

**CENTER FOR DRUG EVALUATION AND  
RESEARCH**

*APPLICATION NUMBER:*

**206356Orig1s000**

**PHARMACOLOGY REVIEW(S)**

**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: 206-356  
Supporting document/s: 0010  
Applicant's letter date: June 22, 2015  
CDER stamp date: June 22, 2015  
Product: ORFADIN (Nitisinone) Oral Suspension  
Indication: For the treatment of hereditary tyrosinemia Type 1 (HT-1).  
Applicant: Swedish Orphan Biovitrum AB (publ); US Agent-Quintiles, Inc.  
Review Division: Division of Gastroenterology product (HFD-180)  
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## **Executive Summary**

### **1.1 Recommendations**

#### **1.1.1 Approvability**

There are no pharm-tox approval issues.

#### **1.1.2 Additional Non Clinical Recommendations**

None

#### **1.1.3 Labeling**

The draft labeling of ORFADIN (Nitisinone) generally conforms to the format specified under 21CFR 201.57(c)(9)(i) through (c)(9)(iii) Requirements for PLLR (Pregnancy and Lactation Labeling Rule) Prescription Drug Labeling.

### **8.1 Pregnancy**

#### **Sponsor's version:**



**Evaluation:** The text should be modified as proposed below.

## 8.1 Pregnancy

### *Risk Summary*

Limited data on nitisinone use in pregnant women are not sufficient to inform any drug associated risk. Animal reproduction studies have been conducted for nitisinone. In these studies, nitisinone was administered to mice and rabbits during organogenesis with oral doses of nitisinone up to 20 and 8 times respectively, the recommended human dose. In mice, nitisinone caused incomplete skeletal ossification of fetal bones and decreased pup survival at doses 0.4 times the recommended human dose, and increased gestational length at doses 4 times the recommended human dose. In rabbits, nitisinone caused maternal toxicity and incomplete skeletal ossification of fetal bones at doses 1.6 times the recommended human dose [see *Data*].

The background risk of major birth defects and miscarriage for the indicated population are unknown. In the U.S. general population, the estimated background risk of major birth defects and miscarriage in clinically recognized pregnancies is 2-4% and 15-20%, respectively.

### *Animal Data*

**8.2** Reproduction studies have been performed in mice at oral doses of about 0.4, 4 and 20 times the recommended human dose (1 mg/kg/day) and in rabbits at oral doses of about 1.6, 4 and 8 times the recommended human dose based on the body surface area. In mice, nitisinone has been shown to cause incomplete skeletal ossification of fetal bones at 0.4, 4 and 20 times the recommended human dose, increased gestational length at 4 and 20 times the recommended human dose, and decreased pup survival at 0.4 times the recommended human dose based on the body surface area. In rabbits, nitisinone caused incomplete skeletal ossification of fetal bones at 1.6, 4 and 8 times the recommended human dose based on the body surface area.

## **13. NONCLINICAL TOXICOLOGY**

### **13.1 Carcinogenesis, Mutagenesis, Impairment of Fertility**

#### **Sponsor's version:**

**Evaluation:** No changes are recommended in this section.

## 1.2 Brief Discussion of Nonclinical Findings

ORFADIN (nitisinone) Capsule was approved in 2002 (NDA 21-232) for the treatment of hereditary tyrosinemia type 1 (HT-1). The current NDA (206-356) is submitted for an oral suspension formulation of nitisinone, and the Applicant did not submit any new nonclinical studies of nitisinone. The new suspension formulation will make administration of nitisinone to infants and children easier. In this new oral suspension formulation, three degradation products, (b) (4)

(b) (4) have been identified. The applicant assessed the genotoxic potential of these impurities using QSAR analysis, and *in vitro* and *in vivo* genotoxicity assays.

The Applicant assessed the genotoxic potential of (b) (4) and (b) (4) through a computational toxicology based assessment using Quantitative Structure-Activity Relationship (QSAR) analysis. Furthermore, bacterial reverse mutation tests were performed to determine the genotoxicity potential of (b) (4) and (b) (4) was also assessed for genotoxic potential using an *in vivo* combined micronucleus and alkaline Comet assay in mice. QSAR analysis showed no mutagenic potential of (b) (4) in the bacterial reverse mutation assay, and (b) (4) was considered to be a non-genotoxic impurity. QSAR analysis predicted (b) (4) to be genotoxic and mutagenic in a bacterial reverse mutation (Ames) test. When (b) (4) was subjected to the *in vitro* bacterial reverse mutation test, weak mutagenic responses were observed both in the absence and in the presence of metabolic activation. However, (b) (4) tested negative in the nitro-reductase deficient TA100NR strain in the Ames test, implicating nitroreduction as the crucial factor of bacterial mutagenicity of (b) (4). The same functional group is present in the parent compound (nitisinone) which is also predicted to be mutagenic. Nitisinone was not positive in the bacterial reverse mutation assay and is regarded as a non-mutagen. The genotoxicity of (b) (4) was further investigated *in vivo* in the mouse bone marrow micronucleus assay and the Comet assay.

Administration of (b) (4) resulted in increased frequencies of micronucleated polychromatic erythrocytes (PCE) in the bone marrow of mice at mid (700 mg/kg/day) and high (1400 mg/kg/day) doses. In the low dose group (350 mg/kg/day) the frequency of micronucleated PCE was not elevated. Despite the observed clastogenic effect of (b) (4) treatment in the bone marrow, no DNA damage could be detected with the Comet assay in the duodenum, liver or blood cells. A fluorescence *in situ* hybridization (FISH)-analysis of the induced micronuclei revealed that the chromosomal lesions were mediated by a clastogenic mechanism, i.e. the majority of the induced micronuclei were centromere-negative. Although, (b) (4) was found to have a genotoxic potential, based on the fact that nitisinone itself has positive genotoxicity findings, the presence of the

impurity would not add additional risk for cancer (ICH M7). The Guidance also recommends that these “impurities could be controlled at acceptable levels for non-mutagenic impurities”. In genotoxicity studies with nitisinone, it was negative in the Ames test and the *in vivo* mouse liver unscheduled DNA synthesis (UDS) test. Nitisinone was positive in the mouse lymphoma cell (L5178Y/TK<sup>+/+</sup>) forward mutation test and in an *in vivo* mouse bone marrow micronucleus test.

A fertility study of Nitisinone was conducted in male and female mice at oral dose of 5, 50 and 250 mg/kg/day. Male mice were administered 4 weeks prior to mating through the mating period and female mice were treated 2 weeks prior to mating through gestation Day 7. An increased pre-coital interval was noticed in females in the  $\geq 50$  mg/kg/day dose groups and an increased post-implantation loss was seen in the 250 mg/kg/day dose group.

In a embryofetal development study in mice, nitisinone was administered orally to pregnant mice at doses of 5, 50, 250 mg/kg/day from gestation Day 7 through 16. There was incomplete skeletal ossification of fetal bones at doses of  $\geq 5$  mg/kg/day. The pregnant mice that received  $\geq 50$  mg/kg/day exhibited an increased gestation length, and pup survival rate decreased by 9% compared to 5% in the untreated control. In an embryofetal development study in rabbits, nitisinone was administered orally to the pregnant animals at doses of 5, 12 and 25 mg/kg/day from gestation Day 7 through 19. Maternal toxicity and incomplete skeletal ossification of fetal bones were observed at all doses ( $\geq 5$  mg/kg/day).

## 2 Drug Information

### 2.1 Drug: ORFADIN (Nitisinone) oral suspension.

#### 2.1.1 CAS Registry Number (Optional)

104206-65-7

#### 2.1.2 Generic Name

Nitisinone

#### 2.1.3 Code Name

None

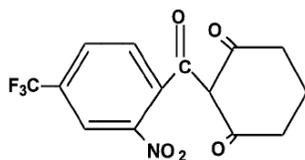
#### 2.1.4 Chemical Name

2-[2-nitro-4-(trifluoromethyl)benzoyl]cyclohexane-1,3-dione.

#### 2.1.5 Molecular Formula/Molecular Weight

C<sub>14</sub>H<sub>10</sub>F<sub>3</sub>NO<sub>5</sub> / 329.23

#### 2.1.6 Structure



### 2.1.7 Pharmacologic class

Enzyme (hydroxyphenyl-pyruvate dioxygenase or HPPD) inhibitor.

## 2.2 Relevant IND/s, NDA/s, and DMF/s

NDA: 21-232: Swedish Orphan Biovitrum AB (publ), Stockholm, Sweden; for the treatment of hereditary tyrosinemia type 1.

## 2.3 Clinical Formulation

### 2.3.1 Drug Formulation

The drug product is supplied in a brown 100 mL medicinal glass Type III bottle sealed with a child resistant cap. Each bottle contains 90 mL of suspension. The composition of the drug product is shown in the Applicant's Table below.

**Table 2.3.P - 1 Composition of the drug product**

Ingredient	Quantity (mg)	Function	Reference to standards
Nitisinone	4.0	Active substance	In-house specification
Hydroxypropyl methylcellulose (HPMC)	(b) (4)		Ph. Eur./NF
Glycerol		Ph. Eur./USP	
Polysorbate 80		Ph. Eur./NF	
Sodium benzoate		Ph. Eur./NF	
Citric acid monohydrate		Ph. Eur./USP	
Trisodium citrate dihydrate		Ph. Eur./USP	
Strawberry aroma		In-house specification	
Water, purified		Ph. Eur./USP	

<sup>a</sup> pH (b) (4)

### 2.3.2 Comments on Novel Excipients

No novel excipients were used in the formulation of the drug product.

### 2.3.3 Comments on Impurities/Degradants of Concern

The known impurities that are tested as part of the Nitisinone oral suspension can be classified into three categories: organic impurities, inorganic (b) (4). The known process related impurities are presented in the Table below.

Table 3.2.S.4.1-1 Specification

Parameter	Test Method (SOP No.)	Acceptance Criteria
Description	Appearance	A white to yellowish-white, crystalline powder. Practically insoluble in water, soluble in 2M sodium hydroxide, sparingly soluble in methanol and ethanol.
Identification		
- IR	Ph.Eur. 2.2.24 and USP <851>, (IA---0179)	Complies with reference spectrum (b) (4)
- Melting point		(b) (4)
Assay	HPLC (b) (4) (b) (4)	Contains not less than (b) (4)% and not more than (b) (4)%, calculated with reference to the (b) (4) substance.
Impurities		
- Organic impurities	HPLC (b) (4)	(b) (4)
- Inorganic impurities	(b) (4)	Not more than (b) (4)
	(b) (4)	Not more than (b) (4) ppm. Not more than (b) (4) ppm. Not more than (b) (4) %.
		(b) (4)
		(b) (4)
Microbial contamination	Total viable aerobic count (Ph. Eur. 2.6.12 and USP <61>).	Not more than (b) (4) micro-organisms per gram

Organic impurities are from (b) (4) Inorganic impurities are controlled and are not more than from (b) (4) as per the USP <221> (b) (4) that could be potentially present in the (b) (4), (b) (4) was routinely evaluated by a validated GC method. Based on ICH Q3C (R4) guidance, these impurities are within the acceptable limits.

Potential primary and secondary degradation products of nitisinone in Orfadin oral suspension are shown in the Applicant's Table below.

**Table 2.3.P - 26 Potential primary and secondary degradation products of nitisinone in the drug product**

Abbreviation	Chemical name	Molecular mass (g/mole)	Molecular formula	Structural formula
<b>Primary degradation products</b>				
(b) (4)				

(b) (4) is a known impurity/degradation product in the drug substance as well as in the currently approved solid oral dosage form. (b) (4) and (b) (4) are the main degradation products of nitisinone in Orfadin oral suspension. The stability studies showed the level of (b) (4) are below the reporting limit corresponding to (b) (4)

(b) (4)  
 (b) (4)  
 (b) (4)  
 Stability testing of the suspension has revealed two new degradation products in Orfadin oral suspension, the (b) (4)  
 (b) (4)  
 (b) (4) under the (b) (4) environment of the formulation. In the 9-month stability study, the total amount of the (b) (4)  
 (b) (4)  
 (b) (4)

The stability specification used for the drug product (ORFADIN) is shown in the Applicant's Table below.

**Table 3.2.P.8.1 - 4 Stability specification for drug product**

Test	Method	Acceptance Criteria
Appearance	Visual inspection	White slightly viscous opaque suspension
Related substances, % of labeled amount	(b) (4)	(b) (4)
(b) (4)		(b) (4)
Any unspecified		
Total		
pH	USP <791>	
Particle size distribution	(b) (4)	
D <sub>10</sub> , µm		For information
D <sub>50</sub> , µm		For information
D <sub>90</sub> , µm		For information
Sodium benzoate, % of labeled amount	(b) (4)	(b) (4)
Resuspendability, % of labeled amount <sup>b</sup>	Ph. Eur. 2.9.27 (b) (4)	
Uniformity of mass of delivered doses from (b) (4) containers	Ph. Eur. 2.9.27	To comply
Assay, % of labeled amount	(b) (4)	(b) (4)
Dissolution after (b) (4) minutes, Q = (b) (4) % LC	USP <711> (b) (4)	To comply
Microbiological quality	USP <61>	
TAMC, CFU/mL		NMT (b) (4)
TYMC, CFU/mL		NMT (b) (4)
Specified microorganisms	USP <62>	(b) (4)
(b) (4)		(b) (4)
Antimicrobial effectiveness testing	USP <51>	Complies

<sup>a</sup> The acceptance criterion corresponds to the total amount of (b) (4)  
 (b) (4)

In the QSAR analysis, (b) (4) was predicted negative for mutagenicity. However, (b) (4) was predicted positive. (b) (4) was identified as a weak bacterial mutagen in Ames test. However, (b) (4) tested negative in the nitro-reductase deficient TA100NR strain. In in vivo micronucleus assay, (b) (4) induced an increase in micronucleated polychromatic erythrocytes of the bone marrow of mice, most likely via a clastogenic mechanism. Despite the observed clastogenic effect of (b) (4) treatment in the bone marrow, no DNA damage could be detected with the Comet assay in duodenum, liver or blood cell tissues. In addition, the parent compound itself is genotoxic. Thus, an acceptable intake of (b) (4) was calculated based on a permitted daily exposure (PDE) by the applicant.

The PDE for (b) (4) was calculated to be (b) (4) µg/day (details of calculation are shown below).

$$\text{PDE} = \text{NOAEL} \div (\text{F1} \times \text{F2} \times \text{F3} \times \text{F4} \times \text{F5} \times \text{adjustment factor}^*)$$

F1=5 (rat-to-human)

F2= 10 (account for human variability between individuals)

F3=1 (results from chronic life-time study)

F4=10 (severe toxicity, i.e. non-genotoxic carcinogenicity)

F5=1 (NOAEL established)

\*Adjustment factor of 10 for exposures before 2 years of age and an adjustment factor of 3 for exposures between 2 and less than 16 years of age. The adjustment factor for adults is 1 (FDA guidance for genotoxicity and carcinogenicity impurities 2008).

NOAEL of (b) (4) was (b) (4) µg/day adopted from in vivo mouse bone marrow micronucleus assay.

$$\text{PDE of (b) (4)} = \text{(b) (4) mg/kg/day} \div (5 \times 10 \times 1 \times 10 \times 1 \times 1) = \text{(b) (4)}$$

The anticipated daily intake of total amount of (b) (4) will be (b) (4) ug/day (b) (4) kg body weight patient. Thus, the anticipated daily intake of (b) (4) from Orfadin oral suspension is much lower than the calculated PDE and is within acceptable limits.

## 2.4 Proposed Clinical Population and Dosing Regimen

Orfadin (Nitisinone) oral suspension is indicated for the treatment of hereditary tyrosinemia type 1 (HT-1). The recommended (b) (4) dose is 1 mg/kg/day divided into 2 daily doses followed by maximum dose of 2 mg/kg/day. One mL oral suspension contains 4 mg nitisinone.

## 2.5 Regulatory Background

A Type B pre-NDA teleconference was held on July 9, 2013 regarding new oral suspension formulation of Orfadin.

### 3 Studies Submitted

1. Nitisinone: Potential Genotoxic Degradants Risk Assessment Report evaluated by the QSAR analysis, using Derek Nexus 2.0 and Leadscope models (Study # TKT-2013-007).
2. (b) (4) Bacterial Reverse Mutation Assay (Study # UJP0002).
3. (b) (4) Bacterial Reverse Mutation Assay (Study # 8302339).
4. (b) (4) Mouse micronucleus and alkaline comet assay (Study # 8302340).

#### 3.1 Studies Reviewed

All studies mentioned in section 3.0 were reviewed.

#### 3.2 Studies Not Reviewed

None

#### 3.3 Previous Reviews Referenced

None.

### 4 Pharmacology

The applicant did not submit any pharmacology studies of Nitisinone.

### 5 Pharmacokinetics/ADME/Toxicokinetics

The applicant did not submit any pharmacokinetic studies with Nitisinone.

### 6 General Toxicology

No single- or repeated-dose toxicology study reports were submitted.

### 7 Genetic Toxicology

No genetic toxicology studies of nitisinone were submitted in this NDA. The proposed oral suspension formulation revealed the presence of two novel degradation products following storage, i.e. (b) (4)

(b) (4) Another potential degradation product of nitisinone is (b) (4) which is only formed in Orfadin oral suspension at exaggerated storage conditions (b) (4) and not at normal (b) (4) or accelerated (b) (4) storage conditions. The Applicant has submitted a report on the assessment of potential genotoxicity of (b) (4) and (b) (4) evaluated by the QSAR

analysis (Section 7.4). Furthermore, the applicant has performed genotoxicity studies of (b) (4) and (b) (4) to support a hazard assessment of the impurity. Genotoxicity studies of these impurities are reviewed below.

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

**Study title:** (b) (4) **Bacterial Reverse Mutation Test**

Study no.: UJP0001

Study report location: Electronic submission

Conducting laboratory and location: (b) (4)

Date of study initiation: March 5, 2014

GLP compliance: No

QA statement: No

Drug, lot #, and % purity: Lot # P094HH2, purity (b) (4)%; lot # P114AS2, purity (b) (4)%

#### Key Study Findings

Bacterial reverse mutation assays were conducted in *Salmonella typhimurium* (TA1537, TA1535, TA100, TA98) and *Escherichia coli* (WP2uvrA) with (b) (4) at concentrations of 50-5,000 µg/plate in the presence or absence of metabolic activation. (b) (4) showed mutagenic potential in the bacterial reverse mutation test for the tester strain TA100 in the presence and absence of S-9 mix, under plate incorporation conditions.

## Methods

- Strains: *Salmonella typhimurium* strains: TA1537, TA1535, TA100, TA98 and *E. coli* WP2uvrA
- Concentrations in definitive study: 50, 150, 500, 1500 or 5000 µg/plate. Under plate incorporation conditions, the concentrations were 500, 1500, 2500, 3500 or 5000 µg/plate for TA100 in the presence of S-9 mix.
- Basis of concentration selection: A dose range finding study to determine the toxicity of (b) (4) was carried out using TA98 and WP2uvrA strains in the presence and absence of S-9 mix. The dose levels were 1.5, 5, 15, 50, 150, 500, 1500 or 5000 µg/plate.
- Negative control: Dimethyl sulfoxide (DMSO)
- Positive control: 4-Nitroquinoline-N-oxide (4NQO), 9-aminoacridine (9AA), 2-nitrofluorene (2NF), 2-aminoanthracene (2AA) and Sodium Azide (SA).
- Formulation/Vehicle: DMSO
- Incubation & sampling time: Plates were incubated at 37°C for approximately 48 h for all strains in the presence or absence of metabolic activation (S9).

## Study Validity

The study was validated by the incidence of spontaneous revertants in the solvent controls within the range of historical values. A test was considered to be positive if the test item induced dose-related statistically significant increases in numbers of revertants compared with negative controls scored. A test was considered to be negative if the test item produced no greater increases in revertants for any strain.

## Results

(b) (4) batch P094HH2 showed a dose-related statistically significant increase in revertant numbers for TA100 at 500, 1500 and 5000 µg/plate in the absence of S-9 mix. There was a statistically significant increase in revertant numbers for TA100 at 5000 µg/plate in the presence of S-9 mix, which was outside of the laboratory historical background range. However, there were no dose-related or statistically significant increases in revertant numbers observed for any other strain at any dose of (b) (4) batch P094HH2, in the presence or absence of S-9 mix, under plate incorporation conditions. Data is presented in the Applicant's Table below.

**Table 1 - Mean Number of Revertants Per Plate - Experiment 1 Batch P094HH2- Plate Incorporation**

Strain	% S-9 mix v/v	Dose level of test item (µg/plate)						PC
		0	50	150	500	1500	5000	
TA1535	0	18.0	10.7	14.7	13.3	15.3 srl	rl	724.7
TA98	0	26.0	30.3	26.7	39.3	29.7	20.0 srl	91.0
TA100	0	160.3	162.7	175.0	200.3*	223.0*	282.3* srl	854.0
WP2uvrA	0	32.7	38.0	41.7	40.3	32.7	36.0 srl	713.0
Strain	% S-9 mix v/v	Dose level of test item (µg/plate)						PC
		0	15	50	150	500	1500	
TA1537	0	7.7	9.3	9.0	9.3	7.7	5.0 srl	176.3

Strain	% S-9 mix v/v	Dose level of test item (µg/plate)						PC
		0	50	150	500	1500	5000	
TA1535	10	10.7	11.0	11.3	8.0	17.3	11.0	127.0
TA1537	10	10.7	15.7	15.0	11.7	4.7	8.7	250.7
TA98	10	41.0	35.0	47.0	32.7	33.0	27.0	390.3
TA100	10	190.0	169.3	166.7	192.7	233.0	359.7*	1773.7
WP2uvrA	10	40.0	38.7	39.3	44.3	41.3	44.7	82.0

\* = p &lt; 0.01

PC: positive control

rl : reduced background bacterial lawn

srl : slightly reduced background bacterial lawn

Another experiment was conducted with TA100 strain only in the presence of S-9 mix using the plate incorporation method, in order to further investigate the potential positive response observed in the above experiment. The result showed that there was a dose-related statistically significant increase in the revertant numbers in TA100 at 3500 and 5000 µg/plate in the presence of S-9 mix, which were outside of the laboratory historical background range. Data is presented in the Applicant's Table below.

**Table 2 - Mean Number of Revertants Per Plate - Experiment 2 Batch P094HH2- Plate Incorporation**

Strain	% S-9 mix v/v	Dose level of test item (µg/plate)						PC
		0	500	1500	2500	3500	5000	
TA100	10	159.7	187.3	229.3	238.3	314.0*	323.0*	2680.0

\* = p &lt; 0.01

PC: positive control

In another study, a batch of (b) (4) (P114AS2) with high purity ((b) (4)%) was tested in TA100 in the presence and absence of S-9 mix. The result showed that there was a dose-related statistically significant increase in revertant numbers for TA100 at 1500 and 5000 µg/plate in the absence of S-9 mix. There was a statistically significant increase in revertant numbers at 5000 µg/plate in the presence of S-9 mix. In both cases, the increase at 5000 µg/plate was outside of the laboratory historical background range. Data are presented in the Applicant's Table below.

**Table 3 - Mean Number of Revertants Per Plate - Experiment 3 Batch P114AS2- Plate Incorporation**

Strain	% S-9 mix v/v	Dose level of test item (µg/plate)						PC
		0	50	150	500	1500	5000	
TA100	0	145.7	145.3	166.0	188.0	234.7*	294.3*	786.0

Strain	% S-9 mix v/v	Dose level of test item (µg/plate)						PC
		0	50	150	500	1500	5000	
TA100	10	178.7	195.3	187.3	220.7	219.0	346.7*	3287.0

\* = p &lt; 0.01

PC: positive control

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

Study no.: 8302339

Study report location: Electronic submission

Conducting laboratory and location:

(b) (4)

Date of study initiation: May 9, 2014

GLP compliance: Yes

QA statement: Yes

Drug, lot #, and % purity: Lot # P142AS2-P5, purity (b) (4)%

**Key Study Findings**

To exclude the possibility of nitro reductase (NR) involvement in the activation of (b) (4) the bacterial reverse mutation was tested in NR deficient strain TA100.

(b) (4) was used at concentrations of 5-5,000 µg/plate in the presence or absence of metabolic activation. In this test, (b) (4) tested clearly negative in the nitro-reductase (NR) deficient TA100NR strain, indicating that the bacterial nitro-reductase is crucial for the activation of (b) (4) to a DNA-reactive species.

## Methods

Strains: *Salmonella typhimurium* strains: TA100 and TA100NR

Concentrations in definitive study: 5, 16, 50, 160, 500, 1600 or 5000 µg/plate.

Basis of concentration selection: A dose range finding study to determine the toxicity of (b) (4)

Negative control: Dimethyl sulfoxide (DMSO)

Positive control: Sodium Azide (SA) and Metronidazole (MTZ).

Formulation/Vehicle: DMSO

Incubation & sampling time: Plates were incubated at 37<sup>0</sup>C for approximately 48 h for all strains in the presence or absence of metabolic activation (S9).

## Study Validity

The study was validated by the incidence of spontaneous revertants in the solvent and positive controls within the range of historical values, and the assay results were evaluated as positive if the number of revertant colonies in the groups treated with the test substance was at least twice the number in the group treated with the solvent (that is, at least twice the number of spontaneous revertant colonies) and if this increase in the number of revertant colonies was found to be concentration- dependent.

## Results

(b) (4) was negative in the Ames test for the nitro-reductase (NR) deficient TA100NR strain, indicating that the bacterial nitro-reductase is crucial for the activation of (b) (4) to a DNA-reactive species. The Applicant did not provide the study data in the study report.

## Study title: (b) (4) Bacterial Reverse Mutation Test

Study no.: UJP0002

Study report location: Electronic submission

Conducting laboratory and location: (b) (4)

Date of study initiation: March 5, 2014

GLP compliance: No

QA statement: No

Drug, lot #, and % purity: Lot # F173AS1, purity (b) (4)%; lot # P094HHI, purity- (b) (4)%

### Key Study Findings

In the bacterial reverse mutation assays in *Salmonella typhimurium* and *Escherichia coli*, (b) (4) at concentrations of 1.5-5,000 µg/plate did not induce gene mutation with or without metabolic activation.

### Methods

- Strains: *Salmonella typhimurium* strains: TA1535, TA1537, TA100, TA98 and *E. coli* WP2uvrA
- Concentrations in definitive study: 1.5, 5, 15, 50, 150, 500, 1500 or 5000 µg/plate.
- Basis of concentration selection: The doses selected for the mutation test were based on testing up to the regulatory maximum dose level of 5000 µg/plate.
- Negative control: Dimethyl sulfoxide (DMSO)
- Positive control: 4-Nitroquinoline-N-oxide (4NQO), 9-aminoacridine (9AA), 2-nitrofluorene (2NF), 2-aminoanthracene (2AA) and Sodium Azide (SA).
- Formulation/Vehicle: DMSO
- Incubation & sampling time: Plates were incubated at 37<sup>0</sup>C for approximately 48 h for all strains in the presence or absence of metabolic activation (S9).

### Study Validity

The study was validated by the incidence of spontaneous revertants in the solvent controls within the range of historical values, and the significant increase in numbers of revertant colonies in the positive control articles. All necessary criteria for a valid study, including appropriate phenotypic confirmations and vehicle/positive control response were fulfilled.

### Results

There were no dose-related or statistically significant increases in revertant numbers observed in any strain at any dose of (b) (4) in the presence or absence of S-9 mix, under plate incorporation conditions. Data are presented in the applicant's Table below.

**Table 1 - Mean Number of Revertants Per Plate - Plate Incorporation**

Strain	% S-9 mix v/v	Dose level of test item ( $\mu\text{g}/\text{plate}$ )						PC
		0	50	150	500	1500	5000	
TA1535	0	12.3	14.7	15.0	9.7	5.7	6.0 ppt	720.0
TA1537	0	15.0	14.0	15.3	7.0	3.7	5.0 ppt srl	245.7
TA98	0	39.7	45.7	45.0	43.3	32.7	32.0 ppt	102.0
TA100	0	176.0	203.0	183.7	169.0	145.3	117.7 ppt	764.0
WP2uvrA	0	36.7	32.7	38.3	38.7	34.7	31.3 ppt	1005.7

Strain	% S-9 mix v/v	Dose level of test item ( $\mu\text{g}/\text{plate}$ )						PC
		0	50	150	500	1500	5000	
TA1535	10	8.3	12.7	8.7	7.7	7.3	3.0 ppt	145.7
TA1537	10	15.7	16.0	10.3	6.5	7.3	3.0 ppt srl	143.0
TA98	10	36.3	32.7	35.7	40.0	30.0	20.0 ppt	744.3
TA100	10	187.0	187.7	191.0	167.0	147.3	110.3 ppt	1648.7
WP2uvrA	10	39.7	41.3	46.3	35.3	32.3	26.0 ppt	81.7

PC: positive control  
ppt : compound precipitation  
srl : slightly reduced background bacterial lawn

### 7.3 *In Vivo* Clastogenicity Assay in Rodent (Micronucleus Assay)

**Study title:** (b) (4) **Mouse Micronucleus and Alkaline Comet Assay.**

Study no: 8302340  
 Study report location: Electronic submission  
 Conducting laboratory and location: (b) (4)  
 Date of study initiation: May 1, 2014  
 GLP compliance: Yes  
 QA statement: Yes  
 Drug, lot #, and % purity: Batch # P142AS2-P5; purity: (b) (4) %

#### Key Study Findings

In the *in vivo* micronucleus and Comet assays, (b) (4) was administered orally (gavage) for 3 days at doses of 350, 700 and 1400 mg/kg/day to male Crl:CD-1 (ICR) mice. (b) (4) induced an increase in micronucleated polychromatic erythrocytes of the bone marrow of male mice at doses of 700 and 1400 mg/kg/day. In the same animals (b) (4) did not induce DNA damage in the liver, duodenum or blood cells when dosed up to 1400 mg/kg/day, as analyzed by the Comet assay.

#### Methods

Doses in definitive study: 350, 700 and 1400 mg/kg/day  
 Frequency of dosing: Once daily for 3 days  
 Route of administration: Oral gavage  
 Dose volume: 14 mL/kg for (b) (4) groups and 10 ml/kg for positive control.  
 Formulation/Vehicle: 7.5 mg/mL carboxymethyl cellulose, 1 mg/mL Tween 80 in 50 mM citrate buffer.  
 Species/Strain: Crl:CD-1 (ICR) mice.  
 Number/Sex/Group: 6 males/group and 3 for positive control  
 Satellite groups: None  
 Basis of dose selection: The highest dose was selected from the dose range finding study based on maximum tolerated dose.  
 Negative control: Vehicle  
 Positive control: Cyclophosphamide (CPA) 40 mg/kg, single oral administration dosed at 24 hours, sampled at 48 hours (Micronucleus End Point).  
 Ethyl methanesulfonate (EMS) 150 mg/kg, single oral administration dosed at 45 hours, sampled at 48 hours (Comet End Point).

#### Study Validity

The vehicle control data were generally comparable with the laboratory's historical vehicle control data. The positive control treatment (cyclophosphamide) resulted in a

statistically significant increase in group mean micronucleated polychromatic erythrocytes (MNPCE) over the concurrent vehicle control response. Thus, the micronucleus assay is accepted as a valid assay.

The vehicle control data for liver and blood were generally comparable with the laboratory's historical vehicle control data. The vehicle control group mean and individual animal data for duodenum were outside the historical vehicle control data ranges. However, there were limited historical vehicle control data in mice as a comparator. The data were subsequently compared to rat historical vehicle control data ranges and were found to be well within range. Therefore, the data were deemed acceptable. The positive control Ethyl methanesulfonate (EMS) induced a marked increase in Comet parameters in all tissues compared to the concurrent vehicle control. Thus, the Comet assay was considered to be valid.

## Results

Mice treated with (b) (4) exhibited group mean percentage of polychromatic erythrocytes (PCE) values that were similar to the concurrent vehicle control group and which were generally comparable with the laboratory's historical control data. There was no evidence of any test article-induced toxicity to the bone marrow (as would usually be indicated by a notable decrease in %PCE values compared to the vehicle control group or dose dependent decrease). Following the initial scoring of 2000 PCE, there were statistically significant, dose related increases in the number of mean micronucleated polychromatic erythrocytes (MNPCE) at 700 and 1400 mg/kg/day compared to vehicle control mean MNPCE count. It was therefore decided to score the same slides again to determine the MNPCE count over 4000 PCE. Following the repeat analysis, animals displayed inter-group heterogeneity at the intermediate and high doses of 700 and 1400 mg/kg/day. Therefore the percentages of MNPCE in each treated group were compared with vehicle control. Treatment of mice with (b) (4) induced statistically significant ( $p \leq 0.05$ ) increases in mean MNPCE frequencies compared to the vehicle control at 700 and 1400 mg/kg/day. There was also a statistically significant linear trend ( $p \leq 0.01$ ) indicating a dose response. The range of individual MNPCE frequencies for animals dosed at 700 and 1400 mg/kg/day were mainly either at the top end or exceeded the laboratory's historical control distribution, and the group mean percentage of MNPCE at 700 and 1400 mg/kg/day were more than 2-fold higher than the concurrent vehicle control group and exceeded the 95% historical vehicle control reference range. The low dose of 350 mg/kg/day (b) (4) induced group mean MNPCE frequencies were not statistically different from the concurrent vehicle control and all of these animals were considered to fall within the laboratory's historical control MNPCE distribution. Data are presented in the Applicant's Table below.

**Text Table 1**  
 (b) (4) **Summary of Micronucleus Data**

Group / Treatment (mg/kg/day)	PCE Scored	Number of MN PCE	% PCE	MN / 2000 PCE	% MN PCE	SD	Heterogeneity		Wilcoxon test vs vehicle control	
							X <sup>2</sup>	S	P- value	S
1M / Vehicle (0)	24000	31	65.73	2.58	0.13	0.04	2.49	NS	-	-
2M / (350)	24000	34	62.87	2.83	0.14	0.04	2.71	NS	0.3030	NS
3M / (700)	24000	80	64.43	6.67	0.33	0.21	26.89	P <sub>≤</sub> 0.001	0.0152	P<0.05
4M / (1400)	24000	78	63.00	6.50	0.33	0.19	22.69	P <sub>≤</sub> 0.001	0.0292	P<0.05
6M / CPA (40)	12000	228	58.53	38.00	1.90	0.22	-	-	0.0119	P<0.05

Terpstra-Jonckheere trend test (Groups 1, 2, 3, 4; upper tail) P-value: 0.0024 (P<0.01)

Thus, (b) (4) induced micronuclei in the polychromatic erythrocytes of the bone marrow in male mice treated at 700 and 1400 mg/kg/day.

The Comet assay of liver, duodenum and blood showed that there was no dose-related increase in percentage clouds cells following treatment with (b) (4). Thus the result showed that treatment with (b) (4) did not cause excessive DNA damage that could have interfered with Comet analysis. Group mean percentage of tail intensity and tail moment values for all groups of animals treated with (b) (4) (all tissues) were generally comparable with the concurrent group mean vehicle control data. All individual animal data at all dose levels were generally consistent with the concurrent vehicle control animals and generally fell within the laboratory's historical control. These data confirm that (b) (4) did not induce DNA damage in the liver, duodenum or blood cells of treated male mice. Data are presented in the Applicant's Tables below.

(b) (4) **Text Table 2**  
**Summary of Group Mean Comet Data – Liver**

Group / Treatment (mg/kg/day)	Total No. Cells Scored	Tail Intensity		Tail Moment		Mean %Clouds
		Mean	SEM	Mean	SEM	
1M / Vehicle (0)	900	1.19	0.69	0.12	0.07	2.26
2M / (b) (4) (350)	900	0.71	0.21	0.08	0.02	1.51
3M / (700)	900	0.75	0.18	0.10	0.02	1.09
4M / (1400)	900	1.44	0.80	0.16	0.08	1.72
5M / EMS (150)	450	10.84	2.07	1.38	0.26	1.93

(b) (4) **Text Table 3**  
**Summary of Group Mean Comet Data – Duodenum**

Group / Treatment (mg/kg/day)	Total No. Cells Scored	Tail Intensity		Tail Moment		Mean %Clouds
		Mean	SEM	Mean	SEM	
1M / Vehicle (0)	750	0.77	0.12	0.08	0.02	11.54
2M / (b) (4) (350)	900	0.36	0.12	0.04	0.01	8.90
3M / (700)	750	0.82	0.25	0.10	0.03	10.09
4M / (1400)	900	0.40	0.07	0.04	0.01	6.11
5M / EMS (150)	450	2.92	0.64	0.32	0.05	10.11

(b) (4) **Text Table 4**  
**Summary of Group Mean Comet Data – Blood**

Group / Treatment (mg/kg/day)	Total No. Cells Scored	Tail Intensity		Tail Moment		Mean %Clouds
		Mean	SEM	Mean	SEM	
1M / Vehicle (0)	900	1.05	0.36	0.11	0.04	0.11
2M / (b) (4) (350)	900	1.09	0.37	0.12	0.04	0.55
3M / (700)	900	0.73	0.18	0.08	0.02	0.00
4M / (1400)	900	1.26	0.23	0.13	0.02	0.55
5M / EMS (150)	450	9.67	1.42	1.13	0.19	5.44

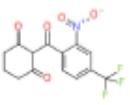
## 7.4 Other Genetic Toxicity Studies

The Applicant has submitted a report on the potential genotoxicity of Nitisinone and their impurities, which are evaluated by the QSAR analysis (Derek Nexus and Leadscope model).

**Study: Nitisinone: Potential Genotoxic Degradants Risk Assessment Report (TKT-2013-007).**

**Method:** The potential bacterial mutagenesis of nitisinone and their degradant (listed in the Applicant's table below) were predicted by two Quantitative Structure-Activity Relationship (QSAR) prediction methodologies. They are Derek Nexus (Lhasa Ltd., version 3.0.1, knowledge base Derek KB 2012 1.0) and Leadscope Model Applier (Leadscope Inc., version 1.6.0-17).

## 3.1 Compounds

Compound 1	
Nitrofurantoin	
CAS # 104206-65-7	
Compound 2	

(b) (4)	(b) (4)
CAS # None found	
Compound 3	
(b) (4)	
(b) (4)	
Compound 4	
(b) (4)	
(b) (4)	
Compound 5	
(b) (4)	
CAS # None found	
Compound 6	
(b) (4)	
(b) (4)	

**Results:** The Derek Nexus and Leadscope Model predicted that both (b) (4)

are positive for bacterial mutagenicity. Positive for mutagenesis was originated from the aromatic nitro function, which is similar to nitrofurantoin. However, nitrofurantoin was negative in Ames test (ORFADINE, Nitrofurantoin, 2002). Thus, the Applicant concluded that (b) (4)

are not likely to possess a potential for bacterial mutagenicity and considered as a non-genotoxic degradants. These degradants are NMT (b) (4)% in the drug product, and meet the thresholds qualification according to ICH Q3B guidelines.

Both Derek Nexus and Leadscope Model predicted non-genotoxic for (b) (4). Furthermore, in the Nitrofurantoin drug product it is NMT (b) (4)% and meet the thresholds qualification according to ICH Q3B guidelines.

The Derek Nexus Model predicted negative for bacterial mutagenicity for (b) (4), but Leadscope Model predicted positive for mutagenicity in the Microbial Mut model., (b) (4) in the drug product and considered as not mutagenic and meet the thresholds qualification according to ICH Q3B guidelines.

The safety evaluation of (b) (4) is calculated based on the PDE (Permitted Daily Exposure). The highest level of (b) (4) was NMT (b) (4)% in oral suspension. The anticipated maximum exposure to (b) (4) would be (b) (4) µg/day based on the maximum daily administration of (b) (4) body weight). (b) (4) is negative in the bacterial mutagenicity (Ames) test. (b) (4) is not mutagenic and in a 2-year combined chronic toxicity/carcinogenicity study in rats, the no-observed-effect level (NOEL) of (b) (4) mg/kg/day (b) (4) (b) (4)).

The PDE for (b) (4) was calculated to be (b) (4) µg/day (details of calculation are shown below) which is much higher than the anticipated maximum daily intake of (b) (4) µg/day. Therefore, there is a reasonable assurance of safety for the (b) (4) in nitisinone oral suspension.

$$\text{PDE} = \text{NOEL} \div (\text{F1} \times \text{F2} \times \text{F3} \times \text{F4} \times \text{F5} \times \text{adjustment factor}^*)$$

F1=5 (rat-to-human)

F2= 10 (account for human variability between individuals)

F3=1 (results from chronic life-time study)

F4=10 (severe toxicity, i.e. non-genotoxic carcinogenicity)

F5=1 (NOAEL established)

PDE of (b) (4) µg/day.

## 8 Carcinogenicity

No carcinogenicity studies were submitted.

## 9 Reproductive and Developmental Toxicology

No reproductive and developmental toxicology studies were submitted.

## 10 Special Toxicology Studies

No special toxicology studies were submitted.

## 11 Integrated Summary and Safety Evaluation

In the current submission, the applicant is seeking approval of nitisinone oral suspension for the treatment of hereditary tyrosinemia type 1 (HT-1). Orfadin capsules have been approved in USA in 2002 for the treatment of hereditary tyrosinemia type 1 (HT-1) under NDA 21-232. (b) (4)

(b) (4) The recommended maximum oral daily dose is 2 mg/kg/day divided into two daily doses.

In this 505 (b)(1) NDA, the applicant relied on the Agency's previous assessment of safety of nitisinone. In the current suspension formulation, the Applicant identified three degradation products , (b) (4)

(b) (4) have been identified. Among them, (b) (4) is a known impurity/degradation product in the drug substance as well as in the current solid oral dosage form. The potential genotoxicity profiles of these compounds were determined by QSAR analysis and AMES tests. (b) (4) was further investigated in vivo in mice using bone marrow micronucleus assay and the Comet assay. (b) (4) was negative in QSAR and Ames test. However, (b) (4) was determined to be positive in QSAR analysis, AMES test and bone marrow micronucleus assay. But (b) (4) was negative in the nitro-reductase deficient TA100NR strain in Ames test and no DNA damage could be detected with the Comet assay in duodenum, liver or blood cell tissue. (b) (4) contains the same functional group as parent compound (nitisinone), which is also predicted to be mutagenic in the mouse lymphoma cell (L5178Y/TK<sup>+/−</sup>) forward mutation test and in an *in vivo* mouse bone marrow micronucleus test. According to ICH M7 guidance (2015), if the drug substance itself is mutagenic, exposure to a mutagenic impurity in these cases would not significantly add to the cancer risk of the drug substance. The Guidance also recommends that these "impurities could be controlled at acceptable levels for non-mutagenic impurities". Thus, there are no safety concerns for the impurities/degradants in the drug product, and no pharm-tox related safety issues were identified for ORFADIN (nitisinone) oral suspension.

## 12 Appendix/Attachments: None

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/s/  
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DINESH C GAUTAM  
03/09/2016

SUSHANTA K CHAKDER  
03/09/2016