

酢酸イソプロピルのラットを用いた  
吸入によるがん原性試験報告書

試験番号：0610

# APPENDICES

## APPENDICES

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APPENDIX 1-1

IDENTITY OF ISOPROPYL ACETATE  
IN THE 2-YEAR INHALATION STUDY

## IDENTITY AND IMPURITY OF ISOPROPYL ACETATE IN THE 2-YEAR INHALATION STUDY

Test Substance : Isopropyl acetate (Wako Pure Chemical Industries, Ltd.)

A. Lot No. : KLE3931

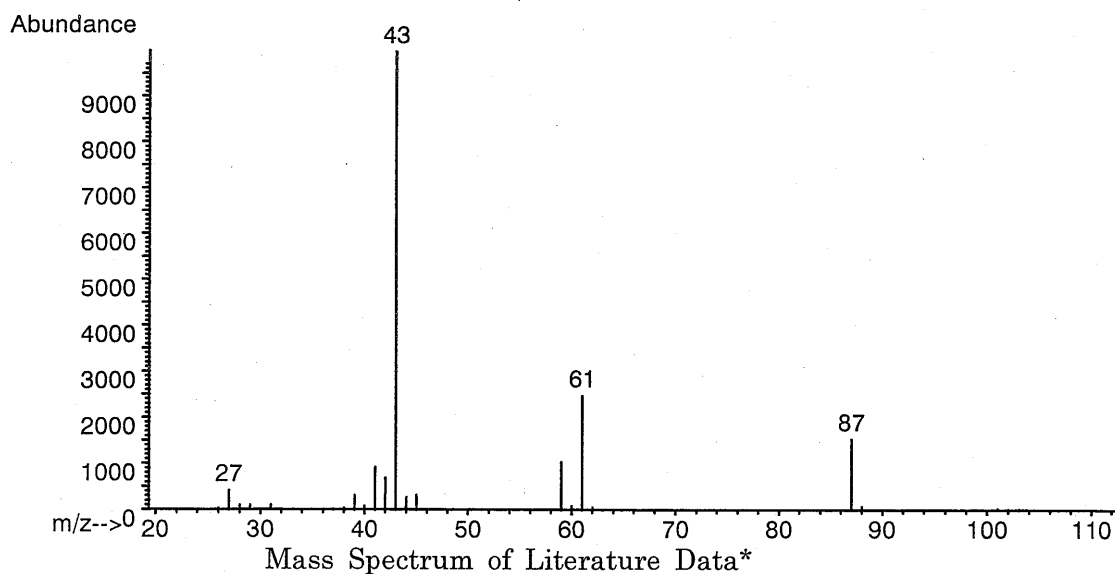
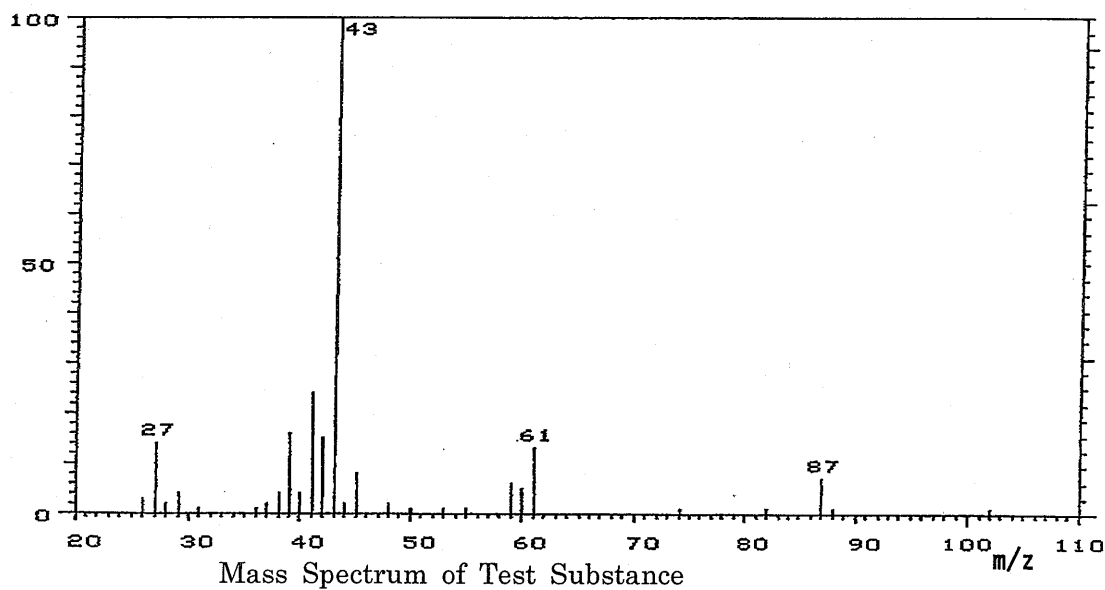
## 1. Spectral Data

Mass Spectrometry

Instrument : Hitachi M-80B Mass Spectrometer

Ionization : EI (Electron Ionization)

Ionization Voltage : 70eV



Result: The mass spectrum was consistent with literature spectrum.

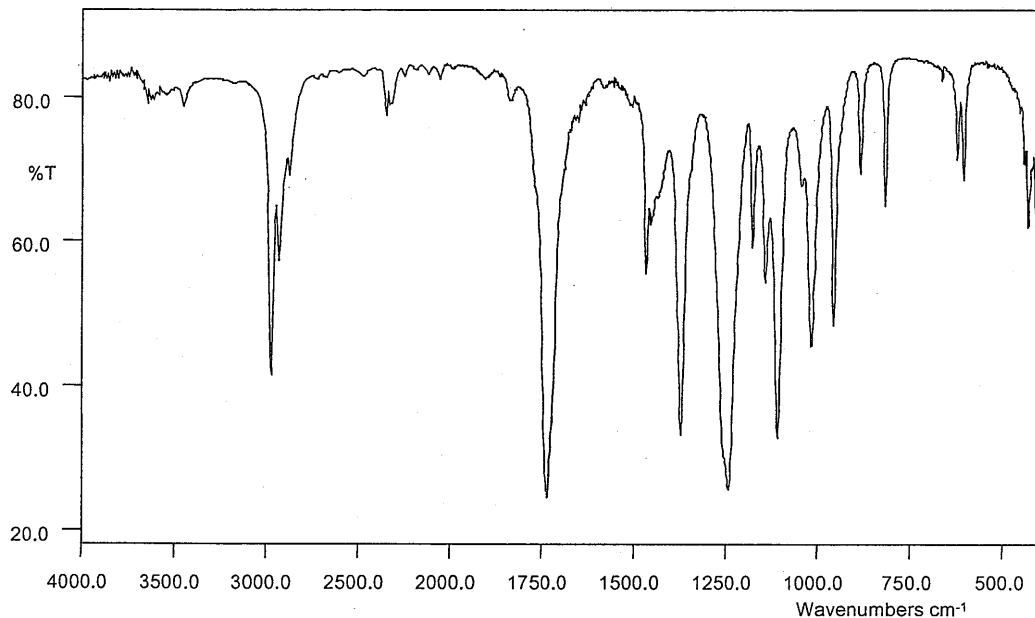
(\*McLafferty FW, ed. 1994. Wiley Registry of Mass Spectral Data. 6th ed. New York, NY:John Wiley and Sons.)

Infrared Spectrometry

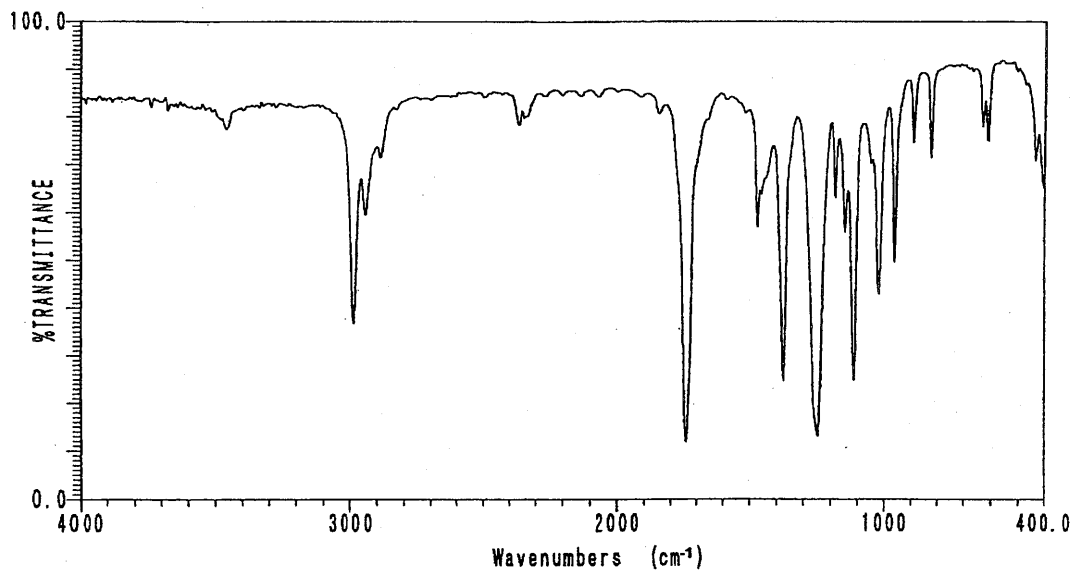
Instrument : Shimadzu FTIR-8200PC Infrared Spectrometer

Cell : KBr Liquid Cell

Resolution : 4  $\text{cm}^{-1}$



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. Impurity

Instrument : Agilent Technologies 5890A Gas Chromatograph  
Column : Methyl Silicone ( 0.53 mm  $\phi$   $\times$  60 m)  
Column Temperature: 80° C  
Flow Rate : 15 mL/min  
Detector : FID (Flame Ionization Detector)  
Injection Volume : 1  $\mu$ L

Sample Name	Peak No.	Area (%)	Peak Name
	1	0.031	2-Propanol
Test Substance	2	99.969	Isopropyl acetate

Result: Gas chromatography indicated one major peak (peak No. 2) and one impurity. The impurity (peak No. 1) was identified as 2-propanol by comparing GC-MS with that of standard sample. The amount of 2-propanol in the test substance was 0.031% (The quantity value by the standard sample was 0.031%.) with a gas chromatograph.

3. Conclusion: The test substance was identified as isopropyl acetate by mass spectrum and infrared spectrum. Gas chromatography indicated one major peak (isopropyl acetate) and one impurity. The impurity was 2-propanol in the test substance.

## IDENTITY AND IMPURITY OF ISOPROPYL ACETATE IN THE 2-YEAR INHALATION STUDY

Test Substance : Isopropyl acetate (Wako Pure Chemical Industries, Ltd.)

B. Lot No. : EWH6219

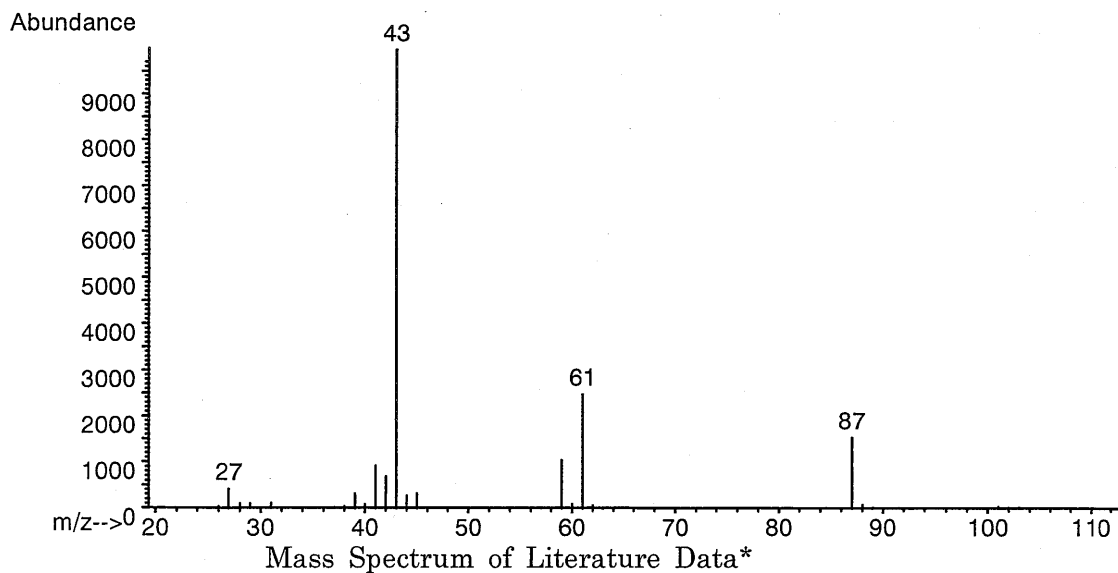
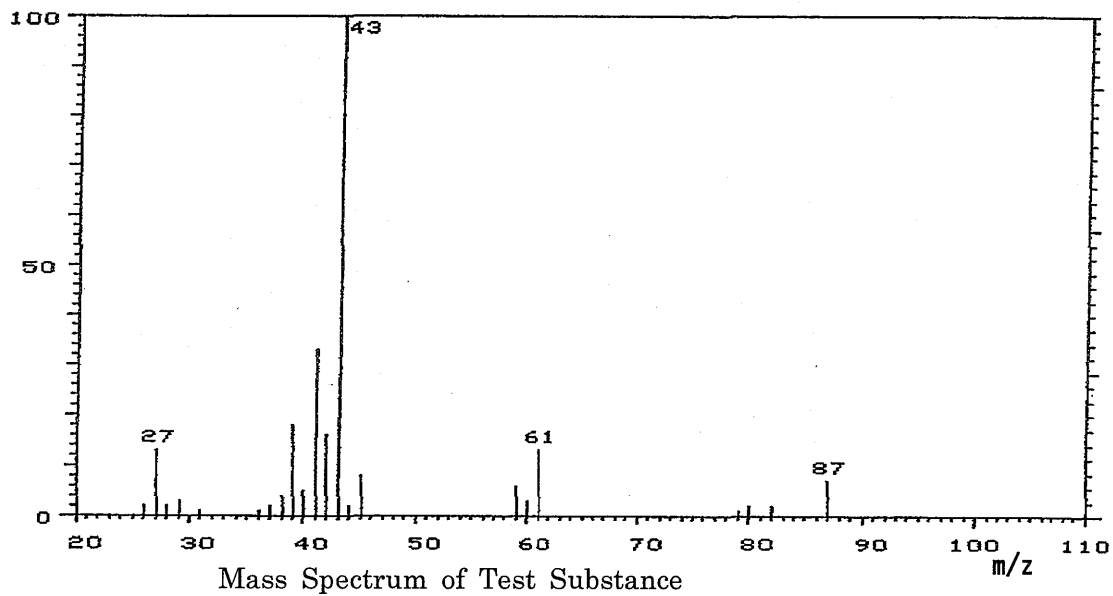
## 1. Spectral Data

Mass Spectrometry

Instrument : Hitachi M-80B Mass Spectrometer

Ionization : EI (Electron Ionization)

Ionization Voltage : 70eV



Result: The mass spectrum was consistent with literature spectrum.

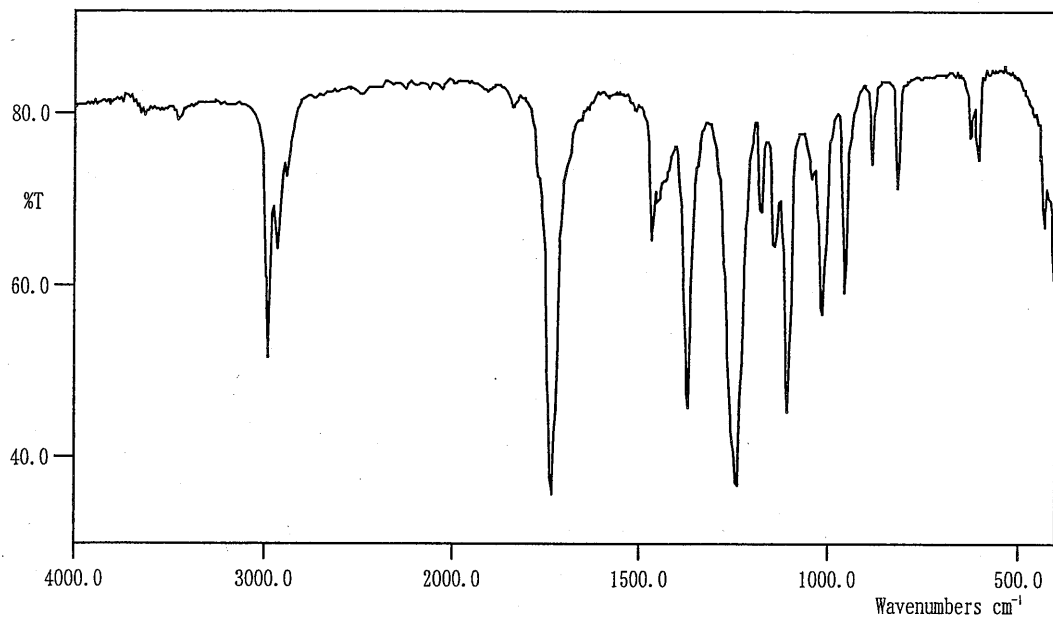
(\*McLafferty FW, ed. 1994. Wiley Registry of Mass Spectral Data. 6th ed. New York, NY:John Wiley and Sons.)

Infrared Spectrometry

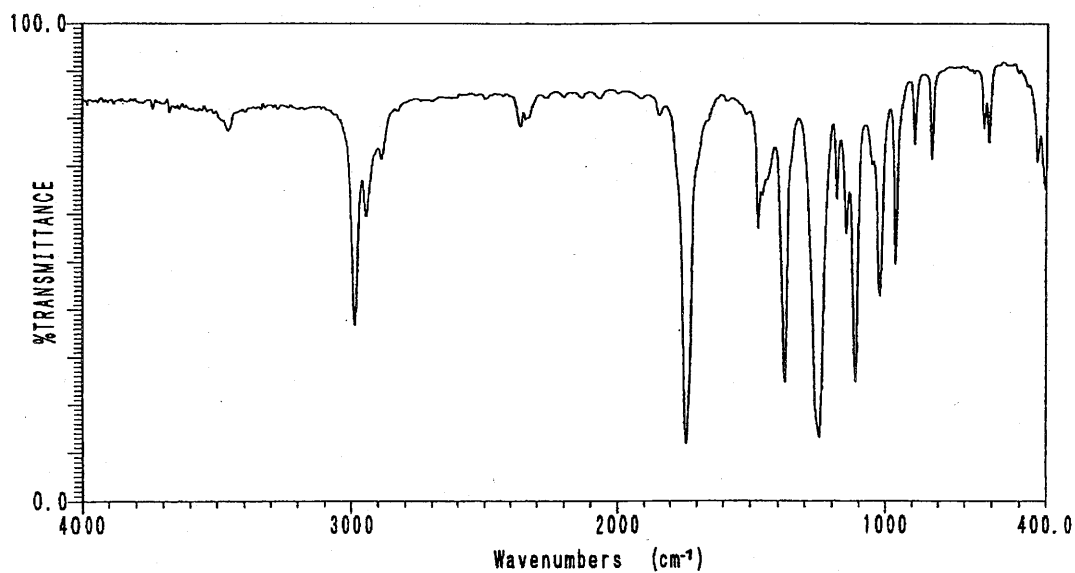
Instrument : Shimadzu FTIR-8200PC Infrared Spectrometer

Cell : KBr Liquid Cell

Resolution : 4  $\text{cm}^{-1}$



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. Impurity

Instrument : Agilent Technologies 5890A Gas Chromatograph  
Column : Methyl Silicone ( 0.53 mm  $\phi$   $\times$  60 m)  
Column Temperature: 80° C  
Flow Rate : 15 mL/min  
Detector : FID (Flame Ionization Detector)  
Injection Volume : 1  $\mu$ L

Sample Name	Peak No.	Area (%)	Peak Name
	1	0.039	2-Propanol
Test Substance	2	99.961	Isopropyl acetate

Result: Gas chromatography indicated one major peak (peak No. 2) and one impurity. The impurity (peak No. 1) was identified as 2-propanol by comparing GC-MS with that of standard sample. The amount of 2-propanol in the test substance was 0.039% (The quantity value by the standard sample was 0.032%.) with a gas chromatograph.

3. Conclusion: The test substance was identified as isopropyl acetate by mass spectrum and infrared spectrum. Gas chromatography indicated one major peak (isopropyl acetate) and one impurity. The impurity was 2-propanol in the test substance.

IDENTITY AND IMPURITY OF ISOPROPYL ACETATE IN THE 2-YEAR INHALATION STUDY

Test Substance : Isopropyl acetate (Wako Pure Chemical Industries, Ltd.)

C. Lot No. : DPP3664

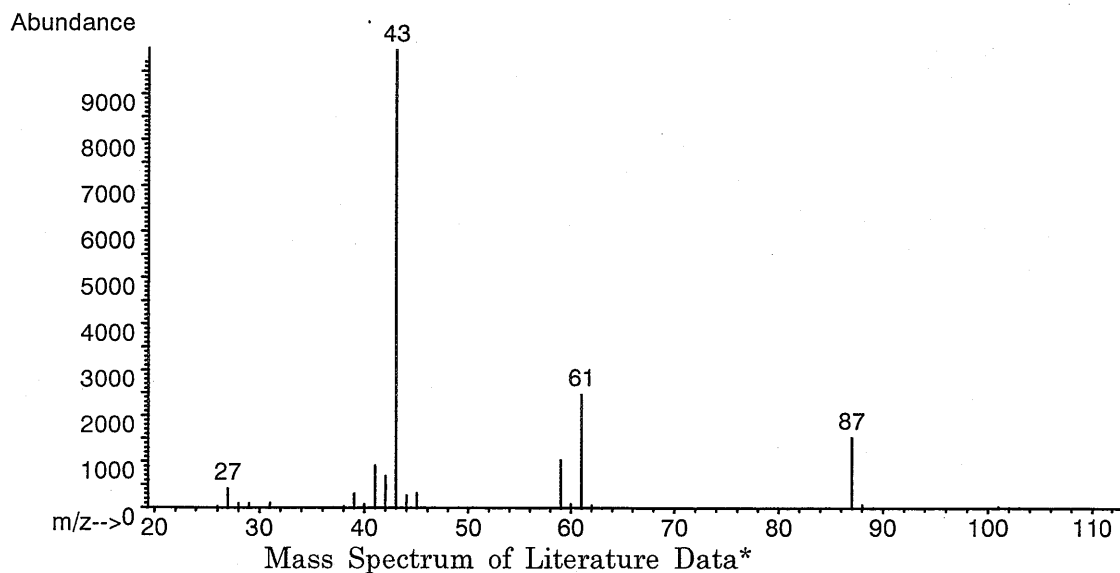
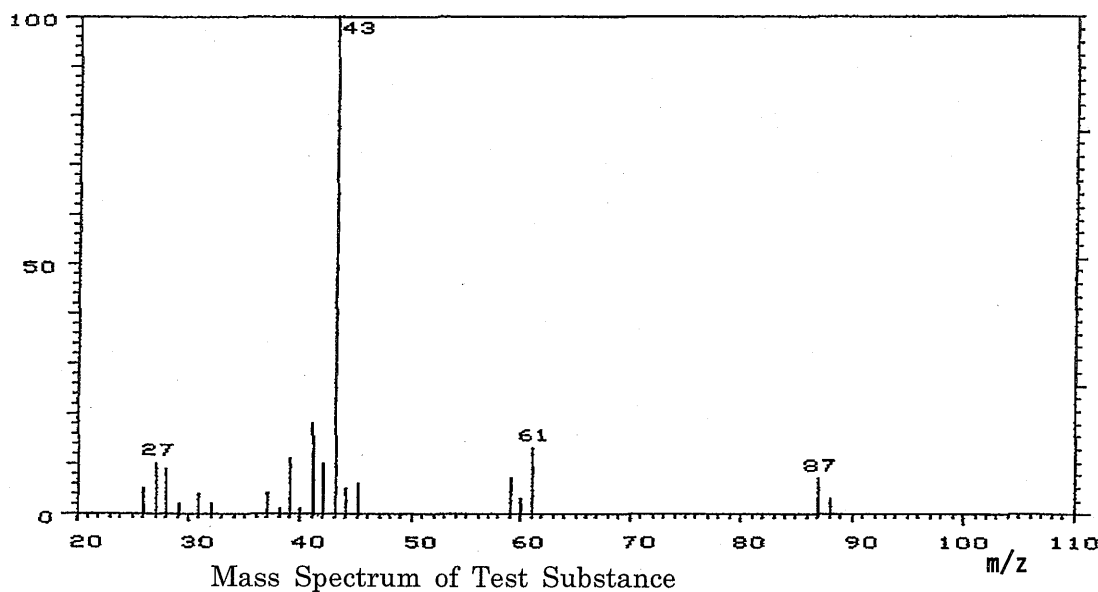
1. Spectral Data

Mass Spectrometry

Instrument : Hitachi M-80B Mass Spectrometer

Ionization : EI (Electron Ionization)

Ionization Voltage : 70eV



Result: The mass spectrum was consistent with literature spectrum.

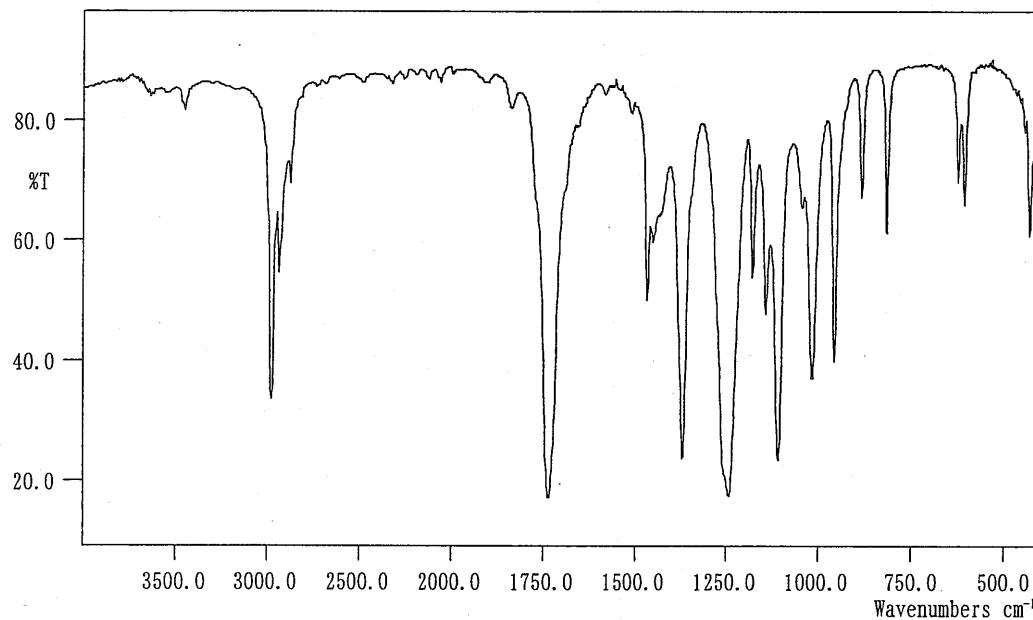
(\*McLafferty FW, ed. 1994. Wiley Registry of Mass Spectral Data. 6th ed. New York, NY:John Wiley and Sons.)

Infrared Spectrometry

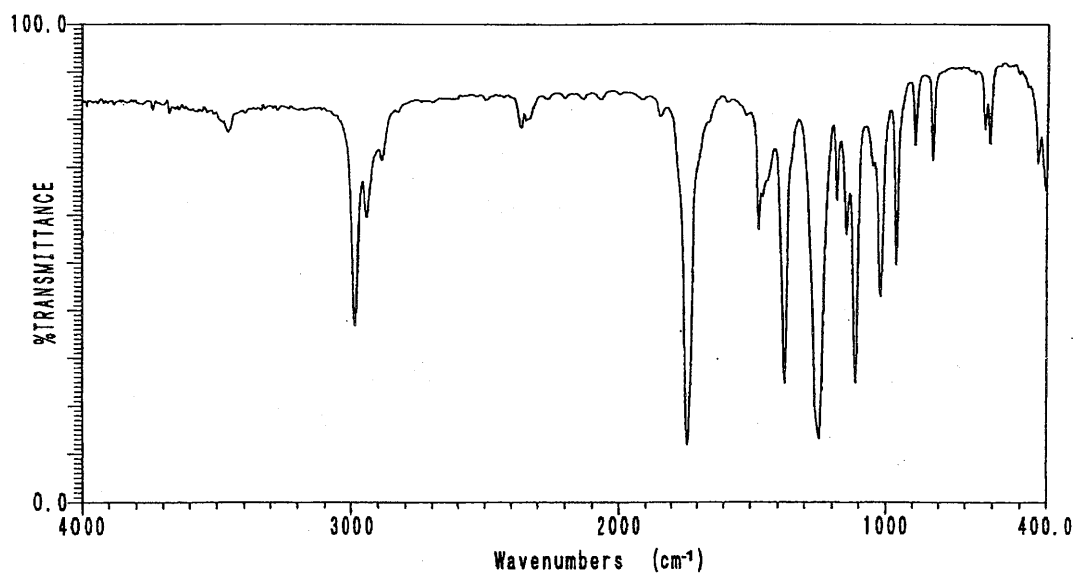
Instrument : Shimadzu FTIR-8200PC Infrared Spectrometer

Cell : KBr Liquid Cell

Resolution : 4  $\text{cm}^{-1}$



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. Impurity

Instrument : Agilent Technologies 5890A Gas Chromatograph  
Column : Methyl Silicone ( 0.53 mm  $\phi$   $\times$  60 m)  
Column Temperature: 80° C  
Flow Rate : 15 mL/min  
Detector : FID (Flame Ionization Detector)  
Injection Volume : 1  $\mu$ L

Sample Name	Peak No.	Area (%)	Peak Name
	1	0.038	2-Propanol
Test Substance	2	99.962	Isopropyl acetate

Result: Gas chromatography indicated one major peak (peak No. 2) and one impurity. The impurity (peak No. 1) was identified as 2-propanol by comparing GC-MS with that of standard sample. The amount of 2-propanol in the test substance was 0.038% (The quantity value by the standard sample was 0.038%) with a gas chromatograph.

3. Conclusion: The test substance was identified as isopropyl acetate by mass spectrum and infrared spectrum. Gas chromatography indicated one major peak (isopropyl acetate) and one impurity. The impurity was 2-propanol in the test substance.

IDENTITY AND IMPURITY OF ISOPROPYL ACETATE IN THE 2-YEAR INHALATION STUDY

Test Substance : Isopropyl acetate (Wako Pure Chemical Industries, Ltd.)

D. Lot No. : DPF2284

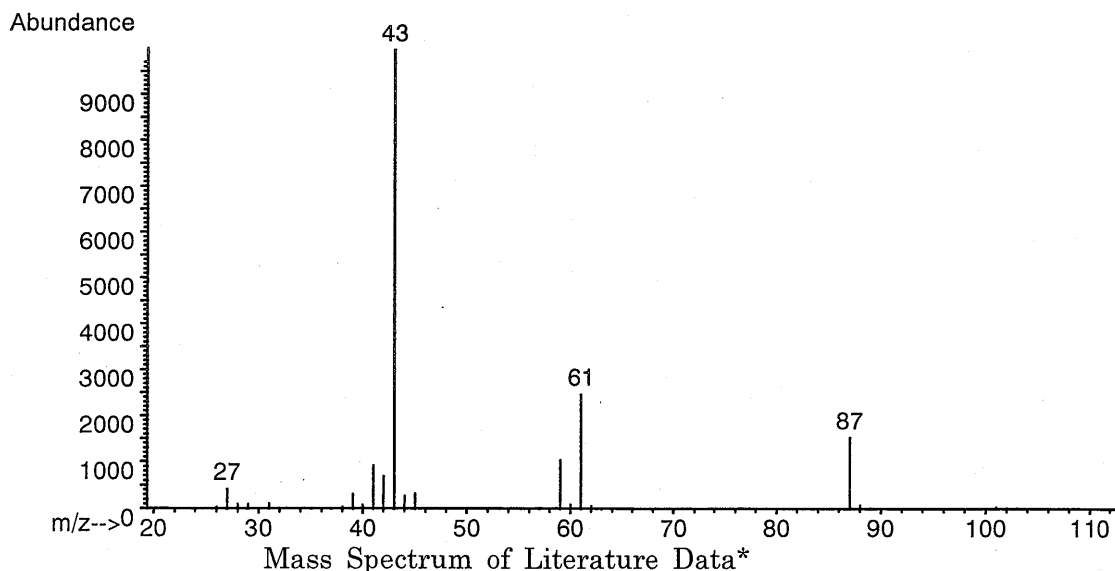
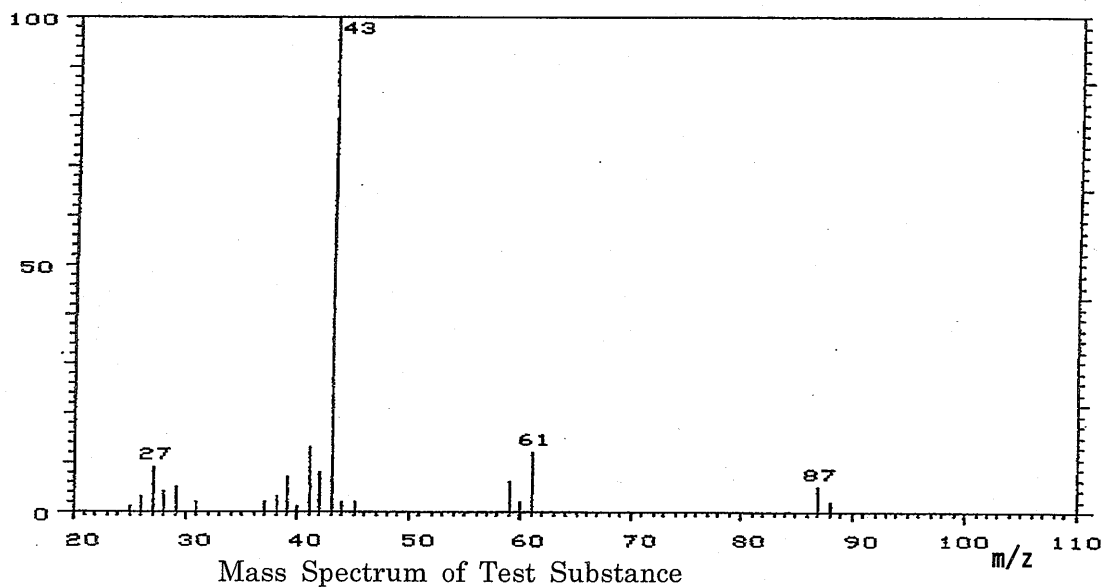
1. Spectral Data

Mass Spectrometry

Instrument : Hitachi M-80B Mass Spectrometer

Ionization : EI (Electron Ionization)

Ionization Voltage : 70eV



Result: The mass spectrum was consistent with literature spectrum.

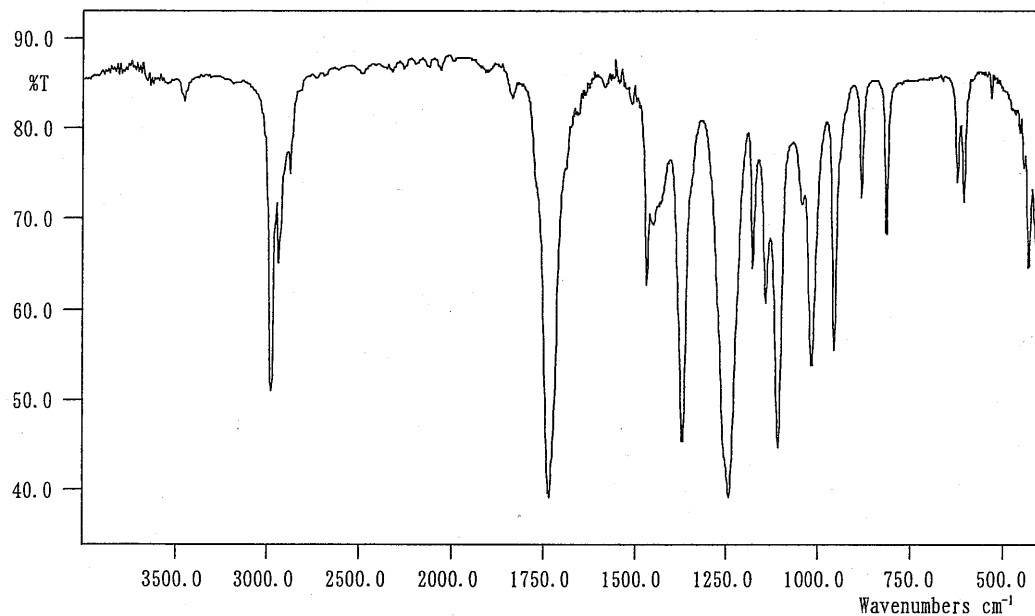
(\*McLafferty FW, ed. 1994. Wiley Registry of Mass Spectral Data. 6th ed. New York, NY:John Wiley and Sons.)

Infrared Spectrometry

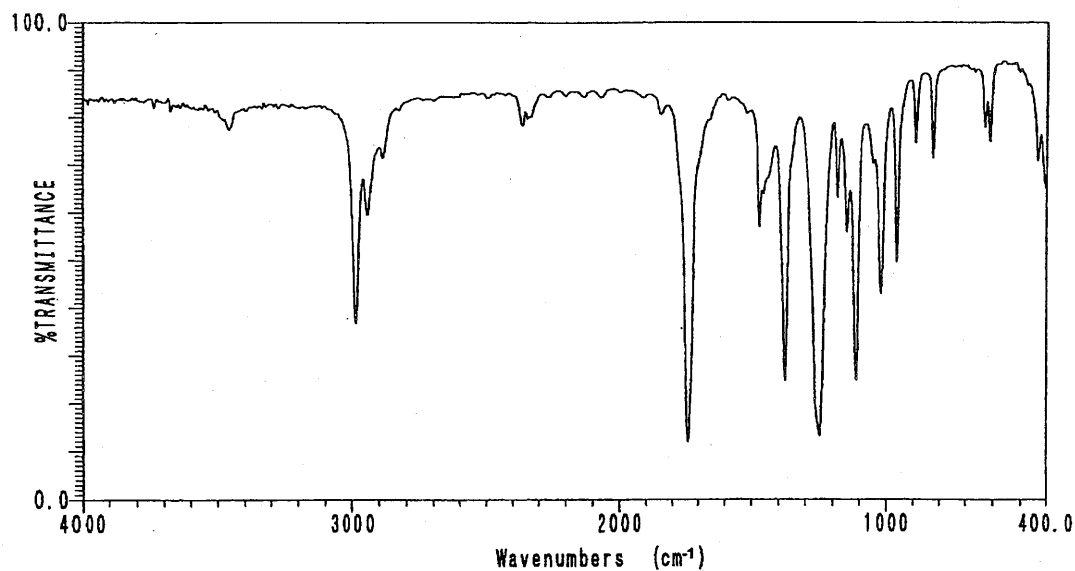
Instrument : Shimadzu FTIR-8200PC Infrared Spectrometer

Cell : KBr Liquid Cell

Resolution : 4  $\text{cm}^{-1}$



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. Impurity

Instrument : Agilent Technologies 5890A Gas Chromatograph  
Column : Methyl Silicone ( 0.53 mm  $\phi$   $\times$  60 m)  
Column Temperature: 80° C  
Flow Rate : 15 mL/min  
Detector : FID (Flame Ionization Detector)  
Injection Volume : 1  $\mu$ L

Sample Name	Peak No.	Area (%)	Peak Name
	1	0.044	2-Propanol
Test Substance	2	99.956	Isopropyl acetate

Result: Gas chromatography indicated one major peak (peak No. 2) and one impurity. The impurity (peak No. 1) was identified as 2-propanol by comparing GC-MS with that of standard sample. The amount of 2-propanol in the test substance was 0.044% (The quantity value by the standard sample was 0.044%.) with a gas chromatograph.

3. Conclusion: The test substance was identified as isopropyl acetate by mass spectrum and infrared spectrum. Gas chromatography indicated one major peak (isopropyl acetate) and one impurity. The impurity was 2-propanol in the test substance.

## IDENTITY AND IMPURITY OF ISOPROPYL ACETATE IN THE 2-YEAR INHALATION STUDY

Test Substance : Isopropyl acetate (Wako Pure Chemical Industries, Ltd.)

E. Lot No. : TSK3141

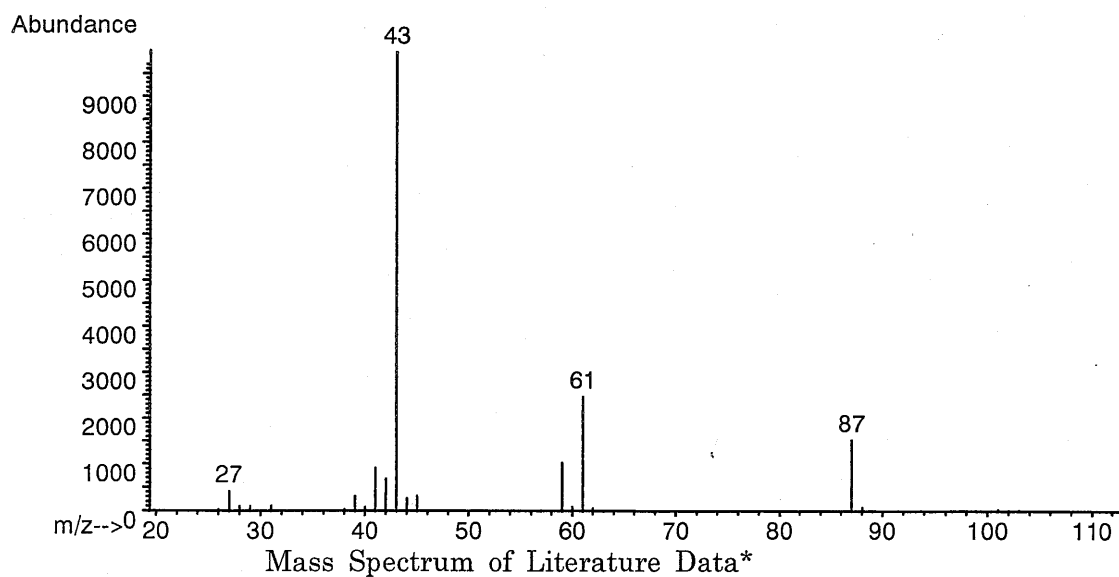
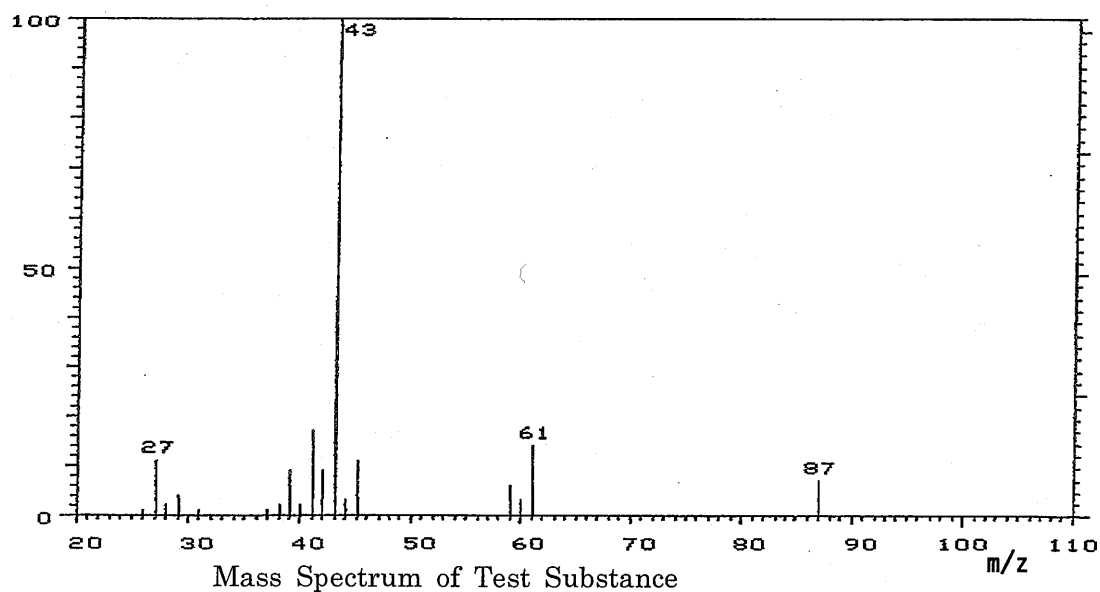
## 1. Spectral Data

Mass Spectrometry

Instrument : Hitachi M-80B Mass Spectrometer

Ionization : EI (Electron Ionization)

Ionization Voltage : 70eV



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.)

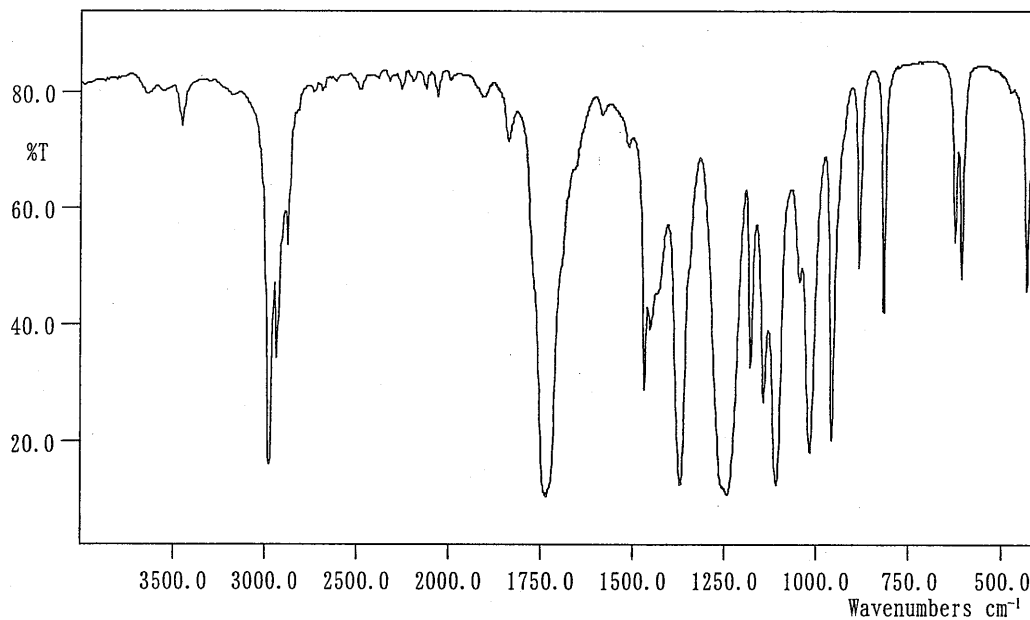


Infrared Spectrometry

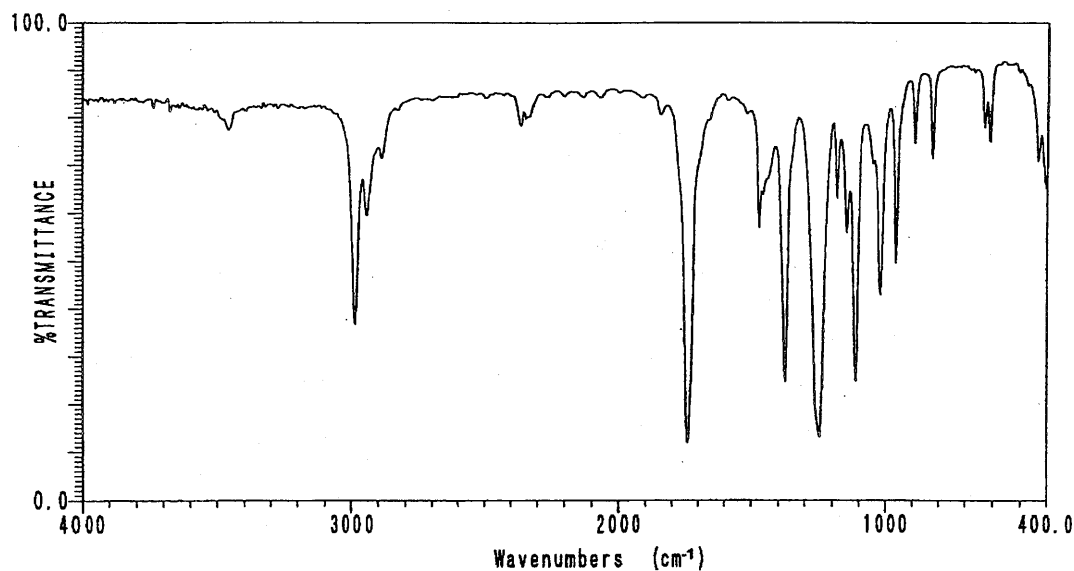
Instrument : Shimadzu FTIR-8200PC Infrared Spectrometer

Cell : KBr Liquid Cell

Resolution : 4  $\text{cm}^{-1}$



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. Impurity

Instrument : Agilent Technologies 5890A Gas Chromatograph  
Column : Methyl Silicone ( 0.53 mm  $\phi$   $\times$  60 m)  
Column Temperature: 80° C  
Flow Rate : 15 mL/min  
Detector : FID (Flame Ionization Detector)  
Injection Volume : 1  $\mu$ L

Sample Name	Peak No.	Area (%)	Peak Name
	1	0.044	2-Propanol
Test Substance	2	99.956	Isopropyl acetate

Result: Gas chromatography indicated one major peak (peak No. 2) and one impurity. The impurity (peak No. 1) was identified as 2-propanol by comparing GC-MS with that of standard sample. The amount of 2-propanol in the test substance was 0.044% (The quantity value by the standard sample was 0.029%.) with a gas chromatograph.

3. Conclusion: The test substance was identified as isopropyl acetate by mass spectrum and infrared spectrum. Gas chromatography indicated one major peak (isopropyl acetate) and one impurity. The impurity was 2-propanol in the test substance.

APPENDIX 1-2

STABILITY OF ISOPROPYL ACETATE  
IN THE 2-YEAR INHALATION STUDY

## STABILITY OF ISOPROPYL ACETATE IN THE 2-YEAR INHALATION STUDY

Test Substance : Isopropyl acetate (Wako Pure Chemical Industries, Ltd.)

A. Lot No. : KLE3931

## 1. Gas Chromatography

Instrument : Agilent Technologies 5890A Gas Chromatograph

Column : Methyl Silicone ( 0.53 mm  $\phi$   $\times$  60 m)

Column Temperature: 80° C

Flow Rate : 15 mL/min

Detector : FID (Flame Ionization Detector)

Injection Volume : 1  $\mu$ L

Date (date analyzed)	Peak No.	Retention Time (min)	Area (%)
2005.12.13	1	1.872	0.031
	2	3.817	99.969
2006.01.18	1	1.868	0.030
	2	3.818	99.970

Result: Gas chromatography indicated one major peak (peak No.2) and one impurity (peak No. 1 < 0.1% of total area) analyzed on 2005.12.13 and one major peak (peak No.2) and one impurity (peak No. 1 < 0.1% of total area) analyzed on 2006.1.18. No new trace impurity peak in the test substance analyzed on 2006.1.18 was detected.

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

## STABILITY OF ISOPROPYL ACETATE IN THE 2-YEAR INHALATION STUDY

Test Substance : Isopropyl acetate (Wako Pure Chemical Industries, Ltd.)

B. Lot No. : EWH6219

## 1. Gas Chromatography

Instrument : Agilent Technologies 5890A Gas Chromatograph

Column : Methyl Silicone ( 0.53 mm  $\phi$   $\times$  60 m)

Column Temperature: 80° C

Flow Rate : 15 mL/min

Detector : FID (Flame Ionization Detector)

Injection Volume : 1  $\mu$ L

Date (date analyzed)	Peak No.	Retention Time (min)	Area (%)
2006.01.10	1	1.866	0.039
	2	3.820	99.961
2006.07.21	1	1.863	0.039
	2	3.794	99.961

Result: Gas chromatography indicated one major peak (peak No.2) and one impurity (peak No. 1 < 0.1% of total area) analyzed on 2006.1.10 and one major peak (peak No.2) and one impurity (peak No. 1 < 0.1% of total area) analyzed on 2006.7.21. No new trace impurity peak in the test substance analyzed on 2006.7.21 was detected.

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

## STABILITY OF ISOPROPYL ACETATE IN THE 2-YEAR INHALATION STUDY

Test Substance : Isopropyl acetate (Wako Pure Chemical Industries, Ltd.)

C. Lot No. : DPP3664

## 1. Gas Chromatography

Instrument : Agilent Technologies 5890A Gas Chromatograph

Column : Methyl Silicone ( 0.53 mm  $\phi$   $\times$  60 m)

Column Temperature: 80° C

Flow Rate : 15 mL/min

Detector : FID (Flame Ionization Detector)

Injection Volume : 1  $\mu$ L

Date (date analyzed)	Peak No.	Retention Time (min)	Area (%)
2006.07.14	1	1.859	0.038
	2	3.789	99.962
2007.03.05	1	1.858	0.038
	2	3.767	99.962

Result: Gas chromatography indicated one major peak (peak No.2) and one impurity (peak No. 1 < 0.1% of total area) analyzed on 2006.7.14 and one major peak (peak No.2) and one impurity (peak No. 1 < 0.1% of total area) analyzed on 2007.3.5. No new trace impurity peak in the test substance analyzed on 2007.3.5 was detected.

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

## STABILITY OF ISOPROPYL ACETATE IN THE 2-YEAR INHALATION STUDY

Test Substance : Isopropyl acetate (Wako Pure Chemical Industries, Ltd.)

D. Lot No. : DPF2284

## 1. Gas Chromatography

Instrument : Agilent Technologies 5890A Gas Chromatograph

Column : Methyl Silicone ( 0.53 mm  $\phi$   $\times$  60 m)

Column Temperature: 80° C

Flow Rate : 15 mL/min

Detector : FID (Flame Ionization Detector)

Injection Volume : 1  $\mu$ L

Date (date analyzed)	Peak No.	Retention Time (min)	Area (%)
2007.02.27	1	1.858	0.044
	2	3.789	99.956
2007.10.11	1	1.881	0.055
	2	3.983	99.945

Result: Gas chromatography indicated one major peak (peak No.2) and one impurity (peak No. 1 < 0.1% of total area) analyzed on 2007.2.27 and one major peak (peak No.2) and one impurity (peak No. 1 < 0.1% of total area) analyzed on 2007.10.11. No new trace impurity peak in the test substance analyzed on 2007.10.11 was detected.

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

## STABILITY OF ISOPROPYL ACETATE IN THE 2-YEAR INHALATION STUDY

Test Substance : Isopropyl acetate (Wako Pure Chemical Industries, Ltd.)

E. Lot No. : TSK3141

## 1. Gas Chromatography

Instrument : Agilent Technologies 5890A Gas Chromatograph

Column : Methyl Silicone ( 0.53 mm  $\phi$   $\times$  60 m)

Column Temperature: 80° C

Flow Rate : 15 mL/min

Detector : FID (Flame Ionization Detector)

Injection Volume : 1  $\mu$ L

Date (date analyzed)	Peak No.	Retention Time (min)	Area (%)
2007.10.01	1	1.881	0.044
	2	3.980	99.956
2008.01.07	1	1.881	0.043
	2	3.984	99.957

Result: Gas chromatography indicated one major peak (peak No.2) and one impurity (peak No. 1 < 0.1% of total area) analyzed on 2007.10.1 and one major peak (peak No.2) and one impurity (peak No. 1 < 0.1% of total area) analyzed on 2008.1.7. No new trace impurity peak in the test substance analyzed on 2008.1.7 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 ISOPROPYL ACETATE

ENVIRONMENTAL CONDITIONS OF INHALATION CHAMBER IN THE 2-YEAR  
INHALATION STUDY OF ISOPROPYL ACETATE

Group Name	Temperature (°C)	Humidity (%)	Ventilation Rate (L/min)		Air Change (time/h)	
	Mean ± S.D.	Mean ± S.D.	Mean ± S.D.* <sup>1</sup>	Mean ± S.D.* <sup>2</sup>	Mean* <sup>1</sup>	Mean* <sup>2</sup>
Control	23.0 ± 0.2	54.8 ± 2.5	763.3 ± 5.2	1514.3 ± 18.9	6.0	12.0
1000 ppm	23.0 ± 0.2	54.6 ± 1.8	764.5 ± 3.9	1520.7 ± 16.6	6.0	12.0
2000 ppm	23.0 ± 0.2	53.3 ± 2.1	764.0 ± 4.4	1521.5 ± 17.8	6.0	12.0
4000 ppm	23.0 ± 0.2	53.1 ± 2.5	765.1 ± 3.6	1517.0 ± 16.4	6.0	12.0

\* 1: Exposure period      \* 2: After exposure period

## APPENDIX 3

METHODS, UNITS AND DECIMAL PLACE FOR  
HEMATOLOGY AND BIOCHEMISTRY IN THE 2-YEAR  
INHALATION STUDY OF ISOPROPYL ACETATE

METHODS, UNITS AND DECIMAL PLACE FOR HEMATOLOGY AND BIOCHEMISTRY  
IN THE 2-YEAR INHALATION STUDY OF ISOPROPYL ACETATE

Item	Method	Unit	Decimal place
<b>Hematology</b>			
Red blood cell (RBC)	Light scattering method <sup>1)</sup>	$\times 10^6/\mu\text{L}$	2
Hemoglobin(Hgb)	Cyanmethemoglobin method <sup>1)</sup>	g/dL	1
Hematocrit(Hct)	Calculated as $\text{RBC} \times \text{MCV}/10$ <sup>1)</sup>	%	1
Mean corpuscular volume(MCV)	Light scattering method <sup>1)</sup>	fL	1
Mean corpuscular hemoglobin(MCH)	Calculated as $\text{Hgb}/\text{RBC} \times 10$ <sup>1)</sup>	pg	1
Mean corpuscular hemoglobin concentration (MCHC)	Calculated as $\text{Hgb}/\text{Hct} \times 100$ <sup>1)</sup>	g/dL	1
Platelet	Light scattering method <sup>1)</sup>	$\times 10^3/\mu\text{L}$	0
Reticulocyte	Light scattering method <sup>1)</sup>	%	1
White blood cell(WBC)	Light scattering method <sup>1)</sup>	$\times 10^3/\mu\text{L}$	2
Differential WBC	Pattern recognition method <sup>2)</sup> (Wright staining)	%	0
<b>Biochemistry</b>			
Total protein(TP)	Biuret method <sup>3)</sup>	g/dL	1
Albumin (Alb)	BCG method <sup>3)</sup>	g/dL	1
A/G ratio	Calculated as $\text{Alb}/(\text{TP} - \text{Alb})$ <sup>3)</sup>	-	1
T-bilirubin	Azobilirubin method <sup>3)</sup>	mg/dL	2
Glucose	GlcK·G-6-PDH method <sup>3)</sup>	mg/dL	0
T-cholesterol	CE·COD·POD method <sup>3)</sup>	mg/dL	0
Triglyceride	MGLP·GK·GPO·POD method <sup>3)</sup>	mg/dL	0
Phospholipid	PLD·ChOD·POD method <sup>3)</sup>	mg/dL	0
Aspartate aminotransferase (AST)	JSCC method <sup>3)</sup>	IU/L	0
Alanine aminotransferase (ALT)	JSCC method <sup>3)</sup>	IU/L	0
Lactate dehydrogenase (LDH)	SFBC method <sup>3)</sup>	IU/L	0
Alkaline phosphatase (ALP)	GSCC method <sup>3)</sup>	IU/L	0
$\gamma$ -Glutamyl transpeptidase ( $\gamma$ -GTP)	JSCC method <sup>3)</sup>	IU/L	0
Creatine kinase (CK)	JSCC method <sup>3)</sup>	IU/L	0
Urea nitrogen	Urease·GLDH method <sup>3)</sup>	mg/dL	1
Creatinine	Jaffé method <sup>3)</sup>	mg/dL	1
Sodium	Ion selective electrode method <sup>3)</sup>	mEq/L	0
Potassium	Ion selective electrode method <sup>3)</sup>	mEq/L	1
Chloride	Ion selective electrode method <sup>3)</sup>	mEq/L	0
Calcium	OCPC method <sup>3)</sup>	mg/dL	1
Inorganic phosphorus	PNP·XOD·POD method <sup>3)</sup>	mg/dL	1

1) Automatic blood cell analyzer (ADVIA120 : Siemens Healthcare Diagnostics Inc.)

2) Automatic blood cell differential analyzer (MICROX HEG-120NA : OMRON Corporation)

3) Automatic analyzer (Hitachi 7080 : Hitachi, Ltd.)