

Acute Toxicity Study of β -APM in Rats and Mice

Y. Takasaki

Yutaka Takasaki

(Study director)

Central Research Laboratories, Life Science Laboratories,
Ajinomoto Co., Inc.

214 Maeda-cho, Totsuka-ku, Yokohama, Japan

Contents

QAU statement	Page 2
Abstract	Page 3
Sign of personnel	Page 4
General information	Page 5
Control and experimental articles	Page 7
Animal management	Page 10
Experimental procedure	Page 12
Results	Page 15
Discussion	Page 20

Quality Assurance Statements for Final Report

This final report was checked by Quality Assurance Unit (QAU) with GLP regulations of Life Science Laboratories, Central Research Laboratories, Ajinomoto Co., Inc.

There was no difference discovered between practices used in conducting the study and those required by GLP regulations.

Date of inspection

Date of report to study director
and manager

July 29, 1983

August 9, 1983

August 9, 1983

August 29, 1983

August 29, 1983

Sep. 9, 1983

Date

T. Hirahara

Tsuneo Hirahara

Quality Assurance Unit

Life Science Laboratories, Central Research Laboratories
Ajinomoto Co., Inc.

Abstract

Acute toxicity study of β -APM, a by product of APM (α -L-aspartyl-L-phenylalanine-methyl ester), was performed in male and female rats and mice.

LD50 values of β -APM were evaluated more than 5.0g/kg in these strains, respectively.

Study director

Sep. 9, 1983

Date

Y. Takasaki

Yutaka Takasaki

Life Science Laboratories, Central Research
Laboratories, Ajinomoto Co., Inc.

214 Maeda-cho, Totsuka-ku, Yokohama, Japan

Research scientist

Sep. 9, 1983

Date

Y. Abe

Yasuji Abe

Life Science Laboratories, Central Research
Laboratories, Ajinomoto Co., Inc.

214 Maeda-cho, Totsuka-ku, Yokohama, Japan

Sep. 9, 1983

Date

K. Yokoi

Kyoko Yokoi

Life Science Laboratories, Central Research
Laboratories, Ajinomoto Co., Inc.

214 Maeda-cho, Totsuka-ku, Yokohama, Japan

General Information

1. Protocol No. and Title

No.8351 ; Acute Toxicity Study of β -APM in Rats

No.8352 ; Acute Toxicity Study of β -APM in Mice

2. Objective

Oral acute toxicity study of β -APM, a by-product of Aspartame (α -L-aspartyl-L-phenylalanine-methyl ester), is performed in male and female rats and mice and referred to safety evaluation of Aspartame.

3. Sponsor

Jiro Kirimura

Director of Products Safety & Assesment Dept., Ajinomoto Co.,Inc.

1-5-8, Kyobashi, Chuo-ku, Tokyo, Japan

4. Location of study, raw data and final report

Life Science Laboratories, Central Research Laboratories,

Ajinomoto Co., Inc.

214, Maeada-cho, Totsuka-ku, Yokohama, Japan

5. Personnel

Study director ; Yutaka Takasaki

Research scientist ; Yasuji Abe

Kyoko Yokoi

6. Schedule

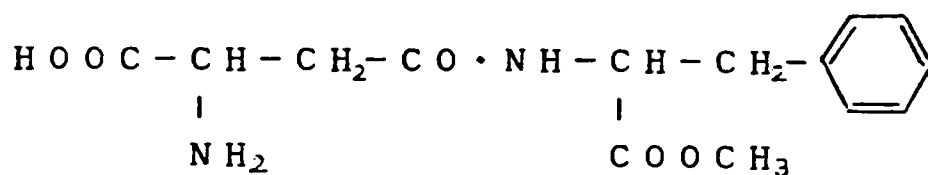
Start of study	:	July,	25,	1983
Animal Purchase	:	July,	27,	1983
Administration	:	August	2,	1983
Autopsy	:	August	16,	1983
Final report	:	August	30,	1983
Final report in English	:	September	9,	1983

Control and Experimental Articles

1. Experimental articles

1) Chemical name and chemical structure

α -L-aspartyl-L-phenylalanine-methyl ester



2) Chemical Abstracts Service Registry Numbers

No. 22839-61-8

3) Lot. No.

No. 830708

4) Source and manufacturer

Process Engineering Laboratories, Central Research Laboratories,
Ajinomoto Co., Inc.

1-1, Suzuki-cho, Kawasaki-ku, Kawasaki, Japan

5) Physical and chemical features

Molecular formula	: $\text{C}_{14}\text{H}_{19}\text{N}_2\text{O}_5$
Molecular weight	: 294.31
Appearance	: white and crystalline powder
Stability	: relatively stable at room temp.
Solubility	: 0.74%/100g H_2O

6) Purity (determined by manufacturer)

More than 99%

7) Storage condition

Stored in dry-seal at room temp.

2. Control articles

Sterile distilled water

Lot. No.2J870

Otsuka Pharmaceutical Co., Ltd., Japan

3. Sample preparation

The article was dissolved and/or suspended in the above-mentioned distilled water in final concentration of 10.0%(w/v) with agate mortar and pestle. All sample solutions were prepared just before administration at Life Science Laboratories, Central Research Laboratories, Ajinomoto co., Inc.

4. Analysis of concentration of test article

Concentration of test article was assayed by amino acid auto analyzer in Process Engineering Laboratories, Central Research Laboratories, Ajinomoto Co., Inc.

Animal Management

1. Species, strain and sex

Rat ; Male and female Sprague-Dawley rat

Mouse ; Male and female ICR mouse

2. Justification for selection of species

Rat and mouse are considered to be one of the most useful animals for acute toxicity studies.

3. Source of supply

Charles River Japan Inc.

795, Shimofurusawa, Atsugi-shi, Kanagawa, Japan

4. Number of animals purchased

4 weeks old rat ; 50 in male and 51 in female

3 weeks old mouse ; 36 in each sex

5. Number of animals used

Rat ; 30 in each sex

Mouse ; 30 in each sex

6. Age at administration and body weight range

Rat ; 5 weeks old male ; 112 - 130g

female ; 91 - 111g

Mouse ; 4 weeks old male ; 18.0- 21.7g

female ; 16.1- 20.2g

7. Housing condition in acclimation period

5 or 6 rats were housed in a wire-mesh suspended cage and 20, 11 or 10 mice in a poly carbonate cage bedded with pine chips.

8. Diet and water

Rats and mice were offered a commercial solid chow (CRF-1, ORIENTAL YEAST CO., LTD., JAPAN) and tap-water ad libitum. It is not considered likely that any contaminants which might be in the food and water affect the outcome of the study. But analysis of diet was performed in Bioresearch Laboratory, Institute For Biological Science, ORIENTAL YEAST CO., LTD., Japan and Japan Food Research Laboratories, Japan, and water in THE WATER LABORATORY, WATER WORKS VIEROW, THE CITY OF YOKOHAMA, Japan.

9. Environment condition

Rats and mice were kept in animal room No.209 and 211, respectively in Life Science Laboratories, Central Research Laboratories, Ajinomoto Co., Inc. These animal rooms were maintained at $23 \pm 1^{\circ}\text{C}$ temp., $55 \pm 10\%$ moist., 12L12D lightening condition and all fresh air condition of 12 cycle per hour.

Experimental Procedure

1. Acclimation period

1 week

2. Route of administration

Forced intubation with metallic gastric catheter

3. Justification of route

Intended condition of use in Aspartame is per os in human, and β -APM, a by-product, was treated by po.

4. Procedure for treatment

In rats and mice, control and test articles were intubated at 0.25ml and/or 0.5ml per 10g B.W. with 5.0ml or 10.0ml glass syringe and metallic gastric catheter (NATSTUME SEISAKUSHO CO., LTD., Japan).
(SOP NO. 530)

5. Duration and number of treatment

Single treatment

6. Randomization and identification of animal

After 1 week of acclimation period, healthy and well growing animals were selected at random.

Rats were housed in a wire-mesh suspended cage in each 5 and mice in a poly carbonate cage bedded with pine chips in each 10. Animals were identified by picric acid applied (SOP No. 510). Each cage was also identified by label written protocol No., articles, dosage and treatment date. More than two cages were not opened at the same time not to mistake animals.

7. Dosage and volume

1) Rat (Male and female)

Group	Dosage(g/kg)	Volume (ml/100g B.W.)	No. of animals
1	0	5.0	10
2	2.5	2.5	10
3	5.0	5.0	10

2) Mouse (Male and female)

Group	Dosage(g/kg)	Volume (ml/10g B.W.)	No. of animals
1	0	0.5	10
2	2.5	0.25	10
3	5.0	0.5	10

8. Justification of dosage level

Dosage level were proffered in the same fashion in acute toxicity study of Aspartame performed in Life Science Laboratories, Central Research Laboratories, Ajinomoto Co., Inc.

9. Clinical observation

Acute toxic signs for 2hrs just after treatment and death and general signs were observed for 2 weeks (SOP No. 6000). Body weights were recorded at 0hr, 24hrs, 1 and 2 weeks after treatment (SOP No. 54).

10. Autopsy

At 2 weeks after treatment, all survivors were sacrificed and main organs were grossly examined (SOP No. 6000).

11. Statistical analysis

LD50 in rats and mice were evaluated from rate of death at 2 weeks in Life Science Laboratories, Central Research Laboratories, Ajinomoto Co., Inc. (SOP No. 6000).

Results

No circumstances were encountered which affected the quality of integrity of the study.

1. Acute toxic signs

No specific acute toxic signs was observed in all male and female rats and mice.

2. Survival and mortality after 2 weeks

1) Rat

(Male)

Dosage g/kg	Time and No. of survival *)					Mortality
	0	2	24	168	336 hrs	
0	10	10	10	10	10	0 %
2.5	10	10	10	10	10	0 %
5.0	10	10	10	10	10	0 %

(Female)

Dosage g/kg	Time and No. of survival *)					Mortality
	0	2	24	168	336 hrs	
0	10	10	10	10	10	0 %
2.5	10	10	10	10	10	0 %
5.0	10	10	10	10	10	0 %

*) : Results of representative time were shown.

2) Mouse

(Male)

Dosage g/kg	Time and No. of survival *)					Mortality
	0	2	24	168	336 hrs	
0	10	10	10	10	10	0 %
2.5	10	10	10	10	10	0 %
5.0	10	10	10	10	10	0 %

(Female)

Dosage g/kg	Time and No. of survival *)					Mortality
	0	2	24	168	336 hrs	
0	10	10	10	10	10	0 %
2.5	10	10	10	10	10	0 %
5.0	10	10	10	10	10	0 %

*) ; Results of representative time were shown.

3. Body weight change

There was no difference between control and treated groups in male and female rats and mice.

1) Rat

(Male)

Dosage	Time after treated and body weight (g)			
	0	24	168	336 hrs
0	121.2±3.4	a) 133.2±3.9	184.3±6.6	239.7±8.1
2.5	122.0±2.7	128.8±5.5	177.6±12.5	236.1±18.6
5.0	119.6±6.5	126.9±5.5	174.5±10.8	237.9±15.2

(Female)

Dosage	Time after treated and body weight (g)			
	0	24	168	336 hrs
0	98.3±4.0	a) 104.6±3.7	139.1±6.8	169.1±7.3
2.5	101.3±4.1	107.7±4.2	138.1±4.5	161.9±6.6
5.0	100.5±4.7	106.2±6.1	138.2±7.7	165.9±11.7

a) ; expressed as mean ± SD

2) Mouse

(Male)

Dosage	Time after treated and body weight (g)			
	0	24	168	336 hrs
0	20.3±0.9 a)	22.8±1.2	28.2±1.6	30.9±1.7
2.5	19.8±1.0	22.5±1.2	27.4±1.9	29.9±1.9
5.0	20.0±1.2	22.7±1.0	28.2±1.0	31.2±1.0

(Female)

Dosage	Time after treated and body weight (g)			
	0	24	168	336 hrs
0	17.8±1.2 a)	19.2±1.1	21.9±0.9	23.5±1.4
2.5	18.3±1.1	19.3±0.7	22.8±1.0	24.2±1.9
5.0	18.6±0.7	20.2±0.8	22.8±1.3	24.1±1.6

a) ; expressed as mean ± SD

4 Gross findings at autopsy

No abnormal finding was observed in all male and female rats and mice contained controls.

5. LD50 value of β -APM

The LD50 value of β -APM were evaluated more than 5.0g/kg body weight in male and female rats and mice in all cases.

Discussion

β -APM, a by-product of Aspartame, is produced in preservation of Aspartame, this article has not sweetness unlike Aspartame. In acute toxicity study of Aspartame in rats and mice by po treatment, formerly performed in this laboratory, the LD50 values were more than 5.0g/kg in any species and only transient light depressomotor was observed in rats and mice just after treatment. In this acute toxicity study of β -APM, no acute toxic sign was found and growth after treatment for 2 weeks in dosed groups was compared with that in controls, and the LD50 values were more than 5.0g/kg in male and female rats and mice like Aspartame. So, there is no disparity between these two articles in acute toxicity. Quantity of β -APM produced from Aspartame is very small in usually intended strage, depending on the storage condition. Hence, produce of β -APM is no trouble acute-toxicologically in the safety evaluation of Aspartame.