

ジフェニルアミンのマウスを用いた
経口投与による13週間毒性試験（混餌試験）報告書

試験番号：0670

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APPENDIX 1-1

IDENTITY OF DIPHENYLAMINE
IN THE 13-WEEK FEED STUDY

IDENTITY OF DIPHENYLAMINE IN THE 13-WEEK FEED STUDY

Test Substance : Diphenylamine (Wako Pure Chemical Industries, Ltd.)

Lot No. : SDH5697

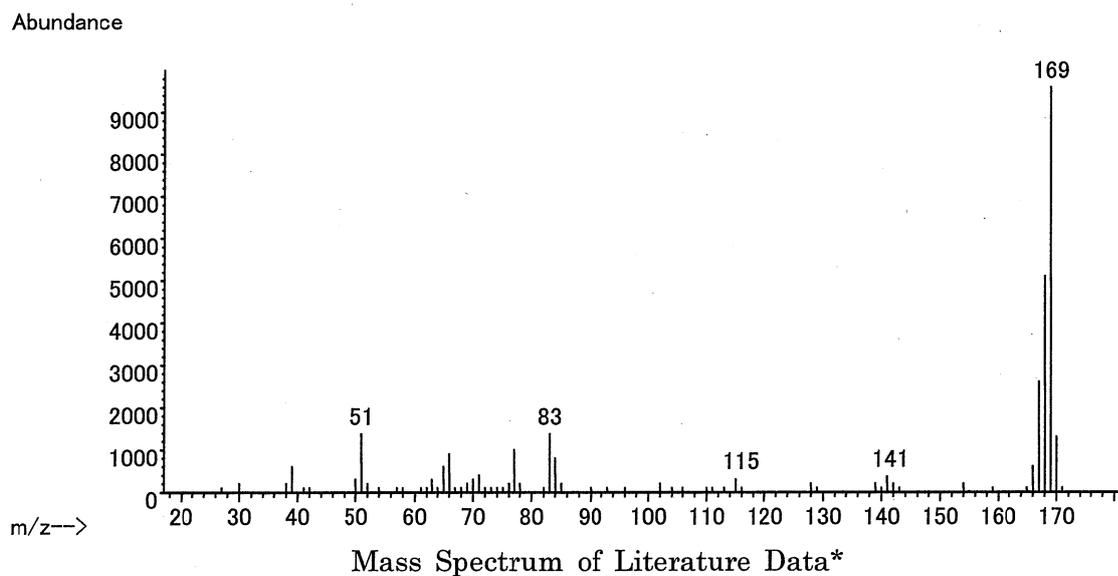
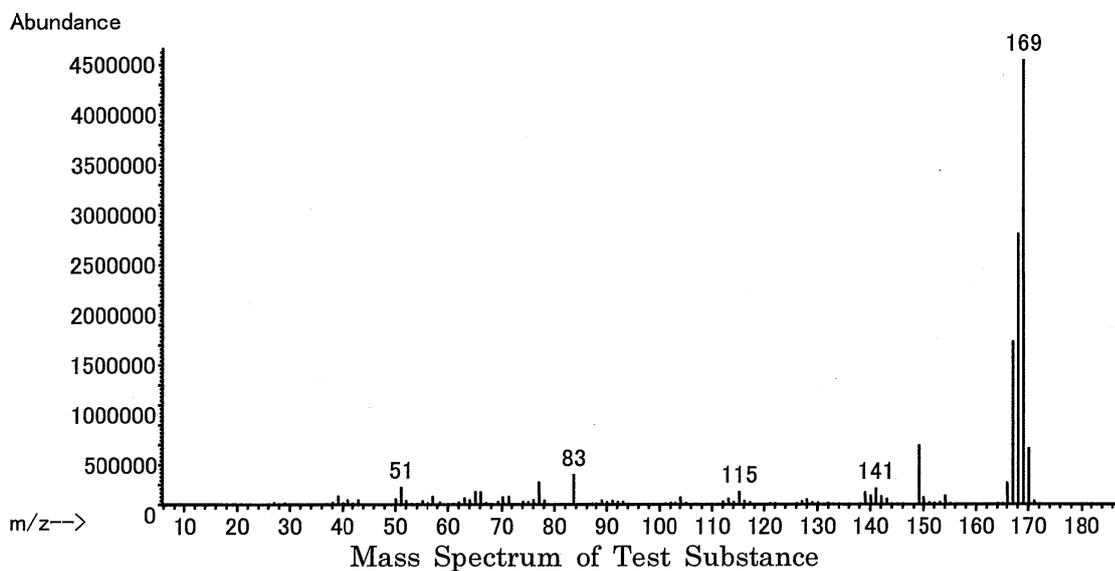
1. Spectral Data

Mass Spectrometry

Instrument : Agilent Technologies 5973N Mass Spectrometer

Ionization : EI (Electron Ionization)

Ionization Voltage : 70eV



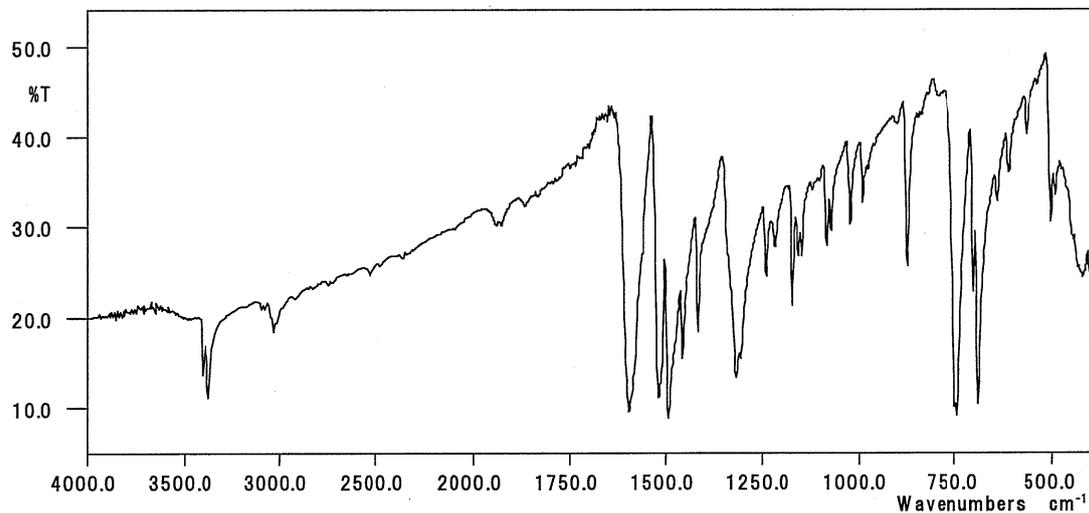
Result: The mass spectrum was consistent with literature spectrum.

(*McLafferty FW, ed. 1994. Wiley Registry of Mass Spectral Data. 6th ed. New York, NY:John Wiley and Sons.)

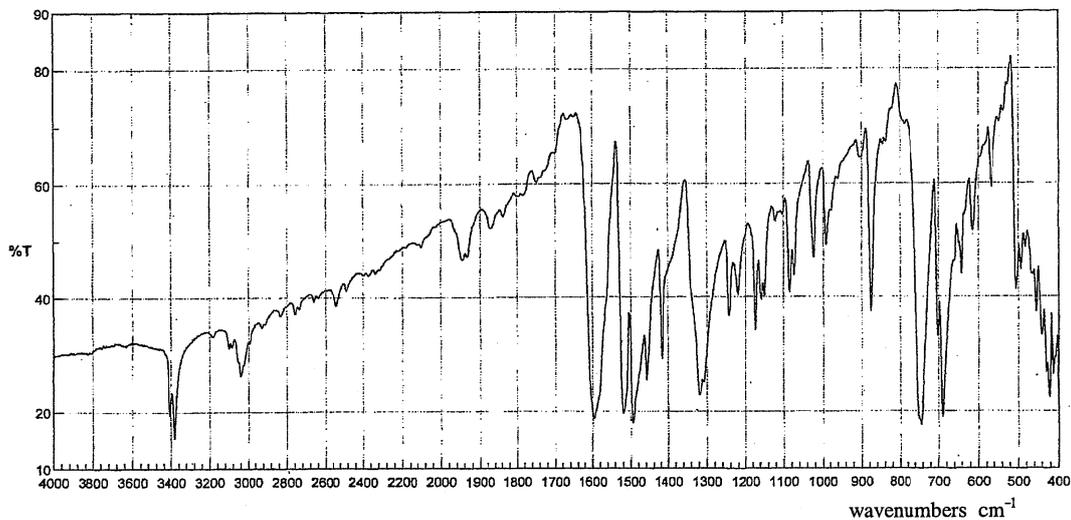
Infrared Spectrometry

Instrument : Shimadzu FTIR-8200PC Infrared Spectrometer

Cell : KBr

Resolution : 2 cm^{-1} 

Infrared Spectrum of Test Substance



Infrared Spectrum of Literature Data*

Result: The infrared spectrum was consistent with literature spectrum.
(*Performed by Wako Pure Chemical Industries, Ltd.)

2. Conclusion: The test substance was identified as diphenylamine by mass spectrum and infrared spectrum.

APPENDIX 1-2

STABILITY OF DIPHENYLAMINE
IN THE 13-WEEK FEED STUDY

STABILITY OF DIPHENYLAMINE IN THE 13-WEEK FEED STUDY

Test Substance : Diphenylamine (Wako Pure Chemical Industries, Ltd.)

Lot No. : SDH5697

1. High Performance Liquid Chromatography

Instrument : Shimadzu LC-10 High Performance Liquid Chromatograph

Column : TSK-GEL ODS-80TM (4.6 mm ϕ \times 15 cm)

Column Temperature: 40 °C

Flow Rate : 1 mL/min

Mobile Phase : Acetonitrile : Distilled Water = 70 : 30

Detector : UV (285 nm)

Injection Volume : 10 μ L

| Date analyzed | Peak No. | Retention Time (min) | Area (%) |
|---------------|----------|-------------------------|-------------|
| 2006.10.05 | 1 | 4.411 | 100 |
| 2007.01.22 | 1 | 4.410 | 100 |

Result: High performance liquid chromatography indicated one major peak (peak No.1) analyzed on 2006.10.5 and one major peak (peak No.1) analyzed on 2007.1.22. No new trace impurity peak in the test substance analyzed on 2007.1.22 was detected.

2. Conclusion: The test substance was stable for the period that the test substance had been used for the study.

APPENDIX 1-3

CONCENTRATION OF DIPHENYLAMINE
IN FORMULATED DIETS IN THE 13-WEEK FEED STUDY

CONCENTRATION OF DIPHENYLAMINE IN FORMULATED DIETS IN THE 13-WEEK FEED STUDY

Analytical Method : The samples were analyzed by high performance liquid chromatography.

Instrument : Shimadzu LC-10 High Performance Liquid Chromatograph

Column : TSK-GEL ODS-80TM (4.6 mm ϕ \times 15 cm)

Column Temperature: 40 °C

Flow Rate : 1 mL/min

Mobile Phase : Acetonitrile : Distilled Water = 70 : 30

Detector : UV (285 nm)

Injection Volume : 10 μ L

| Date Analyzed | Target Concentration | | | | |
|---------------|-------------------------------------|-----------|------------|------------|-------------|
| | 256 ^a | 640 | 1600 | 4000 | 10000 |
| 2006.10.17 | 264 ^b (103) ^c | 666 (104) | 1650 (103) | 4150 (104) | 10300 (103) |

^a ppm

^b ppm (Mean measured concentration.)

^c % (Mean measured concentration/target concentration \times 100.)

APPENDIX 1-4

HOMOGENEITY OF DIPHENYLAMINE
IN FORMULATED DIETS IN THE 13-WEEK FEED STUDY

HOMOGENEITY OF DIPHENYLAMINE IN FORMULATED DIETS IN THE 13-WEEK FEED STUDY

Analytical Method : The samples were analyzed by high performance liquid chromatography.

Instrument : Shimadzu LC-10 High Performance Liquid Chromatograph

Column : TSK-GEL ODS-80TM (4.6 mm ϕ \times 15 cm)

Column Temperature: 40 °C

Flow Rate : 1 mL/min

Mobile Phase : Acetonitrile : Distilled Water = 70 : 30

Detector : UV (285 nm)

Injection Volume : 10 μ L

| | Target Concentration | | | | |
|-----------------------|----------------------|------|------|------|-------|
| | 256 ^a | 640 | 1600 | 4000 | 10000 |
| Coefficient Variation | 3.22 ^b | 1.61 | 2.34 | 2.24 | 1.37 |

^a ppm

^b % (n=7)

APPENDIX 1-5

STABILITY OF DIPHENYLAMINE
IN FORMULATED DIETS IN THE 13-WEEK FEED STUDY

STABILITY OF DIPHENYLAMINE IN FORMULATED DIETS IN THE 13-WEEK
FEED STUDY

Analytical Method : The samples were analyzed by high performance liquid chromatography.

Instrument : Shimadzu LC-10 High Performance Liquid Chromatograph

Column : TSK-GEL ODS-80TM (4.6 mm ϕ \times 15 cm)

Column Temperature: 40 °C

Flow Rate : 1 mL/min

Mobile Phase : Acetonitrile : Distilled Water = 70 : 30

Detector : UV (285 nm)

Injection Volume : 10 μ L

| Date Analyzed | Target Concentration | |
|-------------------------|-------------------------|---------------|
| | 100 ^a | 10000 |
| 2006.09.08 | 99.0 (100) ^b | 10100 (100) |
| 2006.09.16 ^c | 96.0 (97.0) | 10000 (99.0) |
| 2006.09.16 ^d | 100 (101) | 10100 (100) |

^a ppm

^b % (Percentage was based on the concentration at the date of preparation.)

^c Animal room samples

^d Cold storage samples

APPENDIX 2

METHODS, UNITS AND DECIMAL PLACE FOR
HEMATOLOGY AND BIOCHEMISTRY IN THE
13-WEEK FEED STUDY OF DIPHENYLAMINE

METHODS, UNITS AND DECIMAL PLACE FOR HEMATOLOGY AND BIOCHEMISTRY
IN THE 13- WEEK FEED STUDY OF DIPHENYLAMINE

| Item | Method | Unit | Decimal place |
|--|---|---------------------------|---------------|
| Hematology | | | |
| Red blood cell (RBC) | Light scattering method ¹⁾ | $\times 10^6/\mu\text{L}$ | 2 |
| Hemoglobin(Hgb) | Cyanmethemoglobin method ¹⁾ | g/dL | 1 |
| Hematocrit(Hct) | Calculated as $\text{RBC} \times \text{MCV} / 10^3$ ¹⁾ | % | 1 |
| Mean corpuscular volume(MCV) | Light scattering method ¹⁾ | fL | 1 |
| Mean corpuscular hemoglobin(MCH) | Calculated as $\text{Hgb} / \text{RBC} \times 10^3$ ¹⁾ | pg | 1 |
| Mean corpuscular hemoglobin concentration (MCHC) | Calculated as $\text{Hgb} / \text{Hct} \times 100$ ¹⁾ | g/dL | 1 |
| Platelet | Light scattering method ¹⁾ | $\times 10^3/\mu\text{L}$ | 0 |
| Methemoglobin | Van Assendelft method ²⁾ | % | 1 |
| White blood cell(WBC) | Light scattering method ¹⁾ | $\times 10^3/\mu\text{L}$ | 2 |
| Differential WBC | Pattern recognition method ³⁾ (Wright staining) | % | 0 |
| Biochemistry | | | |
| Total protein(TP) | Biuret method ⁴⁾ | g/dL | 1 |
| Albumin (Alb) | BCG method ⁴⁾ | g/dL | 1 |
| A/G ratio | Calculated as $\text{Alb} / (\text{TP} - \text{Alb})$ ⁴⁾ | — | 1 |
| T-bilirubin | Azobilirubin method ⁴⁾ | mg/dL | 2 |
| Glucose | GlcK·G-6-PDH method ⁴⁾ | mg/dL | 0 |
| T-cholesterol | CE·COD·POD method ⁴⁾ | mg/dL | 0 |
| Triglyceride | MGLP·GK·GPO·POD method ⁴⁾ | mg/dL | 0 |
| Phospholipid | PLD·ChOD·POD method ⁴⁾ | mg/dL | 0 |
| Aspartate aminotransferase (AST) | JSCC method ⁴⁾ | IU/L | 0 |
| Alanine aminotransferase (ALT) | JSCC method ⁴⁾ | IU/L | 0 |
| Lactate dehydrogenase (LDH) | SFBC method ⁴⁾ | IU/L | 0 |
| Alkaline phosphatase (ALP) | GSCC method ⁴⁾ | IU/L | 0 |
| γ -Glutamyl transpeptidase (γ -GTP) | JSCC method ⁴⁾ | IU/L | 0 |
| Creatine kinase (CK) | JSCC method ⁴⁾ | IU/L | 0 |
| Urea nitrogen | Urease·GLDH method ⁴⁾ | mg/dL | 1 |
| Sodium | Ion selective electrode method ⁴⁾ | mEq/L | 0 |
| Potassium | Ion selective electrode method ⁴⁾ | mEq/L | 1 |
| Chloride | Ion selective electrode method ⁴⁾ | mEq/L | 0 |
| Calcium | OCPC method ⁴⁾ | mg/dL | 1 |
| Inorganic phosphorus | PNP·XOD·POD method ⁴⁾ | mg/dL | 1 |

1) Automatic blood cell analyzer (ADVIA120 : Bayer Corporation)

2) Spectrophotometer (UV-240 : Shimadzu Corporation)

3) Automatic blood cell differential analyzer (MICROX HEG-120NA : OMRON Corporation)

4) Automatic analyzer (Hitachi 7080 : Hitachi, Ltd.)