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APPLICATION NUMBER:

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NON-CLINICAL 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:	210852
Supporting document/s:	1
Applicant's letter date:	September 28, 2017
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Product:	Cyclophosphamide injection
Indication:	Malignant diseases
Applicant:	Dr. Reddy's Laboratories Limited Plot No. P1 to P9, Q1 to Q5, Phase-III, VSEZ, Duvvada Disakhapatnam, Andhara Pradesh, India, 530046 Srinivasa Rao Dr. Reddy's Laboratories, Inc. 107 College Road East Princeton, NJ 08540
Review Division:	Division of Hematology Oncology Toxicology (DHOT) for Division of Oncology Products 1 (DOP1)
Reviewer:	Claudia P Miller, PhD
Team Leader:	Tiffany Ricks, PhD (Acting)
Division Director:	John K Leighton, PhD, DABT (DHOT) Julia Beaver, MD (Acting; DOP1)
Project Manager:	Fatima Rizvi, PharmD

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1 Executive Summary

1.1 Introduction

On September 28, 2017, Dr. Reddy's Laboratories Limited (Dr. Reddy's) submitted a new drug application (NDA) under section 505(b)(2) of the Federal Food, Drug and Cosmetic Act (FDCA) for Cyclophosphamide Injection 500 mg/mL, 1 g/2 mL and 2 g/4 mL. Dr. Reddy's intends to rely on the FDA's previous findings of safety and efficacy for the listed drug, Cytoxan® (cyclophosphamide for injection) under NDA 012142. Of note, this product is discontinued. Dr. Reddy's proposed formulation is different from the listed drug (LD) formulation in terms of dosage (from being a solution versus dry powder), and that it uses dehydrated alcohol, as an excipient. The Applicant submitted animal pharmacokinetic and toxicology studies in rodents in support of this NDA.

1.2 Brief Discussion of Nonclinical Findings

Dr. Reddy's provided a study report for a single dose, bridging toxicity study in male rats (Study No. P264/SE/065) to compare pharmacokinetics between Dr. Reddy's product and the LD. Findings from this study showed comparable decreases in body weight with both products. Pharmacokinetic results showed similar exposures with Dr. Reddy's product versus the LD. The Applicant's formulation uses dehydrated alcohol as a vehicle. Volume of distribution and clearance results were similar between test-article and reference product, suggesting ethanol did not influence disposition of kinetics of Dr. Reddy's product versus LD.

Dr. Reddy's conducted a 4-week repeat-dose comparative study with their product without or with spiking of impurities compared to the LD in CD-1 mice (Study No. SE/64/01). Animals were administered vehicle, 50 mg/kg or 100 mg/kg of products via tail injection. The purpose of this GLP study was to qualify the levels of impurities in Dr. Reddy's product when administered once weekly, for a total of 4 doses. Hence, one arm received Dr. Reddy's product spiked with impurities to enrich their final levels to (b) (4)% for impurities A, C and D and (b) (4)% for impurity B. In the main study, one male administered with 100 mg/kg Dr. Reddy's product spiked with impurities died at Day 14. This animal had reduced body weight and histopathology showed hepatocellular degeneration and necrosis and necrotic foci with bacterial colonies and multifocal thrombus in liver. The Applicant suggested death was incidental due to multifocal thrombus formation in the liver with subsequent liver degeneration and necrosis as indicated in the pathology report. It should be noted that the toxicokinetic group receiving Dr. Reddy's product spiked with impurities also had two early decedents, a male at 100 mg/kg was found dead on Day 12 and a male at 50 mg/kg was euthanized due to moribundity. These deaths were considered by the Applicant to be related to stress due to blood sampling. Overall, results showed changes in hematology parameters and major target organs of toxicity were nonreversible effects on spleen (minimal to moderate extramedullary hematopoiesis) and testes (minimal to marked degeneration/atrophy). Findings were similar across all groups administered with Dr.

Reddy's product, Dr. Reddy's product enriched with impurities or LD. Thus, the impurities were qualified based on the percent present in the toxicology drug lot.

A comparative intravenous local tolerance study in rabbits showed that a single administration at the absolute human dose of 50 mg/kg/body weight of Dr. Reddy's product at the strength dose of 20 mg/mL was tolerated and comparable to LD.

An in vitro hemolysis assay in human whole blood indicated Dr. Reddy's product and LD were not hemolytic.

1.3 Recommendations

1.3.1 Approvability

From the nonclinical perspective, this NDA is recommended for approval.

1.3.2 Additional Non Clinical Recommendations

None.

1.3.3 Labeling

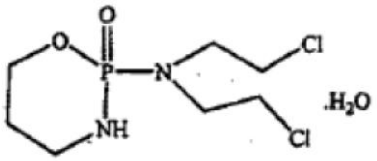
Refer to the final approved Prescribing Information for the final labeling. Minor changes were made to the lactation recommendation in the highlights, Sections 8.2 and 17 to reflect DOP1 practices with PLLR content and formatting.

2 Drug Information

2.1 Drug

CAS Registry Number (Optional)	6055-19-2
Generic Name	Cyclophosphamide
Code Name	None
Chemical Name	N,N-bis (2-chloroethyl)-N'-(3-hydroxypropyl) phosphordiamidic acid cyclic ester monohydrate
Molecular Formula/Molecular Weight	$C_7H_{17}Cl_2N_2O_3P$/ 279.09 (monohydrate) g/mol

Structure or Biochemical Description



(Excerpted from Applicant's submission)

Pharmacologic Class Alkylating agent

2.2 Relevant INDs, NDAs, BLAs and DMFs

NDA 012142 for Cytoxan.

2.3 Drug Formulation

The drug product is a clear, colorless to yellow color solution, packaged in USP (b) (4) tubular glass vials. It is available as 500 mg/vial, 1 g/vial and 2 g/vial.

Table 1: Composition of cyclophosphamide injection drug product

Ingredients	Reference to Quality Standards	Pharmaceutical Function	Quantity/vial (mg/mL)		
			500 mg/mL	1 g/2 mL	2 g/4 mL
Cyclophosphamide monohydrate	USP	Active pharmaceutical ingredient	534.5 mg#	1069 mg@	2138 mg$
Dehydrated Alcohol	USP	Vehicle	(b) (4)		

q.s. - quantity sufficient
534.5 mg of Cyclophosphamide monohydrate USP equivalent to 500 mg of Cyclophosphamide
@ 1069 mg of Cyclophosphamide monohydrate USP equivalent to 1000 mg of Cyclophosphamide
$ 2138 mg of Cyclophosphamide monohydrate USP equivalent to 2000 mg of Cyclophosphamide

(Excerpted from Applicant's submission)

2.4 Comments on Novel Excipients

Dehydrated alcohol is the sole excipient/vehicle used in the proposed drug product. Experience exists with the use of this excipient in the industry, and it complies with the requirements of USP. However, the Applicant did not provide sufficient information in their submission regarding alcohol levels. To include alcohol content in the label, the following information request was sent out to the Applicant on 12 March 2018:

Provide a table with the maximum daily intake of ethanol in g/kg expected from Cyclophosphamide Injection for each dosage in the label (i.e. 50 mg/kg, 40 mg/kg, 15 mg/kg, 10 mg/kg etc.) that will be administered to patients.

Applicant's response (22 March 2018):

We acknowledge the Agency's comment. As recommended by Agency, a table with the maximum daily intake of ethanol in g/kg expected from Cyclophosphamide injection for each dosage in the label that will be administered to patients is provided below:

Product Name	Dosage in label (Drug content)	Corresponding Ethanol content in the dose	Dosing Schedule as per prescribing information (Label)	Ethanol administered per day
Cyclophosphamide injection			(b) (4)	

(Excerpted from Applicant's submission)

The amount of ethanol that would be administered to the patient as per the dosage recommended in Cyclophosphamide injection label are calculated based on the total quantity of ethanol used in drug product batch.

Reviewer's Assessment:
The information provided is acceptable. For detailed calculations provided by the Applicant, refer to response to information request eCTD 0006. The amount of ethanol administered per day is less than other oncology FDA-approved ethanol containing products (i.e. docetaxel).

2.5 Comments on Impurities/Degradants of Concern

During the review of this NDA, the CMC reviewer requested from the Pharmacology/Toxicology team to confirm if impurities were qualified by nonclinical studies at the specified level. Impurities were qualified with general toxicology study # SE/64/01 based on the percent present in the toxicology drug lot. In addition, ICH Q3B and S9 allow for flexibility in impurity limits for anticancer therapeutics intended to treat advanced disease. The specified impurities are acceptable based on patient population, intermittent dosing, and the total daily intake (TDI) of impurities in ICH Q3B being for daily administration. As such the daily intake of impurities would be within the impurity limits of 0.2% to 3 mg TDI.

2.6 Proposed Clinical Population and Dosing Regimen

- Proposed clinical populations:
  - Malignant lymphomas: Hodgkin's disease, lymphocytic lymphoma, mixed-cell type lymphoma, histiocytic lymphoma, Burkitt's lymphoma
  - Multiple myeloma
  - Leukemias
  - Mycosis fungoides
  - Neuroblastoma
  - Adenocarcinoma of ovary, retinoblastoma, breast carcinoma
- Dosing regimen:
  - Malignant Diseases (Adult and Pediatric Patients):

- Intravenous: Initial course for patients with no hematologic deficiency – 40 mg/kg to 50 mg/kg in divided doses over 2 to 5 days. Other regimens include 10 mg/kg to 15 mg/kg given every 7 to 10 days or 3 mg/kg to 5 mg/kg twice weekly

2.7 Regulatory Background

Under PIND 129593, written responses were provided to the Applicant by the FDA on July 27, 2016 regarding the proposed plan for submission of a 505(b)(2) application and to gain input on clinical, non-clinical, CMC and bioequivalence issues. At this time, the FDA notified the Applicant that it did not appear that their drug product would satisfy the criteria for a bio waiver. The Applicant submitted follow up questions regarding the bio bridge request, which were addressed by the FDA on March 3, 2017. On September 28, 2017, Dr. Reddy's Laboratories Limited submitted this original 505(b)(2) NDA for cyclophosphamide injection 500 mg/mL, 1 g/2 mL and 2 g/4 mL.

3 Studies Submitted

3.1 Studies Reviewed

Study No.	Study Title
P264/SE/065	Single dose intravenous comparative pharmacokinetics study of Dr. Reddy's product, cyclophosphamide injection and reference product, cyclophosphamide for injection in male Sprague Dawley rats
SE/64/01	Dr. Reddy's product, cyclophosphamide injection 500 mg/mL (enriched with impurities & without impurities) and reference product, cyclophosphamide for injection USP 500 mg: Four weeks repeated dose comparative (once weekly) intravenous toxicity and toxicokinetic study in CD-1 mice with two weeks recovery period.
VLL/0616/G/T046	Cyclophosphamide Injection (2 g/4 mL) (Dr. Reddy's Product) and Cyclophosphamide for Injection (2 g/vial) (Reference Product): In Vitro Hemolysis Assay in Human Whole Blood
U15111	Cyclophosphamide Injection (Dr. Reddy's Product, RTU) and Endoxan (RLD, Cyclophosphamide Powder for Injection: Comparative Intravenous Local Tolerance Study in New Zealand White Rabbits

3.2 Studies Not Reviewed

Study No.	Study Title
P264/SE/038	Single dose comparative intravenous toxicity study of Dr. Reddy's product, cyclophosphamide injection (RTU) and reference product, cyclophosphamide for injection (powder) in CD-1 mice
P264/SE/040	4 weeks repeated dose (twice weekly) comparative intravenous toxicity study of Dr. Reddy's product, cyclophosphamide injection

	(RTU) and reference produce, cyclophosphamide for injection (powder) in CD-1 mice
P264/SE/048	Cyclophosphamide Injection (RTU) (spiked with impurities): two week repeated dose (once weekly) intravenous toxicity study in CD-1 mice
S14234	Cyclophosphamide Injection (Dr. Reddy's Product, RTU) and Endoxan (RLD, Cyclophosphamide powder for Injection: Comparative Intravenous Local Tolerance Study in New Zealand White Rabbits

3.3 Previous Reviews Referenced

None.

4 Pharmacology

The Applicant did not submit any pharmacology studies.

5 Pharmacokinetics/ADME/Toxicokinetics

5.1 PK/ADME

Study title: Single dose intravenous comparative pharmacokinetics study of Dr. Reddy's product, cyclophosphamide injection and reference product, cyclophosphamide for injection in male Sprague Dawley rats

Study no.:	P264/SE/065
Study report location:	eCTD Section 4.2.2.7.
Conducting laboratory and location:	(b) (4)
Date of study initiation:	October 5, 2016
GLP compliance:	No
QA statement:	Yes
Drug, lot #, and % purity:	Cyclophosphamide injection -RTU (Dr. Reddy's Product) 2 g/4mL, EH15027, 97.6%; Cyclophosphamide injection USP 500 mg/mL, 5G040G, 100.4%

Key Study Findings

- Decreased body weight was observed with both formulations of cyclophosphamide
- Comparative ratios between Dr. Reddy's product and reference product of PK parameters ranged between 0.94 to 1.04

- Ethanol did not influence disposition of kinetics, based on comparable volume distribution and clearance between Dr. Reddy's product versus LD

Methods

Doses:	Vehicle, 50 and 100 mg/kg
Frequency of dosing:	Once
Route of administration:	IV
Dose volume:	7.71 mL/kg
Formulation/Vehicle:	0.9% w/v sodium chloride
Species/Strain:	Rats/Sprague Dawley
Number/Sex/Group:	10 males/group
Age:	8 to 9 weeks
Weight:	263 ± 19.90g
Satellite groups:	None
Unique study design:	None
Deviation from study protocol:	None were considered to have had a major impact on the validity and interpretation of study results.

Table 2: Study design and allocation of male rats in Study No. P264/SE/065

Group No.	Treatment	Batch No.	Group Colour Code	Dose (mg/kg)	Strength (mg/mL)	Dose volume (mL/kg)	Number of Rats
G1	Reference Product, Cyclophosphamide for Injection	5G040G	Green	154.2	20	7.71	1-10
G2	Dr. Reddy's Product Cyclophosphamide Injection	EH15027	Blue	154.2	20	7.71	11-20

(Excerpted from Applicant's submission)

Justification for Dose Selection

Based on findings observed in a non-GLP, single dose toxicity study in rats using Dr. Reddy's cyclophosphamide for injection (Study# P264/SE/064). The human equivalent dose (HED) of 154.2 mg/kg was selected for this study. The selected dose is equivalent to human maximum daily dose of 25 mg/kg/day of cyclophosphamide.

Observations

Clinical signs	Twice daily after dosing
Body weight	Day 1 (pre-dose) and prior to the last blood sampling at 24 h time point on Day 2
Blood sampling	5 min, 30 min, 1, 1.5, 2, 4, 6, 8, and 24 h post-dosing

Necropsy/Gross Pathology	At the end of last blood collection

Results

Mortality

None.

Clinical Signs

Unremarkable.

Body Weights

- Decreased body weight was noted in Group 1 and Group 2 on Day 2, compared to Day 1.
- There was no statistically significant difference in body weight changes between Group 1 and Group 2 animals.

Table 3: Summary of body weight changes in Study No. P264/SE/065

Group	-	Day 1	Day 2
G1	Mean	262.85	238.38
	SD	21.40	17.38
	N	10	10
G2	Mean	263.00	241.43
	SD	19.90	15.64
	N	10	10

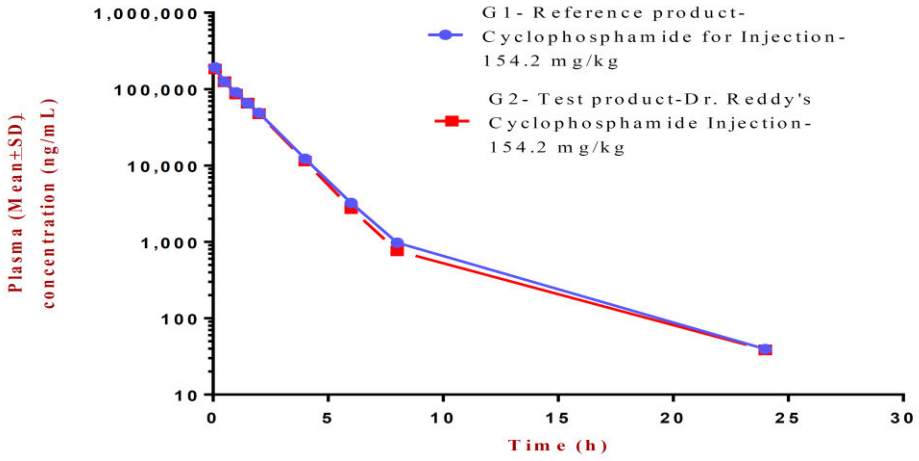
SD: Standard Deviation; N: No. of animals

G1: Reference Product, Cyclophosphamide for Injection @ 154.2 mg/kg
G2: Dr. Reddy's Product Cyclophosphamide Injection@ 154.2 mg/kg

(Excerpted from Applicant's submission)

Toxicokinetics

Figure 1: Comparative PK profiles between test-product and reference product in Study No. P264/SE/065



(Excerpted from Applicant's submission)

Table 4: Summary of PK parameters for Dr. Reddy's Cyclophosphamide and reference product in rats in Study No. P264/SE/065

PK Parameter	G1-Reference product-Cyclophosphamide for Injection	G2-Test product-Dr. Reddy's Cyclophosphamide Injection
$T_{1/2}$ (h)	3.006 ± 0.4788	3.095 ± 0.4031
T_{max} (h)	0.083 ± 0.0000	0.083 ± 0.0000
C_{max} (ng/mL)	192900.065 ± 11080.8062	182824.963 ± 11125.4932
C_0 (ng/mL)	210434.193 ± 13666.8888	197410.906 ± 13882.474
$AUC_{(0-24\ h)}$ (h*ng/mL)	293533.841 ± 44793.4606	282056.849 ± 23386.7685
$AUC_{(0-inf)}$ (h*ng/mL)	293717.072 ± 44796.4405	282240.983 ± 23443.8883
Vd (mL/kg)	2406.116 ± 682.122	2491.779 ± 389.7125
Cl (mL/h/kg)	536.762 ± 86.1122	549.81 ± 46.5488
MRT (h)	1.558 ± 0.1899	1.518 ± 0.1162

(Excerpted from Applicant's submission)

Table 5: Comparative ratios of PK parameters between Dr. Reddy's Cyclophosphamide and reference product in rats in Study No. P264/SE/065

PK Parameters	Test /Reference ratio
C_0 (ng/mL)	0.94
C_{max} (ng/mL)	0.95
AUC $_{(0-24)}$ (ng*h/mL)	0.96
AUC $_{(0-Inf)}$ (ng*h/mL)	0.96
Cl (mL/h/kg)	1.02
Vd (mL/kg)	1.04
$T_{1/2}$ (h)	1.03
T_{max} (h)	1.00
MRT (h)	0.97

(Excerpted from Applicant's submission)

Dosing Solution Analysis

Not reported.

6 General Toxicology

6.2 Repeat-Dose Toxicity

Study title: Dr. Reddy's product, cyclophosphamide injection 500 mg/mL (enriched with impurities & without impurities) and reference product, cyclophosphamide for injection USP 500 mg: Four weeks repeated dose comparative (once weekly) intravenous toxicity and toxicokinetic study in CD-1 mice with two weeks recovery period.

Study no.:	SE/64/01
Study report location:	eCTD Section 4.2.3.7.6.
Conducting laboratory and location:	(b) (4)
Date of study initiation:	April 1, 2016
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	Cyclophosphamide injection 500 mg/mL, CYI-2-500-F-006 (enriched with impurities), 88.9%; Cyclophosphamide injection 500 mg/mL, EH15019, 97.9%; Cyclophosphamide injection USP 500 mg/mL, 5G040G, 100.4%

Key Study Findings

- One death in male mouse at 100 mg/kg Dr. Reddy's product enriched with impurities on Day 14. Mouse presented a weight loss of -13.6%. Cause of death was hepatocellular degeneration and necrosis and necrotic foci with bacterial colonies and multifocal thrombus in liver in histopathology.
- Major target organs of toxicity in mice administered either formulation of cyclophosphamide were nonreversible effects on spleen (minimal to moderate extramedullary hematopoiesis) and testes (minimal to marked degeneration/atrophy).

Methods

Doses:	Vehicle, 50 and 100 mg/kg
Frequency of dosing:	Once weekly (total 4 injections)
Route of administration:	IV
Dose volume:	10 mL/kg
Formulation/Vehicle:	0.9% w/v sodium chloride
Species/Strain:	CD-1 mice
Number/Sex/Group:	10/sex/group
Age:	11 to 12 weeks
Weight:	31.79 to 37.60 g Males; 24.98 to 28.77 g Females
Satellite groups:	See Table 6
Unique study design:	None
Deviation from study protocol:	None were considered to have had a major impact on the validity and interpretation of study results.

Table 6: Impurities spiking levels in cyclophosphamide injection (enriched with impurities)

Impurities	Impurity % as per CoA	Impurity Spiking Level to Stock Solution (%)	Target Impurity Level to be qualified (%)
Impurity A	(b) (4)	(b) (4)	(b) (4)
Impurity B			
Impurity C	Detected by TLC method** (b) (4)		
Impurity D		-	

**: Based on the recommendation of the Sponsor, Impurity C was spiked as per target qualification level of (b) (4)% though the % impurity C was detected > (b) (4)% by TLC method (Qualitative in nature) and the prepared formulations were analysed as per validated analytical method.

(Excerpted from Applicant's submission)

Justification for Dose Selection

Based on findings observed in a non-GLP, 2-week, repeat-dose toxicity study in mice using cyclophosphamide spiked with impurities (Study# P264/SE/048); and non-GLP, single dose (Study# P264/SE/038) and 4-week, repeat dose (Study# P264/SE/040) comparative toxicity study of Dr. Reddy's cyclophosphamide for injection and reference product. Doses up to 300 mg/kg doses were tested in these studies. In Study# P264/SE/048, cyclophosphamide spiked with impurities was administered once weekly, for 2-weeks at 0, 100, 200 and 300 mg/kg. Males were more sensitive based on mortality, clinical signs (immobility, deep breathing, hypoactivity), reduced body weight, hematology changes, and changes in thymus and reproductive organs at 200 and 300 mg/kg doses. In Study# P264/SE/040, when administered twice weekly for 4 weeks,

Dr. Reddy's and reference product were tolerated up to highest dose tested, 60 mg/kg. Histopathology findings included changes in testes (tubular degeneration/atrophy), epididymides, thymus (minimal to mild atrophy) and spleen (minimal to mild extramedullary hematopoiesis). Thus, for this study, 50 and 100 mg/kg were selected to for evaluation. Per Applicant, these doses represent equal or double amount when compared to human maximum dose (50 mg/kg) on a body weight basis under clinical conditions.

Table 7: Study Design and allocation of mice

Main Groups:

Group No.	Treatment	Group Color Code	Dose (mg/kg)	Dose Strength (mg/mL)	Dose Volume (mL/kg)	No. of Mice	Sex	Mice Numbers
G1	Vehicle Control (0.9 % NaCl)	Green	0	0	10	10	M	1-10
						10	F	186-195
G2	Cyclophosphamide Injection (Without Impurities)	Blue	50	5	10	10	M	11-20
						10	F	196-205
G3		Red	100	10	10	10	M	21-30
						10	F	206-215
G4	Cyclophosphamide Injection (Enriched with Impurities)	Blue	50	5	10	10	M	31-40
						10	F	216-225
G5		Red	100	10	10	10	M	41-50
						10	F	226-235
G6	Reference Product, Cyclophosphamide for Injection	Blue	50	5	10	10	M	51-60
						10	F	236-245
G7		Red	100	10	10	10	M	61-70
						10	F	246-255

Recovery Groups:

Group No.	Treatment	Group Color Code	Dose (mg/kg)	Dose Strength (mg/mL)	Dose Volume (mL/kg)	No. of Mice	Sex	Mice Numbers
G1R	Vehicle Control (0.9 % NaCl)	Green	0	0	10	10	M	71-80
						10	F	256-265
G3R	Cyclophosphamide Injection (Without Impurities)	Red	100	10	10	10	M	81-90
						10	F	266-275
G5R	Cyclophosphamide Injection (Enriched with Impurities)	Red	100	10	10	10	M	91-100
						10	F	276-285
G7R	Reference Product, Cyclophosphamide for Injection	Red	100	10	10	10	M	101-110
						10	F	286-295

Toxicokinetics Groups:

Group No.	Treatment	Group Color Code	Dose (mg/kg)	Dose Strength (mg/mL)	Dose Volume (mL/kg)	No. of Mice	Sex	Mice Numbers
G1TK	Vehicle Control (0.9 % NaCl)	Green	0	0	10	3	M	111-113
						3	F	296-298
G2TK	Cyclophosphamide Injection (Without Impurities)	Blue	50	5	10	12	M	114-125
						12	F	299-310
G3TK		Red	100	10	10	12	M	126-137
						12	F	311-322
G4TK	Cyclophosphamide Injection (Enriched with Impurities)	Blue	50	5	10	12	M	138-149
						12	F	323-334
G5TK		Red	100	10	10	12	M	150-161
						12	F	335-346
G6TK	Reference Product, Cyclophosphamide for Injection	Blue	50	5	10	12	M	162-173
						12	F	347-358
G7TK		Red	100	10	10	12	M	174-179, 374,181-185
						12	F	359-370

M: Male; F: Female

(Excerpted from Applicant's submission)

Observations

Clinical signs	Daily
Body weight	Pre-test (Day 1), followed by twice weekly thereafter
Food consumption	Twice weekly
Ophthalmic exams	Direct ophthalmoscopy: pre-test, Day 26, Day 39 (recovery females), Day 40 (recovery males)
Hematology	Day 29 and Day 43
Serum Chemistry	Day 29 and Day 43
Coagulation	Day 29 and Day 43
Urinalysis	Not analyzed
Necropsy	Day 29 and Day 43 (examination of external surfaces, external orifices, abdominal, thoracic and cranial cavities, organs, tissues and site of injection)
Organ Weight	Day 29 and Day 43 (See Table Z in the Appendix for a full list organs)
Histopathology	Day 29 and Day 43 (See Table Z in the Appendix for a full list of evaluated tissues)

Mortality

Main Study Group:

- Male# 45 at 100 mg/kg Dr. Reddy's product enriched with impurities was found dead on Day 14. Mouse presented a weight loss of -13.6%. Gross pathology showed multifocal areas of discoloration of liver, which correlated with hepatocellular degeneration and necrosis and necrotic foci with bacterial colonies and multifocal thrombus in liver in histopathology. The Applicant suggested death was incidental due to multifocal thrombus formation in the liver with subsequent liver degeneration and necrosis.

Toxicokinetic Group:

- Male# 153 at 100 mg/kg Dr. Reddy's product enriched with impurities was found dead on Day 12.
- Male# 148 at 50 mg/kg Dr. Reddy's product enriched with impurities was sacrificed due to moribund conditions on Day 17.
- The Applicant suggested these deaths may be due to blood sampling stress.

Clinical Signs

- Transient reduced body tone on Days 19 to 21, in two male mice (Males# 42 and 48) at 100 mg/kg Dr. Reddy's product enriched with impurities.

Functional Observation Battery

No effects noted with test-article nor reference item.

Body Weights

Transient reduced body weight (-8%; $p < 0.05$ vs. vehicle control) was noted on Day 26 in males treated with 100 mg/kg with cyclophosphamide injection (no enrichment).

Food Consumption

There were transient periods where individual animals treated with cyclophosphamide with or without enrichment of impurities or reference product showed a decrease (up to -19%) or increase (up to +29%) of food intake.

Ophthalmoscopy

No effects noted with test-article nor reference item.

Hematology

Table 8: Summary of hematology changes (% relative to vehicle control) in Study No. SE/64/01

Parameters	Males						Females					
	Dr. Reddy's product		Dr. Reddy's product enriched with impurities		Reference product		Dr. Reddy's product		Dr. Reddy's product enriched with impurities		Reference product	
Dose (mg/kg)	50	100	50	100	50	100	50	100	50	100	50	100
RBC	-11	-14	-12	-14	-14	-16	-	-8	-	-11	-	-9
HGB	-10	-22	-	-14	-12	-16	-	-6	-	-12	-	-9
Hematocrit	-9	-10	-11	-11	-11	-14	-	-	-	-9	-	-
MCV	-	4	-	-	4	-	-	-	-	-	-	-
MCHC	-	-	-	-4	-	-	-	-3	-	-3	-	-3
RDW	-	24	-	25	-	23	9	18	14	19	11	23
WBC	-	-43	-	-58	-54	-50	-	-41	-	-43	-	-49
Platelets	-	-	-	-	-	-	-	29	-	41	-	-
Monocytes	-	254	-	-	-	141	-	-	-	126	-	-
Neutrophils	-42	-	-	-51	-48	-	-	-	-	-	-	-
Lymphocytes	-	-56	-46	-68	-62	-59	-	-49	-	-53	-	-61
Eosinophils	-	-	-	-	-	-51	-	-	-	-	-	-64
Reticulocytes	-	213	-	245	140	215	45	190	64	186	67	199

Recovery

- In males, increase in platelets (48-56%) versus recovery control was noted; In females, increase in MCV (up to 6%), MCH (up to 4%), neutrophils (up to 106%) were observed at end of recovery.

- Reversible or partially reversible parameters in males, included WBC, monocytes, lymphocytes, eosinophils, reticulocytes; and in females, included HGB, MCHC, RDW, monocytes, lymphocytes.
- In females, RBC, WBC and lymphocyte changes with 100 mg/kg Dr. Reddy's product or reference product were not reversible; and reticulocytes in 100 mg/kg Dr. Reddy's product group (other groups reversible)

Clinical Chemistry

- The following statistically significant changes were noted, but they were not dose dependent, differed between sexes, and/or lacked histopathology correlates.
    - A decrease in total protein was noted in males of 100 mg/kg Dr. Reddy's product (-8%), 50 mg/kg reference product (-6%) and females in 100 mg/kg Dr. Reddy's product enriched with impurities (-6%) at end of study; and an increase was noted in recovery males from 100 mg/kg Dr. Reddy's product enriched with impurities (5%) group.
    - A decrease in ALP was observed in females dosed with 100 mg/kg of any product of approximately -24%; and an increase in recovery males at 100 mg/kg Dr. Reddy's product (30%) and 100 mg/kg reference product (24%).
    - Females from 50 mg/kg Dr. Reddy's product group had 23% decrease in cholesterol
    - During recovery, increased glucose was noted in males treated with 100 mg/kg of any product, by approximately 24%

Urinalysis

No urinalysis data was submitted.

Gross Pathology

- Reduced testes size was noted at 100 mg/kg doses: 5/10 mice Dr. Reddy's product, 5/10 of Dr. Reddy's product enriched with impurities, 6/10 reference product. Recovery animals had an incidence of 2/10 mice in each group.
- Reduced thymus in males: 3/10 of Dr. Reddy's product, 3/10 of Dr. Reddy's product enriched with impurities, 4/10 reference product. No abnormalities in thymus were noted after recovery.
- Liver (multifocal area, white discoloration): Present in early decedent, Male#45 at 100 mg/kg Dr. Reddy's product enriched with impurities

Organ Weights

- Table 9 shows statistically significant changes, expressed by percentage change over respective vehicle control for absolute weight. Similar weight changes were observed for changes relative to whole body and brain weight.

Table 9: Organ weight changes (% relative to vehicle control) in Study No. SE/64/01

Organs	Males						Females					
	Dr. Reddy's product		Dr. Reddy's product enriched with impurities		Reference product		Dr. Reddy's product		Dr. Reddy's product enriched with impurities		Reference product	
Dose (mg/kg)	50	100	50	100	50	100	50	100	50	100	50	100
Spleen	48	76	30	85	34	87	-	79	-	82	-	59
Testes	-	-49	-	-34	-14	-36	-	-	-	-	-	-
Prostate+Seminal vesicles+coagulating glands	-17	-27	-27	-22	-25	-19	-	-	-	-	-	-

- At recovery:
  - An increase in liver with gallbladder weight was observed in males with Dr. Reddy's product enriched with or without impurities
  - An increase in thymus organ weight in females treated with Dr. Reddy's product enriched with impurities and males treated with Dr. Reddy's product enriched with impurities or reference product
  - A decrease in epididymides weight in males treated with Dr. Reddy's product
  - Effects on testes and prostate/seminal vesicles/coagulating glands were nonreversible and correlated with histopathology.
  - Effects on spleen were reversible (females) or partially reversible (males) and correlated with histopathology.

Histopathology

Adequate Battery: Yes

Peer Review: Yes

Histological Findings

	Males							Females						
Dose group	G1	G3		G5		G7		G1	G3		G5		G7	
mg/kg	0	50	100	50	100	50	100	0	50	100	50	100	50	100
n	10	10	10	10	10	10	10	10	10	10	10	10	10	10
Liver														
Infiltration, mononuclear, perivascular, focal/multifocal														
Minimal	1		1				1	2				2		1
Moderate										1				
Degeneration/necrosis hepatocellular														
Mild					1									

	Males							Females						
Dose group	**G1**	**G3**		**G5**		**G7**		**G1**	**G3**		**G5**		**G7**	
mg/kg	**0**	**50**	**100**	**50**	**100**	**50**	**100**	**0**	**50**	**100**	**50**	**100**	**50**	**100**
n	**10**	**10**	**10**	**10**	**10**	**10**	**10**	**10**	**10**	**10**	**10**	**10**	**10**	**10**
Necrotic foci with bacterial colonies and multifocal thrombus														
Present					1									
Lungs														
Infiltration, mononuclear, perivascular, focal/multifocal														
Minimal			3				1							
Mild			2				2			1		1		
Congestion, diffused														
Mild					1									1
Spleen														
Extra medullary hematopoiesis														
Minimal		4		4		2		2	5	1	7		5	
Mild		6	3	6		8	2		5	4	3	4	5	3
Moderate			7		7		7			5		4		6
Marked					2		1					2		1
Testes														
Degeneration/atrophy, tubular diffused														
Minimal														
Mild		2	3	2	6	1	2							
Moderate			1				3							
Marked			4		1		2							
Hypertrophy/hyperplasia Leydig cells, diffused			2											
Minimal														
			3		1									
Epididymides														
Reduced sperm, luminal caput/corpus														
Minimal			3		2		1							
Urinary bladder														
Infiltration, mononuclear cells, focal/multifocal/diffuse/ mixed cells														
Minimal	1		4							1		3		
Mild					1							1		1
Moderate					1									
Eyes with optic nerve														
Retinal atrophy														
Present	2		1		1		1	2		2				1
Sternum with marrow														

	Males							Females						
Dose group	**G1**	**G3**		**G5**		**G7**		**G1**	**G3**		**G5**		**G7**	
mg/kg	**0**	**50**	**100**	**50**	**100**	**50**	**100**	**0**	**50**	**100**	**50**	**100**	**50**	**100**
n	**10**	**10**	**10**	**10**	**10**	**10**	**10**	**10**	**10**	**10**	**10**	**10**	**10**	**10**
Increased hematopoesis Minimal			2		2		1							

G1= vehicle; G3 = Dr. Reddy's product without impurities; G5 = Dr. Reddy's product enriched with impurities; G7 = Reference product

Microscopic findings in testes (minimal to moderate degeneration/atrophy, tubular, diffuse) in males, and spleen (minimal to moderate extramedullary hematopoiesis) of both sexes were still present at end of recovery in animals treated with Dr. Reddy's product, Dr. Reddy's product enriched with impurities and reference product.

Toxicokinetics

- Comparisons between Dr. Reddy's product and reference product showed mean C_{max} and AUC ratios ranging between 0.93 to 1.08 and 0.95 to 1.09, respectively.
- Comparisons between Dr. Reddy's product enriched with impurities vs not enriched, showed mean C_{max} and AUC ratios ranging between 0.74 to 0.86 and 0.85 to 1.13, respectively.

Table 10: Toxicokinetics – Comparing Dr. Reddy's Product (without impurities) and reference product in Study No. SE/64/01

Parameters	Day	Dose (mg/kg)	Dr. Reddy's Product, Cyclophosphamide Injection (without impurities)		Cyclophosphamide for Injection (Reference Product)		Parameters Ratio		
			Male	Female	Male	Female	Male[a]	Female[b]	Mean[c]
$AUC_{(0\text{-}6\ hr)}$ (ng·hr/mL)	1	50	13414.822	12437.025	12315.140	11887.523	1.09	1.05	1.07
		100	37245.963	28037.432	36315.225	32349.015	1.03	0.87	0.95
	22	50	12573.081	15584.046	13876.334	12240.600	0.91	1.27	1.09
		100	31185.329	32765.985	37675.425	29555.705	0.83	1.11	0.97
C_{max} (ng/mL)	1	50	53149.792	57625.185	48145.088	56094.792	1.10	1.03	1.07
		100	118617.035	108424.627	110914.583	123152.364	1.07	0.88	0.97
	22	50	53200.681	68565.317	57690.562	55520.182	0.92	1.23	1.08
		100	110226.123	107329.536	121580.690	113024.188	0.91	0.95	0.93

Note: Male[a] or Female[b]: Dr. Reddy's Product, Cyclophosphamide Injection (without impurities)/ Reference Product, Cyclophosphamide for Injection, Mean[c] = Average of Male[a] and Female[b]

(Excerpted from Applicant's submission)

Table 11: Toxicokinetics – Comparing Dr. Reddy's Product (enriched with impurities) and Dr. Reddy's Product (without impurities) in Study No. SE/64/01

Parameters	Day	Dose (mg/kg)	Dr. Reddy's Product, Cyclophosphamide Injection (Enriched with impurities)		Dr. Reddy's Product, Cyclophosphamide Injection (without impurities)		Parameters Ratio		
			Male	Female	Male	Female	Male[a]	Female[b]	Mean[c]
$AUC_{(0\text{-}6\ hr)}$ (ng·hr/mL)	1	50	11977.675	10055.714	13414.822	12437.025	0.89	0.81	0.85
		100	36954.289	29779.104	37245.963	28037.432	0.99	1.06	1.03
	22	50	14884.468	14123.165	12573.081	15584.046	1.18	0.91	1.05
		100	36194.002	36075.928	31185.329	32765.985	1.16	1.10	1.13
C_{max} (ng/mL)	1	50	39811.930	41757.165	53149.792	57625.185	0.75	0.72	0.74
		100	98816.460	95625.986	118617.035	108424.627	0.83	0.88	0.86
	22	50	50197.506	49211.559	53200.681	68565.317	0.94	0.72	0.83
		100	88068.885	99770.139	110226.123	107329.536	0.80	0.93	0.86

Note: Male[a] or Female[b]: Dr. Reddy's Product, Cyclophosphamide Injection (Enriched with impurities)/ Dr. Reddy's Product, Cyclophosphamide Injection (without impurities), Mean[c] = Average of Male[a] and Female[b]

(Excerpted from Applicant's submission)

Dosing Solution Analysis

HPLC Method:

- Reference product (cyclophosphamide for injection) and Dr. Reddy's product, were ± 10% of the nominal concentration when tested on Days 1 and 22.
- Dr. Reddy's product, cyclophosphamide injection enriched with impurities did not meet acceptance criteria. Dosing formulations ranged from (b) (4)% to (b) (4)% on Day 1, and (b) (4)% or (b) (4)% on Day 22.
- The % relative standard deviation (RSD) of % unknown specified impurities ((b) (4) RRTs) was ± (b) (4)% of nominal concentrations on Days 1 and 22.

LC-MS/MS Method to detect impurities A, B, C, D

The following is in reference to cyclophosphamide related impurities A, B, C, and D in Dr. Reddy's product enriched with impurities, unless otherwise noted:

- % RSD of related impurities A, B, C, and D were ± (b) (4)% of study sample analysis on Days 1 and 22.
- For the 5 mg/mL formulation, the mean % accuracy of related impurities were ± (b) (4)% of nominal concentration on Day 1. For the 10 mg/mL formulation, impurities A, C and D were ± (b) (4)% of nominal concentration, and for impurity B it was slightly lower, at (b) (4)% compared to nominal concentration.
- On Day 22 the mean % accuracy of related impurities did not meet acceptance criteria, ranging for impurity A – (b) (4) to (b) (4)%, B – (b) (4)% to (b) (4)%, and D – (b) (4)% to (b) (4)%. Impurity C was within acceptance limits, ± (b) (4)% of nominal concentration, ranging from (b) (4)% to (b) (4)%.

7 Genetic Toxicology

Dr. Reddy's Laboratories Limited did not submit any genetic toxicology data in support of this NDA. These studies were not required as the applicant relied on FDA's previous findings of safety and efficacy for Cytoxan.

8 Carcinogenicity

Dr. Reddy's Laboratories Limited did not submit any carcinogenicity data in support of this NDA. Per ICH S9, carcinogenicity studies are not warranted to support marketing for therapeutics intended to treat patients with advanced cancer.

9 Reproductive and Developmental Toxicology

Dr. Reddy's Laboratories Limited did not submit any reproductive and developmental toxicology data in support of this NDA. These studies were not required as the applicant relied on FDA's previous findings of safety and efficacy for Cytoxan.

10 Special Toxicology Studies

Study Title: Cyclophosphamide Injection (2 g/4 mL) (Dr. Reddy's Product) and Cyclophosphamide for Injection (2 g/vial) (Reference Product): In Vitro Hemolysis Assay in Human Whole Blood/ Study No.VLL/0616/G/T046

This GLP-compliant study assessed the hemolytic potential of Dr. Reddy's product, Cyclophosphamide for Injection compared to reference product, together with (b) (4)% (b) (4)as a positive control and 0.9% sodium chloride injection as a negative control, in human whole blood. Doses tested for test-article and reference product were 500 μg/mL, 100 μg/mL and 50 μg/mL. Based on hemolytic grading, where ≤ 10% is considered non-hemolytic and ≥ 25% is hemolytic, the test-article and reference product were not hemolytic, with an average hemolysis of ≤ 10% at all doses tested. In contrast, positive control achieved an average hemolysis of 97% and negative control did not induce hemolysis.

Study title: Cyclophosphamide Injection (Dr. Reddy's Product, RTU) and Endoxan (RLD, Cyclophosphamide Powder for Injection: Comparative Intravenous Local Tolerance Study in New Zealand White Rabbits

Study no.:	U-15111
Study report location:	eCTD Section 4.2.3.6
Conducting laboratory and location:	(b) (4)
Date of study initiation:	August 14, 2015
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	Test-article: Cyclosphosphamide Injection (Dr. Reddy's Product) 500 mg/mL (RTU), Lot# EH15019, 97.9% purity. Reference product: Cyclosphosphamide for Injection (Endoxan), Lot# 4C166A, 99.4% purity.

Key Study Findings

- Dr. Reddy's Product was tolerated and comparable to reference product.

Methods

Doses:	50 mg/kg
Frequency of dosing:	Single administration
Dose volume:	2.5 mL/kg
Route of administration:	IV (marginal ear vein)
Formulation/Vehicle:	Saline
Species/Strain:	Rabbits/New Zealand White
Number/Sex/Group:	6 males/group
Satellite groups:	None
Unique Study design:	No
Deviation from study protocol:	None were considered to have had a major impact on the validity and interpretation of study results.

Twelve rabbits were randomized in two groups of 6 rabbits. Group#1 (G1) was administered Cyclophosphamide Injection 500 mg/mL formulation (RTU-test article) and Group#2 (G2) received Cyclophosphamide powder for injection (Endoxan-reference product) formulation by intravenous route. The test article or reference product were administered as a single dose in the right marginal ear vein. For control, the left marginal ear vein of each rabbit was injected with equivalent volume (2.5 mL/kg) of normal saline.

Table 12: Study design for Study No. U15111

Group	Route of Administration	Dose (mg/kg)	Formulation Concentration (mg/mL)	Dose Volume (mL/kg)	Number of Animals	Injection Site		Animal Number
						Test/ Reference Item	Normal Saline	
G1 (Dr. Reddy's Product)	Intravenous	50	20	2.5	6	Right Marginal Ear Vein	Left Marginal Ear Vein	Oa0818 to Oa0823
G2 (RLD)		50	20	2.5	6			Oa0824 to Oa0829

(Excerpted from Applicant's submission)

Justification for Dose Selection

The dose selected, 50 mg/kg/body weight was chosen because it represented the absolute human therapeutic dose based on the results of a dose rage finding study (Study No. S14234).

Observations and Results

Mortality

All animal survived to necropsy.

Clinical Signs

- Male# Oa0820 treated with test-article presented hematuria on Days 2 and 3.

Body Weight

Unremarkable

Injection Site

- Male# Oa0818 treated with test-article exhibited a:
  - well defined erythema from Day 1 until it recovered by Day 4.
  - vein engorgement at site on injection from Day 1 until it recovered by Day 2.

Gross Pathology

Unremarkable

Histopathology

Minimal to mild fibroplasia and minimal epidermal crust were noted in two animals treated with test-article on right ear or two animals with saline (left ear of rabbits treated with reference product). These microscopic findings were considered to be procedure related since they were present in left ear (saline treated) of Group#2 rabbits (reference product).

11 Integrated Summary and Safety Evaluation

See Executive Summary.

12 Appendix/Attachments

Table 13: Organ and tissues evaluated in Study No. SE/64/01

Tissue	Organ Weighed	Collected & Preserved	Microscopic Examination
Adrenal glands[+]	X	X	X
Aorta	-	X	X
Bone marrow smear[@]	-	X	X
Brain (Cerebrum, mid brain, cerebellum, medulla/pons)	X	X	X
Cecum	-	X	X
Colon	-	X	X
Diaphragm	-	X	X
Duodenum	-	X	X
Epididymides[^+]	X	X	X
Esophagus	-	X	X
Eyes (with optic nerve)*	-	X	X
Skeletal muscle, Biceps Femoris	-	X	X
Femur with joint**	-	X	X
Gross lesions	-	X	X
Heart	X	X	X
Ileum with Peyer's Patch	-	X	X
Jejunum	-	X	X
Kidneys[+]	X	X	X
Liver with gall bladder	X	X	X
Lung with bronchi [#]	-	X	X
Lymph nodes (mesenteric & mandibular)	-	X	X
Mammary gland	-	X	X
Nerves, sciatic	-	X	X
Ovaries[+]	X	X	X
Oviducts	-	X	X
Pancreas	-	X	X
Parathyroid	-	X	-
Pituitary	-	X	X
Prostate, Seminal vesicles & Coagulating glands	X	X	X
Rectum	-	X	X
Salivary glands	-	X	X
Skin	-	X	X
Spinal cord (cervical, thoracic & lumbar)	-	X	X
Spleen	X	X	X
Sternum with marrow**	-	X	X
Stomach, glandular and non glandular	-	X	X
Testes*[+]	X	X	X
Thymus	X	X	X
Thyroid with parathyroid	-	X	X
Tongue	-	X	X
Trachea	-	X	X
Tail	-	X	-
Urinary bladder	-	X	X
Uterus	X	X	X
Vagina and Cervix	-	X	X
Injection site (tail)**	-	X	X

*: Collected in Modified Davidson's fluid
#: Inflated with fixative and then immersed in formalin
X: Activity carried out
@: Bone marrow smear was prepared from the femur of all animals. All smears were stained with May Grunwald's Giemsa stain after preparation.
**: Bones and tail were decalcified prior to sectioning
+: Paired organs were weighed together
-: Not Applicable

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/s/

CLAUDIA P MILLER
06/18/2018

TIFFANY RICKS
06/19/2018