WOOJUNGBIO

Final report (Summarized)

Dose Range Finding test of NWK A-FV(A) using Sprague-Dawley rats

Study No.	SH123004
Date	June 12, 2023
Study director	Young-June Shin

Contents

1. Introduction

Objective of study	The objective of this dose range finding study was to evaluate the possible toxicity of NWK A-FV(A) after 7-days repeated inhalation exposure in SD rats and to determine the dose levels for the following "28-days (subacute) inhalation study"
Sponsor	NaeWoi Korea Co.Ltd
Study facility	WOOJUNGBIO Inc.
Study No.	SH123004

2. Quality Assurance State

The Study described in this report was a non-GLP study. However, the principles of Good Laboratory Practice were taken into consideration as far as possible, and the study was conducted in accordance with the partially modified TEST Guideline 412 of the OECD.

3. Test article

Name	NWK A-FV(A)		
CAS No.	No data available	Lot No.	AFV20230105
Appearance	Liquid, Yellow color	pH	7.0 – 8.0
Molecular weight	No data available	Purity	No data available
Melting point	100 ℃	Initial boiling point	No data available
Water solubility	Not applicable	Density	1.02
Manufacturing date	March 10, 2023	Expire date	March 9, 2025

4. Methods

Test method	Dose-range finding study: Inhalation
GLP compliance	Non-GLP (Screening)
Animals	SD rat
Number of exposed rats	5 males and 5 females / group
Exposure system	Nose-only inhalation exposure
Aerosol generation	Mist generation
Exposure condition	4 h/day for 5 days
Exposure concentration	Low: 2.19, medium 8.75 and High: 35.00, mg/m^3 a)
Measurement of concentration	Gravimetric analysis, 3 times/day for 5 days for each group
Size distribution	Cascade impactor, 2 times/day for 5 days for each group
Stability/Homogeneity	Conducted on pre-generation test
Clinical pathology	Performed by collected blood from high-concentration and control animals
Analysis of BALF	Analyzed by collected bronchial lavage fluid from high-concentration and control animals

a) The determination of the exposure concentration/condition of the test article is summarized in 8. Limitation

5. Results

Chamber condition	Under regulated condition (table 1.)
Nominal concentration	Sum of nominal concentration measured during total exposure period (table 2.) Low: 26961.81, medium: 43101.85 and high: 75115.74 mg/m^3
Exposure concentration	Mean of exposed concentration Low: 2.08 ± 0.3, medium: 8.33 ± 0.94 and high: 34.4 ± 2.87 mg/m^3 (table 3.)
Size distribution	Low: 0.517 ± 0.024 μm and 1.746 ± 0.026, medium: 0.540 ± 0.012 μm and 1.779 ± 0.042, high: 0.438 ± 0.003 μm and 1.877 ± 0.04 (table 4.)
Stability/homogeneity	Homogeneity: 3.57, 3.74, 13.45 % Stability: 6.49 % (data not shown)
Body weights	No statistical difference between control- and test-group (table 5, 6)
Mortality	No dead animals found (Data not shown)
Clinical observation	No symptoms were observed in all experimental animals (Data not shown)
Necropsy and gross finding	No specific abnormalities were observed in the exposed group compared to the control group (Data not shown)
BALF analysis	No statistical difference in the number of bronchial cells in exposed group compared to the control group. No statistical difference in the expression levels of ABL, LDH and TP in exposed group compared to the control group (Data not shown)

6. Discussion

The aim of this study was to evaluate the toxic effects of NWK A-FV(A) after repeated inhalation exposure to SD rats for 4 hours/day, and to set the exposure concentration of main study (28-days repeated inhalation exposure).

There's no statistical difference on body weights between control- and test-group. Mortality and clinical symptoms were not observed. Include the respiratory systems, no gross findings were observed for all animals at necropsy. Statistical significance was observed in MCV and LYMPH in males and NETU in females in blood tests. Statistical significance was observed in ALB and TBIL in males and TBIL in females in biochemical tests. ALB, LDH, and TP in bronchoalveolar lavage fluid were not different between the control group and the high concentration group. In addition, the number of immune cells in bronchial lavage fluid from high group did not increase statistically significantly compared to the control group.

In summary, although significance was observed between the high concentration group and the control group in several parameters of the blood biochemistry test, the toxicity caused by the test substance was not considered to be significant.

The concentration exposed to the high group is considered as the limit-concentration of generation for the test substance.

7. Table

Table 1. Chamber environmental conditions

Monitoring condition	G1	G2	G3	G4
Temperature (℃)	22.62 ± 0.19	21.18 ± 0.12	19.93 ± 0.27	21.45 ± 0.05
Humidity (%)	68.87 ± 0.95	68.42 ± 0.66	69.17 ± 0.66	68.62 ± 0.79
Differential pressure (pa)	-166.42 ± 6.38	-30.20 ± 13.73	-58.30 ± 4.02	-123.53 ± 7.01
Oxygen (%)	19.80 ± 0.17	20.48 ± 0.08	20.00 ± 0.25	20.08 ± 0.18
Carbon dioxide (ppm)	531.67 ± 67.60	159.58 ± 126.45	491.25 ± 28.71	481.25 ± 27.24

Values are presented as mean ± standard deviation

Table 2. Nominal concentrations

Date	G2 (Low)	G3 (Medium)	G4 (High)
2023-04-28	1.14	26.32	985.17
2023-04-29	1.28	30.03	1078.34
2023-04-30	1.23	29.03	577.98
2023-05-01	1.19	29.43	736.04
2023-05-02	1.76	53.56	1317.20

Unit: mg/m^3

Table 3. Exposure concentrations

Date	G2 (Low)	G3 (Medium)	G4 (High)
2023-04-28	1.93 ± 0.10	8.32 ± 0.69	37.42 ± 3.54
2023-04-29	1.93 ± 0.20	8.86 ± 1.24	34.63 ± 2.08
2023-04-30	1.98 ± 0.47	7.93 ± 0.79	32.71 ± 1.22
2023-05-01	2.33 ± 0.36	7.39 ± 1.31	32.84 ± 3.56
2023-05-02	2.23 ± 0.37	9.16 ± 0.68	38..37 ± 3.97

Unit: mg/m^3
Values are presented as mean ± standard deviation

Table 4. Size distributions

Groups	G2 (Low)	G3 (Medium)	G4 (High)
MMAD (μm)	0.517 ± 0.024	0.540 ± 0.012	0.438 ± 0.003
GSD	1.746 ± 0.026	1.779 ± 0.042	1.877 ± 0.041

Values are presented as mean ± standard deviation

Table 5. Body weights (male)

Groups	G1	G2	G3	G4
1 (1) day	347.32 ± 9.59 (5)	334.99 ± 12.14 (5)	338.76 ± 3.17 (5)	332.95 ± 13.53 (5)
1 (2) day	329.86 ± 11.84 (5)	323.60 ± 13.59 (5)	324.69 ± 4.22 (5)	317.29 ± 13.65 (5)
4 day	337.05 ± 13.49 (5)	332.05 ± 12.93 (5)	329.73 ± 5.37 (5)	325.05 ± 12.27 (5)
5 days	326.39 ± 26.30 (5)	324.52 ± 13.24 (5)	328.00 ± 12.78 (5)	321.63 ± 14.17 (5)
TBW	302.29 ± 13.09 (5)	299.20 ± 15.02 (5)	304.45 ± 2.51 (5)	295.48 ± 14.21 (5)

Unit: g, (n): number of animals
Values are presented as mean ± standard deviation

Table 6. Body weights (female)

Groups	G1	G2	G3	G4
1 (1) day	232.56 ± 11.19 (5)	235.05 ± 15.02 (5)	230.45 ± 11.65 (5)	227.38 ± 11.55 (5)
1 (2) day	219.23 ± 10.60 (5)	222.06 ± 14.80 (5)	216.09 ± 12.37 (5)	216.33 ± 9.34 (5)
4 day	231.92 ± 5.57 (5)	230.43 ± 18.10 (5)	215.06 ± 16.48 (5)	227.09 ± 10.69 (5)
5 days	230.89 ± 5.19 (5)	231.62 ± 16.45 (5)	218.19 ± 14.97 (5)	226.79 ± 15.93 (5)
TBW	208.54 ± 4.95 (5)	208.48 ± 15.06 (5)	201.31 ± 12.41 (5)	202.95 ± 9.75 (5)

Unit: g, (n): number of animals
Values are presented as mean ± standard deviation

8. Limitation of study

The test article of this study was present in the form of a mixture of natural oil components and water, and the oil components showed aggregation with each other, resulting in the formation of a precipitate on the bottom.

As a result of confirming the pre-generation of the test substance, the orifice tube was frequently clogged while using the mist generator due to this aggregation, and thus the standard deviation between the measured values of the exposure concentration was unstable.

On the other hand, in the MMAD and GSD tests, it was confirmed that the generated atmosphere was formed in an inhalable particle size distribution, and that the test substance was in a partially suspended state.

Therefore, for stable generation, the aggregated particles of the test article were removed by filtration, and the toxic effect of the test article was evaluated more accurately by performing inhalation exposure with the remaining substance.

The test article used in this study was filtered through a 5 μm filter paper and diluted in an appropriate ratio (%). As a result, the filtered test article was most stable when diluted to 1/16 (6.25%). (At higher percentages, clogging by aggregation continues to occur)

Accordingly, the concentration (35.0 mg/m^3) of the substance diluted by 1/16 after filtration was set as the limit concentration.