ジフェニルアミンのラットを用いた
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：0669

APPENDICES

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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

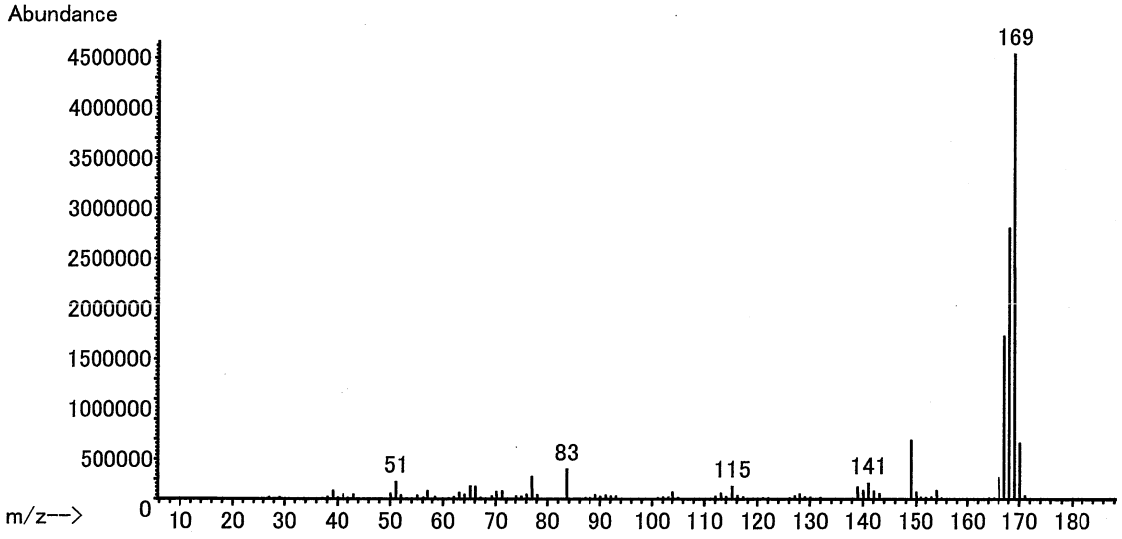
1. Spectral Data

Mass Spectrometry

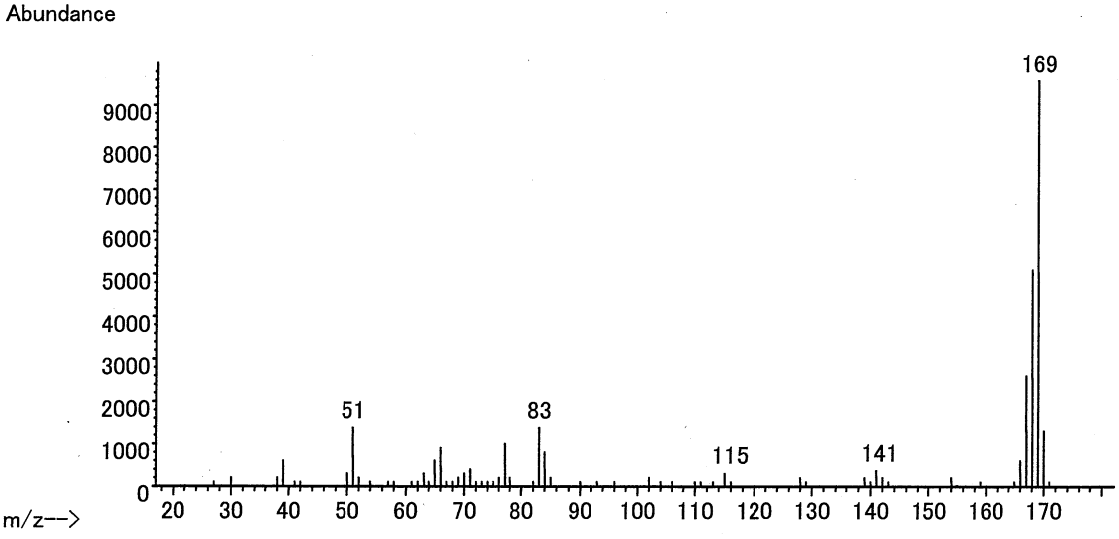
Instrument : Agilent Technologies 5973N Mass Spectrometer

Ionization : EI (Electron Ionization)

Ionization Voltage : 70eV



Mass Spectrum of Test Substance



Mass Spectrum of Literature Data*

Result: The mass spectrum was consistent with literature spectrum.

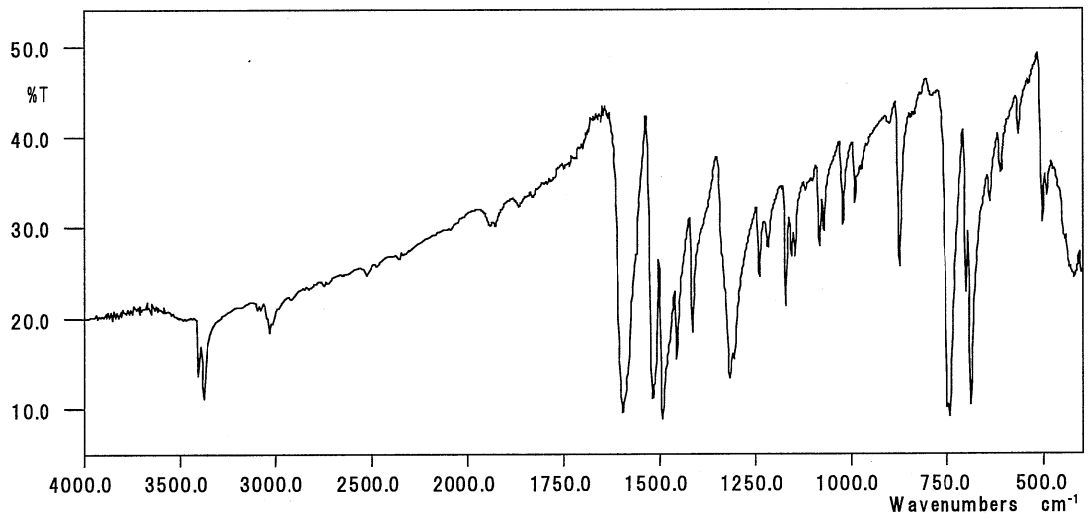
(*McLafferty FW, ed. 1994. Wiley Registry of Mass Spectral Data. 6th ed. New York, NY:John Wiley and Sons.)

Infrared Spectrometry

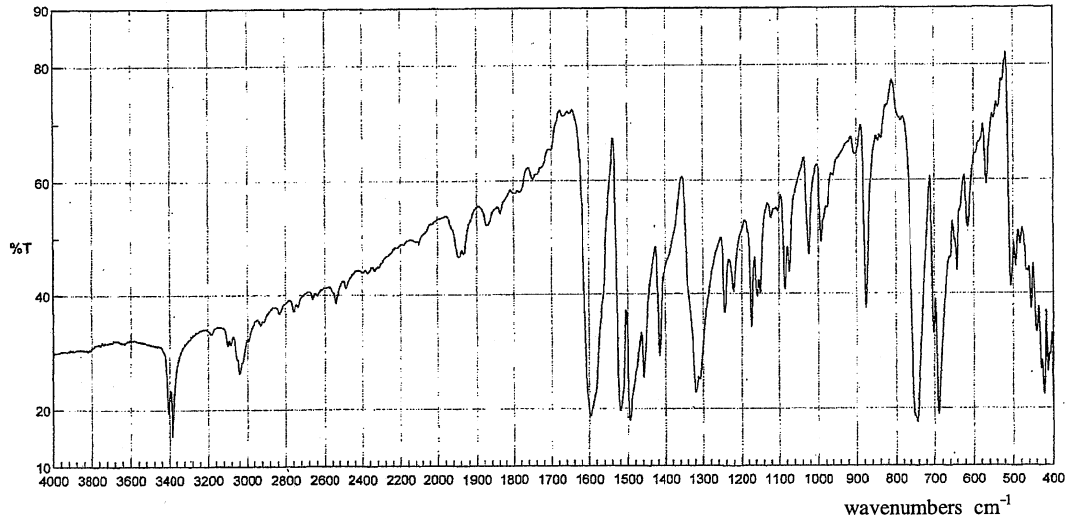
Instrument : Shimadzu FTIR-8200PC Infrared Spectrometer

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 mmϕ × 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 μL

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 mmϕ × 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 μL

	Target Concentration				
Date Analyzed	256[a]	640	1600	4000	10000
2006.10.10	255[b] (99.6)[c]	644 (101)	1630 (102)	4020 (101)	9920 (99.2)

[a] ppm
[b] ppm (Mean measured concentration.)
[c] % (Mean measured concentration/target concentration × 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 mmϕ × 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 μL

	Target Concentration				
	256[a]	640	1600	4000	10000
Coefficient Variation	4.13[b]	2.74	3.21	4.69	2.45

[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 mmϕ × 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 μL

	Target Concentration	
Date Analyzed	100[a]	10000
2006.09.08	99.0 (100)[b]	10100 (100)
2006.09.16[c]	92.7 (93.6)	9690 (95.9)
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)]	$\times 10^6/\mu L$	2
Hemoglobin(Hgb)	Cyanmethemoglobin method [1)]	g/dL	1
Hematocrit(Hct)	Calculated as RBC×MCV/10 [1)]	%	1
Mean corpuscular volume(MCV)	Light scattering method [1)]	fL	1
Mean corpuscular hemoglobin(MCH)	Calculated as Hgb/RBC×10 [1)]	pg	1
Mean corpuscular hemoglobin concentration (MCHC)	Calculated as Hgb/Hct×100 [1)]	g/dL	1
Platelet	Light scattering method [1)]	$\times 10^3/\mu L$	0
Reticulocyte	Light scattering method [1)]	%	1
Methemoglobin	Van Assendelft method [2)]	%	1
White blood cell(WBC)	Light scattering method [1)]	$\times 10^3/\mu L$	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 Alb/(TP−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
Creatinine	Jaffé 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.)