May 7th , 2017

Examination Report

Requester	Co-Aube Sale Corporation
Object to be examined (substance)	PICO
Title	Acute single oral toxicity experiment in rats

This report was issued by the main center in March 14th ,2017
The above substance which was related the experiment had shown the result as follow.

Japan Food Analysis Center
Foundation

Credit & Prepare by.
รณชัย บุญสุริยาธรรม
Boonsuriyatham Ronnachai
Saga University, Japan

Acute Single Oral Toxicity experiment in Rats

Summary

In PICO experiment, there was an experiment in the acute single oral toxicity experiment in rat's topic.

In the experiment group, the dosage of the substance (PICO) was 2,000, 1,000, 300 and 50 mg/kg as well.

In controlled group, the water for injection was used as the controlled solvent in both male and female rats in single oral administration and the period of observation was 14 days.

According to the observation, during observation period, the result did not show the abnormality and mortality. So then, according to the using of LD50 values as an administered solvent in single oral administration in rats, both male and female rats could stand for the dosage which was more than 200mg/kg.

Requester

Co-Aube Sale Corporation

Experiment

PICO

Period of experiment

Heisei 29 April 4th to Heisei 29 May 7th (2017, April 4th to May 19th)

Place of experiment

Japan food analysis center foundation in Tama Laboratory

Tokyo Tama-shi Nagayama 6 chome No. 11 number 10

Head of Experiment team

Japan food analysis center foundation in Tama Laboratory

Department of experiment for safety, experiment for safety section

嶋崎　Shimazaki 智子　Tomoko

Experiment team

Nagai Takeshi 永井　武, Kawamoto Yasuharu　川本　康晴, Ozawa Mirai 小澤　美来, Suzuki Misora　鈴木　美そら

1. Purpose of experiment

The purpose is to examine the specimen in acute oral toxicity in rat experiment.

2. Object to be examined (substance)

PICO

Property; Beige color power

3. Experiment humor and preparation

-Loaded the water for injection as an object to be examined (substance),

-Equalized the Homogenizer (Kinematica) equally,

-Prepared the experiment humor as 100, 50, 10 and 2.5 mg/mL.

4. Animal for experiment

Purchased 5 weeks-old male and female rats type BrlHan : WIST from Japan Clear Corporation. Raised it for 1 week in general condition and confirmed for using in experiment.

The cage which was used as a container for 5 rats must be made of Polycarbonate material. The temperature was 23 Celsius ±2 Celsius and set the light for 12 hours per day. This cage had to be kept in culturing room.

The food was the solid foddle for mouse and rat from the MR Stock Laboratory or Japan agricultural industry corporation and the water was freely adopted (drinking water or tab water).

5. Experimental method

Prepared the substance which had the dosage from 2,000, 1,000, 300 and 50 mg/kg for administered experiment group and the water for injection was set as the solvent for the controlled group. There were 5 rats in each group for experiment. 17 hours before administration, the animals for experiment must be fed, after that measured the weight. The experiment humor was used in experiment group and the water for injection was used in the other one.

Used the feeding tubes, which loaded the humor referred the dosage to the table 1 (administered dosage in each group), to administer the single oral administration in experiment.

The observation period was 14 days, counted the administered day as the first time. Then after that the next day counted one day as one time.

After administered day 7^{th} and day 14^{th}, measured the body weight. According to the variance arrangements for analysis, use 5% of significant level to do statistical analysis.

After the experiment had been finished, all the dead body of experimented animals had been examined.

Table1 experiment group

Administered dosage (mg/kg)	Concentration (mg/mL)	Administer capacity (mL/kg)
2,000	100	20
1,000	50	20
300	15	20
50	2.5	20
0*	0*	20

*controlled group (water for injection was administered)

6. Result of experiment

1. Mortality

During the observation period, there was no mortality in administered group either male or female rats.

2. General condition

During the observation period, there was no abnormality in administered group either male or female rats.

3. Body weight changes

After administered day 7th and day 14th, there was no body weight changes according to the comparison of experiment group and controlled group either male or female rats.

4. Dead body examination

There was no abnormality with dead body of the experimented animals after the observation had been finished either male or female rats.

7. Discussion

The substance had been implemented in acute single oral toxicity experiment in rats.

The result coming from using the 2,000 mg/kg (as the supreme dose), 1,000, 300 and 50 mg/kg of the substance in single oral administration had shown that there was no abnormality and mortality during the observation period. Follow this, the substance which was used in single oral administration in rats at LD50 values, both male and female rats could stand more than 2,000 mg/kg.

8. Reference (bibliography)

OECD Guidelines for the Testing of Chemicals 420 (2001).

Table2 Body weight changes (male)

Administration specimen dose (mg/kg)	Before administration	After administration day 7th	After administration day 14th
2,000	153.7±2.1 (5)	194.2±2.8 (5)	240.4±4.2 (5)
1,000	153.2±3.4 (5)	197.3±6.1 (5)	244.4±11.3 (5)
300	151.6±3.7 (5)	193.0±6.6 (5)	236.4±11.2 (5)
50	151.5±2.8 (5)	197.0±4.0 (5)	239.3±9.4 (5)
0*	151.7±2.8(5)	196.6±4.1 (5)	243.2±8.4 (5)

The average weight value was shown ±the standard deviation (unit : g)
The number in the bracket was shown the number of animals.
*in the contrast group, the water for injection was administered.

Table3 Body weight changes (female)

Administration specimen dose (mg/kg)	Before administration	After administration day 7th	After administration day 14th
2,000	129.3±2.1 (5)	153.7±3.8 (5)	171.4±3.9 (5)
1,000	129.0±3.3 (5)	150.2±5.9 (5)	170.4±8.7 (5)
300	128.1±2.6 (5)	150.4±3.2 (5)	169.2±4.4 (5)
50	129.3±2.1 (5)	150.9±4.5 (5)	171.3±7.1 (5)
0*	128.4±3.5(5)	147.3±5.2 (5)	165.8±6.2 (5)

The average weight value was shown ±the standard deviation (unit : g)
The number in the bracket was shown the number of animals.
*in the contrast group, the water for injection was administered.

Heisei 29, March 31th (2017, March 31th)

Co-Aube Sale corporation

Examination/test report

1. The purpose of examination/test

   To confirm the ability of PICO in rotten fish deodorization

2. Date of the examination/test

   Heisei 29, March 30th (2017, March 30th)

3. Place of examination/test

   Co-Aube Sale corporation

4. Person in charge (responsibility person)

   巽 Tatsumi 貫明 (specific name)

5. Test implement method

1) Mix the PICO 1 gram with 1 Littre of water and leave it more than 10 minutes. ※ The dilution ratio is 500 times, 1,000 times and 2,000 times.

2) Prepare the demolition and natural rotten fish (died more than 15 days)

3) Take the liquid from above rotten fish 2 cc and pour into the deep container. ※ use the 2,400 cc size-container which is made of plastic

4) Use the detector tube to measure the concentration in the first period. (smell component = trim ethylamine) ※ (Gastech corporation GV-100s)

5) Pour the PICO which is diluted by water 50cc in to the container.

6) The below following is the time of concentration measurement which is going to implement.

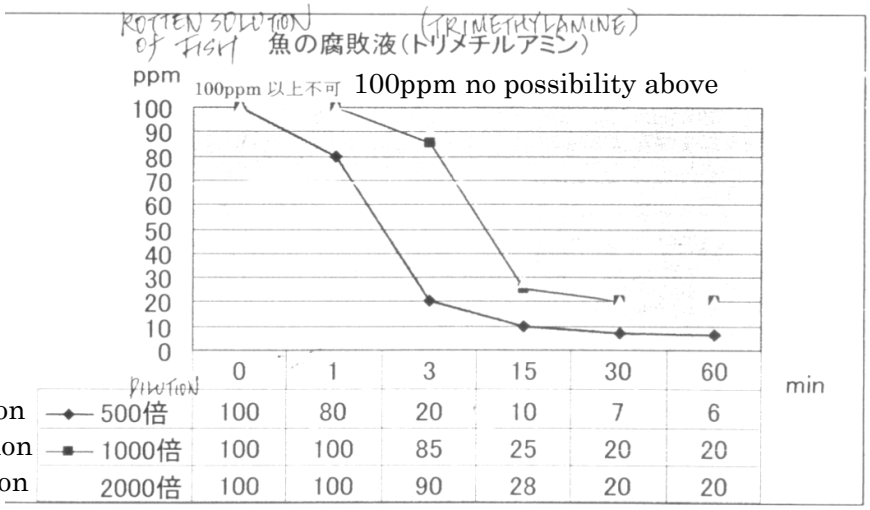
(A) after 1 minute (B) after 3 minutes (C) after 15 minutes (D) after 30 minutes (E) after 60 minutes

6. Test result

According to the graph below, the PICO in after 15 minutes is showing the prosperous effect in deodorization.

Table Graph

Rotten solution of Fish (Tri methylamine)



500dilution

1,000dilution

2,000dilution

7. Comment

This test is implementing that PICO can help the Trim ethylamine in deodorization power very much.

The concentration of PICO at 1,000 times and 200 times do not show the high effective but after 15 minutes it shows sufficient effect.

Anyway, in case of the effect and deodorization power of 500 time dilution, it can be recommended as one of the good and high effective condition also.