

**"Report of Repeated Dose (28 days) Oral
Toxicity Study of NFTT-14-H in rat as per
OECD Guideline No. 407"**

Submitted to:

Aimil Pharmaceuticals (I), Ltd.
Pillar No-208, Main Patel Road, Patel Nagar,
New Delhi – 110008, India

Study Performed and Submitted By:



Accuprec Research Labs Pvt. Ltd.

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Post. Matoda, Tal. Sanand,

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Report of "Repeated Dose (28 days) Oral Toxicity Study of NFTT-14-H in rat as per OECD Guideline No. 407."

Protocol Number	ARL/PRO/PT/17/009
Name of Product	NFTT-14-H
Method to be followed	OECD Guideline for Testing of Chemicals; 407 (2008)
Testing Facility	Accuprec Research Labs Pvt. Ltd. Opp. Zydus Pharmez, Changodar – Bavla Highway, Nr. Matoda Patiya, Post – Matoda, Ahmedabad, Gujarat 382213, India.
Sponsor	Aimil Pharmaceuticals (I), Ltd. Pillar No-208, Main Patel Road, Patel Nagar, New Delhi – 110008
Study Period	28 Days
Turn Around Period	60 Days
Report Number	ARL/3769/2017
Report Date	14/10/2017

Prepared By (Sign/Date)	Reviewed By (Sign/Date)	Approved By (Sign/Date)
Mr. Kalpesh Nagar (Research Associate)	Dr. Manish Rachchh (CEO)	Dr. Rina Gokani (CSO)
<i>Kalpesh Nagar</i> 14/10/2017	<i>Manish Rachchh</i> 14/10/2017	<i>Rina Gokani</i> 14/10/2017




I. STATEMENT OF COMPLIANCE

We, the undersigned hereby declare that this Protocol No. "ARL/PRO/PT/17/009" entitled "Repeated Dose (28 days) Oral Toxicity Study of NFFT-14-H in rat as per OECD Guideline No. 407." was performed under our supervision in compliance with OECD principle of good laboratory practice. Characterization of the test material was performed by the sponsor. The objective laid down in the study protocol was achieved. No unforeseen circumstances were observed which might affect the quality or integrity of the study.

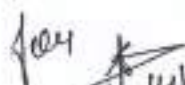
The report represent a true and accurate results obtained. We accept the responsibility for validity of the data, as well as the interpretation, analysis, documentation and reporting of the results.

The report comprises total 18 pages, which includes statement of compliance, quality assurance statement, study personnel detail, experimental design, results, discussion, conclusion, reference and period of archival.

Date: 14/10/2017


14/10/2017

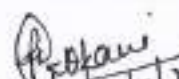
Mr. Kalpesh Nagar
Asst. Study Director


14/10/2017

Mr. Bhargav Gohel
DM, QA


24/10/2017

Dr. Manish Rachchh
Study Director


14/10/2017

Dr. Rina Gokani
Q. A. Head



II. QUALITY ASSURANCE STATEMENT

This study report has been reviewed by the Quality Assurance unit of Accuprec Research Labs Pvt. Ltd., for compliance with the OECD Principles of GLP & ISO 17025, study plan, study data and applicable operating procedures.

This statement confirms that the study report accurately reflects study data. The summary of inspections performed during the course of the study is as follows:

Sr. No.	Type of Inspection	Date of Inspection	Phases of Study inspected
1	Study Based	24/02/2017	Acclimatization of Animal
2	Study Based	01/03/2017	Grouping of animal, body weight, dose preparation and dosing.
3	Study Based	07/03/2017	Body weight, dose preparation and dosing, cage side observation
4	Study Based	14/03/2017	Body weight, dose preparation and dosing, cage side observation
5	Study Based	28/03/2017	Body weight, cage side observation and necropsy (Group I – IV)
6	Study Based	04/04/2017	Body weight, cage side observation (Satellite group)
7	Study Based	11/04/2017	Body weight, cage side observation and necropsy (Satellite group)

Date:

for
14/10/2017
Mr. Bhargav Gohel
DM, QA

R. Gokani
14/10/2017
Dr. Rina Gokani
Q. A. Head



III. ABBREVIATION

GLP	- Good Laboratory Practice
Gm	- Gram
hr	- Hour
ISO	- International Organization for Standardization
Kg	- Kilogram
M	- Male
F	- Female
Mg	- Milligram
CMC	- Carboxy Methyl Cellulose
ppb	- parts per billion
MSDS	- Material Safety Data Sheet
OECD	- The Organization for Economic Co-operation and Development



1. STUDY INFORMATION

Study Protocol Number

: ARL/PRO/PT/17/009

Report Number

: ARL/3769/2017

Study Title

: Repeated Dose (28 days) Oral Toxicity Study of NFFT-14-H in rat as per OECD Guideline No. 407.

Sponsor

: Aimil Pharmaceuticals (I), Ltd.
Pillar No-208, Main Patel Road,
Patel Nagar, New Delhi – 110008

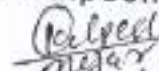
Testing Facility

: Accuprec Research Labs Pvt. Ltd.
Opp. Zydus Pharmez,
Changodar – Bavla Highway,
Nr. Matoda Patiya, Post – Matoda,
Ahmedabad, Gujarat 382213, India.

Name of Asst. Study Director

: Mr. Kalpesh Nagar

Sign of Asst. Study Director

: 
14/10/2017

Name of Study Director

: Dr. Manish A. Rachchh

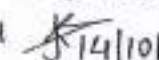
Sign of Study Director

: 
14/10/2017

Name of DM - Q. A.

: Mr. Bhargav Gohel

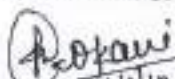
Sign of DM - Q. A.

: 
14/10/2017

Name of Q. A. Head

: Dr. Rina Gokani

Sign of Q. A. Head

: 
14/10/2017



2. SUMMARY

The objective of the study now reported was to evaluate adverse effects of NFFT-14-H after 28 days repeated dose oral administration at 310 mg/kg, 620 mg/kg and 1240 mg/kg in rat. Adverse effect after repeated administration of NFFT-14-H was evaluated using different parameters like gross behavior, feed water consumption, change in body weight, hematology, serum biochemistry and histopathology of organs. The result indicates that, there was no significant sign and symptoms of toxicity up to tested dose level of 1240 mg/kg in rat.

3. INTRODUCTION

3.1. OBJECTIVE

The study was conducted to evaluate Repeated dose (28 days) oral toxicity of NFFT-14-H in rat as per OECD guideline no 407.

4. TEST GUIDELINES:

The Organization for Economic Co-operation and Development (OECD) Guideline for Testing of Chemicals; 407 (2008) - Repeated Dose 28-Day Oral Toxicity Study in Rodents.

5. STUDY PERSONNEL

Study Director	: Dr. Manish A. Rachchh
Asst. Study Director	: Mr. Kalpesh Nagar
Research Assistant	: Ms. Neha Lavle
Research Assistant	: Ms. Kruti Upadhyay
Pathologist	: Mr. Vijaysinh Chauhan
Veterinarian	: Dr. Ashish patel
DM - Q.A.	: Mr. Bhargav Gohel
Q. A. Head	: Dr. Rina Gokani



	weighed on the day of receipt and observed daily for abnormalities if any. Detailed records of acclimatization was maintained in the raw data.
Animal Husbandry	
Test Room	A - 101
Animal House conditions	Lighting : 12 / 12 hour light-dark cycle Temperature: 25 ± 2°C Relative Humidity: 30 to 70% Temperature and relative humidity were recorded Thrice daily.
Animal Housing	Rats were housed 5 per cage in clean, sterilized Polypropylene cages.
Feed and Water	Standard certified rat pellet feed (Manufactured by Keval Sales Corporation, Vadodara) and drinking water treated by reverse osmosis) <i>ad libitum</i> was provided to all animals. Analysis reports for microbial load and contaminants in feed and water and nutrient content of feed was retained with the raw data.
Sanitation	The floor of the experimental room was swept and mopped thrice daily. Cages and bedding material were changed once in three days and water bottles were changed daily. All the experimental procedures were done in a clean environment.
Study Schedule	Allocation of animals : 24/02/2017 Acclimatization : 24/02/2017 Test item administration : 01/03/2017 – 27/03/2017 End of the observation period : 11/04/2017



6. EXPERIMENTAL DESIGN

Details of the methods mentioned in the subsequent section of the study plan are as per current version of standard operating procedures no: ARL/SOP/PT/032-00 of Accuprec Research Labs Pvt. Ltd, Ahmedabad.

6.1. SOURCE OF TEST ITEM:

NFTT-14-H was received from Aimil Pharmaceuticals (I) Ltd.

6.2. EXPERIMENTAL PROCEDURE:

The details of animal grouping and administration of NFTT-14-H is mentioned in below Table 1 as well as in Annexure – 1.

Table 1

Group ID	Drug, Dose, Route and Frequency of Treatment	No. of Animals	Treatment Period
Normal Control	Vehicle (CMC), Oral, <i>o.i.d</i>	5 (M) + 5 (F)	28 days treatment period
Test 1	NFTT-14-H (310 mg/kg, Oral, <i>o.i.d</i>)	5 (M) + 5 (F)	
Test 2	NFTT-14-H (620 mg/kg Oral, <i>o.i.d</i>)	5 (M) + 5 (F)	
Test 3	NFTT-14-H (1240 mg/kg, Oral, <i>o.i.d</i>)	5 (M) + 5 (F)	
Test 4 (Satellite - 1)	Vehicle (CMC), Oral, <i>o.i.d</i>	5 (M) + 5 (F)	
Test 5 (Satellite - 2)	NFTT-14-H (1240 mg/kg, Oral, <i>o.i.d</i>)	5 (M) + 5 (F)	

6.3. OBSERVATIONS:

6.3.1. Gross behavior (Rearing and crossing) before dosing, 4 hrs after dosing, at 24 hrs., then on 7th, 14th, 21st, and 28th day of dosing)

6.3.2. Food-water consumption (daily)

6.3.3. Change in body weight (before dosing and then every week till 28 days)

6.3.4. Mortality (daily)

6.3.5. Followed at the end of 28th day

6.3.5.1. Hematology

6.3.5.2. Biochemistry (AST, ALT, Creatinine, Uric acid)

- 6.3.5.3. Gross necropsy (7 major organs namely brain, lung, heart, liver, kidney, ovary and testis)
- 6.3.5.4. Histopathology (7 major organs namely brain, lung, heart, liver and kidney, ovary and testis)
- 6.3.5.5. Bioanalytical measurement of Mercury in blood (Using ICP-OES technique) (David et al., 1999)
- 6.3.6. Observation was continued in Satellite groups 1 & 2 for further 14 days as per 6.3.1 to 6.3.4. After 14 days, observations were done as per 6.3.5

6.4. STATISTICAL ANALYSIS:

All data represents in Mean \pm SEM. One way ANOVA followed by Tuckey's test was performed. p value <0.05 was considered to be statistically significant, when compared with control group.



7.0. RESULT

7.1. Change in Body Weight

7.1.1. In Male Rat

- There was no significant change observed in body weight of treated male rats as compared to body weight of normal control group of male rats (**Annexure 2 and 3**).

7.1.2. In Female Rat

- There was no significant change observed in body weight of treated female rats as compared to body weight of normal control group of female rats (**Annexure 4 and 5**).

7.2. Feed and Water Intake

7.2.1. In Male Rat

- There was no significant change in feed and water intake in treated male rats as compared to feed and water intake of normal control group of male rats (**Annexure 6,7, 8 and 9**).

7.2.2. In Female Rat

- There was no significant change in feed and water intake in treated female rats as compared to feed and water intake of normal control group of female rats (**Annexure 10,11, 12 and 13**).

7.3. Gross Behavior

7.3.1. In Male Rat

- There was no significant change in gross behavior in treated male rats as compared to gross behavior of normal control group of female rats (**Annexure 14 and 15**).

7.3.2. In Female Rat

- There was no significant change in gross behavior in treated female rats as compared to gross behavior of normal control group of female rats (**Annexure 16 and 17**).



7.4. Mortality

7.4.1. In Male Rat

- There was no mortality found in male rats during study period (**Annexure 18**).

7.4.2. In Female Rat

- There was no mortality found in female rats during study period (**Annexure 19**).

7.5. Hematology

7.5.1. In Male Rat

- There was no significant change in hemoglobin level, RBC count, WBC count, hematocrit, neutrophils, lymphocytes, eosinophils, monocytes, platelet count, coagulation time, and reticulocyte level in all test groups as compared to values of normal control group after Day 28 (**Annexure 20 and 21**).
- There was no significant change in Hemoglobin level, RBC count, WBC count, hematocrit, neutrophils, lymphocytes, eosinophils, monocytes, platelet count, coagulation time, and reticulocyte level in Test 5 group (Satellite 2) as compared to values of Test 4 (Satellite 1) group after Day 42 (**Annexure 22 and 23**).

7.5.2. In Female Rat

- There was no significant change in hemoglobin, hematocrit, WBC Count, neutrophils, lymphocytes, eosinophils, monocytes, platelet count, coagulation time and reticulocyte level in all test groups as compared to values of normal control group after Day 28 (**Annexure 24 and 25**).
- Significant increase ($p < 0.05$) was observed in RBC count of Test-1 group as compared to normal control group, whereas there was no significant change in RBC count of Test-2 and Test- 3 group as compared with normal control group after Day 28 (**Annexure 24 and 25**).
- There was no significant change in Hemoglobin level, RBC count, WBC count, hematocrit, neutrophils, lymphocytes, eosinophils, monocytes, platelet count, coagulation time, and reticulocyte level in Test 5 group (Satellite 2) as compared to values of Test 4 (Satellite 1) group after Day 42 (**Annexure 26 and 27**).

7.6. Serum Biochemistry

7.6.1. In Male Rat

- There was no significant change in serum biochemical parameters like AST, ALT, uric Acid and creatinine of all test groups as compared to normal control group after Day 28 (**Annexure 28 and 29**).
- There was no significant change in serum biochemical parameters like AST, ALT, uric Acid and creatinine of Test 5 group (Satellite 2) as compared to test 4 group (Satellite 1) after Day 42 (**Annexure 30 and 31**).

7.6.2. In Female Rat

- There was no significant change in serum biochemical parameters like AST, ALT, uric Acid and creatinine of all test groups as compared to normal control group after Day 28 (**Annexure 32 and 33**).
- There was no significant change in serum biochemical parameters like AST, ALT, uric Acid and creatinine of Test 5 group (Satellite 2) as compared to test 4 group (Satellite 1) after Day 42 (**Annexure 34 and 35**).

7.7. Mercury determination in Blood

- Blood samples were tested for mercury determination using ICP-OES (Model No. 5110 Agilent technologies) as per procedure described in articles. [2] Mercury was not found in any group (Detection limit of Instrument was 10 ppb) (**Annexure 36 and 37**).

7.8. Gross Necropsy

- There was no significant change in organ weight in treated group as compared with normal control group of animals (**Annexure 38 and 39**).

7.9. Histopathology

- There was no significant histopathological changes in various organs in treated group as compared with normal control group of animals after day 28 (**Annexure 40**).



8.0. DISCUSSION

In this study, NFFT-14-H was administered orally in Wistar rat for 28 days in three different doses (310 mg/kg, 620 mg/kg and 1240 mg/kg Powder).

In case of male and female rat groups, no significant changes ($p > 0.05$) were observed in case of body weight, gross behavior, feed intake and water intake as compared to respective normal control group of animals.

In case of male and female rat groups, no significant changes ($p > 0.05$) were observed in case of hematological parameters as compared to respective normal control group of animals.

In case of female rats, significant increase ($p < 0.05$) was observed in RBC count (T1 group) as compared with normal control group after Day 28. Apart from that there was no significant changes were observed in hematological parameters.

In case of male and female rat groups, no significant changes ($p > 0.05$) were observed in case of serum parameter estimation as compared to respective normal control group of animals.

Above mentioned changes in hematological parameter did not appear to be drug related as they were variable, not dose dependent and were within normal ranges only.

Further, in case of male and female rat groups, no significant changes ($p > 0.05$) were observed in case of gross necropsy as well as histopathological examination of internal organs. Further, the mercury level was below detection limit after 28 days of dosing as well as after 42 days in case of satellite groups.

In brief, oral administration of NFFT-14-H didn't show any sign of toxicity in rat after daily treatment for 28 days at 310 mg/kg, 620 mg/kg and 1240 mg/kg dose level.



9.0. CONCLUSION

From above results, it can be concluded that 310 mg/kg, 620 mg/kg and 1240 mg/kg of NFFT-14-H, when administered orally for 28 days in rats, produced changes in few hematological, but they were mild in nature and no significant toxicities were observed. It can be concluded that NFFT-14-H (p.o) was safe in rat up to dose level of 1240 mg/kg. This dose was equivalent to four times higher therapeutic dose of human.

10.0. REFERENCE

1. The Organization for Economic Co-operation and Development (OECD) Guideline for Testing of Chemicals; 407 (2008) - Repeated Dose 28-Day Oral Toxicity Study in Rodents.
2. David E. Nixon, Mary F. Burritt, Thomas P. Moyer. The determination of mercury in whole blood and urine by inductively coupled plasma mass spectrometry. Spectrochimica Acta Part B 54 (1999) 1141-1153.

