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APPENDICES

APPENDICES

APPENDIX 1-1

IDENTITY OF 4-CHLORO-2-NITROANILINE IN

THE 2-WEEK FEED STUDY

IDENTITY OF 4-CHLORO-2-NITROANILINE IN THE 2-WEEK FEED STUDY

Test Substance   : 4-Chloro-2-nitroaniline (Tokyo Chemical Industry Co., Ltd.)

Lot No.   : GJ01

1. Spectral Data

Mass Spectrometry

Instrument   : Hitachi M-80B Mass Spectrometer

Ionization   : EI (Electron Ionization)

Ionization Voltage   : 70eV
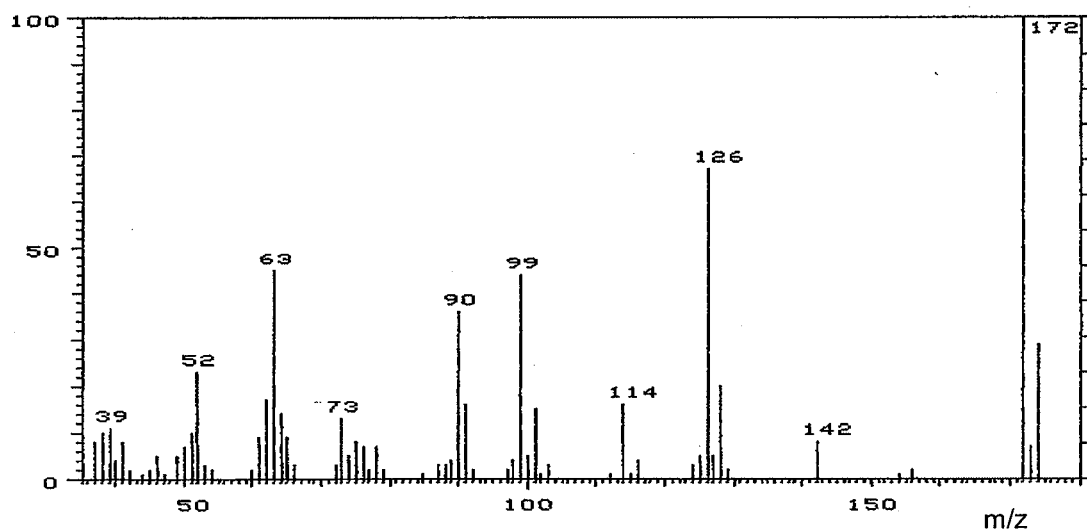
Mass Spectrum of Test Substance
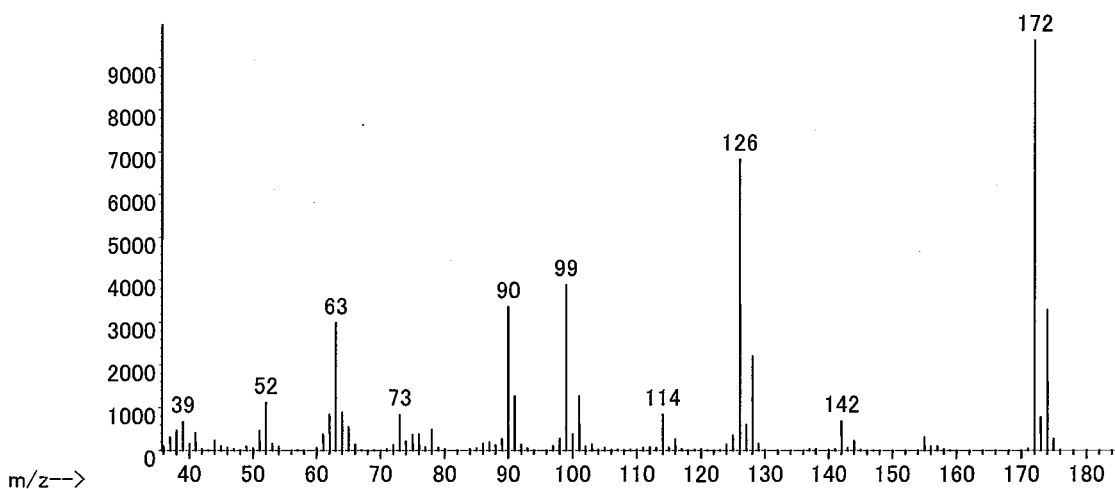
Mass Spectrum of Literature Data*

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.)

<u>Infrared Spectrometry</u>

Instrument   : Shimadzu FTIR-8200PC Infrared Spectrometer

Cell   : KBr

Resolution   : 4 cm^{-1}
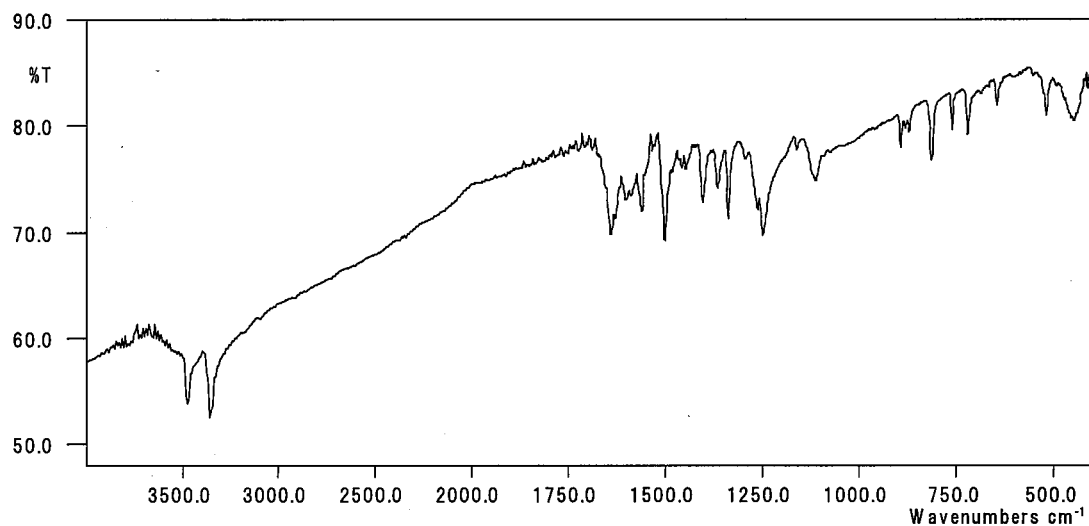
Infrared Spectrum of Test Substance
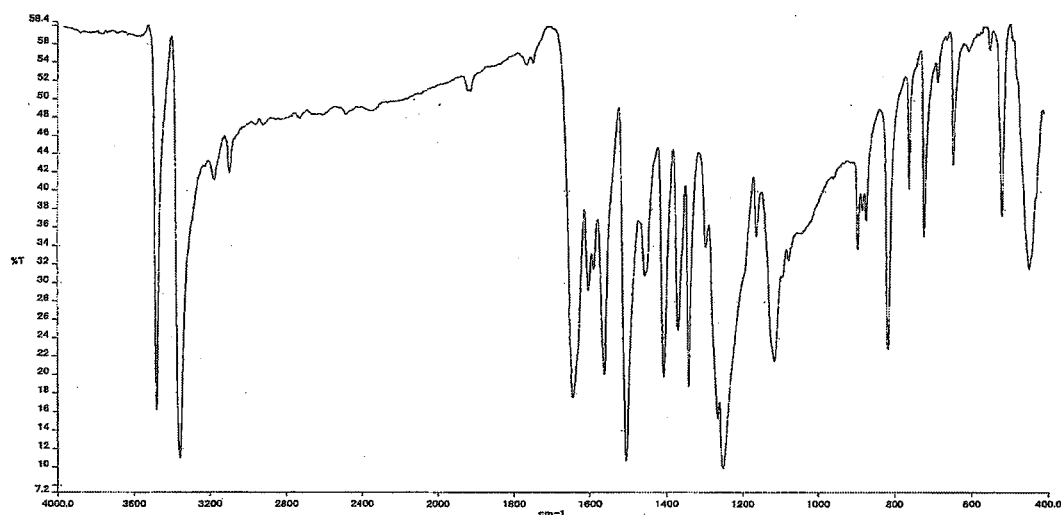
Infrared Spectrum of Literature Data*

Result: The infrared spectrum was consistent with literature spectrum.
       (*Performed by Tokyo Chemical Industry Co., Ltd.)

2. Conclusion: The test substance was identified as 4-chloro-2-nitroaniline by mass spectrum
              and infrared spectrum.

APPENDIX 1-2

STABILITY OF 4-CHLORO-2-NITROANILINE IN

THE 2-WEEK FEED STUDY

STABILITY OF 4-CHLORO-2-NITROANILINE IN THE 2-WEEK FEED STUDY

Test Substance   : 4-Chloro-2-nitroaniline (Tokyo Chemical Industry Co., Ltd.)

Lot No.   : GJ01

1. High Performance Liquid Chromatography

    Instrument   : Shimadzu LC-10 High Performance Liquid Chromatograph

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

    Column Temperature: 40 ° C

    Flow Rate   : 1 mL/min

    Mobile Phase   : Acetonitrile : 5mmol SDS solution (Phospholic acid pH2.2) = 70 : 30

    Detector   : UV (405 nm)

    Injection Volume   : 10 μL

Date analyzed	Peak No.	Retention Time (min)	Area (%)
2009.06.30	1	3.054	100
2009.07.24	1	3.057	100

    Result: High performance liquid chromatography indicated one major peak (peak No.1) analyzed on 2009.6.30 and one major peak (peak No.1) analyzed on 2009.7.24 No new trace impurity peak in the test substance analyzed on 2009.7.24 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 4-CHLORO-2-NITROANILINE IN

FORMULATED DIETS IN THE 2-WEEK FEED STUDY

CONCENTRATION OF 4-CHLORO-2-NITROANILINE IN FORMULATED DIETS IN THE 2-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 $^\circ$ C

Flow Rate : 1 mL/min

Mobile Phase : Acetonitrile : 5mmol SDS solution (Phospholic acid pH2.2) = 70 : 30

Detector : UV (405 nm)

Injection Volume : 10 μL

Date Analyzed	Target Concentration				
	2500[a]	5000	10000	20000	30000
2009.07.08	2650[b] (106)[c]	5370 (107)	10100 (101)	20800 (104)	31100 (104)

[a] ppm
[b] ppm (Mean measured concentration.)
[c] % (Mean measured concentration/target concentration $\times$ 100.)

APPENDIX 1-4

HOMOGENEITY OF 4-CHLORO-2-NITROANILINE IN

FORMULATED DIETS IN THE 2-WEEK FEED STUDY

HOMOGENEITY OF 4-CHLORO-2-NITROANILINE IN FORMULATED DIETS IN THE 2-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 : 5mmol SDS solution (Phospholic acid pH2.2) = 70 : 30

Detector : UV (405 nm)

Injection Volume : 10 μL

	Target Concentration				
	2500[a]	5000	10000	20000	30000
Coefficient Variation	6.45[b]	4.50	5.97	4.48	10.3

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

APPENDIX 1-5

STABILITY OF 4-CHLORO-2-NITROANILINE IN

FORMULATED DIETS

STABILITY OF 4-CHLORO-2-NITROANILINE IN FORMULATED DIETS

High Performance Liquid Chromatography

Instrument : Shimadzu LC-10 High Performance Liquid Chromatograph

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

Column Temperature: 40 °C

Flow Rate : 1 mL/min

Mobile Phase : Acetonitrile : 5mM Sodium dodecyl sulfate solution (phosphoric acid pH2.2) = 70 : 30

Detector : UV (405 nm)

Injection Volume : 10 μL

Date Analyzed	Target Concentration	
	100[a]	50000
2008.08.18	107 (100)[b]	49200 (100)
2008.08.26[c]	103 (103)	48100 (97.8)
2008.08.26[d]	105 (98.1)	46000 (93.5)

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

APPENDIX 2

METHODS, UNITS AND DECIMAL PLACE FOR HEMATOLOGY

AND BIOCHEMISTRY IN THE 2-WEEK FEED STUDY OF

4-CHLORO-2-NITROANILINE

METHODS, UNITS AND DECIMAL PLACE FOR HEMATOLOGY AND BIOCHEMISTRY IN THE 2- WEEK FEED STUDY OF 4-CHLORO-2-NITROANILINE

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	Light scattering method [1]	%	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
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)	JSCC 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
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 : Siemens Healthcare Diagnostics Inc.)
2) Spectrophotometer (DU-530 : Beckman Coulter,Inc.)
3) Automatic analyzer (Hitachi 7080 : Hitachi,Ltd.)