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APPENDICES

APPENDICES

APPENDIX 1   1

IDENTITY OF ACROLEIN IN THE 13-WEEK

INHALATION STUDY

IDENTITY OF ACROLEIN IN THE 13-WEEK INHALATION STUDY

Test Substance   : Acrolein (Tokyo Chemical Industry Co., Ltd.)

Lot No.   : KOLRO

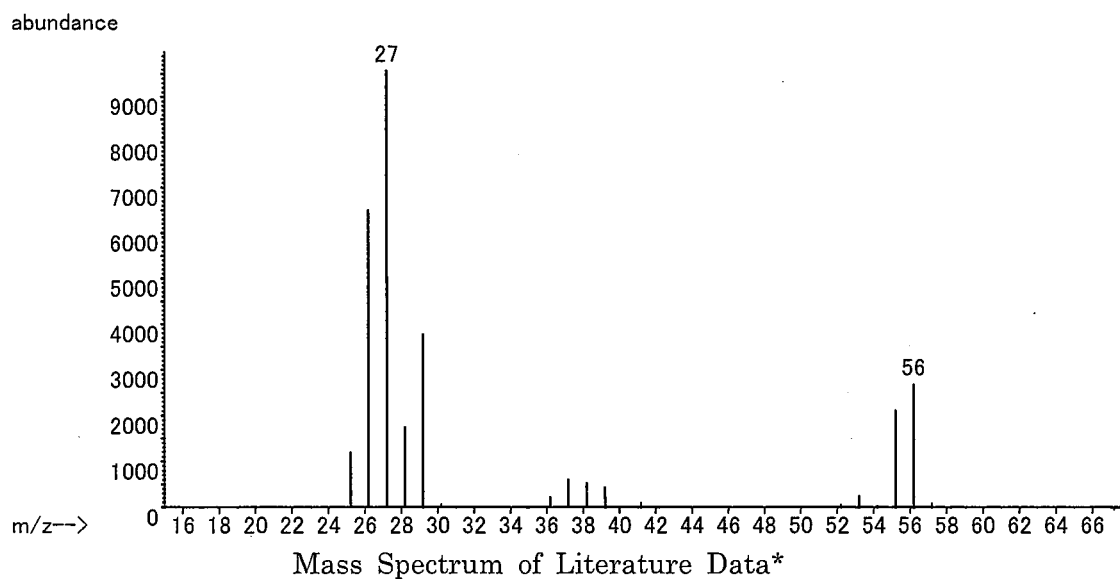## 1. Spectral Data

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.)

2. Conclusion: The test substance was identified as acrolein by mass spectrum.

APPENDIX 1 2

STABILITY OF ACROLEIN IN THE 13-WEEK

INHALATION STUDY

STABILITY OF ACROLEIN IN THE 13-WEEK INHALATION STUDY

Test Substance   : Acrolein (Tokyo Chemical Industry Co., Ltd.)
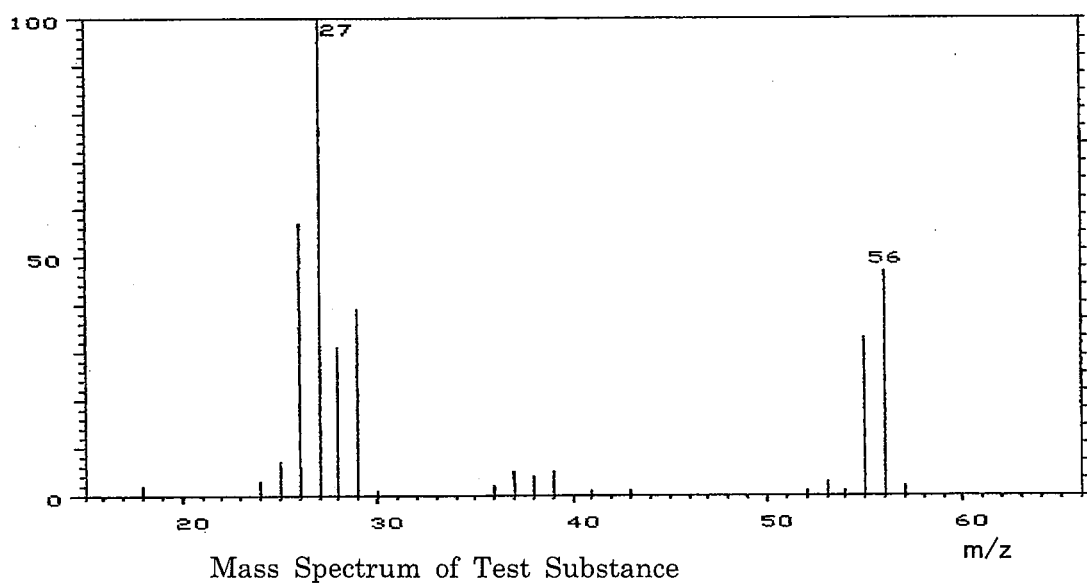
Lot No.   : KOLRO

1. Gas Chromatography

    Instrument   : Agilent Technologies 5890A Gas Chromatograph

    Column   : INNOWAX (0.53 mm ϕ × 60 m)

    Column Temperature : 60° C

    Flow Rate   : 10 mL/min

    Detector   : FID (Flame Ionization Detector)

    Injection Volume   : 1 μL

Date (date analyzed)	Peak No.	Retention Time (min)	Area (%)
2011.09.12	1	2.618	100
2011.12.22	1	2.618	100

Result: Gas chromatography indicated one major peak (peak No.1) analyzed on 2011.9.12 and one major peak (peak No.1) analyzed on 2011.12.22. No new trace impurity peak in the test substance analyzed on 2011.12.22 was detected.

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

APPENDIX 2

ENVIRONMENTAL CONDITIONS OF INHALATION CHAMBER

IN THE 13-WEEK INHALATION STUDY OF ACROLEIN

ENVIRONMENTAL CONDITIONS OF INHALATION CHAMBER
IN THE 13-WEEK INHALATION STUDY OF ACROLEIN

Group Name	Temperature (℃) Mean ± S.D.	Humidity (%) Mean ± S.D.	Ventilation Rate (L/min) Mean ± S.D.	Air Change (time/h) Mean
Control	22.8 ± 0.2	53.5 ± 0.8	212.9 ± 0.9	12.1
0.1 ppm	22.7 ± 0.3	51.2 ± 0.8	212.4 ± 0.8	12.0
0.3 ppm	22.8 ± 0.3	53.3 ± 0.9	213.6 ± 0.8	12.1
1 ppm	22.6 ± 0.3	53.1 ± 0.9	214.1 ± 0.8	12.1
2 ppm	22.6 ± 0.3	53.6 ± 1.1	211.7 ± 0.9	12.0
3 ppm	22.8 ± 0.3	53.3 ± 1.0	212.7 ± 0.9	12.0

APPENDIX 3

METHODS, UNITS AND DECIMAL PLACE FOR

HEMATOLOGY AND BIOCHEMISTRY IN THE 13-WEEK

INHALATION STUDY OF ACROLEIN

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

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$\times$MCV/10 [1]	%	1
Mean corpuscular volume(MCV)	Light scattering method [1]	fL	1
Mean corpuscular hemoglobin(MCH)	Calculated as Hgb/RBC$\times$10 [1]	pg	1
Mean corpuscular hemoglobin concentration (MCHC)	Calculated as Hgb/Hct$\times$100 [1]	g/dL	1
Platelet	Light scattering method [1]	$\times 10^3/\mu$L	0
Reticulocyte	Light scattering method [1]	%	1
Prothrombin time	Quick one stage method [2]	sec	1
Activated partial thromboplastin time (APTT)	Ellagic acid activated method [2]	sec	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$\cdot$G-6-PDH method [3]	mg/dL	0
T-cholesterol	CE$\cdot$COD$\cdot$POD method [3]	mg/dL	0
Triglyceride	MGLP$\cdot$GK$\cdot$GPO$\cdot$POD method [3]	mg/dL	0
Phospholipid	PLD$\cdot$ChOD$\cdot$POD method [3]	mg/dL	0
Aspartate aminotransferase (AST)	JSCC method [3]	U/L	0
Alanine aminotransferase (ALT)	JSCC method [3]	U/L	0
Lactate dehydrogenase (LDH)	JSCC method [3]	U/L	0
Alkaline phosphatase (ALP)	JSCC method [3]	U/L	0
γ-Glutamyl transpeptidase (γ-GTP)	JSCC method [3]	U/L	0
Creatine kinase (CK)	JSCC method [3]	U/L	0
Urea nitrogen	Urease$\cdot$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$\cdot$XOD$\cdot$POD method [3]	mg/dL	1

1) Automatic blood cell analyzer (ADVIA120 : Siemens Healthcare Diagnostics Inc.)
2) Automatic coagulometer (Sysmex CA-5000 : Sysmex Corporation)
3) Automatic analyzer (Hitachi 7080 : Hitachi,Ltd.)