エチレングリコールモノエチルエーテルアセテートの
ラットを用いた吸入によるがん原性試験報告書

試験番号：0774

APPENDICES

APPENDICES

APPENDIX 1-1

IDENTITY OF

ETHYLENE GLYCOL MONOETHYL ETHER ACETATE

IN THE 2-YEAR INHALATION STUDY

IDENTITY OF ETHYLENE GLYCOL MONOETHYL ETHER ACETATE IN THE 2-YEAR INHALATION STUDY

Test Substance : Ethylene glycol monoethyl ether acetate (Wako Pure Chemical Industries, Ltd.)

A. Lot No. : EPF1259

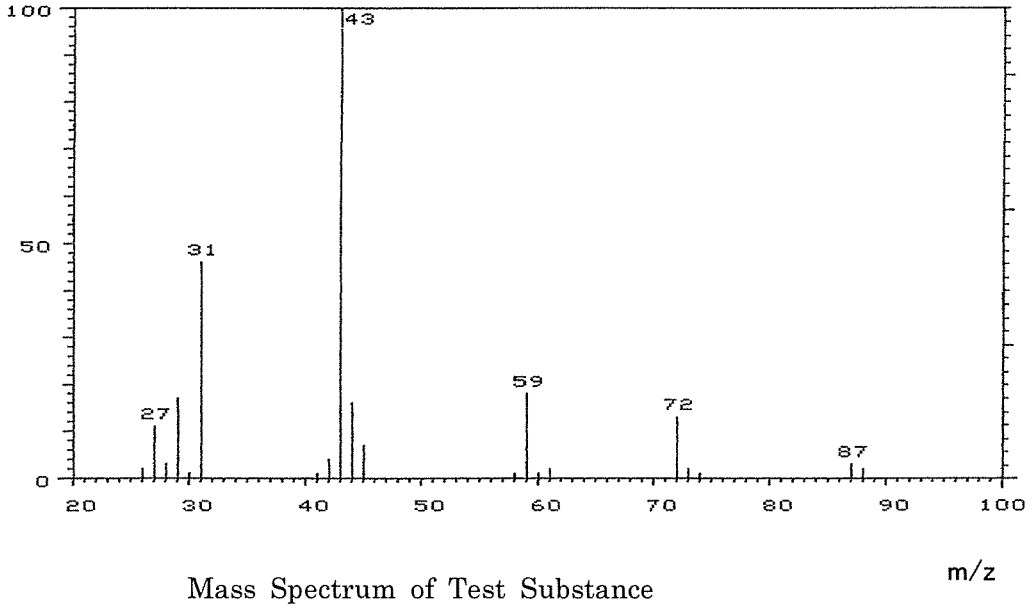
1. Spectral Data

Mass Spectrometry

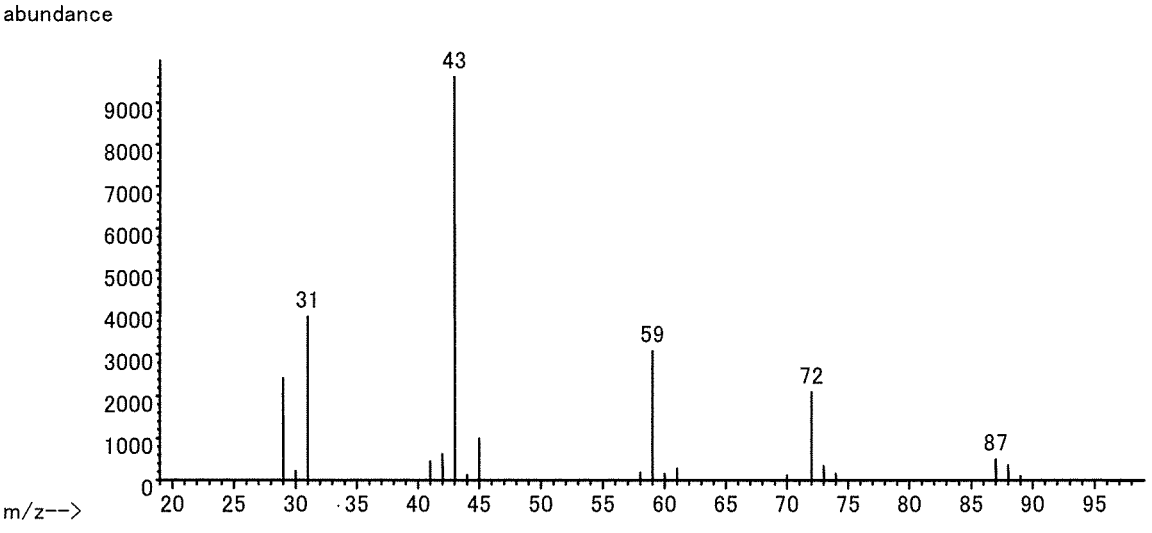
Instrument : Hitachi M-80B Mass Spectrometer

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.

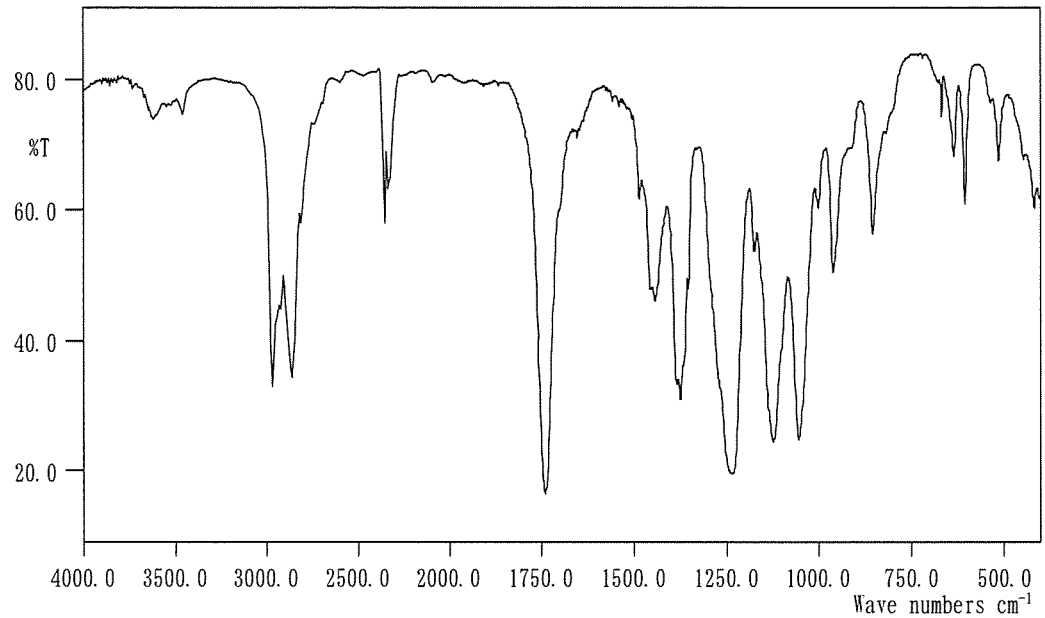
(*McLafferty FW, ed. 1994. Wiley Registry of Mass Spectral Data. 6th ed. New York, NY:John Wiley and Sons.)

Infrared Spectrometry

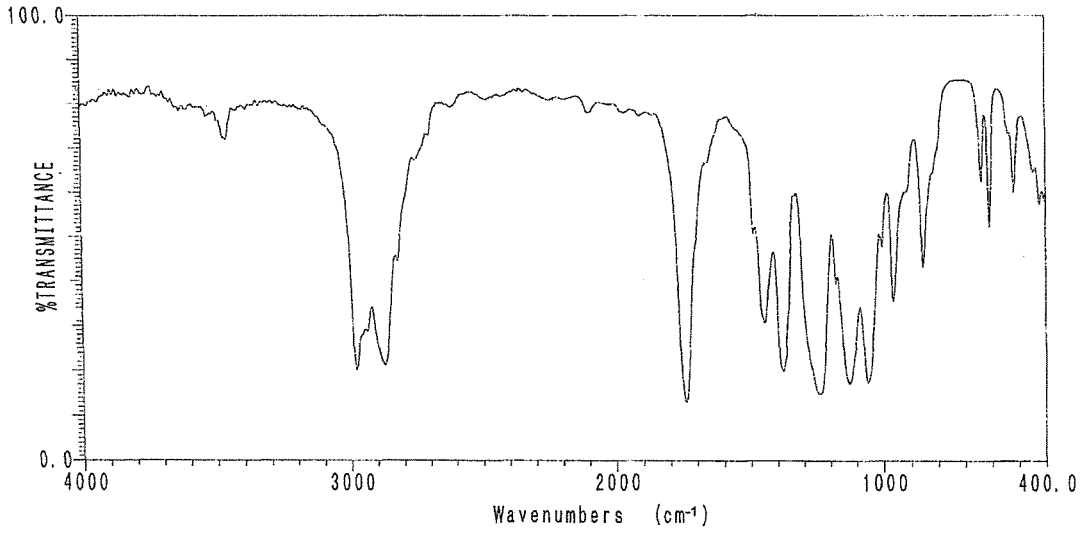
Instrument : Shimadzu FTIR-8200PC Infrared Spectrometer

Cell : KBr Liquid Cell

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 Wako Pure Chemical Industries, Ltd.)

2. Conclusion: The test substance was identified as ethylene glycol monoethyl ether acetate by mass spectrum and infrared spectrum.

IDENTITY OF ETHYLENE GLYCOL MONOETHYL ETHER ACETATE IN THE 2-YEAR INHALATION STUDY

Test Substance : Ethylene glycol monoethyl ether acetate (Wako Pure Chemical Industries, Ltd.)

B. Lot No. : DCM1007

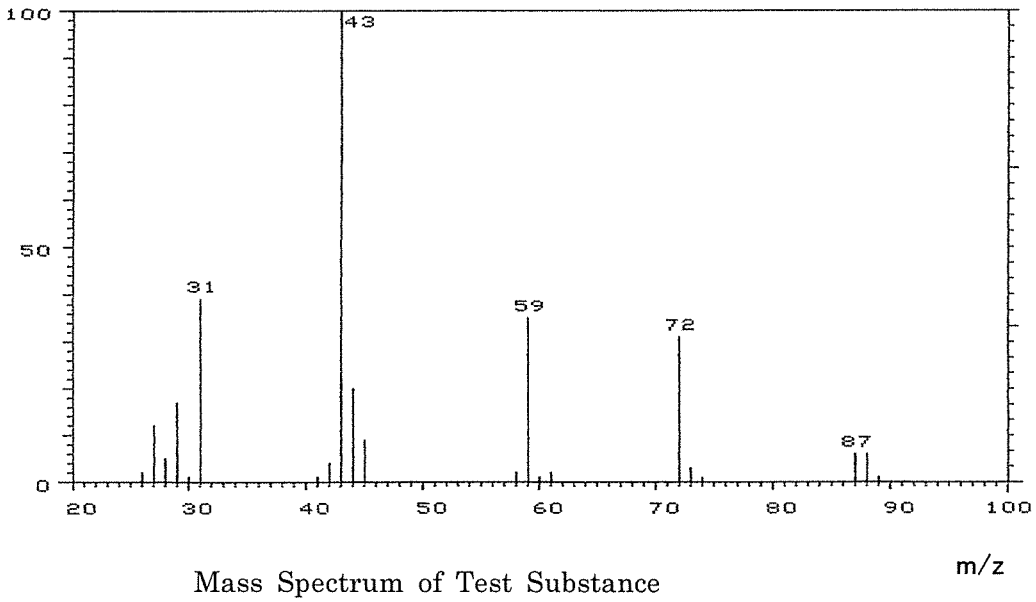
1. Spectral Data

Mass Spectrometry

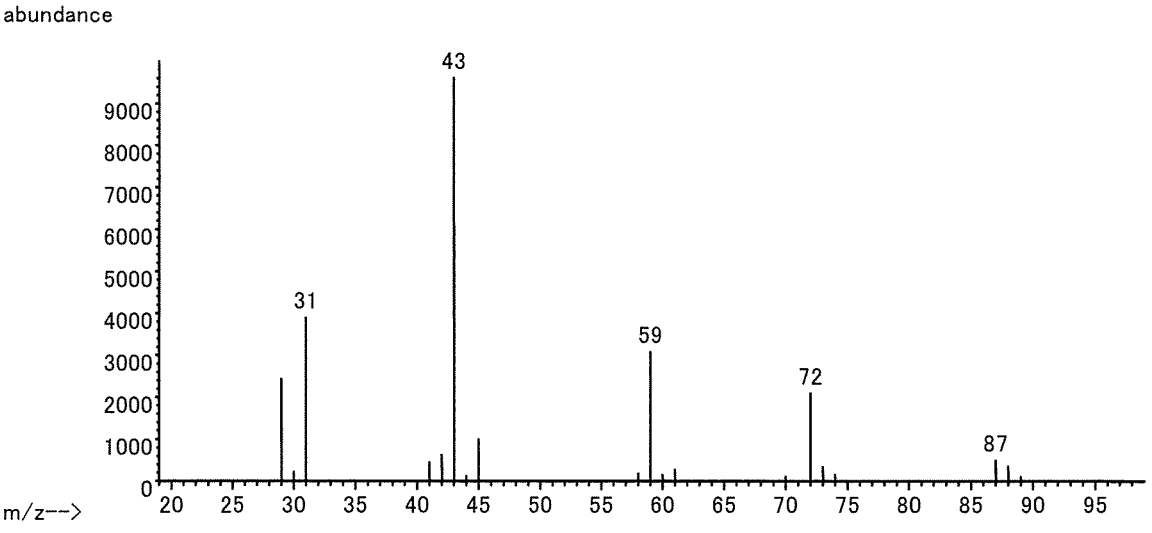
Instrument : Hitachi M-80B Mass Spectrometer

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.

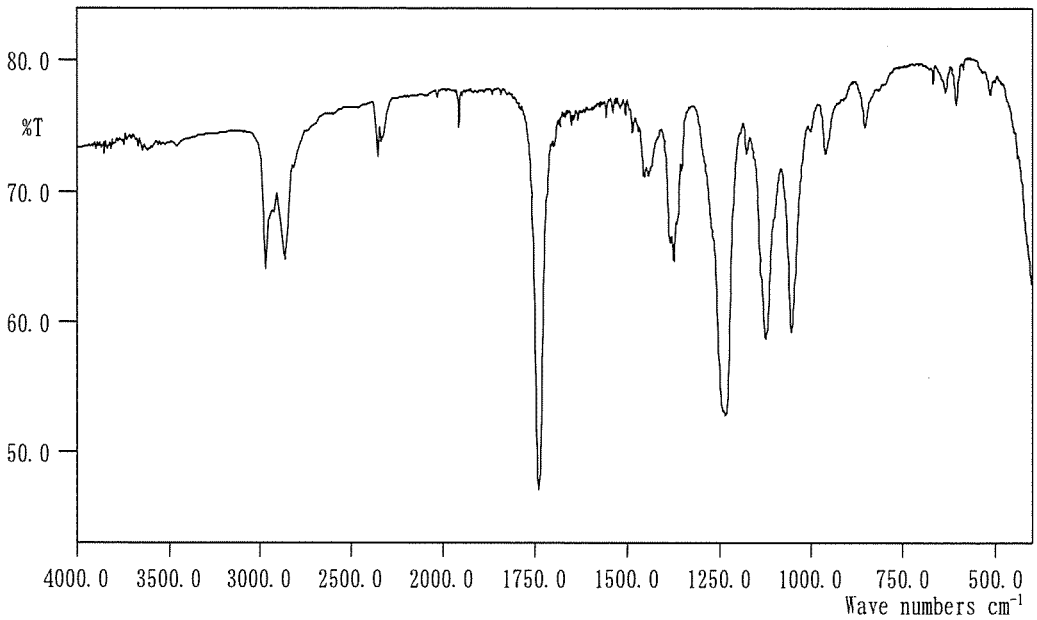
(*McLafferty FW, ed. 1994. Wiley Registry of Mass Spectral Data. 6th ed. New York, NY:John Wiley and Sons.)

Infrared Spectrometry

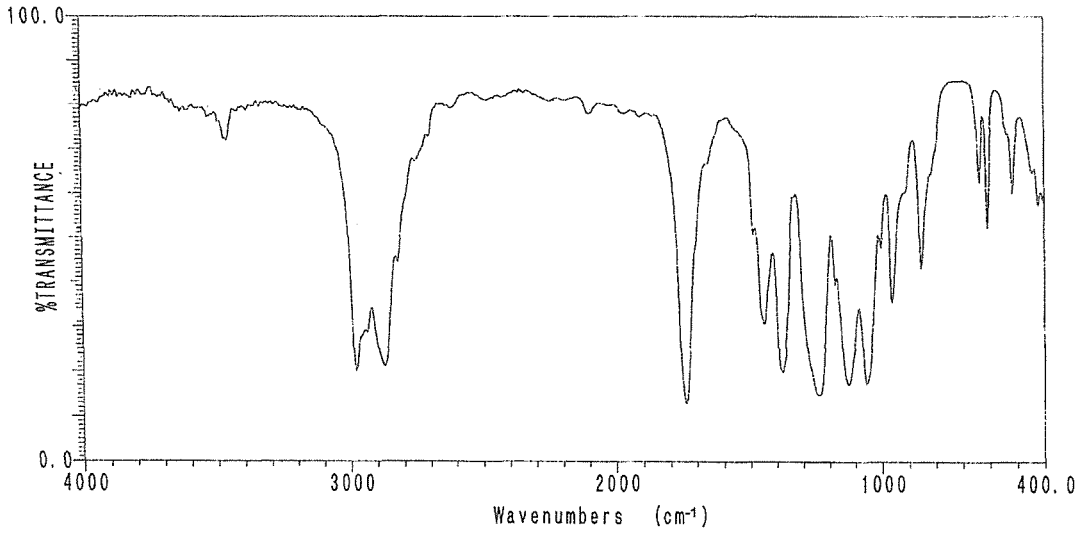
Instrument : Shimadzu FTIR-8200PC Infrared Spectrometer

Cell : KBr Liquid Cell

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 Wako Pure Chemical Industries, Ltd.)

2. Conclusion: The test substance was identified as ethylene glycol monoethyl ether acetate by mass spectrum and infrared spectrum.

IDENTITY OF ETHYLENE GLYCOL MONOETHYL ETHER ACETATE IN THE 2-YEAR INHALATION STUDY

Test Substance : Ethylene glycol monoethyl ether acetate (Wako Pure Chemical Industries, Ltd.)

C. Lot No. : TLN6888

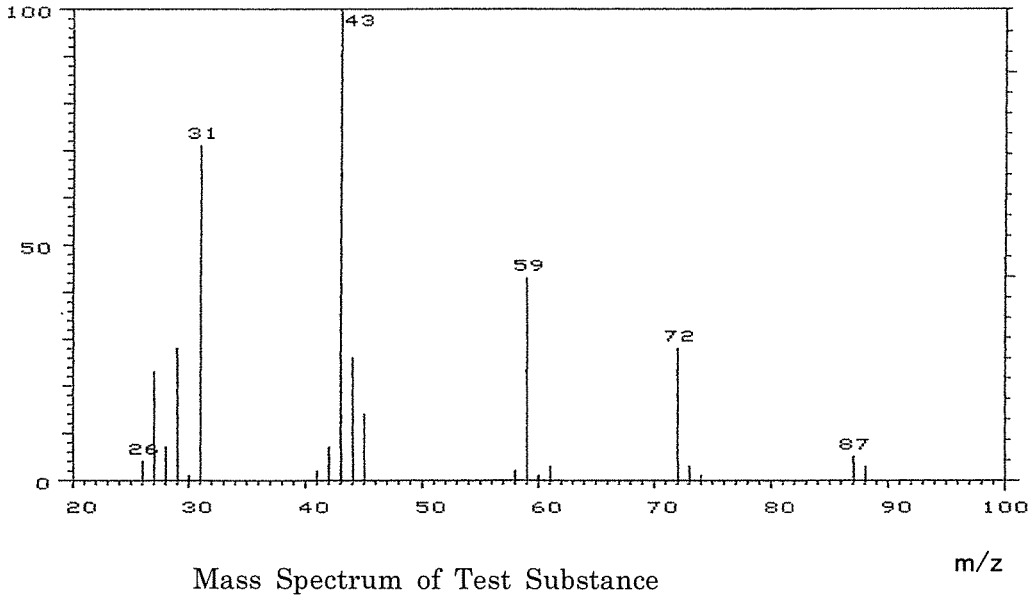
1. Spectral Data

Mass Spectrometry

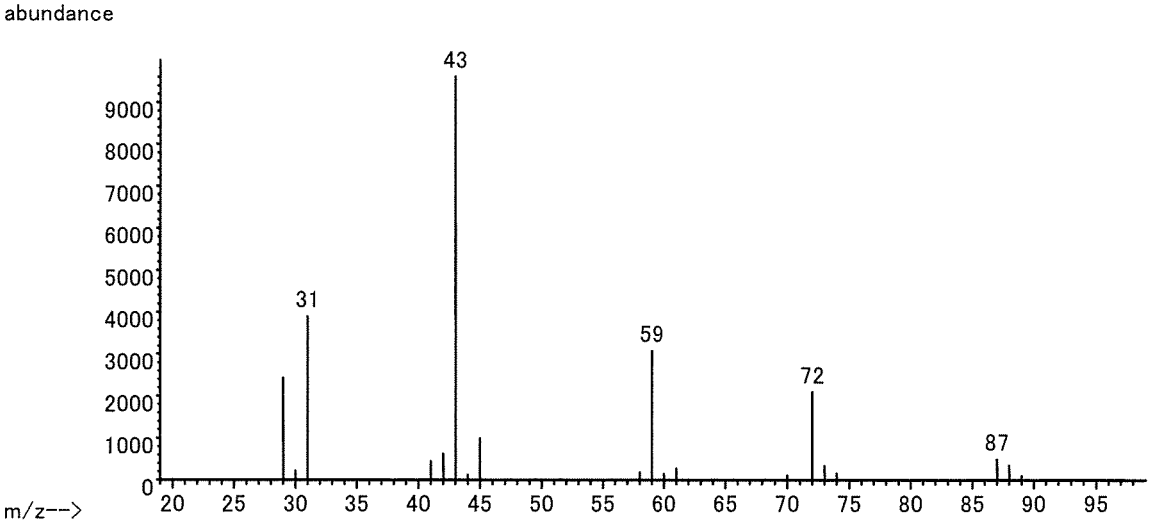
Instrument : Hitachi M-80B Mass Spectrometer

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.

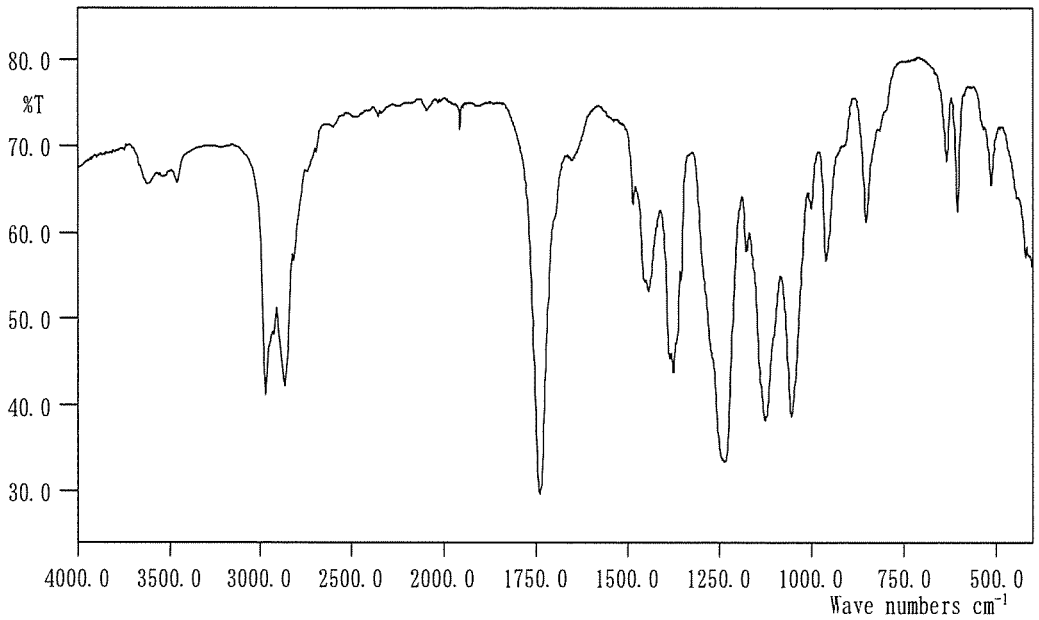
(*McLafferty FW, ed. 1994. Wiley Registry of Mass Spectral Data. 6th ed. New York, NY:John Wiley and Sons.)

Infrared Spectrometry

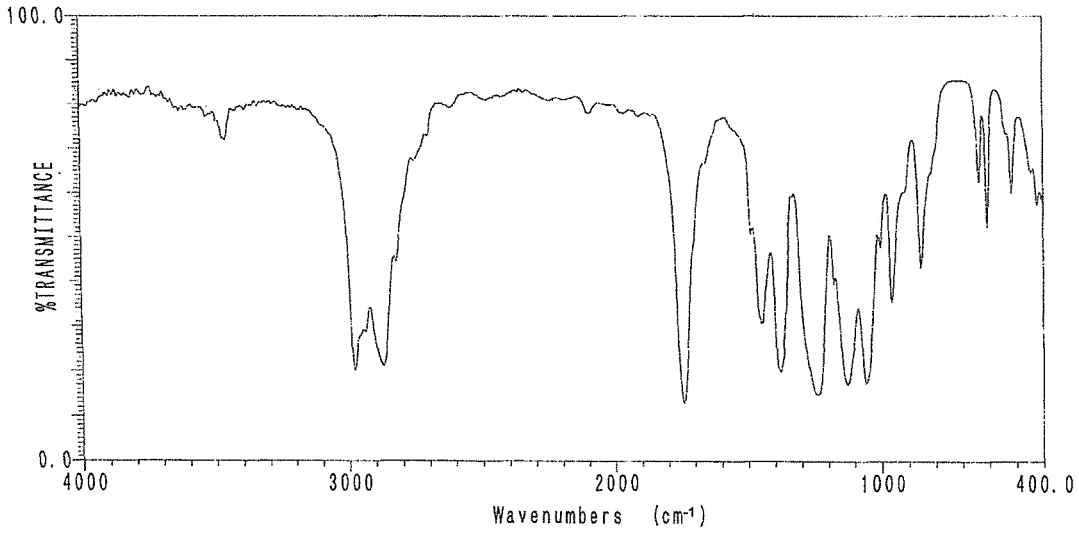
Instrument : Shimadzu FTIR-8200PC Infrared Spectrometer

Cell : KBr Liquid Cell

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 Wako Pure Chemical Industries, Ltd.)

2. Conclusion: The test substance was identified as ethylene glycol monoethyl ether acetate by mass spectrum and infrared spectrum.

APPENDIX 1-2

STABILITY OF

ETHYLENE GLYCOL MONOETHYL ETHER ACETATE

IN THE 2-YEAR INHALATION STUDY

STABILITY OF ETHYLENE GLYCOL MONOETHYL ETHER ACETATE IN THE 2-YEAR INHALATION STUDY

Test Substance : Ethylene glycol monoethyl ether acetate (Wako Pure Chemical Industries, Ltd.)

A. Lot No. : EPF1259

1. Gas Chromatography

Instrument : Agilent Technologies 5890A Gas Chromatograph

Column : INNOWAX (0.53 mmϕ × 60 m)

Column Temperature : 140° C

Flow Rate : 10 mL/min

Detector : FID (Flame Ionization Detector)

Injection Volume : 1 μL

Date (date analyzed)	Peak No.	Retention Time (min)	Area (%)
2011.01.31	1	2.824	100
2011.08.09	1	2.861	100

Result: Gas chromatography indicated one major peak (peak No.1) analyzed on 2011.1.31 and one major peak (peak No.1) analyzed on 2011.8.9. No new trace impurity peak in the test substance analyzed on 2011.8.9 was detected.

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

STABILITY OF ETHYLENE GLYCOL MONOETHYL ETHER ACETATE IN THE 2-YEAR INHALATION STUDY

Test Substance : Ethylene glycol monoethyl ether acetate (Wako Pure Chemical Industries, Ltd.)

B. Lot No. : DCM1007

1. Gas Chromatography

Instrument : Agilent Technologies 5890A Gas Chromatograph

Column : INNOWAX (0.53 mmϕ × 60 m)

Column Temperature : 140° C

Flow Rate : 10 mL/min

Detector : FID (Flame Ionization Detector)

Injection Volume : 1 μL

Date (date analyzed)	Peak No.	Retention Time (min)	Area (%)
2011.08.05	1	2.865	100
2012.05.23	1	2.839	100

Result: Gas chromatography indicated one major peak (peak No.1) analyzed on 2011.8.5 and one major peak (peak No.1) analyzed on 2012.5.23.
No new trace impurity peak in the test substance analyzed on 2012.5.23 was detected.

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

STABILITY OF ETHYLENE GLYCOL MONOETHYL ETHER ACETATE IN THE 2-YEAR INHALATION STUDY

Test Substance : Ethylene glycol monoethyl ether acetate (Wako Pure Chemical Industries, Ltd.)

C. Lot No. : TLN6888

1. Gas Chromatography

Instrument : Agilent Technologies 5890A Gas Chromatograph

Column : INNOWAX (0.53 mmϕ × 60 m)

Column Temperature : 140° C

Flow Rate : 10 mL/min

Detector : FID (Flame Ionization Detector)

Injection Volume : 1 μL

Date (date analyzed)	Peak No.	Retention Time (min)	Area (%)
2012.05.08	1	2.834	100
2013.02.25	1	2.891	100

Result: Gas chromatography indicated one major peak (peak No.1) analyzed on 2012.5.8 and one major peak (peak No.1) analyzed on 2013.2.25. No new trace impurity peak in the test substance analyzed on 2013.2.25 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 2-YEAR INHALATION STUDY OF ETHYLENE GLYCOL MONOETHYL ETHER ACETATE

ENVIRONMENTAL CONDITIONS OF INHALATION CHAMBER IN THE 2-YEAR INHALATION STUDY OF ETHYLENE GLYCOL MONOETHYL ETHER ACETATE

Group Name	Temperature (℃) Mean ± S.D.	Humidity (%) Mean ± S.D.	Ventilation Rate (L/min) Mean ± S.D.	Air Change (time/h) Mean
Control	23.1 ± 0.1	55.1 ± 1.6	1700.7 ± 3.6	12.0
12 ppm	23.1 ± 0.1	55.3 ± 1.8	1699.1 ± 3.3	12.0
50 ppm	23.1 ± 0.1	54.1 ± 2.5	1702.0 ± 4.8	12.0
200 ppm	23.0 ± 0.1	55.1 ± 1.7	1701.6 ± 4.1	12.0

APPENDIX 3

METHODS, UNITS AND DECIMAL PLACE FOR HEMATOLOGY AND BIOCHEMISTRY IN THE 2-YEAR INHALATION STUDY OF ETHYLENE GLYCOL MONOETHYL ETHER ACETATE

METHODS, UNITS AND DECIMAL PLACE FOR HEMATOLOGY AND BIOCHEMISTRY IN THE 2-YEAR INHALATION STUDY OF ETHYLENE GLYCOL MONOETHYL ETHER ACETATE

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	Light scattering method [1]	%	0
Biochemistry			
Total protein(TP)	Biuret method [2]	g/dL	1
Albumin (Alb)	BCG method [2]	g/dL	1
A/G ratio	Calculated as Alb/(TP−Alb) [2]	−	1
T-bilirubin	BOD method [2]	mg/dL	2
Glucose	GlcK·G-6-PDH method [2]	mg/dL	0
T-cholesterol	CE·COD·POD method [2]	mg/dL	0
Triglyceride	MGLP·GK·GPO·POD method [2]	mg/dL	0
Phospholipid	PLD·ChOD·POD method [2]	mg/dL	0
Aspartate aminotransferase (AST)	JSCC method [2]	U/L	0
Alanine aminotransferase (ALT)	JSCC method [2]	U/L	0
Lactate dehydrogenase (LDH)	JSCC method [2]	U/L	0
Alkaline phosphatase (ALP)	JSCC method [2]	U/L	0
γ-Glutamyl transpeptidase (γ-GTP)	JSCC method [2]	U/L	1
Creatine kinase (CK)	JSCC method [2]	U/L	0
Urea nitrogen	Urease·GLDH method [2]	mg/dL	1
Creatinine	Creatinase·SOD·POD method [2]	mg/dL	2
Sodium	Ion selective electrode method [2]	mEq/L	0
Potassium	Ion selective electrode method [2]	mEq/L	1
Chloride	Ion selective electrode method [2]	mEq/L	0
Calcium	OCPC method [2]	mg/dL	1
Inorganic phosphorus	PNP·XOD·POD method [2]	mg/dL	1

1) Automatic blood cell analyzer (ADVIA120 : Siemens Healthcare Diagnostics Inc.)
2) Automatic analyzer (Hitachi 7080 : Hitachi,Ltd.)