West Virginia Chemical Spill: NTP Chemical Procurement, Analysis, and Formulation

Chemical Procurement

The chemicals were acquired from a variety of sources (Table 1).

Table 1. Chemicals Tested in the NTP West Virginia Chemical Spill Studies

Chemical	CASRN	Supplier	COA Purity (%)
1,4-cyclohexanedimethanol	105-08-8	Sigma (St. Louis, MO)	99.9
4-methoxymethylcyclohexanemethanol	98955-27-2	MRI Global (Kansas City, MO)	99.1
4-methylcyclohexanecarboxylic acid	4331-54-8	TCI America (Portland, OR)	99.7
4-methylcyclohexanemethanol	34885-03-5	TCI America (Portland, OR)	99.8
cyclohexanemethanol, 4-((ethenyloxy)methyl)-	114651-37-5	Sigma (St. Louis, MO)	99.8
cyclohexanemethanol, alpha, alpha, 4-trimethyl-	498-81-7	Santa Cruz Biotechnology (Dallas, TX)	≥ 97
dimethyl 1,4-cyclohexanedicarboxylate	94-60-0	Sigma (St. Louis, MO)	99.5
dipropylene glycol phenyl ether	51730-94-0	Dow Chemical (Midland, MI)	99.9
methyl 4-methylcyclohexanecarboxylate	51181-40-9	Toronto Research Chemicals (Toronto, ON)	98.0
phenoxyisopropanol	4169-04-4	TCI America (Portland, OR)	99.7
propylene glycol phenyl ether	770-35-4	Spectrum Chemical MFG Corp (New Brunswick, NJ)	95.4
crude 4-methycyclohexanemethanol	No CASRN	Eastman Chemical Company (Kingsport, TN)	NA*
2-methylcyclohexanemethanol	2105-40-0	MRIGlobal (Kansas City, MO)	98.9
DOWANOL™ DiPPh glycol ether	No CASRN	Dow Chemical (Midland, MI)	84**

* NA = not applicable. Crude 4-methycyclohexanemethanol is a commercial mixture containing >70% 4-methycyclohexanemethanol along with lesser amounts of other chemicals.
** Purity of 84% was based on dipropylene glycol phenyl ether content.

Identity and Purity

4-methylcyclohexanemethanol. 4-methylcyclohexanemethanol was obtained in a single lot (KDY3F) from TCI America (Portland, OR). The identity was confirmed using Fourier transform infrared (Shimadzu, Columbia, MD), and 1H and 13C nuclear magnetic resonance (NMR, Bruker, Woodlands, TX) spectroscopies, as well as gas chromatography/mass spectrometry (GC/MS) and elemental analysis. Purity determined using GC with flame ionization detection (FID) (Agilent, Santa Clara, CA) was > 99.8% with respect to the *cis*- (67.99%) and *trans*- (31.98%) isomers of 4-methylcyclohexanemethanol.

Crude 4-methylcyclohexanemethanol. Identity and purity of lot TP14044373, obtained in two batches, were determined using GC/MS (Agilent, Santa Clara, CA). Two major peaks with a combined total area of 90.35% were found. The *cis*-isomer of 4-methylcyclohexanemethanol accounted for 33.45% and the *trans*-isomer accounted for 56.90% of the crude mixture. There were six other peaks with peak areas > 0.05%, which were tentatively identified by comparison to the National Institute of Standards and Technology (NIST) Mass Spectral Library, as cyclohexanemethanol (1.82%; CASRN: 100-49-2), *cis*- and *trans*- isomers of 1,4-cyclohexanedimethanol (7.15%), 2-ethyl-1-hexanol (0.06%; CASRN: 104-76-7), *cis*-octahydroisobenzofuran (0.13%; CASRN: 13149-01-4), and methyl 4-methylcyclohexanecarboxylate (0.50%). Dimethyl 1,4-cyclohexanedicarboxylate was identified as being present in both batches at less than 1%, although the concentration was not consistent between the two batches.

Propylene glycol phenyl ether. Identity and purity of propylene glycol phenyl ether were determined using GC/MS (Agilent, Santa Clara, CA). This material was composed of two isomers, 1-phenoxypropan-2-ol (83.40%) and 2-phenoxy-1-propanol (16.60%), with the combined areas accounting for 100% of the total chromatographic peak area.

Dipropylene glycol phenyl ether. Identity and purity of dipropylene glycol phenyl ether were determined using GC/MS (Agilent, Santa Clara, CA). This material was composed of a mixture of four isomers of dipropylene glycol phenyl ether, corresponding to three unique chemical structures, which had a combined area accounting for 100% of the total chromatographic peak area.

DOWANOL™ DiPPh glycol ether. Identity and purity of Dowanol™ DiPPh glycol ether, a commercial grade dipropylene glycol phenyl ether, were determined using GC/MS (Agilent, Santa Clara, CA). This material was composed of a mixture of four isomers of dipropylene glycol phenyl ether, corresponding to three unique chemical structures, which had a combined area accounting for 91.7% of the total chromatographic peak area. Nineteen impurities with peak areas > 0.05% were noted, of which three had peak areas of ≥ 0.86%. Three of the impurity peaks, including two of the ~1% impurities, were tentatively identified by comparison to the NIST Mass Spectral Library as phenol (0.11%), 1-phenoxypropan-2-ol (1.92%), and 2-phenoxypropan-1-ol (0.86%). The third major impurity (1.01%) could not be matched to any compound in the NIST Mass Spectral Library.

Methyl 4-methylcylcohexanecarboxylate. Identity and purity were determined by GC/MS (Agilent, Santa Clara, CA). Two components, which were identified as isomers of methyl 4-methylcylcohexanecarboxylate by comparison with the NIST Mass Spectral Library comprised 59.79% and 32.05% of the total area resulting in a purity estimate of 99.29%. Quantification of the individual isomers was not determined. Three impurities were observed, with a combined total area of 0.62%.

1,4-Cyclohexanedimethanol. Identity and purity were determined by GC/MS (Agilent, Santa Clara, CA). Two components, which were identified as *cis*- and *trans*- isomers of 1,4-cyclohexanedimethanol by comparison with the NIST Mass Spectral Library, comprised 47.33% (trans) and 52.31% (cis) of the total area resulting in a purity estimate of 99.63%. Five impurities were observed, with a combined total area of 0.39%.

4-Methylcyclohexanecarboxylic acid. Identity and purity were determined by GC/MS (Agilent, Santa Clara, CA). Identity was confirmed by comparison with the NIST Mass Spectral Library, with a purity of 98.80%. Six impurities were observed, with a combined total area of 1.17%.

Cyclohexanemethanol, 4-((ethenyloxy)methyl)-. Identity and purity were determined by GC/MS (Agilent, Santa Clara, CA). Two components, which were identified as isomers of cyclohexanemethanol, 4-((ethenyloxy)methyl)- by comparison with the NIST Mass Spectral Library comprised 71.75% and 24.23% of the total area resulting in a purity estimate of 96.07%. There was one impurity component with total area of 1.21%, which was tentatively identified as 1,4-cyclohexanedimethanol by comparison with the

NIST Mass Spectral Library. Eleven additional impurities were observed, with a combined total area of 2.61%.

Cyclohexanemethanol, alpha, alpha, 4-trimethly-. Identity and purity were determined by GC/MS (Agilent, Santa Clara, CA). Four components, which were identified as a mixture of isomers of cyclohexanemethanol, alpha, alpha, 4-trimethly- by comparison with the NIST Mass Spectral Library comprised 13.39%, 26.47%, 24.63%, and 31.93% of the total area resulting in a purity estimate of 96.41%. There was one impurity with relative total area of 1.03%, which was tentatively identified as camphol (CASRN 507-70-0) by comparison with the NIST Mass Spectral Library. Seven additional impurities were observed, with a combined total area of 2.23%.

Dimethyl 1,4-cyclohexanedicarboxylate. Identity and purity were determined by GC/MS (Agilent, Santa Clara, CA). The identity was confirmed by comparison to the NIST Mass Spectral Library with a purity of 99.43%. Four impurities were observed, with a combined total area of 0.47%.

4-Methoxymethylcyclohexanemethanol and *2-methylcyclohexanemethanol*. Both compounds were custom synthesized by MRIGlobal (Kansas City, MO), which supplied a certificate of analysis (COA) purity value of 99.1% and 98.9%, respectively, based on GC/MS analysis.

Formulation Preparation, Analysis, and Shipment

Genetic Toxicology Studies

Five gram aliquots of 4-methylcyclohexanemethanol, crude 4-methylcyclohexanemethanol, 1,4-cyclohexanedimethanol, 4-methoxymethylcyclohexanemethanol, dimethyl 1,4-cyclohexanedicarboxylate, methyl 4-methylcyclohexanecarboxylate, propylene glycol phenyl ether, 2-methylcyclohexanemethanol and dipropylene glycol phenyl ether were transferred to amber glass vials and shipped to Integrated Laboratory Systems (ILS, Research Triangle Park, NC).

C. elegans Studies

Five milliliter aliquots of 20 mM solutions of each chemical (Table 1) in dimethylsulfoxide (DMSO) were transferred to amber glass vials and shipped to NIEHS.

Zebrafish Studies

One hundred and fifty microliter aliquots of 40 mM solutions of each chemical in DMSO were prepared in a 96-well plate and shipped to NTP's contract laboratory at Oregon State University (Corvallis, OR).

Dermal Irritancy and Hypersensitivity Studies

Formulations of 4-methylcyclohexanemethanol and crude 4-methylcyclohexanemethanol were prepared at nominal concentrations of 0 (vehicle), 2, 20, and 50% (v/v) and 0 (vehicle), 1, 2, 5, 20, 40, and 80% (v/v), respectively, in acetone:olive oil (4:1, v/v) by the study laboratory (Burleson Research Technologies, Research Triangle Park, NC). An aliquot of each formulation was shipped to the analytical chemistry laboratory for determination of the formulation concentration. For subsequent studies with crude 4-methylcyclohexanemethanol, formulations were prepared by the analytical chemistry laboratory at nominal concentrations of 0 (vehicle), 1, 5, 25, 50, and 75% (v/v) in acetone:olive oil (4:1, v/v) and shipped to Burleson Research Technologies (Research Triangle Park, NC). Formulation concentrations determined by GC/FID are given in Tables 5a, 5b, and 5c.

5-Day Toxicogenomic Studies

Formulations of 4-methylcyclohexanemethanol and crude 4-methylcyclohexanemethanol were prepared at nominal concentrations of 0 (vehicle control), 0.02, 0.2, 2, 20, 60, and 100 mg/mL in corn oil. Propylene glycol phenyl ether formulations were prepared at nominal concentrations of 0 (vehicle), 0.2, 2, 20, 100, 200, and 400 mg/mL in corn oil. Formulation concentrations determined by GC/FID are given in Tables 2a and 2b. Formulations were shipped to Battelle Memorial Institute (Battelle, Columbus, OH).

Prenatal Developmental Toxicity Studies

Dose-Range Finding Study

Formulations of 4-methylcyclohexanemethanol in corn oil were prepared at nominal concentrations of 0 (vehicle control), 75, 150, 300, and 450 mg/mL. Formulation concentrations determined using GC/FID are given in Table 3. The formulations were subsequently shipped to Southern Research Institute (Birmingham, AL). Analysis of animal room samples (the post administration formulation analysis) received from Southern Research Institute is shown in Table 3.

Main Study

Formulations of 4-methylcyclohexanemethanol in corn oil were prepared at nominal concentrations of 0 (vehicle control), 25, 50, 100, and 200 mg/mL. Formulation concentrations determined using GC/FID are given in Table 4. The formulations were subsequently shipped to Southern Research Institute (Birmingham, AL). Two sets of animal room samples were received from Southern Research Institute and the analyses results are given in Table 4.

Formulation Analysis Results

Table 2a. Formulation Analysis Results for 5-Day Toxicogenomic Study of 4-Methylcyclohenaemethanol

Target Concentration (mg/mL)	Formulation Date	Analysis Date	Determined Concentration (mg/mL) Mean (SD*)	Percent of Target Mean (%RSD**)
0	9/8/14	9/8/14	ND***	NA****
0.020	9/8/14	9/8/14	0.0200 (0.0002)	100.0 (1.0)
0.20	9/8/14	9/8/14	0.2041 (0.0023)	102.1 (1.1)
2.0	9/8/14	9/8/14	2.0186 (0.0072)	100.9 (0.4)
20	9/8/14	9/8/14	20.14 (0.04)	100.8 (0.2)
60	9/8/14	9/8/14	60.57 (0.27)	101.0 (0.4)
100	9/8/14	9/8/14	101.53 (0.18)	101.5 (0.2)

*SD = Standard deviation for 3 measurements

**%RSD = % relative standard deviation

***ND = not detected, detection limit of 0.001 mg/mL

****NA = not applicable

Table 2b. Formulation Analysis Results for 5-Day Toxicogenomic Study of Crude 4-Methylcyclohexanemethanol

Target Concentration (mg/mL)	Formulation Date	Analysis Date	Determined Concentration (mg/mL) Mean (SD*)	Percent of Target Mean (%RSD**)
0	9/9/14	9/9/14	ND***	NA****
0.020	9/9/14	9/9/14	0.0165 (0.003) †	82.5 (1.8)†
0.020	9/16/14	9/16/14	0.0197 (0.0003)	98.5 (1.5)
0.20	9/9/14	9/9/14	0.1877 (0.0021)	93.9 (1.2)
2.0	9/9/14	9/9/14	1.962 (0.008)	98.1 (0.4)
20	9/9/14	9/9/14	18.48 (0.29)	92.4 (1.6)
60	9/9/14	9/9/14	58.65 (0.06)	97.8 (0.1)
100	9/9/14	9/9/14	93.77 (1.04)	93.8 (1.1)

*SD = Standard deviation for 3 measurements
**%RSD = % relative standard deviation
***ND = not detected, detection limit of 0.001 mg/mL
****NA = not applicable
† Not used, dose was reformulated

Table 2c. Formulation Analysis Results for 5-Day Toxicogenomic Study of Propylene Glycol Phenyl Ether

Target Concentration (mg/mL)	Formulation Date	Analysis Date	Determined Concentration (mg/mL) Mean (SD*)	Percent of Target Mean (%RSD**)
0	9/10/14	9/10/14	ND***	NA****
0.2	9/10/14	9/10/14	0.2016 (0.0034)	100.9 (1.7)
2	9/10/14	9/10/14	2.009 (0.0057)	100.5 (0.3)
20	9/10/14	9/10/14	20.02 (0.0208)	100.1 (0.1)
100	9/10/14	9/10/14	98.54 (0.21)	98.5 (0.2)
200	9/10/14	9/10/14	198.5 (5.02)	99.2 (2.5)
400	9/10/14	9/10/14	404.8 (1.2)	101.2 (0.3)

*SD = Standard deviation for 3 measurements
**%RSD = % relative standard deviation
***ND = not detected, detection limit of 0.001 mg/mL
****NA = not applicable

Table 3. Formulation Analysis Results for Dose-Range Finding Prenatal Developmental Toxicity Study of 4-Methylcyclohexanemethanol

Target Concentration (mg/mL)	Formulation Date	Analysis Date	Determined Concentration (mg/mL) Mean (SD*)	Percent of Target Mean (%RSD**)
0	8/13/14	8/13/14	ND***	NA****
75	8/13/14	8/13/14	75.82 (0.22)	101.1 (0.3)
150	8/13/14	8/13/14	152.0 (1.07)	101.3 (0.7)
300	8/13/14	8/13/14	303.5 (2.15)	101.2 (0.7)
450	8/13/14	8/13/14	456.0 (6.99)	101.3 (1.5)
0†	8/13/14	9/11/14	3.908 (0.012)[§]	NA****
75†	8/13/14	9/11/14	75.36 (0.44)	100.5 (0.6)
150†	8/13/14	9/11/14	152.1 (0.64)	101.4 (0.4)
300†	8/13/14	9/11/14	302.6 (2.01)	100.9 (0.7)
450†	8/13/14	9/11/14	448.5 (5.80)	99.7 (1.3)

*SD = Standard deviation for 3 measurements

**%RSD = % relative standard deviation

***ND = not detected, detection limit of 0.001 mg/mL

****NA = not applicable

† Animal room sample

[§] The 0 mg/mL formulation was a cloudy yellow mixture with droplets observed at the bottom of the container.

Table 4. Formulation Analysis Results for Main Prenatal Developmental Toxicity Study of 4-Methylcyclohexanemethanol

Target Concentration (mg/mL)	Formulation Date	Analysis Date	Determined Concentration (mg/mL) Mean (SD*)	Percent of Target Mean (%RSD**)
0	10/30/14	10/30/14	ND***	NA****
25	10/30/14	10/30/14	24.78 (0.10)	99.1 (0.4)
50	10/30/14	10/30/14	49.67 (0.27)	99.3 (0.6)
100	10/30/14	10/30/14	99.84 (0.33)	99.8 (0.3)
200	10/30/14	10/30/14	199.3 (0.64)	99.7 (0.3)
0†	10/30/14	12/3/14	ND***	NA****
25†	10/30/14	12/3/14	24.88 (0.02)	99.5 (0.1)
50†	10/30/14	12/3/14	50.57 (0.56)	101.1 (1.1)
100†	10/30/14	12/3/14	100.5 (1.47)	100.5 (1.5)
200†	10/30/14	12/3/14	199.6 (0.31)	99.8 (0.2)
0§	10/30/14	12/10/14	ND***	NA****
25§	10/30/14	12/10/14	25.00 (0.04)	100.0 (0.2)
50§	10/30/14	12/10/14	50.09 (0.52)	100.2 (1.0)
100§	10/30/14	12/10/14	99.83 (0.64)	99.8 (0.6)
200§	10/30/14	12/10/14	200.1 (1.57)	100.0 (0.8)

*SD = Standard deviation for 3 measurements

**%RSD = % relative standard deviation

***ND = not detected, detection limit of 0.001 mg/mL

****NA = not applicable

† Animal room sample set 1

§ Animal room sample set 2

Table 5a. Formulation Analysis Results for Study 1 of Dermal Irritancy and Hypersensitivity Studies of 4-Methylcyclohexanemethanol

Target Concentration (% v/v)	Formulation Date	Analysis Date	Determined Concentration (mg/mL) Mean (SD*)	Percent of Target Mean (%RSD**)
0	12/12/14	12/12-16/14	ND***	NA****
2	12/12/14	12/12-16/14	1.54 (0.006)	77.0 (0.4)
20	12/12/14	12/12-16/14	21.0 (0.08)	105 (0.4)
50	12/12/14	12/12-16/14	44.8 (0.72)	89.6 (1.6)

*SD = Standard deviation for 3 measurements
**%RSD = % relative standard deviation
***ND = not detected, detection limit of 0.001 mg/mL
****NA = not applicable

Table 5b. Formulation Analysis Results for Study 1 of Dermal Irritancy and Hypersensitivity Studies of Crude 4-Methylcyclohexanemethanol

Target Concentration (% v/v)	Formulation Date	Analysis Date	Determined Concentration (mg/mL) Mean (SD*)	Percent of Target Mean (%RSD**)
0	12/12/14	12/12-16/14	ND***	NA****
1	12/12/14	12/12-16/14	0.896 (0.007)	89.6 (0.8)
2	12/12/14	12/12-16/14	2.01 (0.07)	101 (3.3)
5	12/12/14	12/12-16/14	3.84 (0.03)	76.8 (0.7)
20	12/12/14	12/12-16/14	19.2 (0.13)	96.0 (0.7)
40	12/12/14	12/12-16/14	37.1 (1.15)	92.8 (3.1)
80	12/12/14	12/12-16/14	82.0 (3.28)	103 (4.0)

*SD = Standard deviation for 3 measurements
**%RSD = % relative standard deviation
***ND = not detected, detection limit of 0.001 mg/mL
****NA = not applicable

Table 5c. Formulation Analysis Results for Study 2 of Dermal Irritancy and Hypersensitivity Studies of Crude 4-Methylcyclohexanemethanol

Target Concentration (% v/v)	Formulation Date	Analysis Date	Determined Concentration (mg/mL) Mean (SD*)	Percent of Target Mean (%RSD**)
0	3/9-10/15	3/11/15	ND***	NA****
1.0	3/9-10/15	3/11/15	0.985 (0.01)	98.5 (1.3)
5.0	3/9-10/15	3/11/15	5.05 (0.15)	101 (3.1)
25	3/9-10/15	3/11/15	24.4 (1.25)	97.6 (5.1)
50	3/9-10/15	3/11/15	47.6 (1.32)	95.2 (2.8)
75	3/9-10/15	3/11/15	72.5 (2.43)	96.7 (3.4)
0†	3/9-10/15	4/23-24/15	ND***	NA****
1.0†	3/9-10/15	4/23-24/15	1.03 (0.04)	103 (4.1)
5.0†	3/9-10/15	4/23-24/15	5.12 (0.31)	102 (6.1)
25†	3/9-10/15	4/23-24/15	24.6 (2.1)	98.4 (8.4)
50†	3/9-10/15	4/23-24/15	46.6 (0.91)	93.2 (1.9)
75†	3/9-10/15	4/23-24/15	72.4 (2.4)	96.5 (3.4)

*SD = Standard deviation for 3 measurements
**%RSD = % relative standard deviation
***ND = not detected, detection limit of 0.001 mg/mL
****NA = not applicable
† Animal room samples