2-アミノ-4-クロロフェノールのラットを用いた
経口投与によるがん原性試験（混餌試験）報告書

試験番号：0579

APPENDICES

APPENDICES

APPENDIX 1-1

IDENTITY OF 2-AMINO-4-CHLOROPHENOL IN THE 2-YEAR FEED STUDY

IDENTITY OF 2-AMINO-4-CHLOROPHENOL IN THE 2-YEAR FEED STUDY

Test Substance : 2-Amino-4-chlorophenol (Wako Pure Chemical Industries, Ltd.)

A. Lot No. : CEQ0194

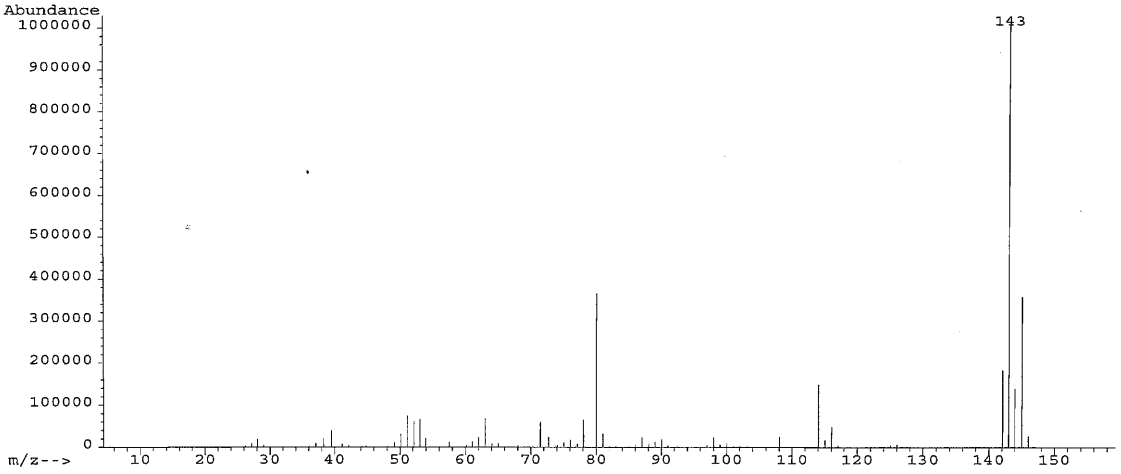
1. Spectral data

Mass Spectrometry

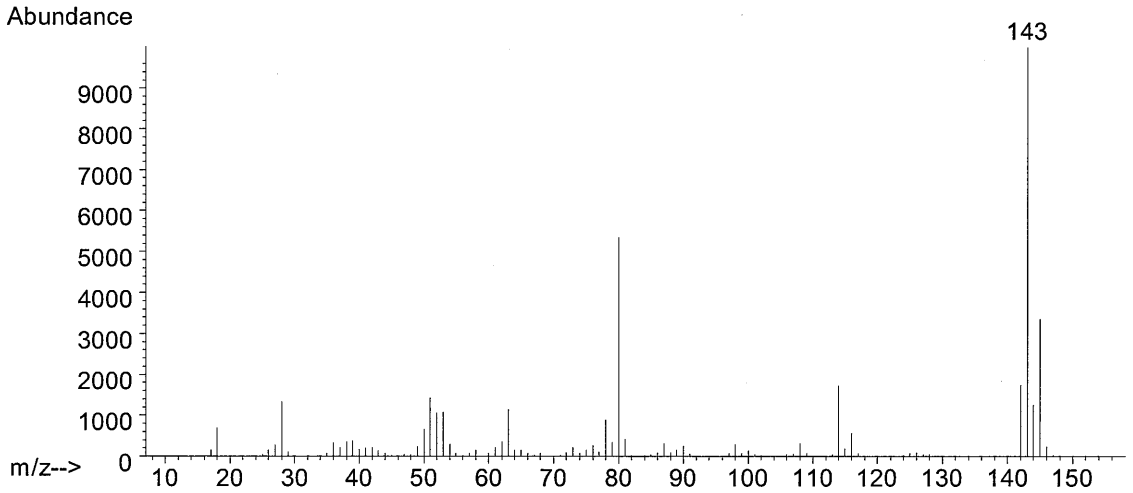
Instrument : Agilent Technologies 5989B Mass Spectrometer

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.

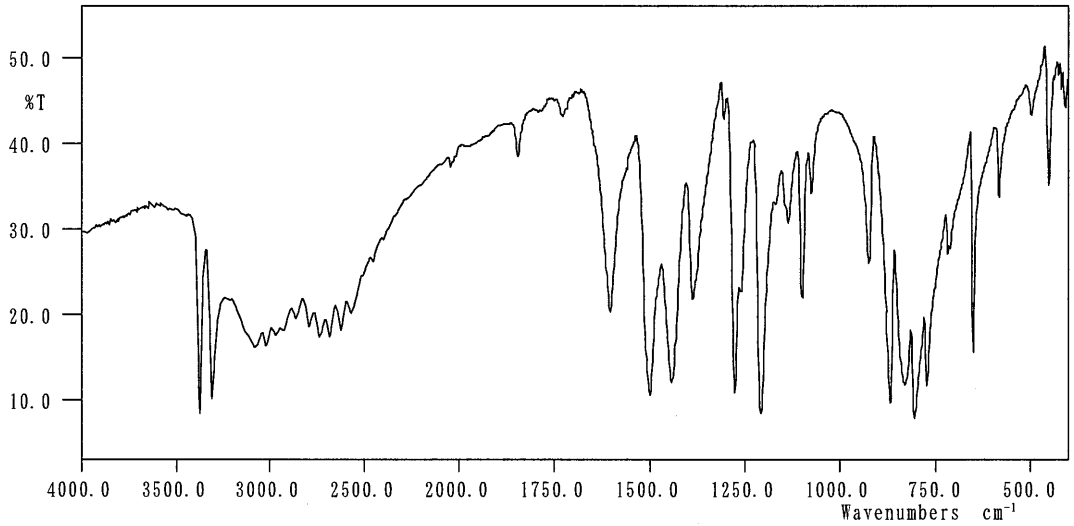
(*McLafferty FW, ed. 1994. Wiley Registry of Mass Spectral Data. 6th ed. New York, NY : John Wiley and Sons.)

Infrared Spectrometry

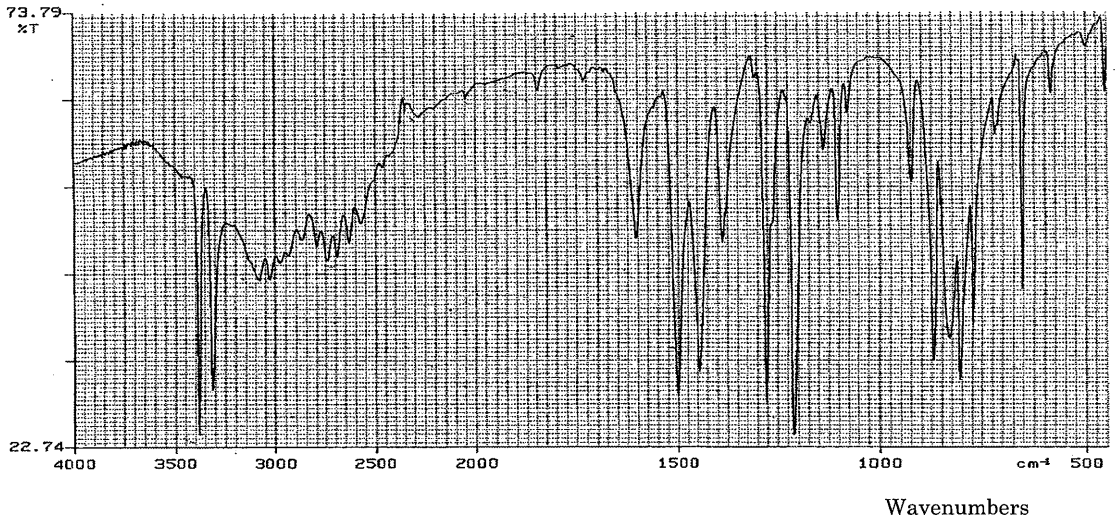
Instrument : Shimadzu FTIR-8200PC Infrared Spectrometer

Cell : KBr

Resolution : 2.0 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 2-amino-4-chlorophenol by mass spectrum and infrared spectrum.

B. Lot No. : SDM0599

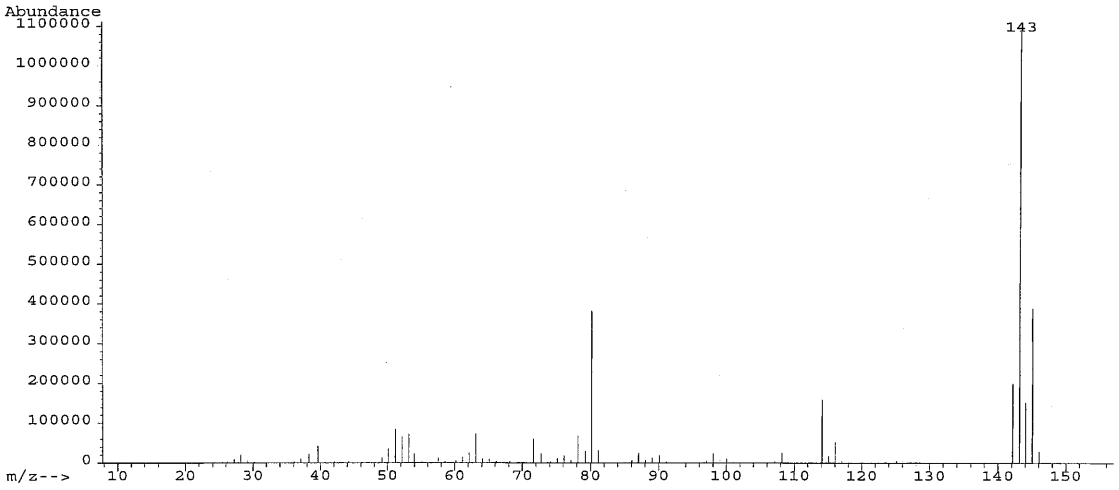
1. Spectral data

Mass Spectrometry

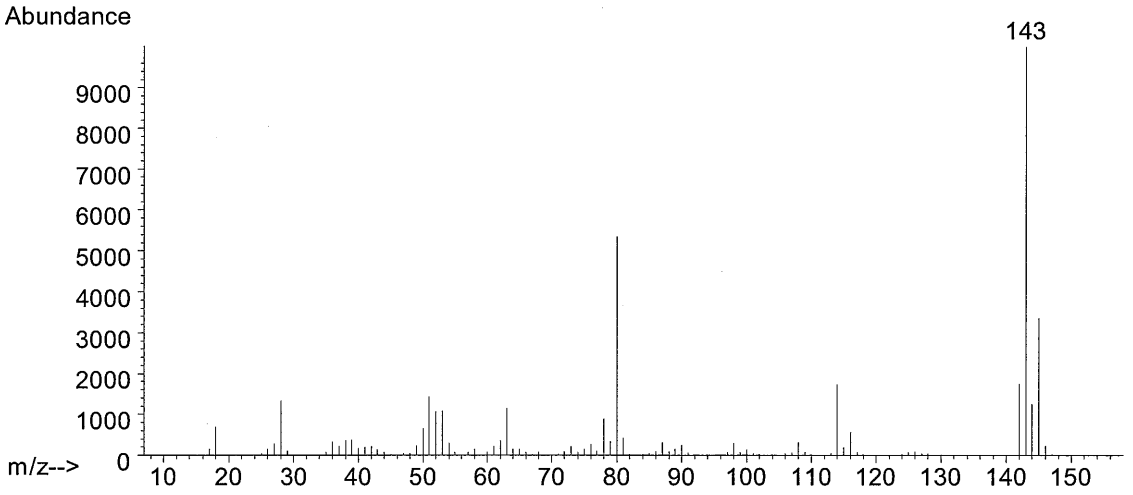
Instrument : Agilent Technologies 5989B Mass Spectrometer

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.

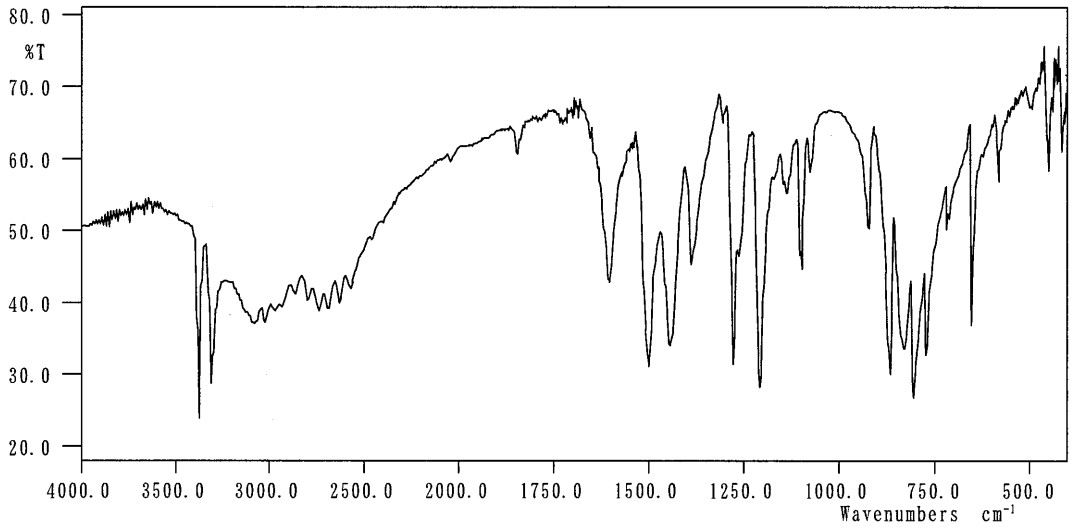
(*McLafferty FW, ed. 1994. Wiley Registry of Mass Spectral Data. 6th ed. New York, NY : John Wiley and Sons.)

Infrared Spectrometry

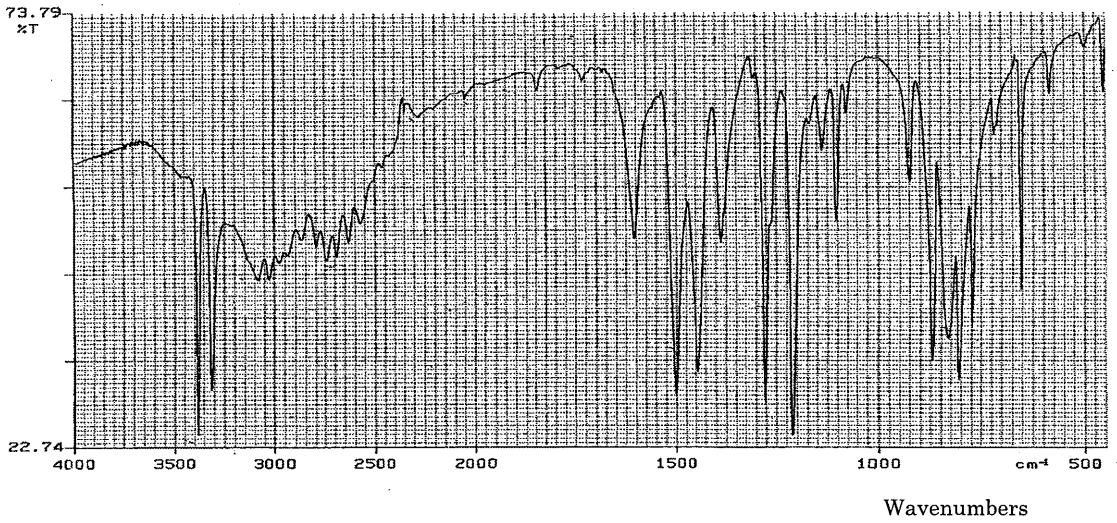
Instrument : Shimadzu FTIR-8200PC Infrared Spectrometer

Cell : KBr

Resolution : 2.0 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 2-amino-4-chlorophenol by mass spectrum and infrared spectrum.

C. Lot No. : LTM0601

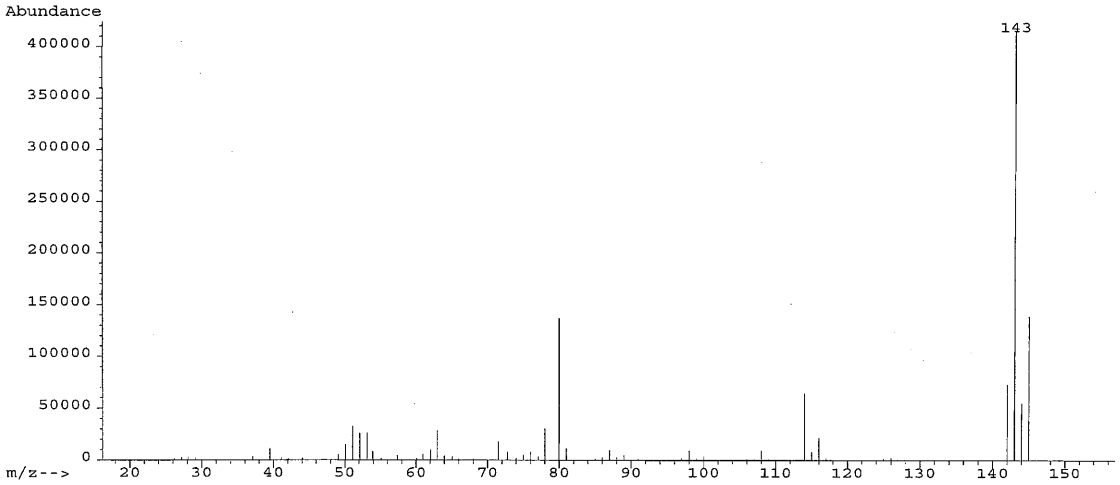
1. Spectral data

Mass Spectrometry

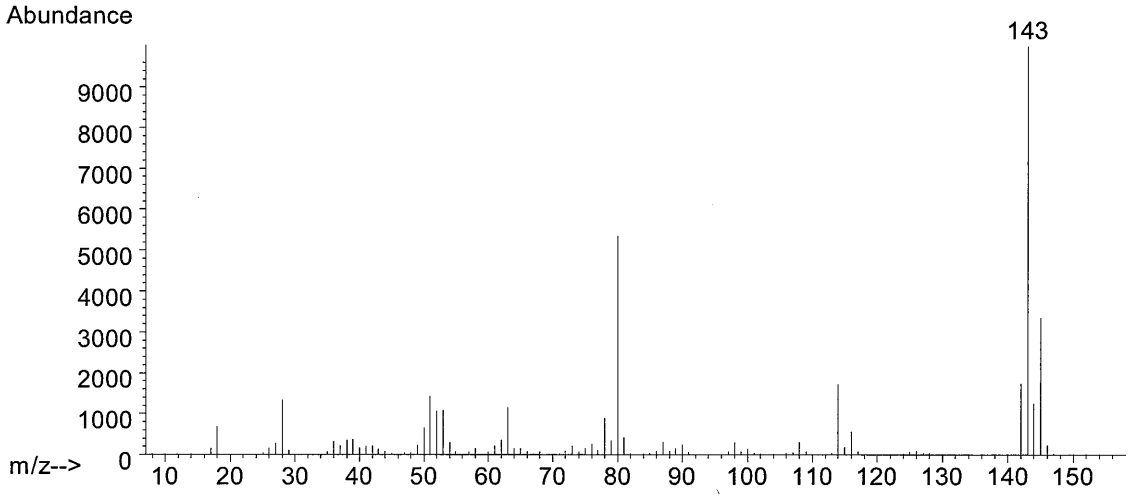
Instrument : Agilent Technologies 5989B Mass Spectrometer

Ionization : EI (Electron Ionization)

Ionization Voltage : 70eV



Mass Spectrum of Test Substance



Mass Spectrum of Literature Data*

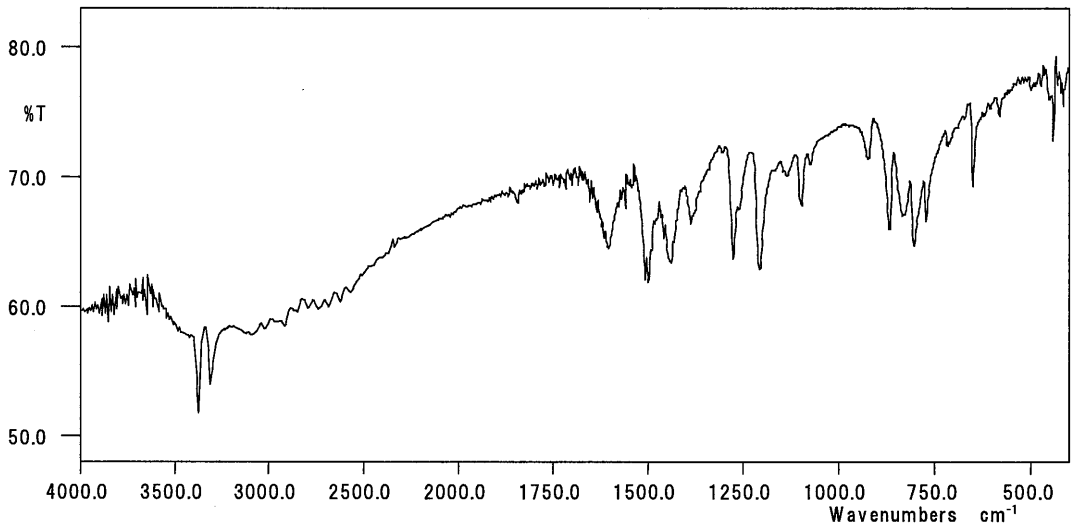
Result: The mass spectrum was consistent with literature spectrum.
(*McLafferty FW, ed. 1994. Wiley Registry of Mass Spectral Data. 6th ed. New York, NY : John Wiley and Sons.)

Infrared Spectrometry

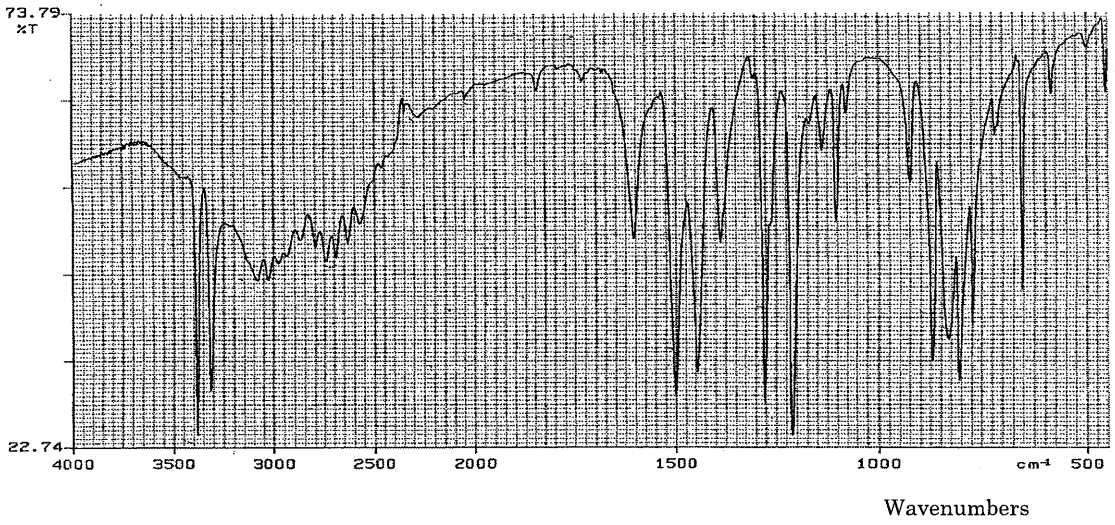
Instrument : Shimadzu FTIR-8200PC Infrared Spectrometer

Cell : KBr

Resolution : 2.0 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 2-amino-4-chlorophenol by mass spectrum and infrared spectrum.

APPENDIX 1-2

STABILITY OF 2-AMINO-4-CHLOROPHENOL IN THE 2-YEAR FEED STUDY

STABILITY OF 2-AMINO-4-CHLOROPHENOL IN THE 2-YEAR FEED STUDY

Test Substance : 2-Amino-4-chlorophenol (Wako Pure Chemical Industries, Ltd.)

A. Lot No. : CEQ0194

1. Gas Chromatography

Instrument : Agilent Technologies 5890A Gas Chromatograph

Column : DB-1 (0.25 mmϕ × 60 m)

Column Temperature: 100 °C → (10 °C/min) → 250 °C (5 min)

Flow Rate : 1 mL/min

Detector : FID (Flame Ionization Detector)

Injection Volume : 1 μL

Date (date analyzed)	Peak No.	Retention Time (min)	Area (%)
2005.03.24	1	13.154	100
2005.09.26	1	13.152	100

Result: Gas chromatography indicated one major peak (peak No.1) analyzed on 2005.3.24 and one major peak (peak No.1) analyzed on 2005.9.26. No new trace impurity peak in the test substance analyzed on 2005.9.26 was detected.

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

B. Lot No. : SDM0599

1. Gas Chromatography

Instrument : Agilent Technologies 5890A Gas Chromatograph

Column : DB-1 (0.25 mmϕ × 60 m)

Column Temperature: 100 °C → (10 °C/min) → 250 °C (5 min)

Flow Rate : 1 mL/min

Detector : FID (Flame Ionization Detector)

Injection Volume : 1 μL

Date (date analyzed)	Peak No.	Retention Time (min)	Area (%)
2005.09.16	1	13.152	100
2006.08.02	1	13.151	100

Result: Gas chromatography indicated one major peak (peak No.1) analyzed on 2005.9.16 and one major peak (peak No.1) analyzed on 2006.8.2. No new trace impurity peak in the test substance analyzed on 2006.8.2 was detected.

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

C. Lot No. : LTM0601

1. Gas Chromatography

Instrument : Agilent Technologies 5890A Gas Chromatograph

Column : DB-1 (0.25 mmϕ × 60 m)

Column Temperature: 100 °C → (10 °C/min) → 250 °C (5 min)

Flow Rate : 1 mL/min

Detector : FID (Flame Ionization Detector)

Injection Volume : 1 μL

Date (date analyzed)	Peak No.	Retention Time (min)	Area (%)
2006.07.13	1	13.156	100
2007.04.27	1	13.157	100

Result: Gas chromatography indicated one major peak (peak No.1) analyzed on 2006.7.13 and one major peak (peak No.1) analyzed on 2007.4.27. No new trace impurity peak in the test substance analyzed on 2007.4.27 was detected.

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

APPENDIX 2-1

CONCENTRATION OF 2-AMINO-4-CHLOROPHENOL IN FORMULATED DIETS IN THE 2-YEAR FEED STUDY

CONCENTRATION OF 2-AMINO-4-CHLOROPHENOL IN FORMULATED DIETS IN THE 2-YEAR 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 : 0.8 mL/min

Mobile Phase : Methanol : Acetonitrile : Phosphoric Acid (5 mM Octanesulfonic Acid Sodium Salt Monohydrate pH2.4) = 1 : 1 : 3

Detector : UV (284 nm)

Injection Volume : 10 μL

	Target Concentration		
Date Analyzed	1280[a]	3200	8000
2005.03.28	1260[b] (98.4)[c]	3150 (98.4)	8140 (102)
2005.06.27	1230 (96.1)	3040 (95.0)	8070 (101)
2005.09.19	1250 (97.7)	3090 (96.6)	7800 (97.5)
2005.12.12	1280 (100)	3230 (101)	8010 (100)
2006.03.06	1280 (100)	3380 (106)	8210 (103)
2006.05.29	1230 (96.1)	3030 (94.7)	7720 (96.5)
2006.08.21	1290 (101)	3250 (102)	8090 (101)
2006.11.13	1230 (96.1)	3010 (94.1)	7500 (93.8)
2007.02.05	1300 (102)	2990 (93.4)	7940 (99.3)

[a] ppm

[b] ppm (Mean measured concentration.)

[c] % (Mean measured concentration/target concentration × 100.)

APPENDIX 2-2

HOMOGENEITY OF 2-AMINO-4-CHLOROPHENOL IN FORMULATED DIETS IN THE 2-YEAR FEED STUDY

HOMOGENEITY OF 2-AMINO-4-CHLOROPHENOL IN FORMULATED DIETS IN THE 2-YEAR 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 : 0.8 mL/min

Mobile Phase : Methanol : Acetonitrile : Phosphoric Acid (5 mM Octanesulfonic Acid Sodium Salt Monohydrate pH2.4) = 1 : 1 : 3

Detector : UV (284 nm)

Injection Volume : 10 µL

	Target Concentration		
	1280[a]	3200	8000
Coefficient Variation	2.03[b]	3.13	3.87

[a] ppm
[b] % (n=7)

APPENDIX 2-3

STABILITY OF 2-AMINO-4-CHLOROPHENOL IN FORMULATED DIETS IN THE 2-YEAR FEED STUDY

STABILITY OF 2-AMINO-4-CHLOROPHENOL IN FORMULATED DIETS IN THE 2-YEAR 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 : 0.8 mL/min

Mobile Phase : Methanol : Acetonitrile : Phosphoric Acid (5 mM Octanesulfonic Acid Sodium Salt Monohydrate pH2.4) = 1 : 1 : 3

Detector : UV (284 nm)

Injection Volume : 10 µL

	Target Concentration		
Date Prepared	Date Analyzed	512[a]	8000
2005.02.24	2005.02.24	484 (100)[b]	7940 (100)
	2005.02.28[c]	438 (90.5)	7610 (95.8)
	2005.03.07[d]	463 (95.7)	7730 (97.4)

[a] ppm
[b] % (Percentage was based on the concentration on date of preparation.)
[c] Animal room samples
[d] Cold storage samples

APPENDIX 3

METHODS, UNITS AND DECIMAL PLACE FOR HEMATOLOGY AND BIOCHEMISTRY IN THE 2-YEAR FEED STUDY OF 2-AMINO-4-CHLOROPHENOL

METHODS, UNITS AND DECIMAL PLACE FOR HEMATOLOGY AND BIOCHEMISTRY IN THE 2-YEAR FEED STUDY OF 2-AMINO-4-CHLOROPHENOL

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
White blood cell(WBC)	Light scattering method [1)]	$\times 10^3/\mu L$	2
Differential WBC	Pattern recognition method [2)] (Wright staining)	%	0
Biochemistry			
Total protein(TP)	Biuret method [3)]	g/dL	1
Albumin (Alb)	BCG method [3)]	g/dL	1
A/G ratio	Calculated as Alb/(TP−Alb) [3)]	−	1
T-bilirubin	Azobilirubin method [3)]	mg/dL	2
Glucose	GlcK·G-6-PDH method [3)]	mg/dL	0
T-cholesterol	CE·COD·POD method [3)]	mg/dL	0
Triglyceride	MGLP·GK·GPO·POD method [3)]	mg/dL	0
Phospholipid	PLD·ChOD·POD method [3)]	mg/dL	0
Aspartate aminotransferase (AST)	JSCC method [3)]	IU/L	0
Alanine aminotransferase (ALT)	JSCC method [3)]	IU/L	0
Lactate dehydrogenase (LDH)	SFBC method [3)]	IU/L	0
Alkaline phosphatase (ALP)	GSCC method [3)]	IU/L	0
γ-Glutamyl transpeptidase (γ-GTP)	JSCC method [3)]	IU/L	0
Creatine kinase (CK)	JSCC method [3)]	IU/L	0
Urea nitrogen	Urease·GLDH method [3)]	mg/dL	1
Creatinine	Jaffé method [3)]	mg/dL	1
Sodium	Ion selective electrode method [3)]	mEq/L	0
Potassium	Ion selective electrode method [3)]	mEq/L	1
Chloride	Ion selective electrode method [3)]	mEq/L	0
Calcium	OCPC method [3)]	mg/dL	1
Inorganic phosphorus	PNP·XOD·POD method [3)]	mg/dL	1

1) Automatic blood cell analyzer (ADVIA120 : Bayer Corporation)
2) Automatic blood cell differential analyzer (MICROX HEG-120NA : OMRON Corporation)
3) Automatic analyzer (Hitachi 7080 : Hitachi,Ltd.)