CENTER FOR DRUG EVALUATION AND RESEARCH

APPLICATION NUMBER:

214313Orig1s000

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 (505)(b)(2) REVIEW AND EVALUATION

Application number:	NDA-214313/Supporting Document #1
Sequence No:	0000 (eCTD)
CDER stamp date:	March 16, 2020
Filing date:	May 15, 2020
Product name:	Norepinephrine Bitartrate in 5% Dextrose Injection
Indication:	Restoration of blood pressure in acute hypotensive states (b) (4)
Applicant:	Baxter Healthcare Corporation (Baxter)
Review Division:	OCHEN-DPT
Reviewer:	Rama Dwivedi
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Review completion Date:	August 12, 2020

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Regulatory Background
Baxter Healthcare Corporation (Baxter) submitted PIND -138572 (Meeting Request –Written Responses; Reference ID: 4334606) to discuss the developmental plan and formulation of the proposed drug products (Norepinephrine Bitartrate in 5% Dextrose Injection). Thereafter, Baxter submitted NDA-214313(Sequence 0000) on March 16, 2020 under section 505(b)(2) of the Federal Food, Drug, and Cosmetic Act. using LEVOPHED (Norepinephrine Bitartrate Injection, USP) as the reference listed drug (RLD), marketed by Hospira Inc. (NDA 007513; approved on

July 13, 1950). The proposed drug product: (Norepinephrine Bitartrate in 5% Dextrose Injection) is a premixed solution of 4mg/250mL and 8mg/250mL (0.016 mg/mL and 0.032 mg/mL) of norepinephrine bitartrate monohydrate in 5% dextrose and provide ready-to-use infusion solutions. Baxter's proposed drug products contain several impurities (Fig.1) in Norepinephrine Bitartrate in 5% Dextrose Injection that are present at a higher level than in the RLD and/or that exceed the appropriate qualification threshold in ICH Harmonized Tripartite Guideline: *Impurities in New Drug Products Q3B(R2)*.

Impurity Structure	Peak ID	Code #
(b) (4)		

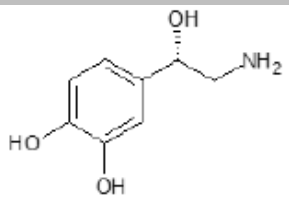
(b) (4)		
	D-Norepinephrine	Enantiomer
Structure Unknown		(b) (4)
Structure Unknown		

Figure 1: Impurities in Baxter's Proposed Drug Products

In accordance with the principles outlined in ICH-M7(R1[1]) and ICH-Q3B (R2[2]) Baxter conducted a (Q)SAR analysis using two complementary methodologies (an expert rule-based and a statistically-based analysis) to assess the mutagenic potential of impurities instead of performing a point mutation/bacterial reverse mutation assay. In addition, a14-day repeated-dose general toxicity (GLP) study in rats was conducted, with a clastogenic (micronucleate erythrocyte formation) endpoint incorporated into the study to qualify several impurities present at a higher level in in Norepinephrine Bitartrate 5% Dextrose Injection compared to the Reference Listed Drug.

The submitted data is reviewed as below.

Impurity Qualification Nonclinical Studies
Norepinephrine Bitartrate in 5% Dextrose Injection: 14-Day Impurity Qualification General Toxicity Study with Bone Marrow Micronucleus Evaluation in Rats

Conducting laboratory and location: (b) (4)

Sponsor Refence No.:
Date of study initiation: 08 February 2019

[1] Assessment and Control of DNA Reactive (Mutagenic) Impurities in Pharmaceuticals to Limit Potential Carcinogenic Risk Guidance for Industry (March 2018) (https://www.fda.gov/media/85885/download)
[2] Impurities in New Drug Products (Revision 2, August 2006) (https://www.fda.gov/regulatory-information/search-fda-guidance-documents/q3br-impurities-new-drug-products-revision-2)

Date of study completion:	26 August 2019
Drug/lot No:	Norepinephrine Bitartrate in 5% Dextrose Injection/BXU533927
Vehicle/lot No:	5% Dextrose Injection/ Y279182
Positive Control/lot No:	Cyclophosphamide/ MKCG5464
RLD/lot No:	Levophed/USP/ 880903A
Dosage:	74 µg/kg/hr.
Species/strain:	Hsd:Sprague Dawley (SD) Rats
Number/sex/group	6 /sex/group
Route:	Intravenous
GLP compliance:	Yes
QA statement:	Yes

Key Study Findings

There was no mortality observed in rats following the intravenous administration of Norepinephrine Bitartrate in 5% Dextrose Injection for 14 days continuous at a dose level of 74 µg/kg/hr.

Lower body weights, correlative decreases in food consumption, changes in clinical chemistry, decreases in spleen weight, and microscopic findings attributed in the heart (arterial medial hypertrophy, myocardial fibrosis, pigmented macrophages, and endocardial hyperplasia) were observed similarly with test article and Levophed.. There was no micronucleus induction observed under the experimental conditions applied, in the polychromatic erythrocytes of the bone marrow in treated rats.

Purpose

To perform a general toxicity study (with chromosomal aberration endpoint) to qualify several impurities present at a higher level in in Norepinephrine Bitartrate 5% Dextrose Injection compared to the Reference Listed Drug (Levophed) as per to the ICH Harmonized Tripartite Guideline: *Impurities in New Drug Products Q3B(R2)*].

Methods

- Dose administration rationale:
  - Maximum d-Enantiomer Exposure to human:

17.3 mg/day is the maximum daily exposure of patients to Norepinephrine Bitartrate in 5% Dextrose Injection solution. The worst-case percentage of the d-enantiomer over product shelf-life is anticipated to be (b) (4)%.

17.3 mg/day × (b) (4) = (b) (4) mg/day ÷ 60 kg = (b) (4) mg/kg/day or (b) (4) µg/kg/day

  - To achieve comparable exposure to d-enantiomer in rats by body surface area, the rat dose should be multiplied by 6.16 (37/6), which is (b) (4) µg/kg/day ((b) (4) µg/kg/day x6.16).

  - At a dose formulation concentration of 16µg/mL and an assumed d-enantiomer concentration of (b) (4) µg/mL (16 µg/mL × (b) (4) = (b) (4) µg/mL), rats needed to be

administered 111 mL/kg/day (or 4.63 mL/kg/hr.) of test article to achieve the target d-enantiomer exposure of (b) (4) µg/kg/day. This volume exposure of 111 mL/kg/day translated into an API exposure of 1778 µg/kg/day or **74 µg/kg/hr** (with 24 hours of continuous IV infusion).

Dose formulations for Groups 1, 3, and 4 were administered daily (for approximately 24 hours) by intravenous infusion via a catheter implanted in a femoral vein at a dose volume of 111 mL/kg/day (4.63 mL/kg/hour). Animals were exposed to test and reference articles (74 µg/kg/hr.) as per following experimental design. The Reference Article used in Group 3 was Levophed (Norepinephrine Bitartrate Injection), the reference drug for this 505(b)(2) application.

Experimental Design

Group	Subgroup	No. of Animals Male	No. of Animals Female	Dose Level (µg/kg/hr)	Dose Concentration µg/mL
1 (Vehicle Control)[a]	1 (Toxicity)	10	10	0	0
2 (Positive Control)[b]	2 (Positive Control)	5	5	N/A	N/A
3 (Reference Article)	1 (Toxicity)	10	10	74	16[c]
4 (Test Article)	1 (Toxicity)	10	10	74	16[d]

a Group 1 was administered vehicle control article only.
b Animals in Group 2 served as positive control animals for micronucleus assay and were not administered any test, reference, or vehicle control article. Animals were administered a single dose on Day 14 of the dosing phase with the positive control article (cyclophosphamide) via oral gavage at a volume of 10 mL/kg, 24 hours (±1.5 hours) prior to bone marrow collection.
c Concentration after diluting the reference article as supplied (1 mg/mL) with 5% Dextrose Injection, USP.
d Concentrations based on test article as supplied.

Animals were examined twice daily for mortality, abnormalities, and signs of pain or distress.

Body weights and food consumption were recorded for each animal twice during the pre-dose phase and prior to dosing on Days 1, 8, and 14 of the dosing phases.

Ophthalmic examinations were conducted once during the pre-dose phase for all animals and once for all experimental animals on Day 8 of the dosing phase. Pupils were dilated with a mydriatic agent prior to examination by a Veterinarian using an indirect ophthalmoscope.

Clinical Observations
Blood samples for hematology, coagulation, clinical chemistry and urine samples for urinalysis were collected from fasted toxicity Animal R0201 (Group 3 toxicity male) when sacrificed on Day 11 of the dosing phase as below:

Hematology Parameters

red blood cell (erythrocyte) count
hemoglobin
hematocrit
mean corpuscular volume
mean corpuscular hemoglobin
mean corpuscular hemoglobin concentration
red cell distribution width
absolute reticulocyte count
platelet count
white blood cell (leukocyte) count
absolute neutrophil count
absolute lymphocyte count
absolute monocyte count
absolute eosinophil count
absolute basophil count
absolute large unstained cell count
blood cell morphology

Coagulation Parameters

prothrombin time
activated partial thromboplastin time
fibrinogen

Clinical Chemistry Parameters

glucose
urea nitrogen
creatinine
total protein
albumin
globulin
albumin:globulin ratio
total cholesterol
triglycerides
total bilirubin
aspartate aminotransferase
alanine aminotransferase
alkaline phosphatase
gamma glutamyltransferase
creatine kinase
calcium
inorganic phosphorus
sodium
potassium
chloride

Urinalysis Parameres

appearance (clarity and color)
volume
specific gravity
pH
protein
urobilinogen
glucose
ketones
bilirubin
blood
microscopic examination of sediment

Terminal Procedures

Animals (positive controls and toxicity) were anesthetized and bone marrow was collected for micronucleus assay on Day 15 of the dosing phase. Following bone marrow collection, animals were exsanguinated and discarded without necropsy. Bone marrow smears (two slides) were prepared from the femur of each animal at the time of sacrifices.

Terminal body weights were recorded for sacrificed animals.

Macroscopic examination of the catheterization sites, external features of the carcass; external body orifices; abdominal, thoracic, and cranial cavities; organs; and tissues was performed.

Urine samples (up to 5 mL) were collected for urinalysis.

The following tissues from each animal were preserved in 10% neutral-buffered formalin, embedded in paraffin, slides were prepared and stained with hematoxylin and eosin for microscopic analyses.

Organ/Tissue			Organ/Tissue		
adrenal (2)	W	P,E	lymph node (mesenteric)		P,E
animal identification			mammary gland (females)		P,E
aorta		P,E	muscle, biceps femoris		P,E
bone, femur with bone marrow (articular surface of the distal end to include stifle joint)		P,E	nasal turbinates		
bone, sternum with bone marrow		P,E	optic nerve (2)[b]		P,E
brain	W	P,E	ovary (2)[a]	W	P,E
catheterization site		P,E	oviduct (2)[a]	W	P,E
cecum		P,E	pancreas		P,E
cervix[a]	W	P,E	pituitary gland	W	P,E
coagulating gland (2) (intact with seminal vesicles)		P,E	prostate	W	P,E
colon		P,E	rectum		P,E
duodenum		P,E	salivary gland (mandibular [2])		P,E
epididymis (2)	W	P,E	sciatic nerve		P,E
esophagus		P,E	seminal vesicle		P,E
eye (2)[b]		P,E	skin/subcutis		P,E
gut-associated lymphoid tissue (GALT) - (Peyer's Patch)		P,E	spinal cord (cervical, thoracic, and lumbar)		P,E
Harderian gland[b]		P,E	spleen	W	P,E
heart	W	P,E	stomach		P,E
ileum		P,E	testis (2)[c]	W	P,E
infusion site		P,E	thymus	W	P,E
jejunum		P,E	thyroid (2 lobes) with parathyroid	W	P,E
kidney (2)	W	P,E	tongue		P,E
lacrimal gland		P,E	trachea		P,E
lesions		P,E	urinary bladder		P,E
liver	W	P,E	uterus[a]	W	P,E
lung with large bronchi		P,E	vagina		P,E
lymph node (mandibular)		P,E			

E = Examined microscopically; P = Processed; W = Weighed.

a Organs weighed together; ovary with oviduct, and uterus with cervix.

b Eyes, optic nerve, and Harderian gland were preserved in Davidson's fixative for approximately 24 hours and then preserved in 50% alcohol.

c Testes were preserved in modified Davidson's fixative for approximately 48 hours and then preserved in 10% neutral-buffered formalin.

Bone Marrow Micronucleus Assay
Bone marrow was extracted on Day 15 of in Groups 1, 3, 4 (10 animals/sex/group) while in Group 2 (positive control for the micronucleus assay, 5 animals/sex) animals on Day 14 and processed by the (b) (4) Clinical Pathology Laboratory for micronucleus analyses, according to SOPs. Both slides from the first five animals/sex/group were stained in Acridine Orange and analyzed by fluorescent microscopy.

Data for each sex were analyzed separately. Analysis of variance (ANOVA) and pairwise comparisons were used to analyze the absolute body weight, body weight change, quantitative food consumption, continuous clinical pathology values, terminal body weight, absolute organ weight, organ: body weight percentage, and organ: brain weight percentage.

Levene's test was done to test the variances between groups. If the group effect of the ANOVA was significant ($P \leq 0.05$), Dunnett's t-test was used for pairwise comparisons between each test article-treated and control group. Group comparisons (Groups 3 and 4 versus Group 1) were evaluated at the 5.0%, two-tailed probability level. If the ANOVA was not significant (P ? 0.05), no further analyses were conducted.

Results
Mortality
There was no mortality observed due to test article (Norepinephrine Bitartrate in 5% Dextrose) and/or vehicle control, and all animals survived to their scheduled sacrifice.

Unscheduled mortality occurred in two animals in Group 3, administered the reference article. One male animal (R0206) was found dead on Day 5, and 2nd animal (R0201) was sacrificed on Day 11 of the dosing phase due to catheter-related inflammation.

Reference article-related heart findings were like those of terminal sacrifice animals, except the moderate myocardial degeneration and necrosis and slight mixed cell inflammation noted for Animal R0201, which were considered secondary to catheter-related inflammation.

Clinical Findings
There were no significant clinical findings observed due to test article (Norepinephrine Bitartrate in 5% Dextrose) or reference article (Levophed).

Red nasal discharge or red discoloration of the hair around the nose was noticed on Days 1 and/or 2 in two males (Animals R0207 and R0208) and one female (Animal R0603) treated with the reference articles.

Another male (Animal R0210) administered the reference article was observed with black discoloration of the haircoat around the right eye on Days 11 and 12. These observations were considered incidental and not related to reference article administration.

Summary of Clinical Findings

Test Article	(dosage)	1	2	3	4
Norepinephrine Bitartrate in 5% Dextrose Injection	µg/kg/hr	-	-	-	74
Reference Article	µg/kg/hr	-	-	74	-
Vehicle Control	µg/kg/hr	0	-	-	-
Positive Control		-	NA	-	-

Phase: Dosing

Category Observation	Group/Subgroup/Sex: Number in Group:	1/1/M 10	3/1/M 10	4/1/M 10	1/1/F 10	3/1/F 10	4/1/F 10
NORMAL							
No remarkable observations		10	10	10	10	10	10
Appearance							
limited use, right hind foot		0	1	0	0	0	0
Discharge							
nasal, red		0	1	0	0	0	0
Skin and pelage							
discolored haircoat, nose, red		0	1	0	0	1	0
discolored haircoat, right periorbital, black		0	1	0	0	0	0
thinning hair coat, periorbital		1	0	0	0	0	0

Ophthalmic Evaluations

There were no ophthalmic findings observed due to test article (Norepinephrine Bitartrate in 5% Dextrose) or reference article.

Indirect Ophthalmoscope

Test Article	(dosage)	1	2	3	4
Norepinephrine Bitartrate in 5% Dextrose Injection	µg/kg/hr	-	-	-	74
Reference Article	µg/kg/hr	-	-	74	-
Vehicle Control	µg/kg/hr	0	-	-	-
Positive Control		-	NA	-	-

Phase: Dosing

Category Observation	Group/Subgroup/Sex: Number in Group:	1/1/M 10	3/1/M 10	4/1/M 10	1/1/F 10	3/1/F 10	4/1/F 10
No visible lesions							
no visible lesions, eyes		10	9	10	10	10	10

Body Weights

Lower body weight changes were observed in both sexes by Day 8 and continuing through Day 14 due to Norepinephrine Bitartrate in 5% Dextrose and reference article when compared to the vehicle control and attributed to reduced food consumption.

However, the magnitude of lower body weight gains and reduced food consumption were similar between the test article (group 4) and reference article (group 3), in both males and females. See tables below for body weight changes.

Body Weight Changes in Males

Test Article	(dosage)	1	2	3	4
Norepinephrine Bitartrate in 5% Dextrose Injection	µg/kg/hr	-	-	-	74
Reference Article	µg/kg/hr	-	-	74	-
Vehicle Control	µg/kg/hr	0	-	-	-
Positive Control		-	NA	-	-

Data Presented in "g"

Group/ Subgroup/ Sex	Phase Day	DSNG 1	DSNG 8	DSNG 14
1/1/M	Mean	301	336	361
	SD	6.9	10.8	14.7
	N	10	10	10
	P(overall)	0.6446	0.0002	<0.0001
3/1/M	Mean	301	315	325
	SD	16.1	14.1	16.6
	N	10	9	8
	P(v1)	-	0.0016*	<0.0001*
4/1/M	Mean	297	312	328
	SD	11.4	11.4	13.8
	N	10	10	10
	P(v1)	-	0.0002*	<0.0001*
	Statistics	A	A	A

* P<=0.05
A = ANOVA and Dunnett's

Body Weight Change in Females

Test Article	(dosage)	1	2	3	4
Norepinephrine Bitartrate in 5% Dextrose Injection	µg/kg/hr	-	-	-	74
Reference Article	µg/kg/hr	-	-	74	-
Vehicle Control	µg/kg/hr	0	-	-	-
Positive Control		-	NA	-	-

Data Presented in "g"

Group/ Subgroup/ Sex	Phase Day	DSNG 1	DSNG 8	DSNG 14
1/1/F	Mean	218	230	238
	SD	7.1	7.3	8.4
	N	10	10	10
	P(overall)	0.5301	0.0473	0.0013
3/1/F	Mean	216	220	222
	SD	4.3	7.2	5.1
	N	10	10	10
	P(v1)	-	0.0279*	0.0006*
4/1/F	Mean	219	226	229
	SD	8.1	9.6	10.6
	N	10	10	10
	P(v1)	-	0.4967	0.0663
	Statistics	A	A	A

* P<=0.05
A = ANOVA and Dunnett's

Changes in Food Consumption in Males

Test Article	(dosage)	1	2	3	4
Norepinephrine Bitartrate in 5% Dextrose Injection	µg/kg/hr	-	-	-	74
Reference Article	µg/kg/hr	-	-	74	-
Vehicle Control	µg/kg/hr	0	-	-	-
Positive Control		-	NA	-	-

Data Presented in "g" Interval X to X

Group/ Subgroup/ Sex	Phase Day	DSNG 1 - 8	DSNG 8 - 14
1/1/M	Mean	173	144
	SD	8.9	11.8
	N	10	10
	P(overall)	0.0041	0.5240
3/1/M	Mean	162	151
	SD	14.1	18.0
	N	9	8
	P(v1)	0.0742	-
4/1/M	Mean	155	150
	SD	11.0	7.3
	N	10	10
	P(v1)	0.0021*	-
	Statistics	A	A

* P<=0.05
A = ANOVA and Dunnett's

Changes in Food Consumption in Females

Test Article	(dosage)	1	2	3	4
Norepinephrine Bitartrate in 5% Dextrose Injection	µg/kg/hr	-	-	-	74
Reference Article	µg/kg/hr	-	-	74	-
Vehicle Control	µg/kg/hr	0	-	-	-
Positive Control		-	NA	-	-

Data Presented in "g" Interval X to X

Group/ Subgroup/ Sex	Phase Day	DSNG 1 - 8	DSNG 8 - 14
1/1/F	Mean	127	105
	SD	8.3	4.1
	N	10	10
	P(overall)	0.1315	0.7661
3/1/F	Mean	119	104
	SD	7.0	8.3
	N	10	10
4/1/F	Mean	123	102
	SD	10.7	7.7
	N	10	10
	Statistics	A	AT

A = ANOVA and Dunnett's
T = Rank-transformed data

Clinical Pathology

There were no effects of Norepinephrine Bitartrate in 5% Dextrose or reference article on clinical pathology parameters on hematology, coagulation, or urinalysis and microscopic correlates. Minor effects due to reference- and Norepinephrine Bitartrate in 5% were of similar magnitude and consisted of mildly higher serum urea nitrogen concentration and mildly lower

sodium and chloride concentrations in both sexes and minimally lower cholesterol and triglyceride concentrations and minimally higher inorganic phosphorous concentration in males.

Organ Weights
An increased heart weight parameters and decreased spleen weight parameters were observed and attributed to the effects of norepinephrine at hyper-physiological levels. Microscopic correlates were noticed in the heart, not the spleen.

Test Article-Related Effects in Organ Weight Parameters

	Norepinephrine Bitartrate in 5% Dextrose Injection					
Sex	Males			Females		
Test Article (µg/kg/hr)	NA	NA	74	NA	NA	74
Reference Article (µg/kg/hr)	NA	74	NA	NA	74	NA
Vehicle Control Article/Diluent (µg/kg/hr)	0	NA	NA	0	NA	NA
Terminal Body Weight (g)	328	92*	92*	218	95*	98
Heart						
Absolute Weight (g)	1.2349	102	107	0.9127	102	110
Body Weight Ratio (%)	0.3766	110*	116*	0.4183	107	112*
Brain Weight Ratio (%)	65.5286	102	108	52.686	100	110*
Spleen						
Absolute Weight (g)	0.6878	82*	89*	0.5761	83*	88
Body Weight Ratio (%)	0.2093	89*	97	0.2638	88	90
Brain Weight Ratio (%)	36.4528	83*	90*	33.2624	82*	88*

NA = Not applicable.
* = Statistically significant difference (absolute or relative) compared with respective vehicle control mean value.
Note: Values for absolute weight and ratio of organ weights (relative to body or brain) for dosed groups expressed as percentage vehicle control mean value.

Macroscopic Observations
There were no macroscopic findings observed due to the reference or Norepinephrine Bitartrate in 5% Dextrose.

Microscopic Observations
Microscopic findings in the heart (arterial medial hypertrophy, myocardial fibrosis, pigmented macrophages, and endocardial hyperplasia) were observed due to reference article and Norepinephrine Bitartrate in 5% Dextrose administration, however, these findings were generally of minimal severity, and the overall incidences of the findings were similar in nature for the reference and Norepinephrine Bitartrate in 5% Dextrose.

The heart findings were considered secondary to hyper-physiological levels of norepinephrine and correlated with increased mean heart weight parameters.

Incidence and Severity of Test Article-Related Microscopic Findings - Terminal Sacrifice

	Norepinephrine Bitartrate in 5% Dextrose Injection					
Sex	Males			Females		
Test Article (µg/kg/hr)	NA	NA	74	NA	NA	74
Reference Article (µg/kg/hr)	NA	74	NA	NA	74	NA
Vehicle Control Article/Diluent (µg/kg/hr)	0	NA	NA	0	NA	NA
Number Examined	10	8	10	10	10	10
Heart						
Hypertrophy, medial, artery						
Minimal	0	8	10	0	10	10
Fibrosis, myocardium						
Minimal	0	3	5	0	5	1
Slight	0	0	0	0	1	1
Macrophages, pigmented						
Minimal	0	2	2	0	3	0
Hyperplasia, endocardium						
Minimal	0	0	2	0	3	3

NA = Not applicable.

Microscopic findings in the catheter and infusion sites were of the types commonly noted in infusion studies, and incidences and severities of the findings were similar for all groups, including vehicle controls.

Micronucleus Analysis

Male and female animals treated with Norepinephrine Bitartrate in 5% Dextrose Injection or the reference article, Levophed, exhibited group mean %PCE and mean MN PCE frequencies that were comparable to the vehicle control group or the laboratory's historical vehicle control data. There were no statistically significant increases in micronucleus frequency for any of the groups receiving the test article, compared to the concurrent vehicle control.

Norepinephrine Bitartrate in 5% Dextrose Injection: Summary of Micronucleus Data – Males

	% PCE		% MN PCE		WRS	
Group/Dose Level (µg/kg/hr)	Mean	SD	Mean	SD	P-value	Significance
01/ Vehicle (0)	37.56	6.86	0.06	0.03	N/A	N/A
02/ Cyclophosphamide (2)	33.08	7.74	1.25	0.33	0.0040	**
03/ Levophed (Norepinephrine Bitartrate) Injection, USP (74)	26.36	12.14	0.06	0.04	0.4206	NS
04/ Norepinephrine Bitartrate in 5% Dextrose Injection (74)	40.56	8.87	0.07	0.03	0.1984	NS

** = $p \leq 0.01$

Norepinephrine Bitartrate in 5% Dextrose Injection: Summary of
Micronucleus Data – Females

	% PCE		% MN PCE		WRS	
Group/Dose Level (µg/kg/hr)	Mean	SD	Mean	SD	P-value	Significance
05/ Vehicle (0)	41.84	6.80	0.09	0.05	N/A	N/A
06/ Cyclophosphamide (2)	28.28	6.25	0.67	0.21	0.0040	**
07/ Levophed (Norepinephrine Bitartrate) Injection, USP (74)	44.84	7.11	0.04	0.01	0.9802	NS
08/ Norepinephrine Bitartrate in 5% Dextrose Injection (74)	45.08	8.87	0.04	0.03	0.9563	NS

** = $p \leq 0.01$

CONCLUSION

Intravenous administration of Norepinephrine Bitartrate in 5% Dextrose (continuous for 24-hrs) and RLD (Levophed) for 14 days at a dose level of 74 µg/kg/hr. was well tolerated in rats. A minor decrease in body weight gains and corresponding food consumption, changes in clinical chemistry, decreases in spleen weight, and microscopic changes in the heart were observed, but the changes were comparable to the reference article (Levophed). There was no micronucleus induction observed in rat bone marrow as a result of the administration of neither RLD nor Norepinephrine Bitartrate in 5% Dextrose solution.

(Q)SAR Analysis Predicting the Mutagenicity Potential of Impurities in Norepinephrine Bitartrate in 5% Dextrose Injection

The proposed drug product Norepinephrine Bitartrate in 5% Dextrose Injection, is a premixed solution of 4 mg/250 mL and 8 mg/250 mL of norepinephrine bitartrate monohydrate in 5% dextrose and contain several impurities that are present at a higher level than the RLD.

As per US FDA (Meeting Request –Written Responses; Reference ID: 4334606) and in accordance with the principles outlined in ICH Harmonized Guideline: *Assessment and Control of DNA Reactive (Mutagenic) Impurities in Pharmaceuticals to Limit Potential Carcinogenic Risk* M7(R1) (Ref 3), Baxter performed an *in silico, (Q)SAR* analysis (BXU539075) using two complementary methodologies (Derek Nexus v.6.0.1., an expert rule-based method and Sarah Nexus v.3.0.0, a statistically-based analysis method) for evaluating the mutagenicity potential of impurities. Both software packages are ICH-M7 compliant and seem to be the most recent software versions. CDER has prior knowledge of the validation and use of both software. The analysis seems valid.

d-norepinephrine analysis using Nexus Derek for mutagenicity *in vitro* in bacterium yielded with "No misclassified or unclassified features" while analysis using Nexus Sarah yielded an overall "Negative with 25% confidence in the prediction" result.

Three supporting hypotheses containing similar examples from the training set were identified. Of the total number of training set example structures: (b) (4) containing the structural alerts were not present in d-norepinephrine (Lhasa Nexus. Nexus v.2.2.1 (Ref. 13).

(b) (4) analysis using Nexus Derek for mutagenicity *in vitro* in bacterium yielded an "INACTIVE" result with "No misclassified or unclassified features" while analysis of (b) (4) using Nexus Sarah yielded an overall "Negative with 29% confidence in the prediction" result.

Five supporting hypotheses containing similar examples from the training set were identified. Of the total number of training set example structures: (b) (4) containing the structural alerts were not present in (b) (4) (Lhasa Nexus. Nexus v.2.2.1 (Ref 13).

(b) (4) analysis using Nexus Derek for mutagenicity *in vitro* in bacterium yielded an "INACTIVE" result with "No misclassified or unclassified features" while analysis of (b) (4) using Nexus Sarah yielded an overall "Negative with 29% confidence in the prediction" result.

Five supporting hypotheses containing similar examples from the training set were identified. Of the total number of training set example structures: (b) (4)

containing the structural alerts were not present in [redacted](Lhasa Nexus. Nexus v.2.2.1 (Ref. 13).

[redacted] analysis using Nexus Derek for mutagenicity *in vitro* in bacterium yielded an "INACTIVE" result that "Contains *misclassified features* defined as features that are also present in a known positive compound for which no alert was raised by Nexus Derek" was identified (Fig. 2). Analysis of [redacted] using Nexus Sarah yielded an overall "Negative with 36% confidence in the prediction" result.

[redacted]

Figure 2: Structural Alerts

Two supporting hypotheses containing similar examples from the training set were identified. Of the total number of training set example structures: [redacted] containing the structural alerts were not present in [redacted] (Lhasa Nexus. Nexus v.2.2.1 (Ref. 13).

[redacted] analysis using Nexus Derek for mutagenicity *in vitro* in bacterium yielded an "INACTIVE" result with "No misclassified or unclassified features" while analysis of [redacted] using Nexus Sarah yielded an overall "Negative with 34% confidence in the prediction" result.

Five supporting hypotheses containing similar examples from the training set were identified. Of the total number of training set example structures: [redacted]; containing the structural alert [redacted], were not present in [redacted] (Lhasa Nexus. Nexus v.2.2.1 (Ref. 13).

[redacted] analysis using Nexus Derek for mutagenicity *in vitro* in bacterium yielded an "INACTIVE" result with "No misclassified or unclassified features" while analysis of [redacted] using Nexus Sarah yielded an overall "Negative with 37% confidence in the prediction" result.

Two supporting hypotheses containing similar examples from the training set were identified. Of the total number of training set example structures: [redacted]; containing the structural alert [redacted], was not present in [redacted] (Lhasa Nexus. Nexus v.2.2.1 (Ref. 13).

[redacted] analysis using Nexus Derek for mutagenicity *in vitro* in bacterium yielded an "INACTIVE" result with "No misclassified or unclassified features" while analysis of [redacted] using Nexus Sarah yielded an overall positive with 17% confidence in the prediction" result.

Three supporting hypotheses containing similar examples from the training set were identified. Of the total number of training set example structures: (b) (4) containing the structural alerts were not present in (b) (4) (Lhasa Nexus. Nexus v.2.2.1 (Ref. 13).

Conclusion

The results of (*Q)SAR* analysis under the experimental conditions tested for several impurities present in the proposed drug product Norepinephrine Bitartrate in 5% Dextrose Injection suggest that the impurities identified are predicted to be negative for mutagenicity *in vitro* in bacterium analysis and do not have risk for mutagenicity potential in the Norepinephrine Bitartrate in 5% Dextrose Injection. Therefore, these impurities should be classified as Class 5 per ICH-M7(R1) as non-mutagenic.

Justification for the Impurities present in Norepinephrine Bitartrate Monohydrate in 5% Dextrose to Human Exposure (Impurity "X" here is used as an example to demonstrate the calculation method):

- Maximum daily patient exposure to Norepinephrine Bitartrate in 5% Dextrose Injection is 17.3 mg/day (Ref 5).
- 0.5% for impurity "X" in Norepinephrine Bitartrate in 5% Dextrose Injection test article used in the 14-day general toxicology study (Ref 3).
- Patient body weight of 60 kg (Ref 6).
- Maximum duration of patient treatment with Norepinephrine Bitartrate in 5% Dextrose Injection of 6 days (Ref 7).

Eq. 1 17.3 mg/day × 0.5% = 0.0865 mg impurity/day ÷ 60 kg = 0.00144 mg/kg/day or 1.44 µg/kg/day × 6 days treatment = 8.64 µg impurity/kg

Conversion of patient exposure to rat "human equivalent" exposure

- Conversion factor of 6.167 to convert human exposure to rat "human equivalent" exposure based on inter-species differences in body surface area (Ref 6).

Eq. 2 8.64 µg impurity/kg ×6.167 = 53.3 µg impurity/kg

The rat "human equivalent" exposure to impurity "X" of 53.3 µg/kg is the minimum exposure in the 14-day general toxicology study to consider impurity "X" qualified.

Actual rat "human equivalent" exposure to impurity "X" achieved in the 14-day general toxicology study

- Norepinephrine Bitartrate in 5% Dextrose Injection test article concentration of 16 µg/mL used in 14-day general toxicology study (Ref 8).
- Average of 0.5% for impurity "X" in Norepinephrine Bitartrate in 5% Dextrose Injection test article during 14-day general toxicology study.
- Norepinephrine Bitartrate in 5% Dextrose Injection infusion rate of 111 mL/kg/day during 14-day general toxicology study (Ref 3).
- Duration of general toxicology study of 14 days (Ref 3).

Eq. 3 16 µg/mL × 0.5% = 0.08 µg impurity/mL ×111 mL/kg/day = 8.88 µg/kg/day × 14 days treatment = 124 µg impurity/kg

For impurity "X", actual rat "human equivalent" exposure to impurity "X" of 124 µg/kg exceeds the minimum rat "human equivalent" exposure of 53.3 µg/kg needed to consider the impurity adequately qualified. Thus, impurity "X" in this example would be considered qualified.

The safety margin of exposure for each impurity in Norepinephrine Bitartrate in 5% Dextrose Injection is given as below. The calculation of each safety margin is based on the proposed specification limit of each impurity in the drug product.

Impurity	Average Impurity in Norepinephrine Bitartrate in 5% Dextrose Injection Test Article, %[1]	Patient Exposure to Impurity, µg/kg[2]	Rat "Human Equivalent" Exposure to Impurity, µg/kg[3]	Actual Rat Exposure to Impurity, µg/kg[4]	Margin of Exposure[5]
d-Norepinephrine					(b) (4)

[redacted]

[1]Ref 8. [2]See section 3.1 and Ref 4. [3]See section 3.2 and Ref 4. [4]See section 3.3 and Ref 4. [5]Margin of Exposure = Actual Rat Exposure to Impurity ÷ Rat "Human Equivalent" Exposure to Impurity. [6]Based on a proposed specification limit of NMT (b) (4) (Ref 9). [7]Based on a proposed specification limit of NMT (b) (4) (Ref 9). [8]Based on a proposed specification limit of NMT (b) (4) (Ref 9). [9]Based on a combined proposed specification limit of NMT (b) (4) (Ref 9). [10]Based on a proposed specification limit of NMT (b) (4) (Ref 9). [11]Based on a proposed specification limit of NMT (b) (4) (Ref 9).

Conclusions and Recommendations

Based on the 14 Days repeated dose toxicology study in rats, and (Q)SAR analysis for mutagenic potential, the identified impurities in the proposed drug product (Norepinephrine Bitartrate in 5% Dextrose Injection) that have a higher threshold than the qualification limit per ICH-Q3B(R2) all have safety margins greater than 1-fold. . Therefore, the proposed drug product specification thresholds for these impurities are considered qualified from the Pharm/Tox perspective.

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