2-メチル-1-プロパノールのラットを用いた
経口投与によるがん原性試験（混水試験）報告書

試験番号：0612

APPENDICES

APPENDICES

APPENDIX 1-1

IDENTITY AND IMPURITY OF 2-METHYL-1-PROPANOL

IN THE 2-YEAR DRINKING WATER STUDY

IDENTITY AND IMPURITY OF 2-METHYL-1-PROPANOL IN THE 2-YEAR DRINKING WATER STUDY

Test Substance : 2-Methyl-1-propanol (Wako Pure Chemical Industries, Ltd.)

A. Lot No. : EWJ4558

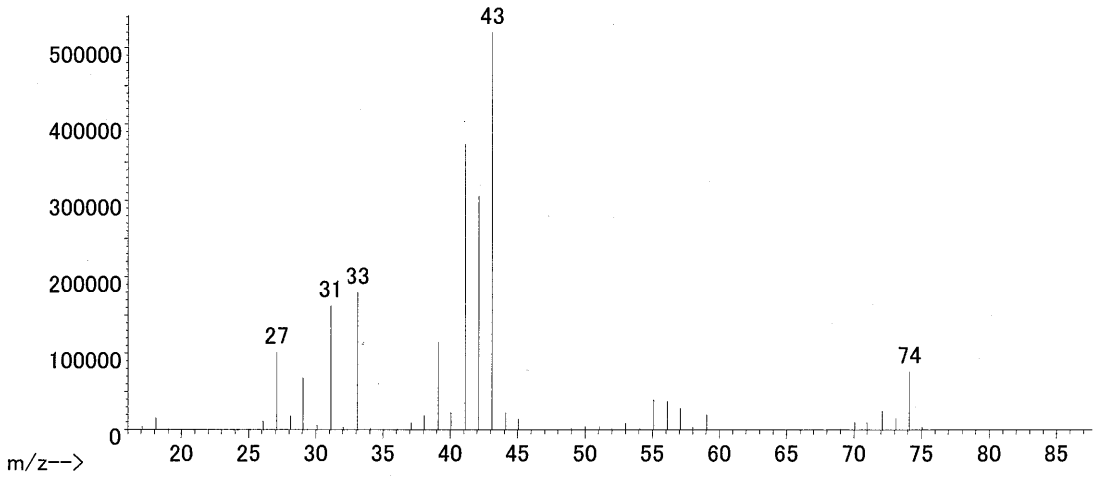
1. Spectral Data

Mass Spectrometry

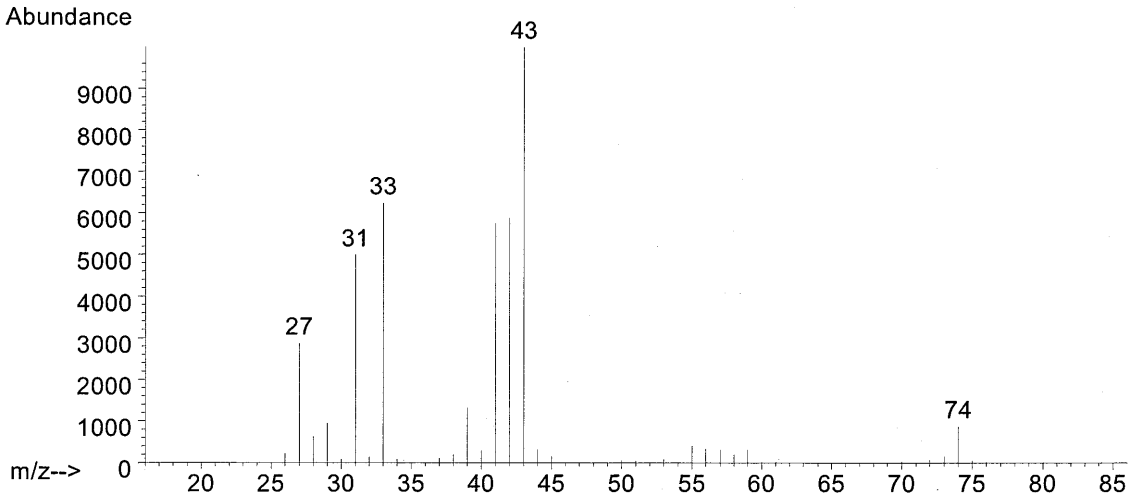
Instrument : Agilent Technologies 5973N Mass Spectrometer

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.

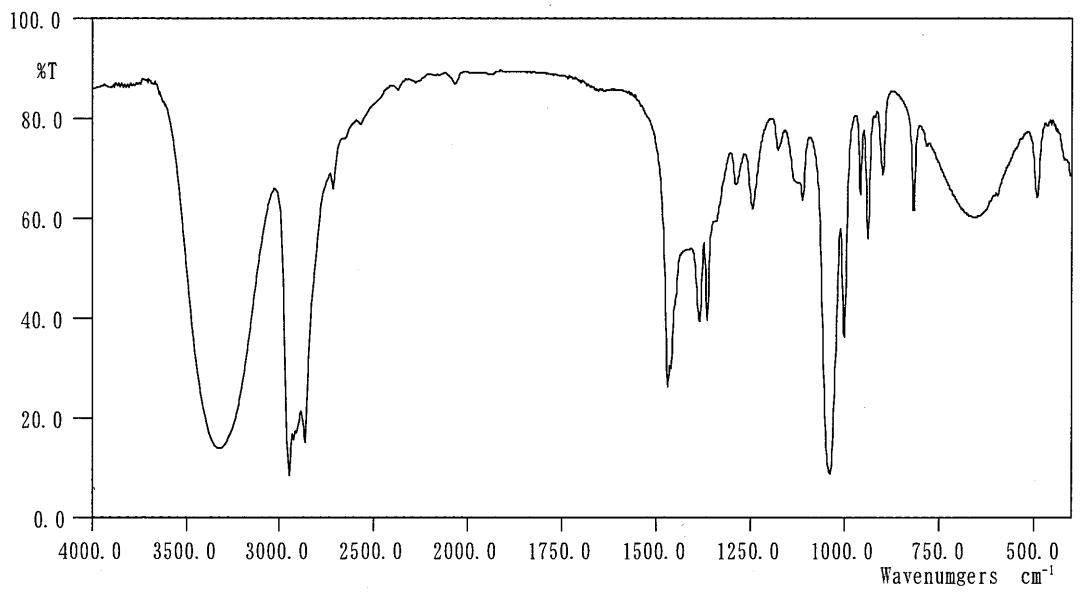
(*McLafferty FW, ed. 1994. Wiley Registry of Mass Spectral Data. 6th ed. New York, NY : John Wiley and Sons.)

Infrared Spectrometry

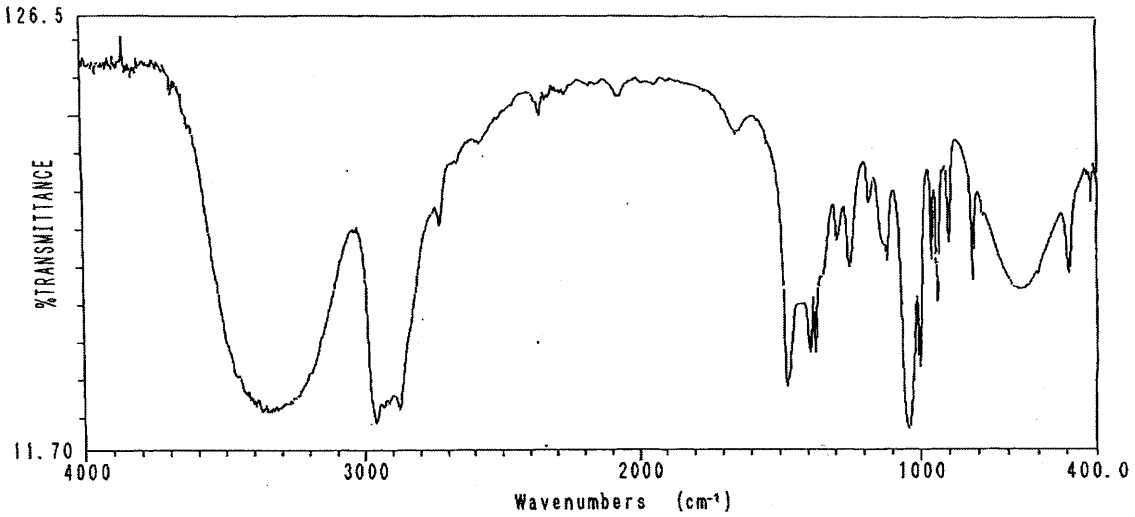
Instrument : Shimadzu FTIR-8200PC Infrared Spectrometer

Cell : KBr Liquid Cell

Resolution : 2 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 : INNOWAX (0.2 mmϕ × 50 m)

Column Temperature: 80 °C (1 min) → (10 °C/min) → 200 °C

Flow Rate : 1 mL/min

Detector : FID (Flame Ionization Detector)

Injection Volume : 1 μL

Sample Name	Peak No.	Area (%)	Peak Name
	1	0.004	Diisobutyl ether
Test Substance	2	99.996	2-Methyl-1-propanol

Result: Gas chromatography indicated one major peak (peak No.2) and one impurity. The impurity (peak No.1) was identified as diisobutyl ether by comparing GC-MS with the standard sample. The amount in the test substance was 0.004% (The quantity value by the standard sample was 0.004%) for diisobutyl ether with a gas chromatograph.

3. Conclusion: The test substance was identified as 2-methyl-1-propanol by mass spectrum and infrared spectrum. Gas chromatography indicated one major peak (2-methyl-1-propanol) and one impurity. The impurity was diisobutyl ether in the test substance.

B. Lot No. : DPL5932

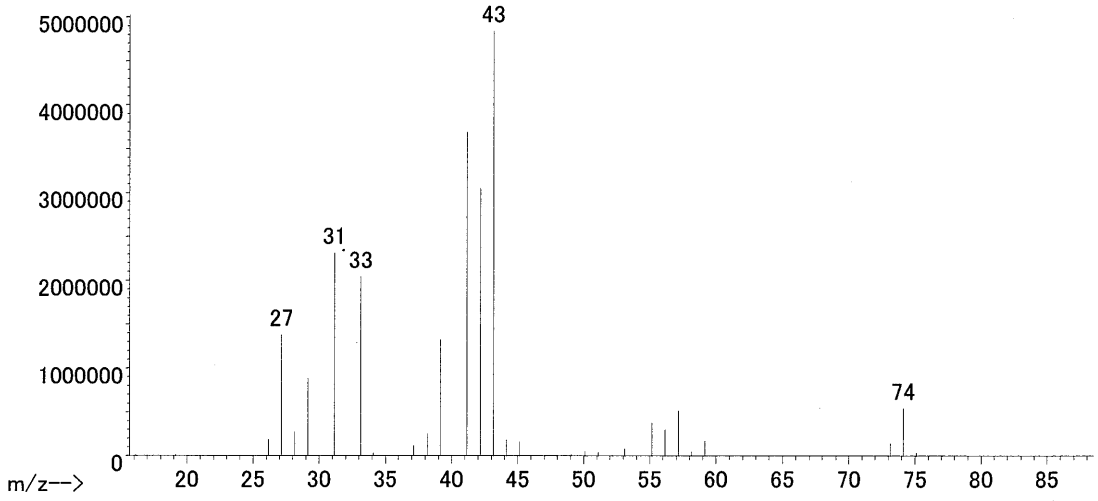
1. Spectral Data

Mass Spectrometry

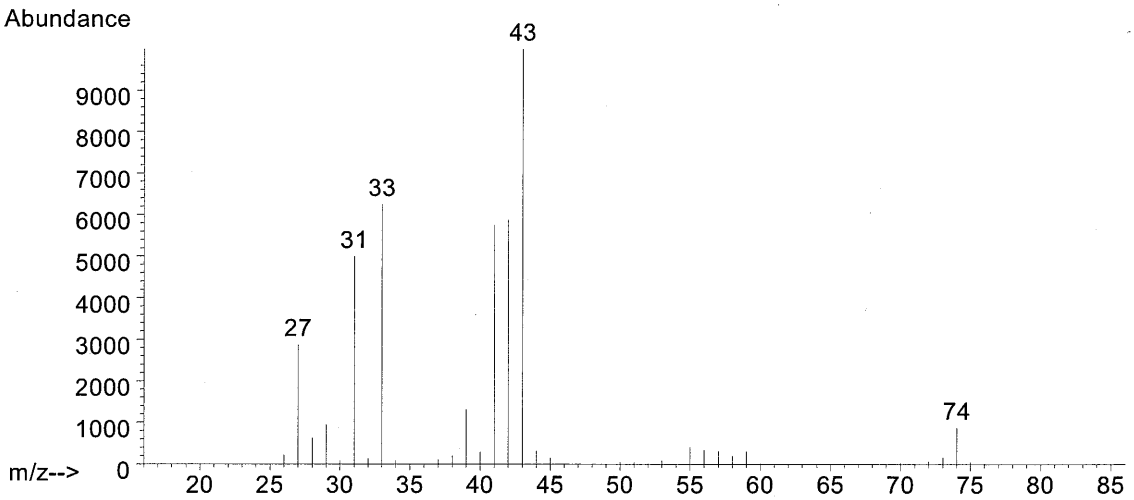
Instrument : Agilent Technologies 5973N Mass Spectrometer

Ionization : EI (Electron Ionization)

Ionization Voltage : 70eV



Mass Spectrum of Test Substance



Mass Spectrum of Literature Data*

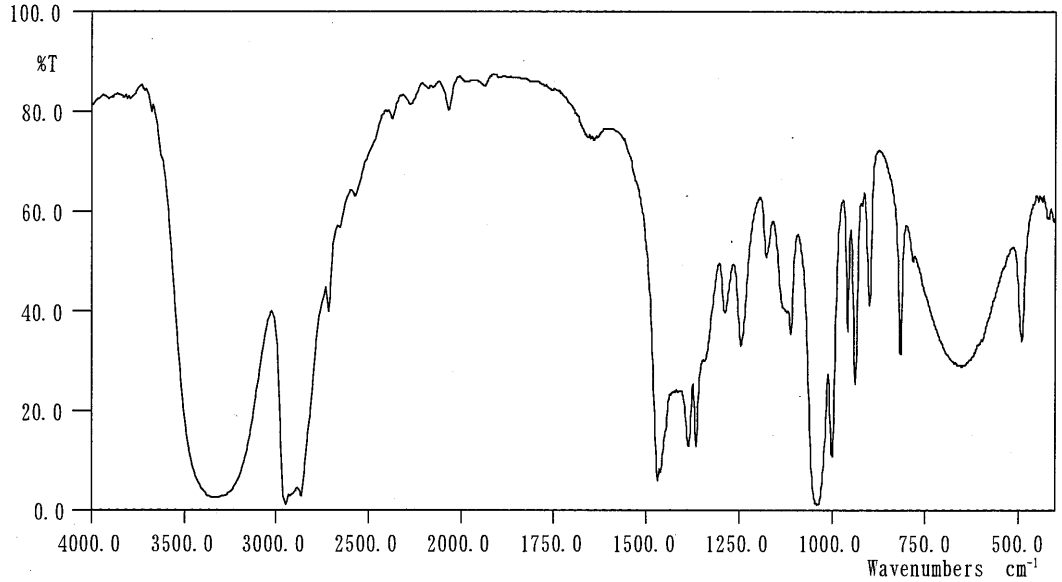
Result: The mass spectrum was consistent with literature spectrum.
(*McLafferty FW, ed. 1994. Wiley Registry of Mass Spectral Data. 6th ed. New York, NY : John Wiley and Sons.)

Infrared Spectrometry

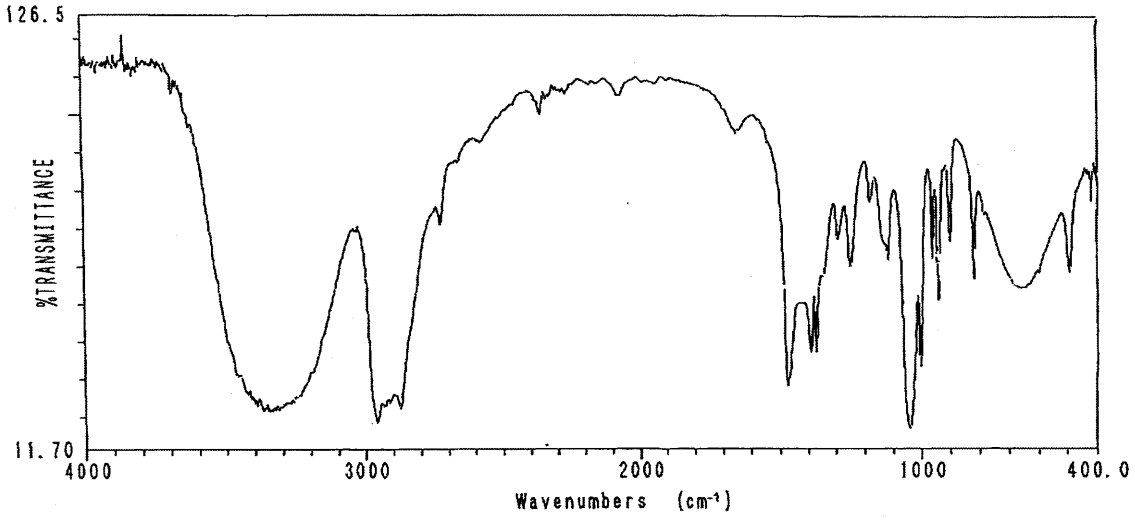
Instrument : Shimadzu FTIR-8200PC Infrared Spectrometer

Cell : KBr Liquid Cell

Resolution : 2 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 : INNOWAX (0.2 mmϕ × 50 m)

Column Temperature: 80 °C (1 min) → (10 °C/min) → 200 °C

Flow Rate : 1 mL/min

Detector : FID (Flame Ionization Detector)

Injection Volume : 1 μL

Sample Name	Peak No.	Area (%)	Peak Name
	1	0.002	Diisobutyl ether
Test Substance	2	99.998	2-Methyl-1-propanol

Result: Gas chromatography indicated one major peak (peak No.2) and one impurity. The impurity (peak No.1) was identified as diisobutyl ether by comparing GC-MS with the standard sample. The amount in the test substance was 0.002% (The quantity value by the standard sample was 0.004%) for diisobutyl ether with a gas chromatograph.

3. Conclusion: The test substance was identified as 2-methyl-1-propanol by mass spectrum and infrared spectrum. Gas chromatography indicated one major peak (2-methyl-1-propanol) and one impurity. The impurity was diisobutyl ether in the test substance.

C. Lot No. : DPE2641

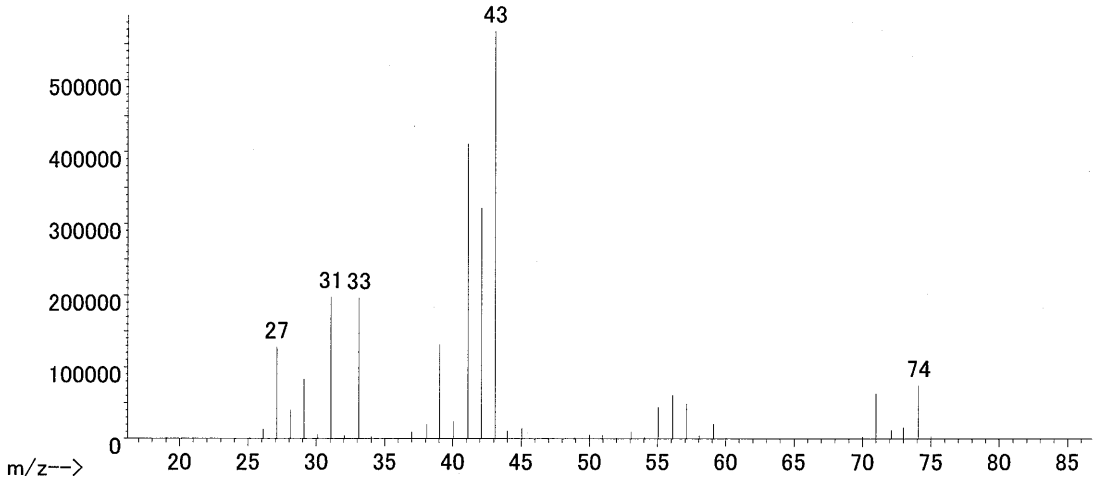
1. Spectral Data

Mass Spectrometry

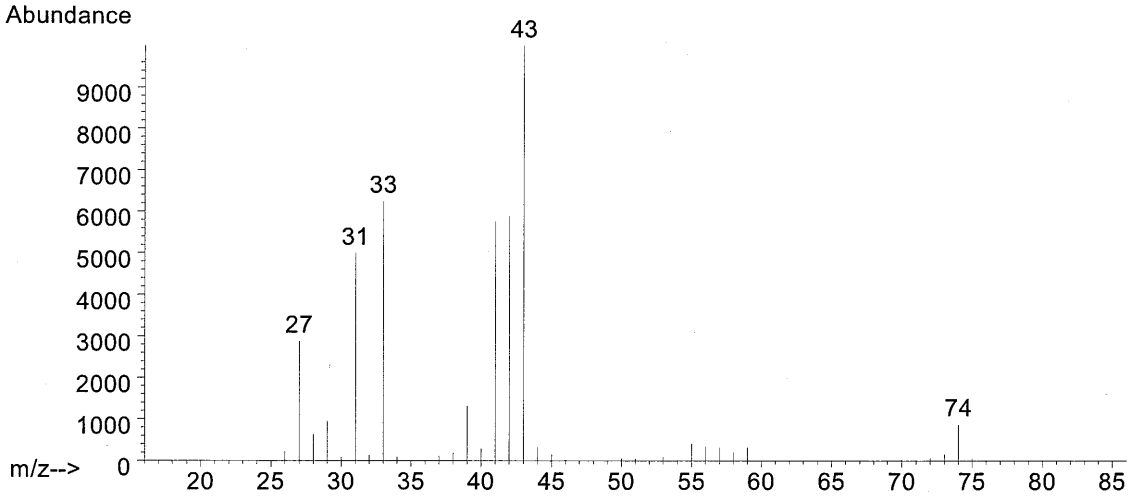
Instrument : Agilent Technologies 5973N Mass Spectrometer

Ionization : EI (Electron Ionization)

Ionization Voltage : 70eV



Mass Spectrum of Test Substance



Mass Spectrum of Literature Data*

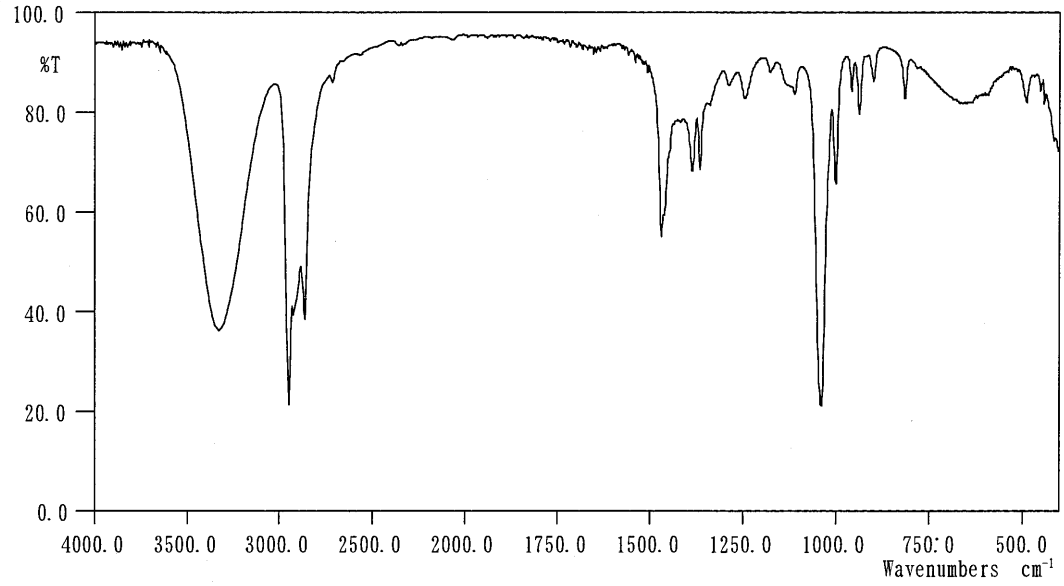
Result: The mass spectrum was consistent with literature spectrum.
(*McLafferty FW, ed. 1994. Wiley Registry of Mass Spectral Data. 6th ed. New York, NY : John Wiley and Sons.)

Infrared Spectrometry

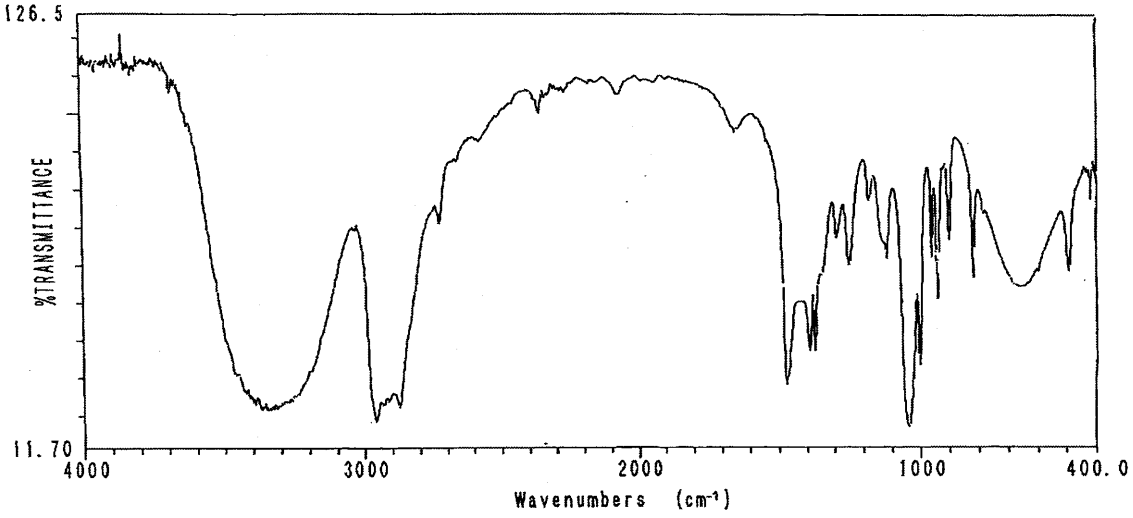
Instrument : Shimadzu FTIR-8200PC Infrared Spectrometer

Cell : KBr Liquid Cell

Resolution : 2 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 : INNOWAX (0.2 mmϕ × 50 m)

Column Temperature: 80 °C (1 min) → (10 °C/min) → 200 °C

Flow Rate : 1 mL/min

Detector : FID (Flame Ionization Detector)

Injection Volume : 1 μL

Sample Name	Peak No.	Area (%)	Peak Name
	1	0.004	Diisobutyl ether
Test Substance	2	99.996	2-Methyl-1-propanol

Result: Gas chromatography indicated one major peak (peak No.2) and one impurity. The impurity (peak No.1) was identified as diisobutyl ether by comparing GC-MS with the standard sample. The amount in the test substance was 0.004% (The quantity value by the standard sample was 0.004%) for diisobutyl ether with a gas chromatograph.

3. Conclusion: The test substance was identified as 2-methyl-1-propanol by mass spectrum and infrared spectrum. Gas chromatography indicated one major peak (2-methyl-1-propanol) and one impurity. The impurity was diisobutyl ether in the test substance.

APPENDIX 1-2

STABILITY OF 2-METHYL-1-PROPANOL

IN THE 2-YEAR DRINKING WATER STUDY

STABILITY OF 2-METHYL-1-PROPANOL IN THE 2-YEAR DRINKING WATER STUDY

Test Substance : 2-Methyl-1-propanol (Wako Pure Chemical Industries, Ltd.)

A. Lot No. : EWJ4558

1. Gas Chromatography

Instrument : Agilent Technologies 5890A Gas Chromatograph

Column : INNOWAX (0.2 mmϕ × 50 m)

Column Temperature: 80 °C (1 min) → (10 °C/min) → 200 °C

Flow Rate : 1 mL/min

Detector : FID (Flame Ionization Detector)

Injection Volume : 1 μL

Date analyzed	Peak No.	Retention Time (min)	Area (%)
2005.12.26	1	3.711	0.004
	2	5.228	99.996
2006.08.31	1	3.711	0.004
	2	5.233	99.996

Result: Gas chromatography indicated one major peak (peak No.2) and one impurity (peak No.1 < 0.01% of total area) analyzed on 2005.12.26 and one major peak (peak No.2) and one impurity (peak No.1 < 0.01% of total area) analyzed on 2006.8.31. No new trace impurity peak in the test substance analyzed on 2006.8.31 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. : DPL5932

1. Gas Chromatography

Instrument : Agilent Technologies 5890A Gas Chromatograph

Column : INNOWAX (0.2 mmϕ × 50 m)

Column Temperature: 80 °C (1 min) → (10 °C/min) → 200 °C

Flow Rate : 1 mL/min

Detector : FID (Flame Ionization Detector)

Injection Volume : 1 μL

Date analyzed	Peak No.	Retention Time (min)	Area (%)
2006.08.16	1	3.708	0.002
	2	5.301	99.998
2007.04.10	1	3.712	0.004
	2	5.232	99.996

Result: Gas chromatography indicated one major peak (peak No.2) and one impurity (peak No.1 < 0.01% of total area) analyzed on 2006.8.16 and one major peak (peak No.2) and one impurity (peak No.1 < 0.01% of total area) analyzed on 2007.4.10. No new trace impurity peak in the test substance analyzed on 2007.4.10 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. : DPE2641

1. Gas Chromatography

Instrument : Agilent Technologies 5890A Gas Chromatograph

Column : INNOWAX (0.2 mmϕ × 50 m)

Column Temperature: 80 °C (1 min) → (10 °C/min) → 200 °C

Flow Rate : 1 mL/min

Detector : FID (Flame Ionization Detector)

Injection Volume : 1 μL

Date analyzed	Peak No.	Retention Time (min)	Area (%)
2007.03.29	1	3.712	0.004
	2	5.240	99.996
2008.02.05	1	3.711	0.004
	2	5.227	99.996

Result: Gas chromatography indicated one major peak (peak No.2) and one impurity (peak No.1 < 0.01% of total area) analyzed on 2007.3.29 and one major peak (peak No.2) and one impurity (peak No.1 < 0.01% of total area) analyzed on 2008.2.5. No new trace impurity peak in the test substance analyzed on 2008.2.5 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 2-METHYL-1-PROPANOL IN FORMULATED WATER IN THE 2-YEAR DRINKING WATER STUDY

CONCENTRATION OF 2-METHYL-1-PROPANOL IN FORMULATED WATER IN THE 2-YEAR DRINKING WATER STUDY

Analytical Method : The samples were analyzed by gas chromatography.

Instrument : Agilent Technologies 5890A Gas Chromatograph

Column : INNOWAX (0.2 mmϕ × 50 m)

Column Temperature: 80 °C (1 min) → (10 °C/min) → 200 °C

Flow Rate : 1 mL/min

Detector : FID (Flame Ionization Detector)

Injection Volume : 1 μL

	Target Concentration		
Date Analyzed	3300[a]	10000	30000
2006.01.05	3300[b] (100)[c]	9760 (97.6)	29300 (97.7)
2006.03.30	3410 (103)	10200 (102)	30600 (102)
2006.06.22	3540 (107)	9560 (95.6)	31800 (106)
2006.09.14	3330 (101)	10100 (101)	30900 (103)
2006.12.07	3170 (96.1)	9530 (95.3)	29200 (97.3)
2007.03.01	3370 (102)	10300 (103)	30800 (103)
2007.05.24	3290 (99.7)	9330 (93.3)	31700 (106)
2007.08.16	3230 (97.9)	9540 (95.4)	32300 (108)
2007.11.08	3360 (102)	9950 (99.5)	32200 (107)

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

APPENDIX 1-4

STABILITY OF 2-METHYL-1-PROPANOL

IN FORMULATED WATER

STABILITY OF 2-METHYL-1-PROPANOL IN FORMULATED WATER

Analytical Method : The samples were analyzed by gas chromatography.

Instrument : Agilent Technologies 5890A Gas Chromatograph

Column : INNOWAX (0.2 mmϕ × 50 m)

Column Temperature: 80 °C (1 min) → (10 °C/min) → 200 °C

Flow Rate : 1 mL/min

Detector : FID (Flame Ionization Detector)

Injection Volume : 1 μL

	Target Concentration	
Date Analyzed	2500[a]	40000
2004.08.12	2450 (100)[b]	40800 (100)
2004.08.16[c]	2250 (91.8)	38300 (93.9)

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

APPENDIX 2

METHODS, UNITS AND DECIMAL PLACE FOR HEMATOLOGY AND BIOCHEMISTRY IN THE 2-YEAR DRINKING WATER STUDY OF 2-METHYL-1-PROPANOL

METHODS, UNITS AND DECIMAL PLACE FOR HEMATOLOGY AND BIOCHEMISTRY IN THE 2-YEAR DRINKING WATER STUDY OF 2-METHYL-1-PROPANOL

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 : Siemens Healthcare Diagnostics Inc.)
2) Automatic blood cell differential analyzer (MICROX HEG-120NA : OMRON Corporation)
3) Automatic analyzer (Hitachi 7080 : Hitachi,Ltd.)