0.935 ± 0.118
		Female	0.13	4.5 (1 - 7)	33.2 ± 7.31	183 ± 26.5	76.4 ± 20.8	2.90 ± 0.911	0.883 ± 0.0369
		Female	0.52	4 (1 - 7)	113 ± 19.8	713 ± 127	276 ± 53.4	2.68 ± 0.681	1.24 ± 0.252
		Female	1.04	7 (1 - 7)	230 ± 76.7	1410 ± 276	537 ± 92.3	2.60 ± 0.261	1.20 ± 0.249

[a] $AUC_{(0-t)}$ Day 136/ $AUC_{(0-t)}$ Day 1

Text Table 21
Summary Mean (±SD) 3,4-DAP Toxicokinetic Parameters in Beagle Dog Plasma Following Oral Administration of 3,4-DAP on Day 273

Analyte	Day	Sex	Dose (mg/kg/day)	T_{max} (hr)	C_{max} (ng/mL)	$AUC_{(0-t)}$ (hr*ng/mL)	$AUC_{(0-6)}$ (hr*ng/mL)	$T_{1/2}$ (hr)	R_{AUC}[a]
3,4-DAP	273	Male	0.13	7 (1 - 7)	35.9 ± 8.55	190 ± 51.0	84.5 ± 26.4	3.65 ± ID	0.976 ± 0.565
		Male	0.52	4.5 (1 - 8)	108 ± 19.0	740 ± 131	292 ± 68.9	2.88 ± 0.340	1.00 ± 0.195
		Male	1.04	4 (1 - 8)	197 ± 36.5	1440 ± 272	560 ± 40.7	2.72 ± 0.291	1.01 ± 0.240
		Female	0.13	1 (1 - 7)	34.9 ± 10.0	184 ± 66.1	94.6 ± 24.9	2.20 ± 0.555	0.910 ± 0.367
		Female	0.52	7 (1 - 8)	112 ± 13.1	758 ± 101	295 ± 15.1	2.73 ± 0.205	1.32 ± 0.275
		Female	1.04	4 (1 - 7)	215 ± 36.2	1280 ± 169	530 ± 52.4	2.69 ± 0.320	1.10 ± 0.221

[a] $AUC_{(0-t)}$ Day 273/ $AUC_{(0-t)}$ Day 1
ID = Insufficient Data

The sponsor's identified NOAELs of 0.52 mg/kg/day (based on reduced body weight or severe CNS effects and death at the HD) in males and 1.04 mg/kg/day (based on severe CNS effects and death at the HD) in females are reasonable.

7 Genetic Toxicology

7.1 *In Vitro* Reverse Mutation Assay in Bacterial Cells (Ames)

Study title: Bacterial Reverse Mutation Assay of 3,4-Diaminopyridine

Study no.:	232809
Study report location:	EDR
Conducting laboratory and location:	(b) (4)
Date of study initiation:	10/26/10
GLP compliance:	Yes (FDA), except Drug characterization
QA statement:	Yes
Drug, lot #, and % purity:	3,4-DAP, lot No. 3057J, 100.29 % pure

Methods

Strains:	*Salmonella typhimurium* strains, TA98, TA100, TA1535, TA1537 *Escherichia coli* strain WP2 *uvrA*
Concentrations in definitive studies:	0, 0.16, 0.31, 0.63, 1.3, 2.5, and 5.0 mg/plate
Basis of concentration selection:	Concentration limit All concentrations were analyzable. No precipitation was observed.
Negative control:	vehicle
Positive control:	+S9: benzo(a)pyrene or cyclophosphamide, 2-aminoanthracine (WPs uvrA) -S9: MMS (WP2 uvrA) 9-AA (TA1537) Sodium azide (TA100, TA1535) 2-nitrofluorene (TA98)
Formulation/Vehicle:	(b) (4)
Incubation & sampling time:	37 ± 2 C for 48 to 72 hr

Study Validity

Generally, standard methodology was used and was stated to follow the "OECD Guideline for Testing of Chemicals - 471, Bacterial Reverse Mutation Test" (OECD, 1997). Both a plate incorporation assay and a preincubation assay were performed in the presence and absence of metabolic activation (Aroclor 1254-induced rat liver S9 purchased from (b) (4). It was noted that a non-standard positive control, MMS, was used for the E. coli strain in the absence of metabolic activation, but literature exists to support its use (e.g., see Kirkland et al., 2016).

Results- Negative

The sponsor's criteria for a positive response were an increase which exceeded the range of the concurrent negative controls, statistical significance, and demonstration of the "concentration-related increase over the range tested and/or a reproducible increase in at least one or more concentrations."

The sponsor stated that cytotoxicity was not observed in the plate incorporation assay. Although statistically significant increases were observed at 5.0 mg/plate in TA1537 in the absence of metabolic activation and at 1.3 and 5.0 mg/plate in the E. coli strain using the plate incorporation method, the sponsor stated that the results lacked a clear concentration-response and/or the increases were not observed in the preincubation test (i.e., were not reproduced; see review below). See the sponsor's summary tables for TA1537 (-S9) and WP2*uvra* (+S9), with their respective historical controls, below. For TA1537, the result at the maximum concentration was <2-fold the concurrent negative control mean (which was lower than the historical control mean) and the mean was within the range of the historical data (means + SD). For WP2*uvra,* it was noted that the negative control mean in the assay was similar to the negative historical control mean provided and the statistically positive concentrations were within approximately 1.5-fold the concurrent negative control and similar to the negative historical control range provided by the sponsor.

3,4-diaminopyridine mg per plate	Colony counts			Mean ± SD
TA1537, - S9				
0	11	10	9	10 ± 1
0.16	14	11	8	11 ± 3
0.31	11	18	4	11 ± 7
0.63	6	8	10	8 ± 2
1.3	14	14	10	13 ± 2
2.5	21	10	10	14 ± 6
5.0	14	18	18	17 ± 2
9-AA, 100 µg	1449	2060	1965	1825 ± 329
WP2 *uvrA* , + S9				
0	41	43	45	43 ± 2
0.16	41	41	46	43 ± 3
0.31	40	38	36	38 ± 2
0.63	46	38	43	42 ± 4
1.3	56	54	57	56 ± 2
2.5	55	57	41	51 ± 9
5.0	68	68	53	63 ± 9
2-AMA, 100 µg	290	269	268	276 ± 12

Appendix IIIA. Historical Control Data

Historical Negative Controls -S9, Plate Incorporation

(Average Revertants per Plate ± Standard Deviation)

Date	TA98	TA100	TA1535	TA1537	WP2 *uvrA*
Sep 3, 10	19 ± 2	120 ± 11	17 ± 5	12 ± 4	29 ± 5
June 4, 10	23 ± 3			10 ± 1	30 ± 3
May 19, 10	31 ± 7			15 ± 4	33 ± 3
Apr 28, 10	24 ± 9	116 ± 11	17 ± 4	13 ± 4	32 ± 8
Jan 7, 10					35 ± 6
Dec 23, 09	32 ± 5	118 ± 15	17 ± 4	12 ± 5	14 ± 1
Dec 22, 09	23 ± 2	122 ± 14	23 ± 2	15 ± 4	
July 7, 09	29 ± 5	113 ± 3	19 ± 4	14 ± 3	22 ± 2
n'	7	5	5	7	7
$\overline{M}$	26	118	19	13	28
S_m	5	3	2	2	7
$S_{\overline{M}}$	2	2	1	1	3
Range	22-30	115-121	17-21	12-14	23-33

n' - number of assays
$\overline{M}$ - combined mean
S_m - standard deviation
$S_{\overline{M}}$ - standard error
Range - with 95% confidence

Historical Negative Controls +S9, Plate Incorporation

(Average Revertants per Plate ± Standard Deviation)

Date	TA98	TA100	TA1535	TA1537	WP2 *uvrA*
Aug 27, 10	36 ± 3	131 ± 10	12 ± 3	14 ± 5	51 ± 5
Apr 29, 10	36 ± 2	139 ± 18	16 ± 2	25 ± 3	46 ± 8
Jan 26, 10			19 ± 7		
Jan 13, 10			19 ± 3		
Jan 7, 10					48 ± 10
Jan 6, 10		148 ± 7			
Dec 30, 10	44 ± 10	130 ± 8	15 ± 4	22 ± 4	
Dec 24, 09	34 ± 2		19 ± 6	20 ± 4	36 ± 2
July 10, 09	39 ± 4	140 ± 12	15 ± 6	15 ± 4	37 ± 5
n'	5	5	7	5	5
$\overline{M}$	38	138	16	19	44
S_m	4	7	3	5	7
$S_{\overline{M}}$	2	3	1	2	3
Range	34-42	131-145	14-18	15-23	38-50

n' - number of assays
$\overline{M}$ - combined mean
S_m - standard deviation
$S_{\overline{M}}$ - standard error
Range - with 95% confidence

A second assay was conducted using the preincubation method. The sponsor stated that in the presence of metabolic activation slight to moderate cytotoxicity was observed "only at the highest concentration," with slight reduction in the background lawn observed "occasionally under a microscope" at 2.5 mg/plate; however, the sponsor stated that all concentrations were analyzable because although the background lawn showed toxicity "the numbers of colonies per plate were not reduced from the concurrent control levels." Statistically significant increases in revertant colonies occurred in TA98 at 0.63 mg/plate and in WP2*uvra* at 0.625 mg/plate in the presence of metabolic activation (see the sponsor's summary tables, below). The assay was considered negative since no clear dose-response was evident. The statistically positive concentration in WP2*uvra* lacked a clear concentration-response and was within the historical negative control range.

3,4-diaminopyridine mg per plate	Colony counts			Mean ± SD
TA98, + S9				
0	32	26	40	33 ± 7
0.16	38	36	28	34 ± 5
0.31	40	33	33	35 ± 4
0.63	46	47	48	47 ± 1
1.3	25	37	28	30 ± 6
2.5	27	22	24	24 ± 3
5.0	37	44	31	37 ± 7
B[α]P, 5 µg	566	669	579	605 ± 56
WP2 *uvrA* , + S9				
0	37	42	41	40 ± 3
0.16	36	40	42	39 ± 3
0.31	43	42	43	43 ± 1
0.63	44	51	48	48 ± 4
1.3	41	56	44	47 ± 8
2.5	47	51	31	43 ± 11
5.0	31	31	38	33 ± 4
2-AMA, 100 µg	294	274	285	284 ± 10

Appendix IIIB. Historical Control Data

Historical Negative Controls +S9, Plate Incorporation

(Average Revertants per Plate ± Standard Deviation)

Date	TA98	TA100	TA1535	TA1537	WP2 *uvrA*
Aug 27, 10	36 ± 3	131 ± 10	12 ± 3	14 ± 5	51 ± 5
Apr 29, 10	36 ± 2	139 ± 18	16 ± 2	25 ± 3	46 ± 8
Jan 26, 10			19 ± 7		
Jan 13, 10			19 ± 3		
Jan 7, 10					48 ± 10
Jan 6, 10		148 ± 7			
Dec 30, 10	44 ± 10	130 ± 8	15 ± 4	22 ± 4	
Dec 24, 09	34 ± 2		19 ± 6	20 ± 4	36 ± 2
July 10, 09	39 ± 4	140 ± 12	15 ± 6	15 ± 4	37 ± 5
n'	5	5	7	5	5
$\overline{M}$	38	138	16	19	44
S_m	4	7	3	5	7
$S_{\overline{M}}$	2	3	1	2	3
Range	34-42	131-145	14-18	15-23	38-50

n' - number of assays
$\overline{M}$ - combined mean
S_m - standard deviation
$S_{\overline{M}}$ - standard error
Range - with 95% confidence

7.2 *In Vitro* Assays in Mammalian Cells

Study title: Mammalian Gene Mutation Assay of 3,4-Diaminopyridine in Mouse Lymphoma Cells

Study no.:	232807
Study report location:	EDR
Conducting laboratory and location:	(b) (4)
Date of study initiation:	10/26/10
GLP compliance:	Yes, except drug characterization
QA statement:	Yes
Drug, lot #, and % purity:	3,4-diaminopyridine, Lot #3057J, 100.29%

Methods

Cell line:	mouse lymphoma cell line L5178Y $TK^{+/-}$ -3.7.2.C
Concentrations in definitive study:	4hr, -S9: 100, 200, 275, 300, 325, 350, 375, 400, 425, and 450 mcg/mL 4 hr, +S9: 200, 300, 400, 450, 475, 500, 525, 550, 575, 600, 625, 650, and 675 mcg/mL 24hr, -S9: 75, 125, 150, 175, 200, 225, 250, 275, 300, 325, 350, and 375 mcg/mL
Basis of concentration selection:	10-20% RTG (cytotoxicity) Small intervals were used based on the steep cytotoxicity curve in preliminary testing
Negative control:	cell culture grade water
Positive control:	-S9: methyl methanesulfonate (in DMSO) hycanthone (in DMSO) +S9: cyclophosphamide
Formulation/Vehicle:	Dissolved in cell culture grade water
Incubation & sampling time:	Plates were incubated for 10- 12 days at 38 ±2°C

Study Validity

The report indicates that the study was consistent with the OECD Guideline 476, dated 1997. Generally, standard methodology was used. The metabolic activation system

was rat liver S9 purchased from (b) (4) Triplicate plates were used. The sponsor's criterion for a positive result was a "concentration-related increase in mutant frequency or a clearly reproducible increase in mutant frequency at a single concentration," usually with a "minimum increase of two-fold in mutant frequency over a typical background mutant frequency."

Results- Positive

The dosing concentrations were within 10% of nominal concentrations.

In the 4 hr, -S9 test, the RTG was <10% at 400 and 450 mcg/mL. RTG was 21.8% at 375 mcg/mL and 32.4% at 425 mcg/mL. The mutant frequency only exceeded 2-fold in cultures showing excessive cytotoxicity (i.e., 400 and 450 mcg/mL); using the GEF (90 x 10^{-6}), increased mutant frequency was observed only at 450 mcg/mL, RTG= 3.1%.

In the 4hr, +S9 test, the RTG was <10% at 650 (calculations used a correction factor at this concentration since an insufficient number of cells were cloned) and 675 mcg/mL. The mutant frequency exceeded 2-fold the background frequency only in the cultures showing excessive cytotoxicity (i.e., 650 and 675 mcg/mL); the same result was reached using the GEF. At the next highest concentration (625 mcg/mL), the RTG was 45.2%; the induced mutant frequency at this concentration was 1.5-fold the concurrent negative control mean.

In the 24 hr, -S9 test, the RTG was 18.1% at the highest concentration tested (350 mcg/mL). Concentration-related increases in induced mutant frequencies were observed at ≥ 250 mcg/mL. See the sponsor's summary table, below. Evaluation based on the GEF also indicated that the criterion for a positive result was met (≥ 300 mcg/mL). It was noted that the concurrent negative control mean (i.e., average mutant frequency of 63) was at the lowest part of the historical negative control range (i.e., average mutant frequency of 63 to 126, with a mean of 93; see the sponsor's table, below). Both large and small colonies were increased, with proportionally greater increases in the numbers of small colonies (see sponsor's figure, below).

24 Hours Treatment without S9

Table 6: TFT-resistant Colony Counts (TFT^r) and Mutant Frequencies

Code	Dose (µg/mL)	TFT^r counts			Mean $TFT^r \pm SD$	Mutant frequency per 10^6 VC	Induced Mutant Frequency	Fold Induction
0	0	45	60	54	53 ± 8	65	2	1.0
00	0	46	54	48	49 ± 4	64	1	1.0
000	0	42	55	51	49 ± 7	59	-4	0.9
Mean Mutant Frequency ± SD = 63 ± 3								
3	75	50	55	66	57 ± 8	75	13	1.2
5	125	50	63	68	60 ± 9	76	13	1.2
6	150	66	88	70	75 ± 12	98	35	1.6
7	175	49	76	73	66 ± 15	82	20	1.3
8	200	56	95	86	79 ± 20	83	20	1.3
9	225	83	85	107	92 ± 13	120	57	1.9
10	250	122	110	113	115 ± 6	134	72	2.1
11	275	95	104	93	97 ± 6	145	82	2.3
12	300	128	130	123	127 ± 4	192	130	3.1
13	325	132	128	134	131 ± 3	221	159	3.5
14	350	165	162	154	160 ± 6	347	285	5.5
MMS	5	352	402	334	363 ± 35	824	762	13.2
HYC	1	255	229	238	241 ± 13	698	635	11.1
HYC	2	192	195	222	203 ± 17	1078	1015	17.2

All concentration of 3,4-DAP were analyzable for mutagenicity

TFT^r Counts = Trifluorothymidine resistant colonies

VC = Viable Counts

Mutant Frequency (MF) = (10^6 x Mean TFT^r) / (5000 x Mean VC)

Induced Mutant Frequency = Mutant Frequency - Mean of Mutant Frequency of negative controls

Fold Induction = Mutant Frequency / Mean of Mutant Frequency of negative controls

Appendix IVA. Historical Control Data

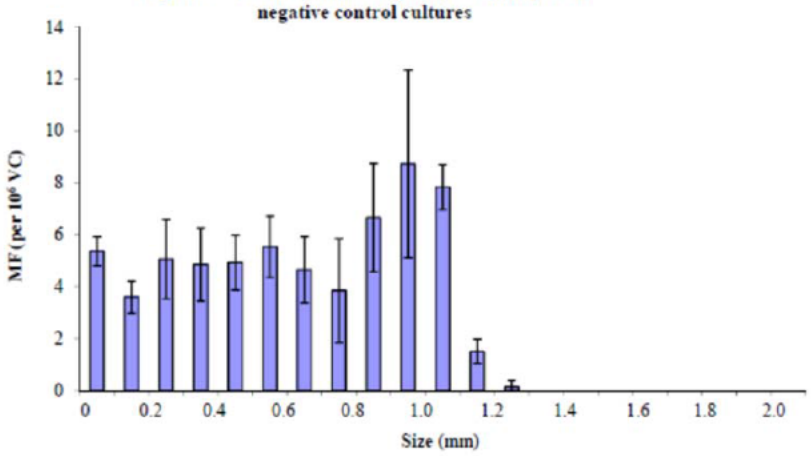
Historical Negative Controls
(Average Mutant Frequency ± Standard Deviation)

Date	Mutant Frequency (per 10^6 Viable Counts)		
	-S9 4 hours	+S9 4 hours	-S9 24 hours
September 8, 04		122 99 123	
July 6, 04			102 63 126
June 23, 04		46 71 61	
June 16, 04	85 73 67		
December 3, 03		39 99	
November 26, 03		88 54 65	
October 22, 03		42 49	
October 1, 03			111 84 73
September 3, 03		94 106 111	
July 2, 03		88 54	
June 25, 03	74 69		
n'	5	18	6
$\overline{M}$	74	78	93
S_m	7	28	24
Range	67-85	39-123	63-126

n' - number of assays
$\overline{M}$ - combined mean
S_m - standard deviation
Range - minimum to maximum

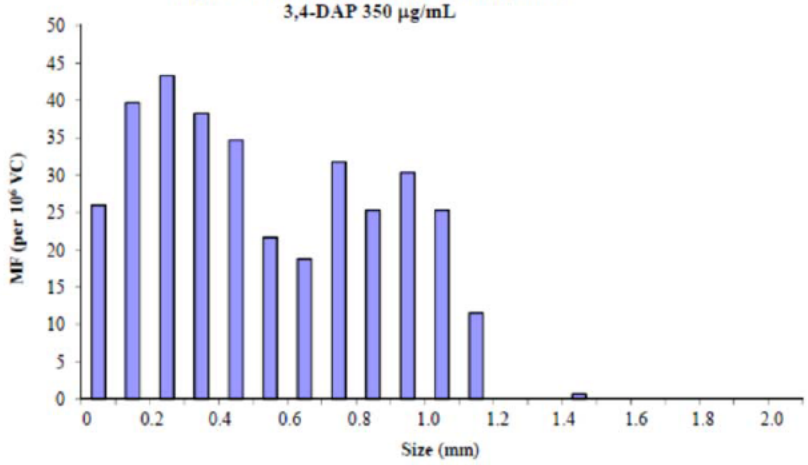
24 Hours Treatment without S9

Figure 12: Mean Mutant Frequency vs Colony Size, negative control cultures



24 Hours Treatment without S9

Figure 13: Mutant Frequency vs Colony Size, 3,4-DAP 350 µg/mL



7.3 *In Vivo* Clastogenicity Assays in Rodent

Study title: *In Vivo* Mouse Micronucleus Test of 3,4-Diaminopyridine

Study no:	232808
Study report location:	EDR
Conducting laboratory and location:	(b) (4)
Date of study initiation:	11/12/2010
GLP compliance:	Yes, except drug characterization
QA statement:	Yes
Drug, lot #, and % purity:	3,4-DAP, Lot #305J (bulk lot 1250), 100.29 % pure

Methods

Doses in definitive study:	0, 5, 10, 20 mg/kg
Frequency of dosing:	Once
Route of administration:	PO (gavage)
Dose volume:	20 mL/kg
Formulation/Vehicle:	Sterile water for injection, USP
Species/Strain:	Swiss albino CD-1 Mice
	(b) (4)
Number/Sex/Group:	21 male mice/group
Basis of dose selection:	The preliminary dose-ranging study in male and female mice (2/sex/group) showed tremors, convulsions, and death in all animals at 40 mg/kg, tremors and death in one F at 30 mg/kg. At 20 mg/kg, animals either appeared normal or showed weight loss (1 M); this dose was considered the MTD.
Negative control:	Sterile water for injection
Positive control:	Cyclophosphamide (70 mg/kg PO)

Study Validity

The report indicates that the study was consistent with the OECD Guideline 474, dated 1997. Male mice were used for the main study because there was no clear sex difference in toxicity. Animals were sacrificed 24, 36, or 48 hours after dosing, and bone marrow was pooled from both femurs. 2000 PCEs were scored for the presence of micronuclei (NCEs were also scored for micronuclei). The PCE:NCE ratio was evaluated based on 200 cells per animal.

Results

The dosing formulations were within 97.4% to 104.3% of nominal concentrations. Based on PCE:NCE ratio, cytotoxicity was not detected. No dose-related increase in micronuclei was observed in PCE at 24, 36, or 48 hours after 3,4-DAP administration, compared to vehicle control (see sponsor's Tables 3,4,and 5, below).

Table 3: 24 Hours

Groups	Polychromatic Erythrocytes				Mean number of Normochromatic with Micronuclei per 2000 Polychromatic	Mean Ratio: Polychromatic / Normochromatic (n=200)
	Scored per 7 animals	with Micronuclei				
		Total	Mean/Mouse	%		
Low Dose (3,4-DAP) (5 mg/kg)	14,000	39	5.6 ± 4.5*	0.3	0.7	0.79
Intermediate Dose (3,4-DAP) (10 mg/kg)	14,000	22	3.1 ± 1.5*	0.2	1.3	0.85
High Dose (3,4-DAP) (20 mg/kg)	14,000	31	4.4 ± 4.4*	0.2	1.3	0.83
Positive Control (Cyclophosphamide)	14,000	857	122 ± 22	6.1	3.4	0.72
Negative Control (Sterile WFI)	14,000	18	2.6 ± 4.3*	0.1	1.3	0.79

* Statistically significant difference (ANOVA; $p \leq 0.05$) when the positive control group was compared to the negative control group and all test groups.
There was no difference between the negative control group and 3,4-DAP test groups.

Table 4: 36 Hours

Groups	Polychromatic Erythrocytes				Mean number of Normochromatic with Micronuclei per 2000 Polychromatic	Mean Ratio: Polychromatic / Normochromatic (n=200)
	Scored per 7 animals	with Micronuclei				
		Total	Mean/Mouse	%		
Low Dose (3,4-DAP) (5 mg/kg)	14,000	11	1.6 ± 0.08**	0.1	0.9	0.79
Intermediate Dose (3,4-DAP) (10 mg/kg)	14,000	6	0.9 ± 0.7*	0.04	0.6	0.80
High Dose (3,4-DAP) (20 mg/kg)	14,000	4	0.6 ± 0.5*	0.03	0	0.77
Positive Control (Cyclophosphamide)	14,000	613	88 ± 30	4.4	2.1	0.83
Negative Control (Sterile WFI)	14,000	11	1.6 ± 2.9*	0.08	1.0	0.85

* Statistically significant difference (ANOVA; $p \leq 0.05$) when the positive control group was compared to the negative control group and all test groups.
There was no difference between the negative control group and 3,4-DAP test groups.

** Statistically significant difference (t-test; $p<0.001$) compared to the positive control group.

Table 5: 48 Hours

Groups	Polychromatic Erythrocytes				Mean number of Normochromatic with Micronuclei per 2000 Polychromatic	Mean Ratio: Polychromatic / Normochromatic (n=200)
	Scored per 7 animals	with Micronuclei				
		Total	Mean/Mouse	%		
Low Dose (3,4-DAP) (5 mg/kg)	14,000	10	1.4 ± 1.0*	0.07	0.9	0.77
Intermediate Dose (3,4-DAP) (10 mg/kg)	14,000	5	0.7 ± 0.8*	0.04	0.6	0.77
High Dose (3,4-DAP) (20 mg/kg)	14,000	9	1.3 ± 1.6*	0.06	0.1	0.74
Positive Control (Cyclophosphamide)	14,000	82	11.7 ± 6.8	0.6	14.7	0.49
Negative Control (Sterile WFI)	14,000	7	1.0 ± 1.3*	0.05	1.9	0.67

* Statistically significant difference ($p \leq 0.05$) when the positive control group was compared to the negative control group and all test groups.
There was no difference between the negative control group and 3,4-DAP test groups.

Study title: In Vivo Chromosome Aberration Test of 3,4-Diaminopyridine

Study no: **247283**
Study report location: EDR
Conducting laboratory and location: (b) (4)
Date of study initiation: 11/7/11
GLP compliance: Yes (FDA), except drug characterization
QA statement: Yes
Drug, lot #, and % purity: 3,4-DAP, lot 305J (bulk lot 1250), 100.2%

Methods

Doses in definitive study:	0, 5, 10, and 20 mg/kg
Frequency of dosing:	Single
Route of administration:	PO
Dose volume:	20 mL/kg
Formulation/Vehicle:	Water for injection
Species/Strain:	Swiss Albino (CD-1) mice (b) (4)
Number/Sex/Group:	6 male mice/group at 18 hr postdose 6 male mice Con, HD 3,4-DAP, PosCon at 42 hr postdose
Negative control:	Water for injection
Positive control:	Cyclophosphamide (50 mg/kg PO; 2.5 mL/kg)

Study Validity

The report indicates that the study was consistent with the OECD Guideline 475, dated 1997. The sponsor selected the maximum dose tested (20 mg/kg) based on the results of the preliminary dose-ranging study for Study 232808, in which tremors, convulsions, and/or death occurred in all animals at 40 mg/kg and one female at 30 mg/kg, and weight loss occurred in one male at 20 mg/kg. Male mice were used for the main study because there was no clear sex difference in toxicity. Animals were sacrificed 18 or 42 hr hours after dosing, and bone marrow was pooled from both femurs; for each vehicle and 3,4-DAP-treated animal, 100 metaphases were scored.

Results

3,4-DAP (20 mg/kg) did not cause demonstrable toxicity at 18 hr postdose; however, some cytotoxicity was observed at 42 hr postdose (i.e., 31% reduction in the RMI). Salivation was the only reported drug-related clinical sign. 3,4-DAP did not cause an increase in structural chromosomal aberrations in male mice at the maximum dose tested.

7.4 Other Genetic Toxicity Studies (metabolite 3-Ac-DAP)

In Vitro Reverse Mutation Assay in Bacterial Cells (Ames)

Study title: Bacterial Reverse Mutation Assay of N-(4-Amino-Pyridin-3-yl) Acetamide

Study no.:	272917
Study report location:	EDR
Conducting laboratory and location:	(b) (4)
Date of study initiation:	8/14/13
GLP compliance:	Yes (FDA), except preliminary testing and drug characterization
QA statement:	Yes
Drug, lot #, and % purity:	N-(4-Amino-pyridin-3-yl) acetamide, lot 1300-95-1, >99% pure

Methods

Strains:	*S. typhim.:* TA98, TA100, TA1535, TA1537 *E.* coli: WP2 *uvrA*
Concentrations in definitive study:	0, 0.31, 0.62 or 0.63, 1.2 or 1.3, 2.5 and 5.0 mg/plate
Basis of concentration selection:	Maximum concentration
Negative control:	vehicle
Positive control:	+S9: benzo(a)pyrene or cyclophosphamide, 2-aminoanthracine (WPs uvrA) -S9: MMS (WP2 uvrA) 9-AA (TA1537) Sodium azide (TA100, TA1535) 2-nitrofluorene (TA98)
Formulation/Vehicle:	cell culture water
Incubation & sampling time:	37 ± 2°C for 48 to 72 hr

Study Validity

Standard methodology was generally used and followed the study design for the "OECD Guideline for Testing of Chemicals - 471, Bacterial Reverse Mutation Test" (OECD, 1997). Both a plate incorporation and preincubation assay were performed in the presence and absence of S9. It was noted that a non-standard positive control, MMS, was used for the E. coli strain in the absence of metabolic activation, but literature exists to support its use (e.g., see Kirkland et al., 2016).

Results

Concentration analysis showed that the formulations were approximately 110% to 117% of the nominal concentrations. Neither precipitates nor cytotoxicity were reported. No clear dose-related increases in revertants were reported in any strain.

In Vitro Assay in Mammalian Cells

Study title: Mammalian Gene Mutation Assay of N-(4-Amino-Pyridin-3-yl) Acetamide In Mouse Lymphoma Cells

Study no.:	275001
Study report location:	EDR
Conducting laboratory and location:	(b) (4)
Date of study initiation:	10/3/13
GLP compliance:	Yes (FDA), except preliminary and osmolality testing and drug characterization
QA statement:	Yes
Drug, lot #, and % purity:	N-(4-Amino-pyridin-3-yl)acetamide, lot 1392-122-1, 99.6% pure

Methods

Cell line:	mouse lymphoma cell line L5178Y $TK^{+/-}$ -3.7.2.C
Concentrations in definitive study:	4 hr +S9 and 4 hr -S9: approximately 0.0195, 0.0391, 0.0782, 0.156, 0.313, 0.625, 1.25, and 2.50 mg/mL (5 mg/mL was cytotoxic) 24 hr -S9: approximately 0.0195, 0.0391, 0.0782, 0.156, 0.313, 0.625, and 1.25 mg/mL (2.5 and 5 mg/mL were cytotoxic)
Basis of concentration selection:	Cytotoxicity was observed at all exposure times at 5 mg/mL. Concentrations up to 2.5 mg/mL were generally acceptable based on cytotoxicity, pH, and osmolality, except 2.5 mg/mL was cytotoxic in the 24 hr exposure assay.
Negative control:	Cell culture grade water
Positive control:	-S9: MMS +S9: cyclophosphamide
Formulation/Vehicle:	Cell culture grade water
Incubation & sampling time:	Incubated at 38 ± 2°C Expression period of 2 days Cloning period of 13/14 days

Study Validity

The report indicates that the study was consistent with the OECD Guideline 476, dated 1997. Standard methodology was used. The metabolic activation system was rat liver

S9 (from SD rats treated with phenobarbital-5, 6-benzoflavone) purchased from [redacted](b) (4) Triplicate plates were used. The sponsor's criterion for a positive result was a "concentration-related increase in mutant frequency or a clearly reproducible increase in mutant frequency at a single concentration," usually with a "minimum increase of two-fold in mutant frequency over a typical background mutant frequency."

Results- Negative

The dosing concentrations were approximately 110% to 120% of nominal concentrations. The RTGs at the maximum concentrations tested were 46% at 4 hr -S9, 15.3% at 4 hr +S9 (52% at 1.25 mg/mL), and 16.8% at 24 hr -S9 (89.6% at 0.63 mg/mL). The RTG in the 4 hr –S9 assay did not meet the 10-20% criterion; however, the 5.0 mg/mL culture was aborted because there were no viable cells on Day 1 of the expression period. At the highest tested concentrations, neither two-fold increases over the negative control mutant frequencies nor increased mutant frequencies evaluated using the GEF (90 x 10^{-6}) were observed in the assays. See the sponsor's summary tables, below.

The mutant frequencies per 10^6 viable counts are presented in the table below.

mg/mL	4 hours -S9
0	75 ± 3
0.0196	67
0.0391	68
0.0782	58
0.156	72
0.313	62
0.626	73
1.25	57
2.50	60
MMS (10 µg/mL)	507
MMS (20 µg/mL)	731

mg/mL	4 hours +S9
0	110 ± 11
0.0195	105
0.0391	129
0.0782	117
0.156	87
0.313	109
0.625	111
1.25	138
2.50	183
CP (1.0 µg/mL)	316
-	-

mg/mL	24 hours -S9
0	72 ± 5
0.0195	81
0.0391	122
0.0782	80
0.156	69
0.313	79
0.625	53
1.25	126
-	-
MMS (5 µg/mL)	598
MMS (10 µg/mL)	1128

8 Carcinogenicity

Carcinogenicity studies have not been conducted; it was previously agreed that these may be submitted post-approval (EoP2 Meeting Minutes, dated 6/17/14).

9 Reproductive and Developmental Toxicology

Reproductive and developmental toxicology studies have not been conducted; it was previously agreed that these may be submitted post-approval (cf., EoP2 Meeting Minutes, dated 6/17/14). A juvenile animal toxicology study has not been conducted.

10 Special Toxicology Studies- Impurities

In Vitro Reverse Mutation Assay in Bacterial Cells (Ames): (b) (4)

Study title: (b) (4) **Bacterial Reverse Mutation Test in Salmonella typhimurium and Escherichia coli**

Study no.:	9601377
Study report location:	(b) (4) EDR
Conducting laboratory and location:	(b) (4)
Date of study initiation:	1/19/16
GLP compliance:	Yes (FDA), except drug characterization (GMP)
QA statement:	Yes
Drug, lot #, and % purity:	(b) (4) lot 1716-51-1, 99.3% pure (from Jacobus)

Methods

Strains:	*S. typhimurium:* TA1535, TA1537, TA98, TA100 *E. coli:* WP2 *uvrA*
Concentrations in definitive study:	0, 1.58, 5.0, 15.8, 50, 158, 500, 1581, 5000 mcg/plate
Basis of concentration selection:	Maximum recommended concentration No precipitation or cytotoxicity was observed.
Negative control:	80% DMSO in water
Positive control:	See the sponsor's Text Table 2, below.
Formulation/Vehicle:	80% DMSO in water
Incubation & sampling time:	37°C for 64 hours and 52 minutes

Text Table 2
Positive Controls for the Assay

Strain	S9	Positive Controls	Concentration µg/plate	Vehicle
TA1535, TA100	0	(b) (4)		Sterile water
TA1537				DMSO
TA98				DMSO
WP2 *uvrA*				DMSO
TA1535	+			DMSO
WP2 *uvrA*				
TA1537, TA98, TA100				DMSO

Study Validity

Standard methodology was used (OECD Guideline 471 and ICH Guideline S2(R1)). The metabolic activation system used was commercially available rat liver S9 fraction (b) (4). The plate incorporation method was used. Concentration analyses showed that the formulations were within (b) (4)% of the nominal concentrations, except the lowest concentration which was (b) (4)% to (b) (4)% of nominal concentration. Concentration-related, reproducible, (b) (4)fold or greater increases in revertants were the sponsor's criteria for a positive response.

Results- Negative

No concentration-related increases in revertants were observed in the presence or absence of metabolic activation.

In Vitro Reverse Mutation Assay in Bacterial Cells (Ames): (b) (4) impurities

Study title: Bacterial Reverse Mutation Assay (b) (4)

Study no.:	282184
Study report location:	EDR
Conducting laboratory and location:	(b) (4)
Date of study initiation:	4/8/14
GLP compliance:	Yes (FDA), except: Preliminary testing Drug formulation analysis
QA statement:	Yes
Drug, lot #, and % purity:	(b) (4) lot JT-742-73 (JPC), 99.5% pure

Methods

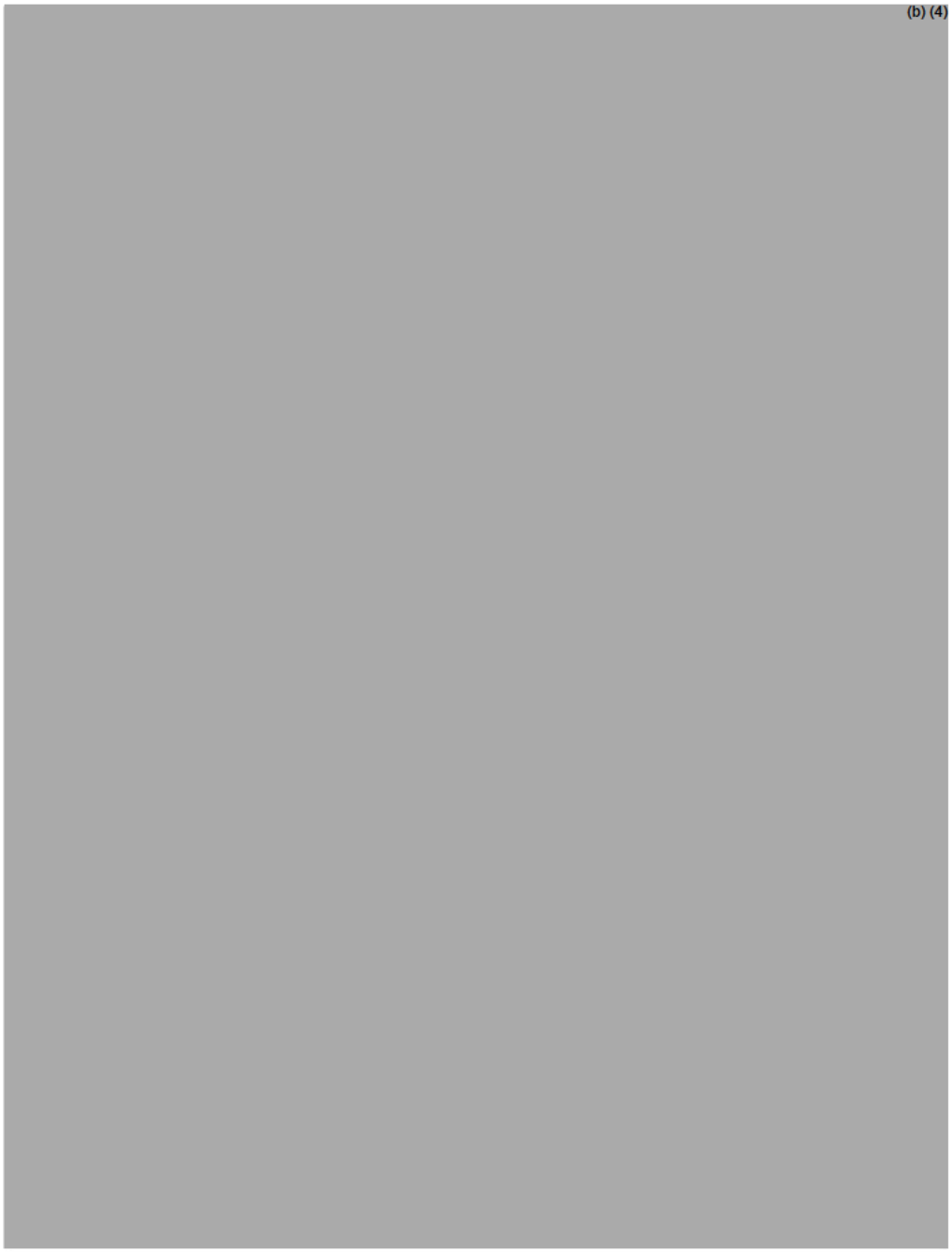
Strains:	*S. typhimurium*.: TA1535, TA1537, TA98, and TA100 *E. coli*: WP2 *uvrA*
Concentrations in definitive study:	0, 0.31, 0.63, 1.3, 2.5, and 5 mg/plate
Basis of concentration selection:	Maximum concentration/ limit dose
Negative control:	DMSO
Positive control:	+S9: (b) (4) (TA98, TA100, TA1537) (WP2 *uvrA*) (b) (4) (TA1535) -S9: (b) (4) TA100, TA1535) (TA98) (b) (4) (WP2 *uvrA*) (b) (4) (TA1537)
Formulation/Vehicle:	DMSO
Incubation & sampling time:	37° ± 2°C for 67 hours

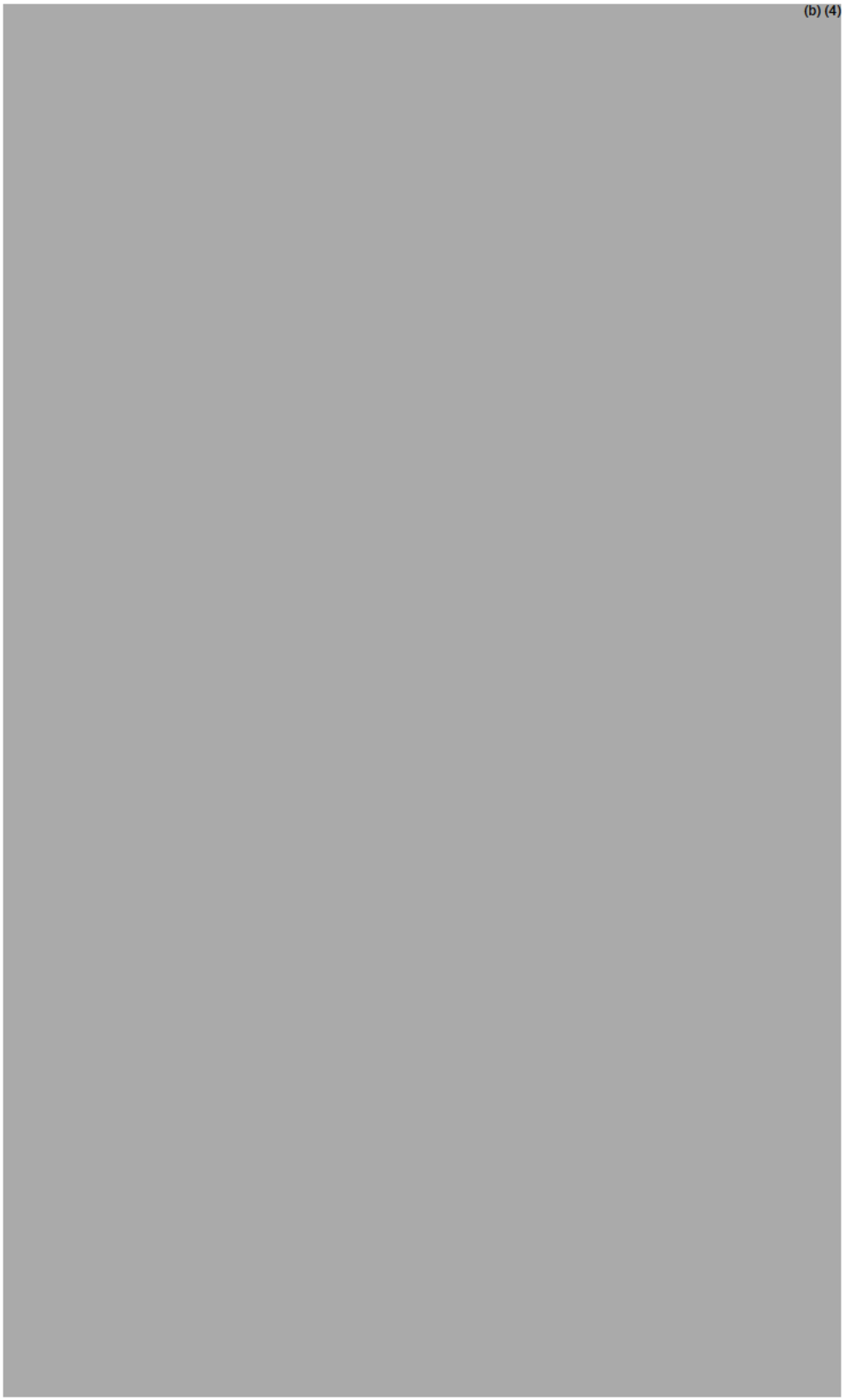
Study Validity

Standard methodology was used. The metabolic activation system was Aroclor 1254-induced SD rat liver S9 fraction purchased from (b) (4) The plate incorporation method was used. The criteria for a positive assay included a statistically significant increase in revertants, with a concentration-response, and comparison to historical controls.

Results- Positive

Cytotoxicity was not observed, except for a slight reduction in revertants for (b) (4) at the maximum concentration tested (i.e., (b) (4) mg/plate); precipitation was not observed. All plates were considered analyzable. (b) (4) caused an increase in revertants in TA98, TA100, and TA 1537 in the absence of metabolic activation, and in TA98 in the presence of metabolic activation. The results for TA100 in the presence of metabolic activation is considered equivocal (although the sponsor consideredTA100 positive based on statistical significance), because none of the results exceed a (b) (4) fold increase for TA 100. The result for TA1537 in the presence of metabolic activation is considered equivocal/positive because a (b) (4) fold increase in revertants was observed at the maximum concentration tested; the sponsor considered the result equivocal because it was not statistically significant. See results tables, below, from the sponsor.


(b) (4)


(b) (4)

Study title: [redacted] **Bacterial Reverse Mutation Test in Salmonella typhimurium and Escherichia coli**

Study no.:	9600771
	[redacted]
Study report location:	EDR
Conducting laboratory and location:	[redacted]
Date of study initiation:	5/29/14
GLP compliance:	Yes (FDA), except: Drug characterization Stability testing
QA statement:	Yes
Drug, lot #, and % purity:	[redacted] lot JT-742-73 (JPC), 100% pure

Methods

Strains:	*S. typhimurium*.: TA1535, TA1537, TA98, and TA100 *E. coli*: WP2 uvrA
Concentrations in definitive study:	0, 1.58, 5.0, 15.8, 50, 158, 500, 1581, and 5000 mcg/plate
Basis of concentration selection:	Maximum concentration/Limit dose
Negative control:	DMSO
Positive control:	+S9: [redacted] (TA1535 and WP2 *uvrA)* [redacted] (TA1537, TA98, TA100) -S9: [redacted] (TA1535, TA100) [redacted] (TA1537) [redacted] (TA98) [redacted] (WP2 *uvrA*)
Formulation/Vehicle:	DMSO
Incubation & sampling time:	37°C for 65 hours and 13 minutes

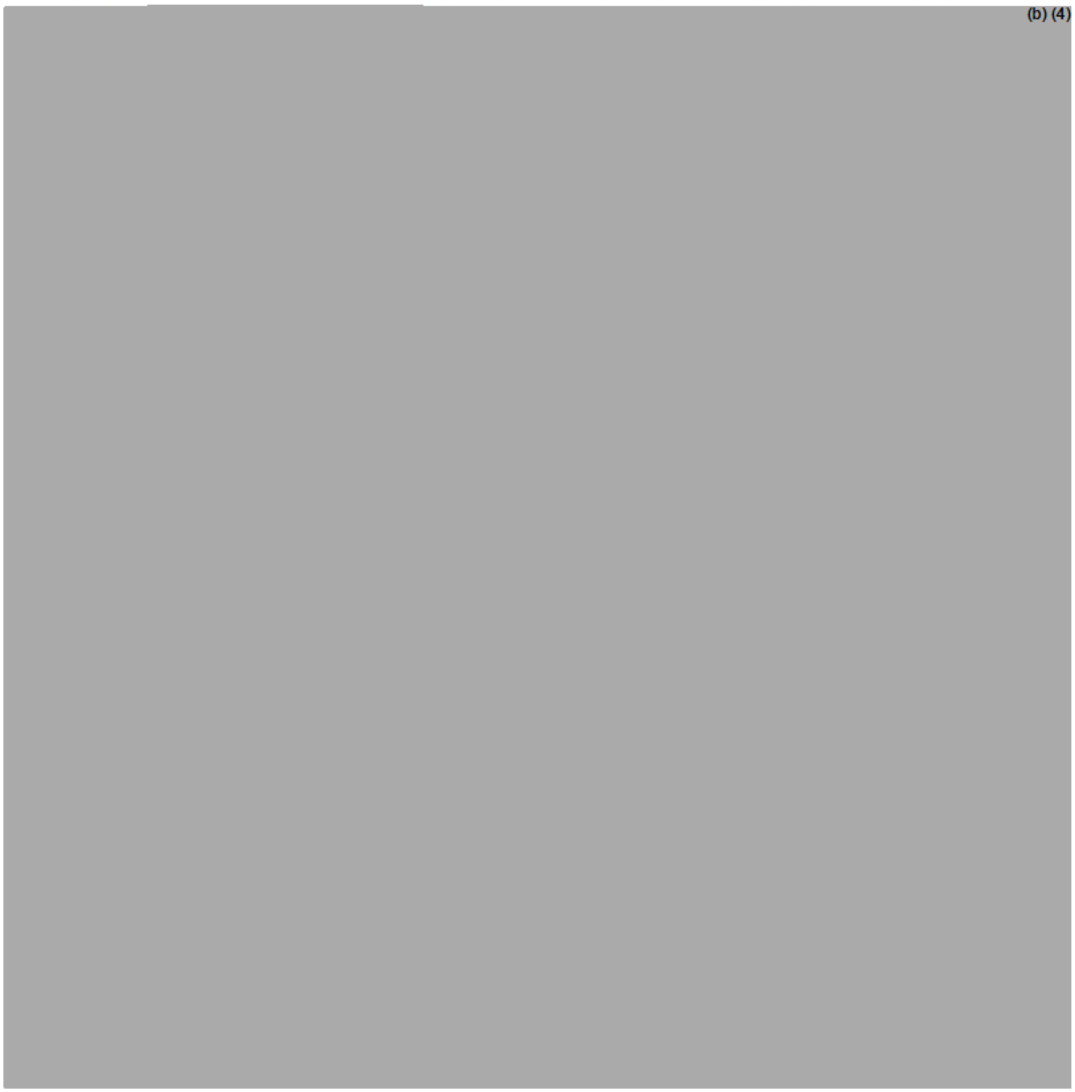
Study Validity

Standard methodology was used. The metabolic activation system was commercially available Aroclor 1254-induced male rat liver S9 fraction [redacted] purchased). The plate incorporation method was used. The assay was considered positive if both of the following criteria were met (from the sponsor):

- The results for the test item show a substantial increase in revertant colony counts, i.e. response ≥ (b) (4) times the concurrent vehicle control level values, with mean value(s) outside the laboratory historical control range (beyond the (b) (4) % tolerance limit). Otherwise results are considered negative.
- The above increase is dose related and/or reproducible i.e. increases must be obtained at more than one experimental point (at least one strain, more than one dose level).

Results- Positive

The concentrations used were within (b) (4) % of the nominal concentration. Neither cytotoxicity or precipitates were reported. Increased revertants were observed in the presence and absence of metabolic activation in strain TA98, and in TA1537 in the absence of metabolic activation. A greater than (b) (4) -fold increase in revertants was also observed in TA1537 in the presence of metabolic activation (which the sponsor considered positive), but this result was similar to the historical control range. See Tables 1 and 2, below, from the sponsor.



(b) (4)

Study title: Data Report for Pharmacology Services

Study no.:	AB25034
Study report location:	EDR
Conducting laboratory and location:	(b) (4)
Date of study initiation:	7/20/14 listed as "study date"
GLP compliance:	No
QA statement:	No
Drug, lot #, and % purity:	(b) (4) lots 1499-26 and JT-742-73, purity not provided

Methods

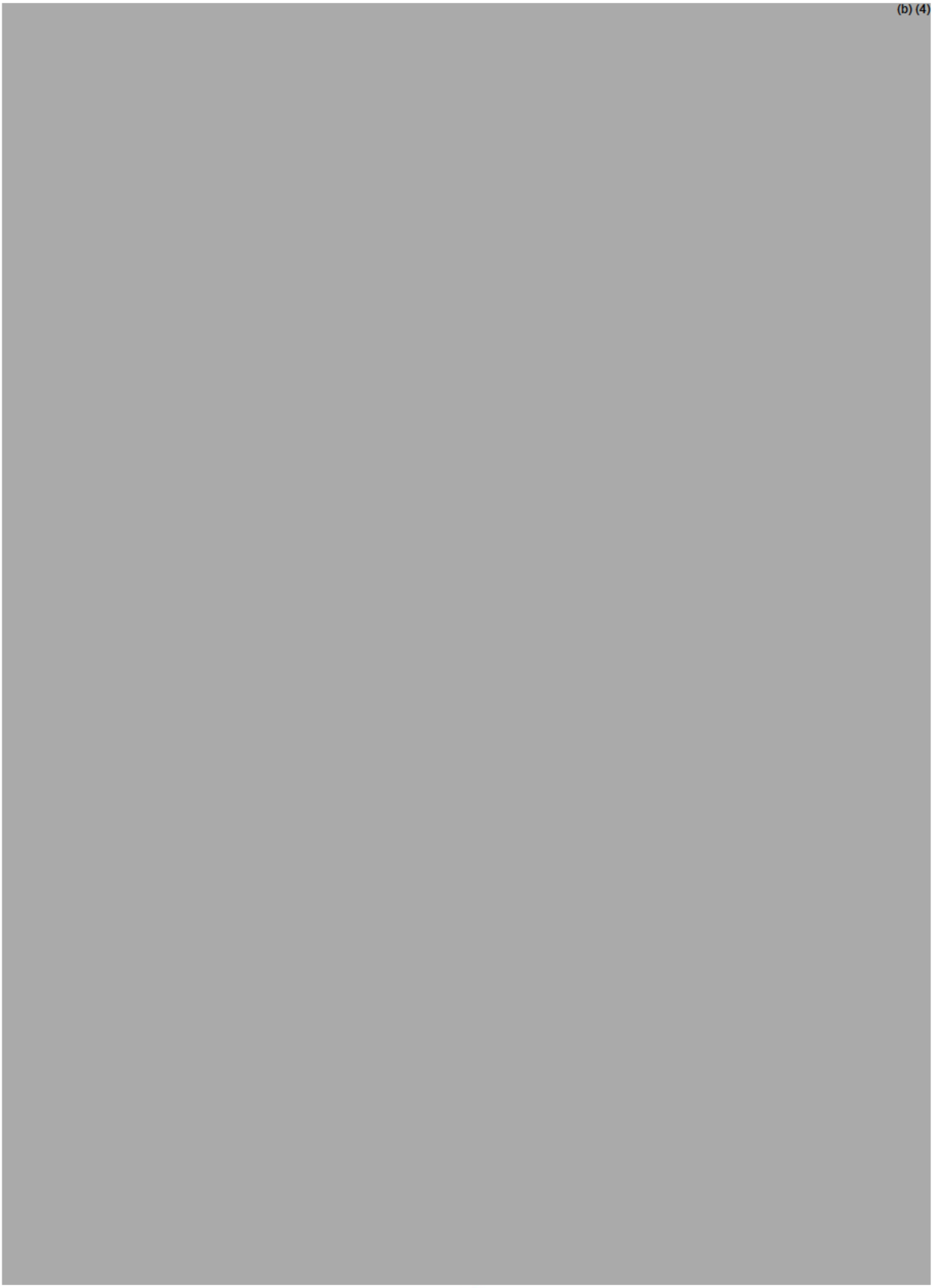
Strains:	*S. typhimurium*: TA98 and TA100
Concentrations in definitive study:	0, 625, 1250, 2500, and 5000 mcg/plate
Basis of concentration selection:	Maximum recommended concentration Lack of cytotoxicity
Negative control:	DMSO
Positive control:	+S9: (b) (4) -S9: (b) (4) (TA98) (b) (4) (TA100)
Formulation/Vehicle:	DMSO
Incubation & sampling time:	37°C for 72 hours

Study Validity

The methodology used in this study was not consistent with OECD Guideline 471. Only two strains were used, and only four concentrations were tested. The metabolic activation system (Aroclor 1254-induced male Sprague Dawley rat liver S9, (b) (4) was purchased commercially; only (b) (4) was used as the positive control in the presence of metabolic activation. The positive control for used for TA98, (b) (4) is not standard; the sponsor provided no support for its use (although data exist; (b) (4) The sponsor defined cytotoxicity as a (b) (4)% or greater reduction in revertants compared to vehicle control.

Results- positive for lot JT-742-73 & negative for lot 1499-26, as conducted

The sponsor conducted this study with two different lots of (b) (4) to assist in the interpretation of the Ames results obtained among the conducted assays. The sponsor defined a positive response as a (b) (4)fold increase in the number of revertants. Lot JT-742-73 was positive in strain TA98 in the presence and absence of metabolic activation (see tables from the sponsor, below).


(b) (4)

Study title: [redacted] **Bacterial Reverse Mutation Test in Salmonella typhimurium and Escherichia coli**

Study no.:	9600918
	[redacted]
Study report location:	EDR
Conducting laboratory and location:	[redacted]
Date of study initiation:	9/16/14
GLP compliance:	Yes (FDA), except: Drug characterization Stability testing
QA statement:	Yes
Drug, lot #, and % purity:	[redacted] lot 1499-131(JPC), 100% pure

Methods

Strains:	*S typhimurium:* TA1535, TA1537, TA98, and TA100 *E. coli:* WP2 *uvrA*
Concentrations in definitive study:	0, 1.58, 5.0, 15.8, 50, 158, 500, 1581, and 5000 mcg/plate
Basis of concentration selection:	Maximum concentration/ limit dose
Negative control:	DMSO
Positive control:	+S9: [redacted] (TA1535 and WP2 *uvrA)* [redacted] (TA1537, TA98, TA100) -S9: [redacted] (TA1535, TA100) [redacted] (TA1537) [redacted] (TA98) [redacted] (WP2 *uvrA*)
Formulation/Vehicle:	DMSO
Incubation & sampling time:	37°C, for 65 hours and 17 minutes

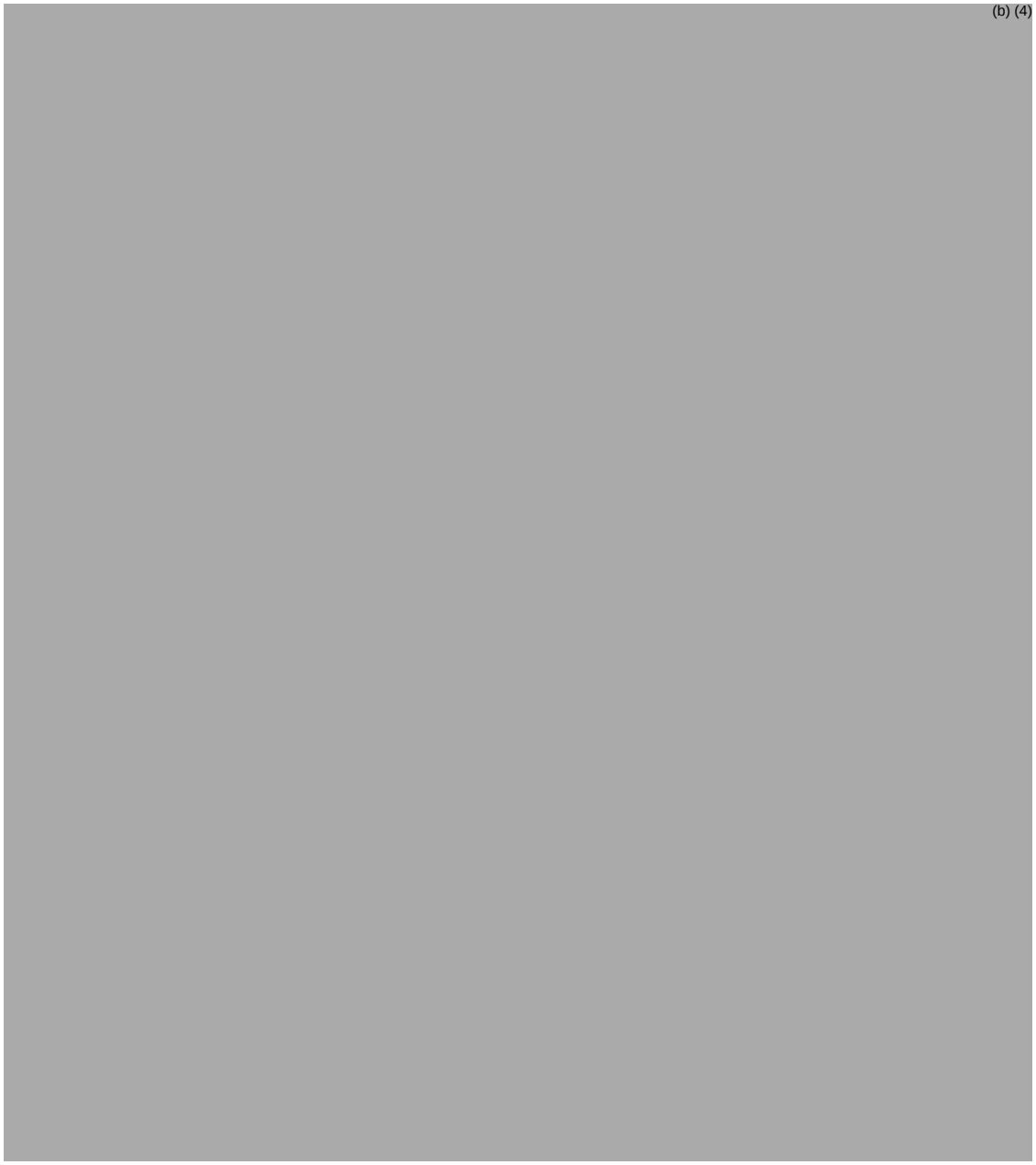
Study Validity

Standard methodology was used. The metabolic activation system was commercially available Aroclor 1254-induced male rat liver S9 fraction [redacted] The plate incorporation and preincubation methods were used. The assay was considered positive if both of the following criteria were met (from the sponsor):

- The results for the test item show a substantial increase in revertant colony counts, i.e. response ≥ (b) (4) times the concurrent vehicle control level values, with mean value(s) outside the laboratory historical control range (beyond the (b) (4) % tolerance limit). Otherwise results are considered negative.
- The above increase is dose related and/or reproducible i.e. increases must be obtained at more than one experimental point (at least one strain, more than one dose level).

Results- Negative

The drug formulation concentrations were within ± (b) (4) % of the nominal concentrations. Cytotoxicity (i.e., reduction in revertants) was observed only in the TA1535 strain in the absence of S9 at the maximum concentration (b) (4) mg/plate); no precipitation was observed in any plate. Five concentrations were used, and although all were considered analyzable by the sponsor, cytotoxicity was suggested by counts at the maximum concentration in a few assays. Increases in revertants meeting the criteria for a positive assay were not observed in any strains in the presence or absence of metabolic activation. In the pre-incubation assay, a (b) (4) -fold increase in revertants was observed in TA98 in the absence of metabolic activation at (b) (4) mg/plate (the maximum concentration; see the sponsor's table, below). Based on the sponsor's historical negative control results provided, revertants for TA98 (-S9) at the maximum concentration ((b) (4) mg/plate) were within the historical control range (b) (4)


(b) (4)

(b) (4)

Study title: **Data Report for Pharmacology Services**

Study no.:	AB30509
Study report location:	EDR
Conducting laboratory and location:	(b) (4)
Date of study initiation:	8/3/15 listed as "study date"
GLP compliance:	No
QA statement:	No
Drug, lot #, and % purity:	(b) (4) lot 1511-123-D, purity not provided
	(b) (4) lot 1595-102-1, purity not provided

Methods

Strains:	*Salmonella typhimurium*: TA98 and TA100
Concentrations in definitive study:	0, 3, 30, 300, and 3000 mcg/plate
Basis of concentration selection:	cytotoxicity
Negative control:	DMSO
Positive control:	+S9: (b) (4) -S9: (b) (4) (TA98) (b) (4) (TA100)
Formulation/Vehicle:	DMSO
Incubation & sampling time:	37°C for 72 hours

Study Validity

Overall, this study was not consistent with OECD Guideline 471. Only two strains were used, and only four concentrations (at (b) (4)x intervals) were tested. The metabolic activation system (Aroclor 1254-induced male Sprague Dawley rat liver S9, (b) (4) was purchased commercially; only (b) (4) was used as the positive control in the presence of metabolic activation. The positive control for used for TA98, (b) (4) is not standard; the sponsor provided no support for its use (although data exist; (b) (4) The sponsor defined cytotoxicity as a (b) (4)% or greater reduction in revertants compared to vehicle control. The sponsor defined a positive response as a (b) (4)fold increase in the number of revertants. A CoA was not provided for either potential impurity.

Results- positive for (b) (4) as conducted

(b) (4) was cytotoxic in TA98 and TA100 in the presence and absence of metabolic activation at (b) (4)mcg/plate. (b) (4) was positive in both strains in the presence and absence of metabolic activation (see tables from the sponsor, below). (b) (4)was positive in TA100 in the absence of metabolic activation at (b) (4)mcg/plate, the maximum concentration that could be analyzed.


(b) (4)

(b) (4)

***In Vitro* Reverse Mutation Assay in Bacterial Cells (Ames):** (b) (4)

Study title: (b) (4) **Bacterial Reverse Mutation Test in Salmonella typhimurium and Escherichia coli**

Study no.:	9602351
Study report location:	EDR
Conducting laboratory and location:	(b) (4)
Date of study initiation:	1/17/19
GLP compliance:	Yes (OECD), except: Drug characterization and stability by the sponsor (GMP)
QA statement:	Yes
Drug, lot #, and % purity:	(b) (4) Lot #1992-105, 100% pure (correction factor 59.4%, free base content)

Methods

Strains:	*S. typhimurium* TA1535, TA1537, TA98, and TA100 *E.coli* WP2 *uvrA*
Concentrations in definitive study:	0, 1.58, 5.0, 15.8, 50, 158, 500, 1581 and 5000 mcg/mL
Basis of concentration selection:	Maximum concentration (limit dose)
Negative control:	DMSO
Positive control:	See the sponsor's Text Table 1, below
Formulation/Vehicle:	DMSO
Incubation & sampling time:	37°C for 64 hr and 2-15 minutes

Text Table 1
Positive Controls for the Assay

Strain	S9	Positive Controls	Concentration ug/plate	Vehicle
TA1535, TA100	0	(b) (4)		Sterile water
TA1537				DMSO
TA98				DMSO
WP2 *uvrA*				DMSO
TA1535	+			DMSO
WP2 *uvrA*				
TA1537, TA98, TA100				DMSO

Study Validity

The plate incorporation method was used. Triplicate plates were used. Aroclor 1254 induced male rat liver fraction was supplied by (b) (4). Cytotoxicity and precipitation were not observed. For the formulation analysis, only concentration was assessed; the mean results were within ± (b) (4)% of the nominal stock solution concentration (b) (4) mcg/mL; i.e., associated with the maximum concentration tested), ± (b) (4)% for (b) (4) mcg/mL solutions, and +1 (b) (4)% for the (b) (4) mcg/mL solutions. The formulations were prepared on the day of use and stored at ambient room temperature until used.

Results- Negative

No cytotoxicity was reported, either by background lawn changes or by revertant counts. No increases were observed in the presence or absence or metabolic activation, with the exception of a single concentration in TA1535 in the absence of metabolic activation. In the initial test, an increase in the revertants was observed for TA1535 at (b) (4) mcg/plate; the increase was (b) (4)-fold the negative control and exceeded the (b) (4)% tolerance limit of the historical control data but was not concentration-related (see selected portion of the summary results Table 1 from the sponsor, below). It was judged equivocal by the study director, and a supplemental assay using concentrations surrounding the concentration at which the increase was previously observed was conducted, with smaller intervals between concentrations. Dose formulation analysis

was not performed for the supplemental assay. The results of the supplemental assay are below (see Table 4, from the sponsor).

Below are the sponsor's Historical Control Data (Jan 2014 to August 2018, including both non-GLP and GLP studies; the number of studies on which the data are based was not provided).

Negative Control

In the supplemental assay, the negative control mean exceeded the (b) (4)% upper tolerance limit of the historical controls, but was within the historical control range.

Overall, the lack of a concentration-related increase in revertants and the results of the supplemental assay indicate the lack of a positive response.

In Vitro Reverse Mutation Assay in Bacterial Cells (Ames): [redacted]

Study title: [redacted] Bacterial Reverse Mutation Test in Salmonella typhimurium and Escherichia coli

Study no.: 9602339
Study report location: EDR
Conducting laboratory and location: [redacted]
Date of study initiation: 1/17/19
GLP compliance: Yes (OECD), except: Drug characterization and stability by the sponsor (GMP)
QA statement: Yes
Drug, lot #, and % purity: [redacted] lot 1992-110-6, 100% pure

Methods

Strains: *S. typhimurium* (TA1535, TA1537, TA98, and TA100)
E. coli WP2 *uvrA*
Concentrations in definitive study: 0, 1.58, 5.0, 15.8, 50, 158, 500, 1581, and 5000 mcg/plate
Basis of concentration selection: Maximum concentration recommended
Negative control: DMSO
Positive control: See the sponsor's table, below
Formulation/Vehicle: DMSO
Incubation & sampling time: 37° for 64 hr and 2 minutes

Text Table 2
Positive Controls for the Assay

Strain	S9	Positive Controls	Concentration µg/plate	Vehicle
TA1535, TA100	0	[redacted]	[redacted]	Sterile water
TA1537				DMSO
TA98				DMSO
WP2 *uvrA*				DMSO
TA1535	+			DMSO
WP2 *uvrA*				
TA1537, TA98, TA100				DMSO

Study Validity

Standard methodology was used. The plate incorporation method was used. The metabolic activation system (i.e., Aroclor-induced male rat liver fraction) was supplied by [redacted] All formulations were within [redacted]% (stock) or [redacted]% (other) concentrations.

Results- Negative

No cytotoxicity or precipitation was reported. No increases in revertants exceeding 2-fold were observed.

11 Integrated Summary and Safety Evaluation

The sponsor has developed 3,4-DAP for [redacted] (b) (4) treatment of Lambert-Eaton Myasthenia.

Pharmacology

Although there has been some debate in recent literature (e.g., Wu et al., 2009), 3,4-DAP (like structurally-related 4-AP) has generally been accepted as a potassium channel blocker (e.g., Kirsch & Narahashi, 1978; Judge & Bever, 2006). 3,4-DAP was shown to block a variety of voltage-gated potassium channels (the observed IC_{50}s ranged from 188 to 1575 mcM). Additionally, 3,4-DAP was shown to inhibit the norepinephrine transporter (IC_{50}= 230 mcM).

The major human metabolite (3-Ac-DAP) showed weak effects on muscarinic receptors, as an antagonist at M_3 (IC_{50}= 2500 mcM) and as an agonist at M_4 and M_5 (EC_{50}= 150 and 200 mcM, respectively).

ADME

In vitro, 3,4-DAP was shown to be metabolized to 3-Ac-DAP in mouse, rat, monkey, and human hepatocyte preparations; dog hepatocytes did not form 3-Ac-DAP. In vivo, metabolite 3-Ac-DAP was shown to be a major human metabolite; rats, but not dogs, also showed in vivo exposure to 3-Ac-DAP.

3,4-DAP was shown to be metabolized primarily by N-acetyl-transferase enzyme NAT-2 and not by cytochrome P450 enzymes. 3,4-DAP and metabolite 3-Ac-DAP were not identified as inducers and/or inhibitors of CYP enzymes and were not clear substrates and/or inhibitors of a number of transporters (e.g., P-gp, BRCP, OCT, OAT).

Comparative TK

3,4-DAP is metabolized to major metabolite 3-Ac-DAP in rats and humans, but not in dogs, the nonrodent toxicity species. Standard TK parameters were estimated in the 6-month chronic toxicity study in rats by comparison to exposures in a rat 7-day bridging TK study. In that study, the calculated mean daily doses tested were somewhat higher than those calculated for the 6-month study (see the sponsor's tables from the 7-day study and the 6-month study, respectively, below):

Average Mean Test Article Consumption (mg/kg/day)

Group No.	Target Dose Level (mg/kg/day)	Calculated Group Mean Dose Level (mg/kg/day)	
		Main Study	
		Males	Females
2	15	18.80	18.83
3	45	57.26	55.24
4	135	121.04	118.80

Text Table 2
Average Mean Test Article Consumption (mg/kg/day)

Group No.	Target Dose Level (mg/kg/day)	Calculated Group Mean Dose Level (mg/kg/day)			
		Main Toxicity Study		Toxicokinetic	
		Males	Females	Males	Females
2	15	10.85	13.85	10.83	13.93
3	45	31.48	42.74	33.20	41.49
4	135	100.72	134.76	107.42	135.01

The estimated exposures achieved in the 6-month study suggested that 3,4-DAP and 3-Ac-DAP were adequately assessed in rats compared to estimated exposures at the MRHD in humans (based on the sponsor's human data below). AUC margins for the estimated exposures achieved at the maximum doses tested were approximately 10- to 20-fold (males, females) for 3,4-DAP and 7- to 8.5-fold (males, females) for 3-Ac-DAP, compared to estimated AUC exposures at a maximum recommended daily dose of 100 mg (based on the human PK summary data from Study JPC 3,4DAP.TQT at 30 mg, below, provided by the Clinical Pharmacology reviewer). To be conservative, the data from SM subjects were used for parent exposure comparisons and data from RM subjects were used for metabolite exposure comparisons, as indicated by the Clinical Pharmacology reviewer.

Table 11-4: Descriptive Statistics for Multiple Dose 3,4-DAP Plasma Pharmacokinetic Parameters following Dose 4 (PK Population)

AP/ Statistic	C_{max} (ng/mL)	$AUC_{(0\text{-}tau)}$ (ng*h/mL)	t_{max} [a] (h)	$t_{1/2}$ (h)	CL/F (L/h)	$RAUC_{(0\text{-}tau)}$	RC_{max}
All Subjects							
n	43	43	43	42	43	43	43
Mean	73.0	145	ND	3.78	497	1.12	0.902
SD	54.7	117	ND	1.02	447	0.293	0.421
CV%	74.9	81.1	ND	27.0	89.9	26.2	46.7
Minimum	6.66	15.5	0.50	1.01	67.4	0.453	0.166
Median	44.8	69.6	0.52	3.98	431	1.14	0.871
Maximum	203	445	2.02	5.15	1930	1.68	2.14
Geo mean	51.0	94.6	ND	3.58	317	1.08	0.794
RM Subjects							
n	3	3	3	2	3	3	3
Mean	19.5	27.6	ND	ND	1140	1.05	0.929
SD	3.56	7.09	ND	ND	290	0.0745	0.125
CV%	18.3	25.7	ND	ND	25.6	7.1	13.4
Minimum	17.2	20.9	0.50	1.25	856	0.985	0.827
Median	17.7	26.9	0.50	ND	1110	1.02	0.891
Maximum	23.6	35.0	0.52	3.40	1440	1.13	1.07
Geo mean	19.3	27.0	ND	ND	1110	1.04	0.923
IM Subjects							
n	21	21	21	21	21	21	21
Mean	33.3	66.7	ND	3.60	740	0.928	0.711
SD	26.5	77.8	ND	1.13	396	0.267	0.442
CV%	79.7	116.7	ND	31.5	53.5	28.8	62.2
Minimum	6.66	15.5	0.50	1.01	97.0	0.453	0.166
Median	28.6	44.0	0.52	3.81	681	0.912	0.605
Maximum	108	309	1.50	4.87	1930	1.56	2.14
Geo mean	26.7	48.3	ND	3.35	621	0.891	0.597
SM Subjects							
n	19	19	19	19	19	19	19
Mean	125	250	ND	4.13	127	1.34	1.11
SD	31.3	66.8	ND	0.644	28.9	0.155	0.326
CV%	25.0	26.8	ND	15.6	22.7	11.5	29.4
Minimum	73.7	162	0.50	2.93	67.4	1.14	0.498
Median	127	247	0.52	4.17	122	1.31	1.05
Maximum	203	445	2.02	5.15	186	1.68	1.66
Geo mean	122	242	ND	4.08	124	1.33	1.06

Note(s): AP = acetylator phenotype (RM = rapid metabolizer, IM = intermediate metabolizer, SM = slow metabolizer); CV% = coefficient of variation; ND = not determined; Geo = geometric; SD = standard deviation.

[a] Only median, minimum, and maximum values are presented for t_{max}.

Source: Table 14.2.7

Table 11-7: Descriptive Statistics for Multiple Dose 3-Ac Plasma Pharmacokinetic Parameters following Dose 4 (PK Population)

AP/ Statistic	C_{max} (ng/mL)	$AUC_{(0\text{-}tau)}$ (ng*h/mL)	t_{max} [a] (h)	$t_{1/2}$ (h)	$RAUC_{(0\text{-}tau)}$	RC_{max}
All Subjects						
n	43	43	43	42	43	43
Mean	467	1490	ND	4.94	1.75	1.44
SD	156	461	ND	0.917	0.274	0.314
CV%	33.4	30.8	ND	18.5	15.6	21.8
Minimum	245	888	0.50	3.30	1.06	0.668
Median	403	1400	1.03	4.94	1.83	1.46
Maximum	774	2530	3.03	6.74	2.36	2.04
Geo mean	442	1430	ND	4.86	1.73	1.40
RM Subjects						
n	3	3	3	3	3	3
Mean	719	2170	ND	4.19	1.92	1.61
SD	95.8	343	ND	0.248	0.145	0.285
CV%	13.3	15.8	ND	5.9	7.6	17.7
Minimum	608	1840	0.50	4.00	1.83	1.42
Median	774	2150	0.50	4.10	1.85	1.47
Maximum	774	2530	1.02	4.47	2.09	1.94
Geo mean	714	2160	ND	4.18	1.92	1.59
IM Subjects						
n	21	21	21	21	21	21
Mean	521	1640	ND	4.70	1.70	1.35
SD	152	450	ND	0.984	0.338	0.359
CV%	29.1	27.5	ND	20.9	19.8	26.5
Minimum	245	888	0.50	3.40	1.06	0.668
Median	580	1630	1.10	4.47	1.83	1.46
Maximum	743	2400	2.05	6.24	2.36	2.01
Geo mean	496	1580	ND	4.60	1.67	1.30
SM Subjects						
n	19	19	19	18	19	19
Mean	367	1220	ND	5.36	1.78	1.51
SD	74.1	277	ND	0.723	0.194	0.243
CV%	20.2	22.7	ND	13.5	10.9	16.1
Minimum	289	906	0.50	3.30	1.47	1.10
Median	367	1190	1.05	5.43	1.70	1.44
Maximum	599	2060	3.03	6.74	2.16	2.04
Geo mean	361	1200	ND	5.31	1.77	1.49

Note(s): AP = acetylator phenotype (RM = rapid metabolizer, IM = intermediate metabolizer, SM = slow metabolizer); CV% = coefficient of variation; ND = not determined; Geo = geometric; SD = standard deviation.
[a] Only median, minimum, and maximum values are presented for t_{max}.
Source: Table 14.2.10

Doses tested in the chronic toxicity study in dogs demonstrated, and were limited by, a steep dose-response for CNS toxicity. C_{max} exposures at the HD, which showed CNS toxicity, were approximately 3- to 4-fold those after a 30 mg dose in humans. At the maximum dose tested (HMD, because the HD was terminated after the first day based on severe CNS toxicity), C_{max} and AUC were approximately 1.5- to 1.7-fold that after a 30 mg dose in humans and the AUC margin at the MRHD. Dogs did not show systemic exposures to metabolite 3-Ac-DAP.

General Toxicology
Acute and subchronic toxicity studies were conducted in mice, rats, and dogs. Doses were primarily limited by body weight reductions in rodents and adverse CNS effects (e.g., convulsions) in dogs.

In the 6-month toxicity study in rats, drug-related reductions in body weight gains were observed at MD (nominally 45 mg/kg; average consumed doses of 32 and 42 mg/kg/day in males and females, respectively) and HD (nominally 135 mg/kg/day; average consumed doses of 101 and 135 mg/kg/day in males and females, respectively). At the end of the dosing period, mean body reduction (compared to controls) were <10% in MD and HD males, and 10% to 15% in MD and HD females. Minimal to mild salivary gland hypertrophy was observed at HD; this finding showed recovery. The incidence and severity of uterine dilatation was also increased in HD females. Based on the mean body weight reductions compared to controls, the NOAEL was the high dose in males (approximately 100 mg/kg was the HD achieved) and the mid dose in females (approximately 43 mg/kg was the MD achieved).

In the 9-month dog toxicity study, the HD (2.1 mg/kg/day BID) resulted in adverse CNS effects (e.g., sustained convulsions) and early sacrifice of 4 (2M, 2F) of twelve animals. The entire HD group was placed on a permanent dosing holiday on D2; tissues from the 4 HD animals sacrificed early were examined microscopically (no target organs were identified in these tissues). In HMDM (1.04 mg/kg/day BID), mean body weight was reduced approximately 11% compared to controls at the end of the dosing period and showed partial recovery. Although not observed in males, individual females administered 1.04 mg/kg/day showed slight reductions in heart rate and shortening of the QTc. The NOAELs were 0.52 mg/kg/day in males (based on mean body weight reductions exceeding 10%; AUC was similar to that at the MRHD), and 1.04 mg/kg/day in females (based on severe CNS signs at 2.1 mg/kg/day in females and males; C_{max} and AUC margins were 1.5- to 1.7-fold those at the MRHD).

Reproductive Toxicology & Carcinogenicity
Reproductive and developmental toxicology and carcinogenicity studies have not been conducted; it was previously agreed that these may be submitted post-approval (EoP2 Meeting Minutes, dated 6/17/14).

Juvenile Animal Toxicology
A juvenile animal toxicology study has not been conducted; this should be conducted Phase 4.

Genotoxicity
3,4-DAP was negative in an Ames assay and in the in vivo mouse micronucleus and chromosomal aberration assays. 3,4-DAP was positive in an in vitro mouse lymphoma assay, with increases in small and large colonies but a proportionally greater increase in small colonies. Given this result, a second in vivo assay with mutagenicity as the endpoint would have been preferable to clarify this result; however, the overall results

suggested clastogenic activity. Notably, the in vitro assays were conducted prior to the implementation of ppm limits for the potentially genotoxic (b) (4) impurities in the 3,4-DAP drug substance (**Impurities**, below).

The major human metabolite (3-Ac-DAP) was negative in the in vitro Ames and mouse lymphoma assays.

Impurities

The sponsor identified several potential process and degradant impurities, including: (b) (4)

(b) (4)

(b) (4) were considered positive for potential mutagenicity in an internal (Q)SAR assessment. However, both impurities were negative in bacterial mutagenicity assays.

12 Appendix/Attachments

Cited References

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(b) (4)
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