Supplementary info to

**Impact of transformation, photodegradation and interaction with glutaraldehyde on the acute toxicity of the biocide DBNPA in cooling tower water**

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**Content**

- Figure S1: Wavelength intensity distribution of sunlight and synthetic sunlight
- Figure S2: The data-processing workflow initialized by ‘patRoon’
- Text S1: The full R-script for data-processing of the non-target screening data for positive ionization
- Figure S3: 24 h LC\(_{50}\) for K\(_2\)Cr\(_2\)O\(_7\)
- Table S1: pH, oxygen content and temperature of EC\(_{50}\) test solutions
- Table S2: pH, oxygen content and temperature of photodegradation toxicity solutions
- Text S2: Detailed info about TP1-MBNPA
- Text S3: Detailed info about TP2-2,2-dibromopropandiamide
- Figure S4: Peak intensities of DBNPA TPs in the dark at t=1
- Figure S5: DBNPA degradation pathways in the dark
- Text S4: Detailed info about TP3-dibromoacetamide
- Text S5: Detailed info about TP4-mz461
- Text S6: Detailed info about TP5-mz153
- Text S7: Detailed info about TP6-mz163
- Text S8: Detailed info about TP7-mz186
- Text S9: Detailed info about TP8-mz148
- Text S10: Detailed info about TP9-mz169
- Text S11: Detailed info about TP10-mz173
- Text S12: Detailed info about TP11-mz206 and TP12-mz206
- Text S13: Detailed info about TP13-mz344
- Text S14: Detailed info about TP14-mz170
- Text S15: Detailed info about GIP1
- Text S16: Detailed info about GIP2
- Text S17: Detailed info about GIP3
- Text S18: Detailed info about GIP4
- Text S19: Detailed info about GIP5
- Figure S6: Peak intensity of GIP2
- Text S20: Detailed info about GIP6
Figure S1. Wavelength intensity distribution for the lamps mimicking sunlight (blue) and actual sunlight (orange). The actual intensities per wavelength are not comparable, since a filter was used to prevent peak overload.
Figure S2. Workflow for non-target data analysis. Workflow-steps are underlined and accompanying software packages are displayed in bold (Wagner et al., 2019a).
# Script automatically generated on Wed May 15 12:16:32 2019

library(patRoon)

# -------------------------# initialization# -------------------------

workPath <- "D:/maXis/Projects/Thomas/Non-target - 15-5/pos"
setwd(workPath)

# Load analysis table
anaInfo <- read.csv("analyses.csv", stringsAsFactors = FALSE, colClasses = "character")

# Subset for proper calibration
anaInfo_manCal <- anaInfo[1:24,]
anaInfo_autoCal <- anaInfo[25:72,]

# Set to FALSE to skip data pre-treatment
doDataPretreatment <- TRUE
if (doDataPretreatment)
{
  setDAMethod(anaInfo_autoCal, "D:/maXis/Projects/Thomas/Non-target - 15-5/pos/20190515-pos.m")
  recalibrarateDAFiles(anaInfo_autoCal)
  convertMSFiles(anaInfo = anaInfo,
                 to = "mzML", algorithm = "bruker", centroid = TRUE)
}

# -------------------------# features# -------------------------

# Find all features.
# NOTE: see manual for many more options
fList <- findFeatures(anaInfo, "openms")

# Group and align features between analysis
fGroups <- groupFeatures(fList, "openms")

# Basic rule based filtering
fGroups <- filter(fGroups, preAbsMinIntensity = 100, absMinIntensity = 5000,
                  relMinReplicateAbundance = 1, maxReplicateIntRSD = 0.75,
                  blankThreshold = 5, removeBlanks = TRUE,
                  retentionRange = c(120, Inf), mzRange = NULL)

# -------------------------# annotation# -------------------------

# Retrieve MS peak lists
avgPListParams <- getDefAvgPListParams(clusterMzWindow = 0.002)
plists <- generateMSPeakLists(fGroups, "mzr", maxMSRtWindow = 5, precursorMzWindow = 4,
                              avgFeatParams = avgPListParams, avgFGroupParams = avgPListParams)

# uncomment and configure for extra filtering of MS peak lists
# plists <- filter(plists, absMSIntThr = NULL, absMSMSIntThr = NULL, relMSIntThr = NULL,
relMSMSIntThr = NULL, topMSPeaks = NULL, topMSMSPeaks = NULL,
deIsotopeMS = FALSE, deIsotopeMSMS = FALSE)

# Calculate formula candidates
formulas <- generateFormulas(fGroups, "genform", plists, relMzDev = 5,
                           adduct = "[M+H]^+", elements = "CHNOPBr",
                           calculateFeatures = TRUE, featThreshold = 0.75)

# Find compound structure candidates
compounds <- generateCompounds(fGroups, plists, "metfrag", method = "CL",
                                dbRelMzDev = 5,
                                fragRelMzDev = 5, fragAbsMzDev = 0.002,
                                adduct = "[M+H]^+", database = "pubchem",
                                maxCandidatesToStop = 2500,
                                extraOpts = list(FilterExcludedElements = c("I",
                                "Si", "B")))
compounds <- addFormulaScoring(compounds, formulas, TRUE)

# Perform automatic generation of components
components <- generateComponents(fGroups, "ramclustr", ionization = "positive")

# reporting
reportCSV(fGroups, path = "report", reportFeatures = FALSE, formulas = formulas,
           compounds = compounds, compoundsNormalizeScores = "max",
           components = components)

reportPDF(fGroups, path = "report", reportFGroups = TRUE, formulas = formulas,
          reportFormulaSpectra = TRUE,
          compounds = compounds, compoundsNormalizeScores = "max",
          components = components, MSPeakLists = plists)

reportMD(fGroups, path = "report", reportPlots = c("chord", "venn", "upset",
          "eics", "formulas"), formulas = formulas,
          compounds = compounds, compoundsNormalizeScores = "max",
          components = components, MSPeakLists = plists,
          selfContained = FALSE, openReport = TRUE)
Figure S3. The mobility of Daphnia magna in the presence of different concentrations of K$_2$Cr$_2$O$_7$ (purple circles), corresponding model fit (black lines) and LC$_{50}$ (+ 95% confidence interval) (yellow circle).
Table S1. Chemical parameters of test solutions of DBNPA used for the determination of the EC\textsubscript{50} for Daphnia magna in cooling tower water and ADaM medium

<table>
<thead>
<tr>
<th>DBNPA concentration (mg/L)</th>
<th>Cooling tower water</th>
<th></th>
<th>ADaM medium</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>pH</td>
<td>Temp (°C)</td>
<td>DO (mg/L)</td>
<td>pH</td>
</tr>
<tr>
<td>0</td>
<td>6.6</td>
<td>20.4</td>
<td>8.94</td>
<td>6.2</td>
</tr>
<tr>
<td>0.25</td>
<td>6.9</td>
<td>20.4</td>
<td>8.96</td>
<td>6.2</td>
</tr>
<tr>
<td>0.5</td>
<td>6.7</td>
<td>20.4</td>
<td>8.89</td>
<td>6.2</td>
</tr>
<tr>
<td>1</td>
<td>6.7</td>
<td>20.5</td>
<td>8.91</td>
<td>6.2</td>
</tr>
<tr>
<td>2.5</td>
<td>6.9</td>
<td>20.5</td>
<td>8.87</td>
<td>6.2</td>
</tr>
<tr>
<td>5</td>
<td>6.9</td>
<td>20.5</td>
<td>8.89</td>
<td>6.3</td>
</tr>
</tbody>
</table>
Table S2

Table S2. Chemical parameters of test solutions from photodegradation experiments used for Daphnia magna toxicity test.

<table>
<thead>
<tr>
<th>Chemical</th>
<th>pH day 1</th>
<th>pH day 3</th>
<th>pH day 5</th>
<th>T (°C) day 1</th>
<th>T (°C) day 3</th>
<th>T (°C) day 5</th>
<th>DO (mg/L) day 1</th>
<th>DO (mg/L) day 3</th>
<th>DO (mg/L) day 5</th>
</tr>
</thead>
<tbody>
<tr>
<td>CTW-L</td>
<td>7.9</td>
<td>7.9</td>
<td>8.0</td>
<td>19.8</td>
<td>20.5</td>
<td>20.3</td>
<td>8.6</td>
<td>8.6</td>
<td>9.1</td>
</tr>
<tr>
<td>CTW-D</td>
<td>7.9</td>
<td>8.0</td>
<td>8.0</td>
<td>19.8</td>
<td>20.2</td>
<td>20.3</td>
<td>8.4</td>
<td>8.6</td>
<td>9.1</td>
</tr>
<tr>
<td>DBNPA-L</td>
<td>7.9</td>
<td>8.0</td>
<td>8.0</td>
<td>19.7</td>
<td>20.3</td>
<td>19.9</td>
<td>8.4</td>
<td>8.5</td>
<td>9.0</td>
</tr>
<tr>
<td>DBNPA-D</td>
<td>8.0</td>
<td>8.0</td>
<td>8.0</td>
<td>19.9</td>
<td>20.4</td>
<td>20.2</td>
<td>8.7</td>
<td>8.7</td>
<td>8.7</td>
</tr>
<tr>
<td>Gluta-L</td>
<td>8.0</td>
<td>8.0</td>
<td>8.0</td>
<td>20.1</td>
<td>20.1</td>
<td>20.0</td>
<td>8.4</td>
<td>8.7</td>
<td>8.7</td>
</tr>
<tr>
<td>Gluta-D</td>
<td>8.0</td>
<td>8.1</td>
<td>8.1</td>
<td>20.1</td>
<td>20.3</td>
<td>20.2</td>
<td>8.0</td>
<td>8.7</td>
<td>8.6</td>
</tr>
<tr>
<td>G_D-L</td>
<td>7.9</td>
<td>8.0</td>
<td>8.0</td>
<td>20.3</td>
<td>19.9</td>
<td>20.4</td>
<td>8.5</td>
<td>8.7</td>
<td>8.8</td>
</tr>
<tr>
<td>G_D-D</td>
<td>7.9</td>
<td>8.1</td>
<td>8.0</td>
<td>20.4</td>
<td>19.9</td>
<td>20.5</td>
<td>8.3</td>
<td>8.7</td>
<td>8.7</td>
</tr>
</tbody>
</table>
Text S2 – Detailed info about TP1-MBNPA

Name: 2-bromo-2-cyanoacetamide (MBNPA)

Origin: Direct transformation product of DBNPA

Formula: $\text{C}_3\text{H}_3\text{BrN}_2\text{O}$

$m/z$: 162.95001 (+); 160.9338 (-)

Smiles: C(#N)C(C(=O)N)Br

Confidence level: 1

Molecular structure:

![Molecular structure of 2-bromo-2-cyanoacetamide](image)

Reasoning: Confirmation with reference standard

MS/MS:
Text S3 – Detailed info about TP2-dibromopropanediamide

Name: 2,2-dibromopropanediamide

Origin: Direct transformation product of DBNPA

Formula: C₃H₄Br₂N₂O₂

m/z: 258.8714 (+)

Smiles: C(=O)(C(=O)N)(Br)BrN

Confidence level: 1

Molecular structure:

![Molecular structure of 2,2-dibromopropanediamide]

Reasoning: Confirmation with reference standard

MS/MS:
Figure S4. Peak intensities of DBNPA transformation products at $t = 1$ in the dark.
Figure S5. Degradation pathways of DBNPA in the dark.
Text S4 – Detailed info about TP3-dibromoacetamide

Name: 2,2-dibromoacetamide
Origin: Direct transformation product of DBNPA
Formula: $\text{C}_2\text{H}_3\text{Br}_2\text{NO}$
$m/z$: 215.86482 (+); 213.85049 (-)
Smiles: $\text{C}(=\text{O})\text{(C(=\text{O})\text{N}(\text{Br})\text{Br})N}$
Confidence level: 1

Molecular structure:

![Molecular structure diagram]

Reasoning: Confirmation with reference standard

MS/MS:

![MS/MS diagram]
**Text S5. Detailed info about TP4-mz461**

**Name:** -

**Origin:** Direct transformation product of DBNPA

**Formula:** $\text{C}_{19}\text{H}_{37}\text{N}_{7}\text{O}_{6}$

**m/z:** 460.5483 (+)

**Smiles:** -

**Confidence level:** 4

**Molecular structure:**

No molecular structure was proposed

**Reasoning:** -

**MS/MS:**

![MS/MS Diagram]
Text S6. Detailed info about TP5-mz151

Name: 

Origin: Photodegradation product of DBNPA

Formula: \( \text{C}_2\text{N}_7\text{O}_2 \)

\( \text{m/z}: \) 153.0043 (-)

Smiles: 

Confidence level: 4

Molecular structure:

No molecular structure was proposed

Reasoning: 

MS/MS:

![MS/MS spectrum](image-url)
Text S7. Detailed info about TP6-mz163

Name: -
Origin: Photodegradation product of DBNPA
Formula: C₂HBrN₂O₂
m/z: 162.9137 (-)
Smiles: -
Confidence level: 4

Molecular structure:

No molecular structure was proposed

Reasoning: -

MS/MS:
Text S8. Detailed info about TP7-mz186

Name: -

Origin: Photodegradation product of DBNPA

Formula: C₈H₅N₅O

m/z: 186.0409 (-)

Smiles: -

Confidence level: 4

Molecular structure:

No molecular structure was proposed

Reasoning: -

MS/MS:
Detailed info about TP8-mz148

Name: -
Origin: Photodegradation product of DBNPA
Formula: 148.0146 (-)
m/z: C₆H₃N₃O₂
Smiles: -
Confidence level: 4
Molecular structure: No molecular structure was proposed

Reasoning: -

MS/MS:
Text S10. Detailed info about TP9-mz169

Name: -

Origin: Photodegradation product of DBNPA

Formula: 169.0139 (-)  

m/z: C_7H_6O_5

Smiles: -

Confidence level: 4

Molecular structure:

No molecular structure was proposed

Reasoning: -

MS/MS:
Text S11. Detailed info about TP10-mz173

Name: -

Origin: Photodegradation product of DBNPA

Formula: $C_{10}H_{6}O_{3}$

m/z: 173.0239 (-)

Smiles: -

Confidence level: 4

Molecular structure:

No molecular structure was proposed

Reasoning: -

MS/MS:
Text S12. Detailed info about TP11-mz206 and TP12mz206

Name: -

Origin: Photodegradation product of DBNPA

Formula: \( C_8H_8N_5O_2 \)

\( m/z: \) 206.0666 (+)

Smiles: -

Confidence level: 4

Molecular structure:

No molecular structure was proposed

Reasoning: -

MS/MS: 

![MS/MS spectrum](image-url)
Detailed info about TP13-mz344

Name: -

Origin: Photodegradation product of DBNPA

Formula: \( C_{12}H_{21}N_7O_5 \)

\( m/z: 344.1688 (+) \)

Smiles: -

Confidence level: 5

Molecular structure:

No molecular structure was proposed

Reasoning: -

MS/MS:

No MS/MS was available
Text S14. Detailed info about TP14-mz170

Name: -

Origin: Photodegradation product of DBNPA

Formula: \( \text{C}_6\text{H}_7\text{N}_3\text{O}_3 \)

\( m/z: \) 170.0559 (+); 168.0411 (-)

Smiles: -

Confidence level: 4

Molecular structure:

No molecular structure was proposed

Reasoning: -

MS/MS:
Text S15. Detailed info about GiP1

Name: -
Origin: Interaction product between DBNPA and glutaraldehyde
Formula: $\text{C}_6\text{H}_9\text{NO}_2$
m/z: 128.07079 (+)
Smiles: -
Confidence level: 5
Molecular structure: No molecular structure was proposed
Reasoning: -

MS/MS:
Text S16. Detailed info about GiP2

Name: -

Origin: Interaction product between DBNPA and glutaraldehyde

Formula: $C_8H_{10}N_2O_3$

$m/z$: 181.06119 (-), 183.07633 (+)

Smiles: -

Confidence level: 3

Molecular structure:

![Molecular structure diagram]

Reasoning: Molecular formula; knowledge on parent compounds formula and structure; knowledge on parent compound’s chemical behaviour. Wagner et al. 2019b

MS/MS:
Text S17. Detailed info about GIP3

Name: 

Origin: Interaction product between DBNPA and glutaraldehyde

Formula: C_8H_{12}N_2O_3

m/z: 183.07678 (-), 185.09193 (+)

Smiles: 

Confidence level: 5

Molecular structure: 

No molecular structure was proposed

Reasoning: 

MS/MS:
Text S18. Detailed info about GiP4

Name: -

Origin: Interaction product between DBNPA and glutaraldehyde

Formula: C_{11}H_{12}N_{4}O_{4}

m/z: 263.0777 (-), 265.0931 (+)

Smiles: -

Confidence level: 3

Molecular structure:

![Molecular structure diagram](image)

Reasoning: Molecular formula; knowledge on parent compounds formula and structure; knowledge on parent compound’s chemical behaviour

MS/MS:
Text S19. Detailed info about GIP5

Name: -

Origin: Interaction product between DBNPA and glutaraldehyde

Formula: $C_{17}H_{30}N_{4}O_{6}$

m/z: 385.20753 (-)

Smiles: -

Confidence level: 5

Molecular structure:

No molecular structure was proposed

Reasoning: -

MS/MS:
Figure S6. Peak intensity of GIP2 (fragment m/z 183.07633) in this study and in Wagner et al. (2019b)
Name: 

Origin: Interaction product between DBNPA and glutaraldehyde

Formula: $\text{C}_{11}\text{H}_{11}\text{N}_{3}\text{O}_{5}$

m/z: 264.06206 (-)

Smiles: 

Confidence level: 5

Molecular structure:

No molecular structure was proposed

Reasoning: 

MS/MS: