Supporting Information (SI)

Enclosure design for flock-level, chronic exposure of birds to air contaminant mixtures

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Alberta Centre for Toxicology Methods for Analysing Passive Samplers

These method summaries are included with permission from the Alberta Centre for Toxicology (ACFT), and include preparation, deployment, and methods of analysing Ogawa samplers (NO_2 and SO_2, Ogawa, USA) and 3M VOC samplers (Organic Vapor Diffusion Monitor 3500, 3M^{TM}, USA), that were used during the research detailed in this thesis. O_3 is included in the methods because it forms part of the ACFT methods, but is not relevant to this thesis. Sampler assembly is per manufacturer instructions and not included here.

SI.1. Ogawa Air Sampler Method Summary - SO_2, NO_2 and O_3

Figure SI.1. Ogawa passive samplers used to measure NO_2 and SO_2 in this study

SI.1.1. Preparation and use procedure

To prepare the blank monitor to use:

- A blank monitor should be included with each set of samples to check for any contamination of the samples. The blank should be opened when the sample is opened.
- Remove a Sampler ID# label and place it on the "Passive Samplers – Log Sheet for Deployment" form enclosed with the monitor.
- Remove the monitor from the brown storage bottle and the re-sealable bag, and then immediately place back in the bag and in the bottle.
- Close the storage bottle with the lid provided.
- Store the blank monitor refrigerated (4℃) while other monitors are being deployed.

To prepare the monitor for use:

- Remove a Sampler ID# label and place it on the "Passive Samplers – Log Sheet for Deployment" form.
- Record the following information on the form "Passive Samplers – Log Sheet for Deployment":
  - Participant ID#
  - Sampling Start Date
  - Comments (e.g., temperature, relative humidity, rain, etc.)
- Remove the sampler from the re-sealable bag. Secure the sampler in the desired location.
  - As a personal monitor, it should be worn near the worker's breathing zone (e.g., front of the shirt collar).
  - As an area monitor, hang it away from walls, corners, table tops or other regions where air movement in the area may be limited.
- Replace the re-sealable bag in the brown storage bottle and tighten the cap securely. Retain it for later use.

To prepare the monitor at the end of sampling:

- Remove the sampler from the sampling location and place it in the re-sealable bag, then in the brown storage bottle, and tighten the cap securely.
- Record the Sampling End Date and Sampling End Time. Ensure the "Passive Samplers – Log Sheet for Deployment" form is filled out appropriately.
- Transport the monitor(s), the blank and the completed form to ACFT within 48 hours. Store the monitors in refrigerator (4℃) if delay is anticipated

SI.1.2. Laboratory analysis

Adapted from: F14027 Ogawa Air Sampler Method Summary Version 140505

Introduction:

Alberta Centre for Toxicology (ACFT) purchases the Ambient Air Passive Air Samplers and Collection Pads for SO_2, NO_2, and O_3 from the Ogawa & Co. ACFT washes all the reusable sampler parts and assembles the pads into the samplers. Exposed samplers are analyzed at ACFT or RTI International (3040 Cornwallis Road, Bldg 6, RTP, NC 27709).

Air Sampler Extraction at (ACFT):

SO_2 Samplers: Each sampler is disassembled and the pad is removed from the holder. The pad is placed in a culture tube. A volume of 5 mL of extraction solvent (Type I water with 2 μg/mL HPO_4^{2-} as internal standard) is added to the tube using a Brinkman bottle top dispenser. The culture tube is then capped and inverted every 5 minutes for 30 minutes. After 30 minutes, 60 μL of 30% hydrogen peroxide is pipetted into the culture tube. The tube is inverted slowly every half an hour for 2 hours. Two mL of the extract is transferred into a Dionex IC vial, capped, and injected on the Dionex IC along with a set of calibrators and controls.

NO_2 Samplers: Each sampler is disassembled and the pad is removed from the holder. The pad is placed in a culture tube. A volume of 8 mL of extraction solvent (Type I water with 2 μg/mL HPO_4^{2-} as internal standard) is added to the tube using a Brinkman

bottle top dispenser. The culture tube is capped and placed on a tube rocker to shake for 15 minutes. Approximately 1.5 mL of the extract is transferred into a Dionex IC vial, capped, and injected on the Dionex IC along with a set of calibrators and controls.

O_3 Samplers: Each sampler is disassembled and the two pads are removed from the holder. The pads are placed in a culture tube. A volume of 5 mL of extraction solvent (Type I water with 2 µg/mL HPO_4^{2-} as internal standard) is added to the tube using a Brinkman bottle top dispenser. The culture tube is capped and placed on a tube rocker to shake for 15 minutes. Approximately 1.5 mL of the extract is transferred into a Dionex IC vial, capped, and injected on the Dionex IC along with a set of calibrators and controls.

Analysis of Ogawa Air Sampler Extractions at ACFT:

Ion Chromatography in conjunction with chemical suppression and conductivity detection is used for the separation and determination of the anions in Passive Air Samplers. The system used is a Dionex DX500 Ion Chromatograph comprised of the following: a Dionex LC25 chromatography cven, a Dionex AS40 automated sampler, a Dionex CD20 conductivity detector, a Dionex GP40 gradient pump, as well as a Metrohm 753 suppressor module.

Separation of the anions is performed on a Dionex IonPac AS12A analytical column (4 x 200 mm) using a Dionex IonPac AG12A guard column (4 x 50 mm). The mobile phase consists of 2.7 mM sodium carbonate (Na_2CO_3) and 0.3 mM sodium bicarbonate ($NaHCO_3$), which is filtered through a 0.22 µm filter. 100 µL of sample is injected onto the IC column with a flow rate of 1.5 mL/min. Data is collected over an 11 minutes run time. The anions are identified according to their retention times and elution order. An internal calibration is used to determine the anion concentrations.

The anions detected are (in order of elution):

1. Nitrite (NO_2^-): for the NO_2 Passive Samplers
2. Nitrate (NO_3^-): for the O_3 Passive Samplers
3. Sulfate (SO_4^{2-}): for the SO_2 Passive Samplers

SI.1.3. Data Analysis at ACFT

The results are collected as a concentration in μg/mL and converted into ng/sampler by multiplying the concentration measured by the amount of extraction solvent used in the extraction and a conversion factor of 1000 to give concentrations in ng/sampler.

- For SO_2, SO_4 concentration (ng/sampler) = SO_4 (μg/mL) * 5 (mL) * 1000
- For NO_2, NO_2 concentration (ng/sampler) = NO_2 (μg/mL) * 8 (mL) * 1000
- For O_3, NO_3 concentration (ng/sampler) = NO_3 (μg/mL) * 5 (mL) * 1000

LOQ: The limit of quantitation for this method is 0.1 μg/mL for Nitrite (NO_2^-), Nitrate (NO_3^-), and Sulfate (SO_4^{2-}). Value below 0.1 μg/mL will be reported as information only for research study.

Linearity: This method is linear up to 50 μg/mL for the anions. Dilutions are performed on any sample with a concentration greater than 50 μg/mL.

SI.1.4. Data Analysis at RIT International

The equations for calculating concentration in ppb (by volume) are based on the following protocols from the Ogawa website www.ogawausa.com and they are attached as references with this summary:

1. NO, NO_2, NO_X AND SO_2 SAMPLING PROTOCOL USING THE OGAWA SAMPLER (PAGE 23, 28)
2. PROTOCOL FOR OZONE MEASUREMENT USING THE OZONE PASSIVE SAMPLER BADGE (PAGE 14)

From protocol 1 (page 23), SO_2 concentration (ppb) = αSO_2 * WSO_2 / t

where:

- WSO_2 is the sulfate quantity (in ng) collected on the SO_2 sampler multiplying the molecular weight of SO_2 and divided by molecular weight of SO_4.
- $WSO_2 = WSO_4$ (in ng) * 64.062/96.06 (Molecular weight of SO_2 = 64.062 g/mole; Molecular weight of SO_4 = 96.06 g/mole)
- αSO_2 is the ppb concentration conversion coefficients (ppb-min/ng). At 20℃ the value of $\alpha SO_2 = 39$.
- t is sample collection time in minutes.

From protocol 1 (page 28), NO_2 concentration (ppb) = αNO_2 * WNO_2 / t

where:

- WNO_2 is the nitrite quantity (in ng) collected on the NO_2 sampler,
- αNO_2 is the ppb concentration conversion coefficients (ppb-min/ng). At 20℃ the value of $\alpha NO_2 = 56$.
- t is sample collection time in minutes.

From protocol 2 (page 14), O_3 concentration (ppb) = (18.09) * 1000 * WNO_3 / t

where:

- WNO_3 is the nitrate quantity (in μg) collected on the O_3 sampler,
- 18.09 is the conversion coefficient from NO_3 to O_3. Derivation of this coefficient is detailed on page 14.
- "1000" is the conversion factor from ppm to ppb.
- t is sample collection time in minutes.

SI.2. Volatile Organic Compounds (VOC) in Air Sampler Method Summary

3M^{TM} Organic Vapor Diffusion Monitor 3500 (Figure A-2)

Figure SI.2. 3M^{TM} Organic Vapor Diffusion Monitor 3500 used for measuring VOC concentrations in this study

SI.2.1. Preparation and use procedure

To prepare the blank monitor to use:

- A blank monitor should be included with each set of samples to check for any contamination of the samples. The blank should be opened when the sample is opened.
- Remove a Sampler ID# label and place it on the back of the monitor.
- Remove another Sampler ID# label and place it on the "Passive Samplers – Log Sheet for Deployment" form enclosed with the monitor.
- Remove the plastic ring and white film from the monitor. Immediately snap the elution cap (with plugs) onto the main monitor body. Be sure the two port plugs are secured.
- Return the blank monitor into the can and close the can with the plastic lid provided.
- Store the blank monitor refrigerated (4℃) while other monitors are being deployed.

To prepare the monitor for use:

- Remove a Sampler ID# label and place it on the back of the monitor.
- Remove another Sampler ID# label and place it on the "Passive Samplers – Log Sheet for Deployment" form enclosed with the monitor.
- Record the following information on the form "Passive Samplers – Log Sheet for Deployment":
    - Participant ID#
    - Sampling Start Date
    - Comments (e.g. temperature, relative humidity, rain, etc.)
- DO NOT remove the white film and plastic ring. Secure the monitor in the desired location.
- As a personal monitor, it should be worn near the worker's breathing zone (e.g. front of the shirt collar).
- As an area monitor, hang it away from walls, corners, table tops or other regions where air movement in the area may be limited.
- Close the can with the plastic lid provided. Retain it for later use.

To prepare the monitor at the end of sampling:

- Remove the plastic ring and white film from the monitor. Immediately snap the elution cap (with plugs) onto the main monitor body. Be sure the two port plugs are secured. Return the monitor to the can. Close the can with the plastic lid provided.
- Record the Sampling End Date and Sampling End Time. Ensure the "Passive Samplers – Log Sheet for Deployment" form is filled out appropriately.
- Transport the monitor(s), the blank and the completed form to ACFT within 48 hours. Store the monitors in refrigerator (4℃) if delay is anticipated.

SI.2.2. Laboratory analysis

VOC Sample Extraction:

Each passive monitor is filled with 1.5 mL of carbon disulfide solvent and allowed to sit for 30 minutes. The carbon disulfide is then transferred from the badge into a glass vial and securely capped.

Analysis of VOCs:

The samples are injected onto a gas chromatograph (GC) for compound separation followed by detection by a mass spectrometer (MS). The system used is a Hewlett-Packard GC-6890/MS-5973. Separation is performed on an HP 19091V-402 capillary column, 25m x 200µm x 1.12µm at an oven temperature range of 40°C - 140°C. Total run time is 17.5 minutes and sample injection volume is 1µL.

The following compounds are detected:

- Hexane
- 3-Methylhexane
- Benzene
- Heptane
- Toluene
- Octane
- Ethylbenzene
- m,p-Xylenes
- Nonane
- o-Xylene
- n-Propylbenzene
- Decane
- Limonene
- n-Butylbenzene

SI.2.3. Data analysis

Calculations: The GC/MS gives VOC concentrations in µg/mL. These concentrations are multiplied by the extraction volume of 1.5 mL and a conversion factor of 1000 to give concentrations in ng/badge.

The formula for converting ng/badge to µg/m^3 is

$$\text{Analyte } (\mu g/m^3) = \frac{\text{Analyte concentration (ng/badge) x 1000}}{\text{Time exposed (min) x Sampling Rate (mL/min)}}$$

Where Time exposed for a 7 day period = 10080 min

Compound	Sampling Rate from 3M Manufacturer (mL/min)	LOQ (ng/badge)	LOQ for 7-day Exposed Period (ug/m^3)
Hexane	32.0	150	0.47
Methylhexane	28.9	150	0.51
Benzene	35.5	150	0.42
Heptane	28.9	150	0.51
Toluene	31.4	150	0.47
Octane	26.6	150	0.56
Ethylbenzene	27.3	150	0.55
m,p-Xylenes	27.3	300	1.09
o-Xylene	27.3	150	0.55
Nonane	24.6	150	0.60
Decane	23.1	150	0.64
Limonene	21.9	150	0.68
N-Propylbenzene	24.6	150	0.60
N-Butylbenzene	22.4	150	0.66

LOQ: The limit of quantitation for this method is 0.2µg/mL for *m,p*-Xylenes and 0.1 µg/mL for the remaining compounds. Any value below 0.1µg/mL is reported as 0 µg/mL. The method detection limit for *m,p*-Xylenes is 300 ng/sampler and 150 ng/sampler for the remaining VOC's.

Linearity: This method is linear up to 50 µg/mL for all compounds. Dilutions are performed on any sample with a concentration greater than 50 µg/mL.