ACCUMULATION OF FINE PARTICLES ONTO THREE CONIFEROUS SPECIES

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ABSTRACT

ROBERT L. BUSH, JR.: Accumulation of fine particles onto three coniferous species. (Under the direction of Dr. David Leith)

There is concern that airborne contaminants generated by applying sewage sludge to land for agricultural purposes may pose significant health risks to nearby residents. Polybrominated diphenyl ethers (PBDEs) have been shown to be both a persistent class of organic chemicals in sewage sludge and to accumulate in the epicuticular wax of coniferous vegetation. Thus, local coniferous species may passively sample PBDEs and provide information related to the atmospheric dispersion of contaminants associated with land-applied sewage sludge. This work uses a controlled exposure chamber to simultaneously subject three conifer species (loblolly pine, short leaf pine and eastern red cedar) native to central North Carolina to a Rhodamine-6G aerosol, which acts as a surrogate for particle-bound PBDEs. Accumulation as a function of exposure for each species is evaluated. Findings suggest that, to normalize particle accumulation across the three species, a factor of 1.35 should be applied to cedar results reported in ng/g. Additionally, the ratio of projected area to mass is used to explain interspecies variations. This information is useful to studies that employ local vegetation as passive samplers of particle-bound contaminants such as those evaluating community exposures to land-applied sewage sludge.

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This thesis is dedicated to my mother, Carol Baysinger, for giving me the opportunity to pursue my ambitions freely and for teaching me the meaning of responsibility. I truly would not be in the position I am today without her love and guidance, which she has given since I can remember.

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LIST OF ABREVIATIONS AND SYMBOLS

BDE-209	brominated diphenyl ether congener 209
β	beta coefficient
C_p	particle concentration (g/cm^3)
CPC	condensation particle counter
D_s	diameter of sampling probe (cm)
g	gravitational constant (cm/s^2)
GSD	geometric standard deviation (cm)
HEPA	high-efficiency particulate air
ID	inside diameter (cm)
K_e	erosion rate constant
K_{oa}	octanol-air partition coefficient
LPM	liters per minute
MDL	method detection limit
MMD	mass median diameter (cm)
PBDE	polybrominated diphenyl ether
Q	volumetric flow rate (cm^3/s)
R6G	rhodamine-6G
SMPS	scanning mobility particle sizer
SVOC	semi-volatile organic compound
POPs	persistent organic pollutants
QMA	quartz microfiber-A

v_p	particle deposition velocity (cm/s)
WWTP	wastewater treatment plant
τ	particle relaxation time (s)

I. INTRODUCTION

The United States Environmental Protection Agency (U.S. EPA) defines sewage sludge as the solid, semi-solid, or liquid residue generated during the treatment of domestic sewage in a wastewater treatment plant (WWTP) (U.S. EPA, 1993). Pollutant limits, management practices, and standards associated with the disposal of sewage sludge are regulated by EPA under Title 40 Code of Federal Regulations, Part 503. Accordingly, sewage sludge may be applied to land, disposed on a surface site such as a landfill, or burned in an incinerator. Government officials consider landfills to be a misuse of valuable real estate and incineration to be an expensive option that requires extensive engineering controls to manage regulated air toxics (Hale, 2001; U.S. EPA, 1999). Therefore, land-application of sewage sludge has become the most widespread method for disposal.

The high content of organic and inorganic plant nutrients in the treated sludge fertilizes soils, increases crop yields, and provides economic incentives and savings to local farmers (NRC, 2002). In 2004, an estimated 7.2 million dry tons of sewage sludge were generated in the US, of which 55% were either directly applied to land or stored for future land-applications. Of the fraction applied to land, 74% was spread on farmland for agricultural purposes (NEBRA, 2007). Land-application is clearly the most prevalent method of sludge disposal and its use will probably increase as surface sites decline and air emissions standards become more stringent.

The chemical and biological contents of sewage sludge are dependent on the influent sources of the WWTP, which in addition to residential waste may include waste from various industries, commercial establishments, storm water run-offs from roads and agricultural lands, and groundwater infiltration (NRC, 2002). The Part 503 rule establishes pollutant limits for nine toxic metals (arsenic, cadmium, copper, lead, mercury, molybdenum, nickel, selenium, zinc), and pathogen requirements based on two bacterial indicators (*Salmonella spp*., and fecal coliform), both of which are easily removed by standard treatment processes (U.S. EPA, 1993; Snyder, 2005). However, the current criteria for monitoring total pathogen content underestimate the infectious risk to the public from emerging pathogens, especially norovirus and adenovirus (Viau et al., 2011).

Other studies have identified significant levels of endotoxin in soil and in airborne particulates generated during land-applications of sludge (Brooks et al., 2007; Paez-Rubio et al., 2007). Toxic organic chemicals that are unregulated by the Part 503 rule have also been detected in sewage sludge. These chemicals include a multitude of persistent organic pollutants (POPs), flame retardants, personal care products, pharmaceuticals, pesticides, plasticizers, and surfactants (Harrison et al., 2006). Because the toxicological and pathogenic profiles of the unregulated chemicals and viruses in land-applied sludge are unknown, the dispersal of sludge on farmlands presents an important public health concern.

Aside from direct incidental ingestion, inhalation of particulate matter generated from application processes poses the greatest threat to public health (Pillai and Ricke, 2002; Viau et al., 2011). However to date, there have been limited community-based epidemiological studies investigating the health effects associated with airborne contaminants generated by land applications of sewage sludge. A 2007 survey of 437 residents residing within one mile

of permitted application fields in Ohio reported significantly elevated symptoms of excessive tearing, abdominal bloating, jaundice, skin ulcers, dehydration, weight loss, and general weakness compared to the control population (Khuder et al., 2007). The researchers also found the incidence of bronchitis, upper respiratory infection, and giardiasis was significantly more prevalent in the exposed population. However, numerous study limitations are acknowledged by the authors that reduce the power of these results. The majority of evidence related to human health effects comes from community members who cite land-applied sewage sludge as the source of malodors, various health impairments, and livestock losses (Clap and Orlando, 2003; Harrison and Oakes, 2002). The limited research on land-applied sewage sludge, with a particular focus on aerosol transport, emerging pathogens, and community-based epidemiological studies, demonstrates the need for risk-assessment and quantitative exposure assessment studies.

A persistent class of chemicals found in sewage sludge includes polybrominated diphenyl ethers, or PBDEs (Hale et al., 2001, Öberg et al., 2002). PBDEs are semi-volatile organic compounds (SVOCs) commonly used as flame retardants in textiles, electrical appliances, circuit boards, building materials, and polyurethane foams (IPCS, 1994). A total of 209 individual chemical congeners are classified by their chemical structure and degree of bromination, with volatility generally decreasing with increasing bromination (Harner and Shoeib, 2002). In addition to sewage sludge, PBDEs have been identified in freshwaters, soil, household dust, biological samples, and indoor and outdoor air (Harrad et al., 2006; Johnson and Olson, 2001; Oros et al., 2005; Wang et al., 2005; Wilford et al., 2004).

Due to their extreme lipophilicity, PBDEs in ambient air readily partition to the epicuticular wax of coniferous vegetation (Kylin, 1996), which is composed of various long

chain organic molecules such as esters, polyesters, and paraffins (Tulloch, 1976). The subsequent contaminant accumulation depends on the octanol-air partition coefficient (K_{oa})—a measure of a contaminant's affinity for lipids—and the lipid content and specific surface area of the plant species (Simonich and Hites, 1995). Coniferous vegetation has thus been used to monitor the spatial distribution of PBDEs in the atmosphere, and to assess the dispersive patterns associated with site-specific releases (St. Amand et al., 2008; Zhao et al., 2009).

PBDEs are deposited from the atmosphere to the foliar surface by gas-phase and/or particle-bound deposition. Researchers have found highly significant correlations between log K_{oa} values and a given congener's preference for either the particle or the gas phase. St. Amand et al. (2008) concluded that, on average, PBDE congeners with log K_{oa} above 11 were found sorbed to particles, while congeners with log K_{oa} below 11 were found in the gas-phase. Consequently, high volatility congeners (log K_{oa}<11) have greater potential for long-range atmospheric transport, while less volatile congeners (log K_{oa}>11) are more indicative of local sources, because their atmospheric removal is determined by the deposition processes of their bound particles (Gouin et al., 2004). Field studies have shown that PBDE congener 209 (BDE-209) with its log K_{oa} of 15 binds strongly to aerosol particles in ambient air (St. Amand et al., 2008; Gouin et al., 2004). Consequently, BDE-209 has reduced potential for long-range atmospheric transport and increased potential as a monitor of local source pollution by deposition onto nearby foliar surfaces.

In theory, coniferous vegetation could be a valuable exposure assessor when studying the health implications associated with land-applications of sewage sludge. Foliage adjacent to permitted application sites could adsorb the atmospheric release of BDE-209 present in the

sewage sludge. By sampling and analyzing the foliage for BDE-209, information that correlates with community exposure could be revealed. To improve the geographic distribution of pine samplers, it is favorable to use all coniferous species native to the study region. Although mechanisms for interspecies variations in accumulation have been suggested (*i.e.*, specific surface area and lipid content), a need exists to quantitate these differences through experimentation.

Thus, the aims of this work were: (1) to evaluate the accumulation of a BDE-209 surrogate aerosol to the foliage of three coniferous species native to central North Carolina and (2) to evaluate the relationship between surrogate accumulation and projected area of the foliage.

II. EXPERIMENTAL METHODS

Study design

Extraction of PBDEs from foliage is expensive, time-consuming, and requires advanced instrumentation and analytical procedures. Thus, an easily quantifiable marker was desired to serve as a proxy for exposure and accumulation of particle-bound BDE-209. Rhodamine-6G (R6G), a solid fluorescent organic dye commonly employed in water flow studies, was selected as the surrogate for BDE-209. After exposure and extraction, concentration of R6G on the needles could be easily measured using basic fluorescence spectroscopy.

Conifer species

Loblolly pine (*Pinus taeda),* short leaf pine (*Pinus echinata*), and eastern red cedar (*Juniperus virginiana*) were selected for study due to their prevalence in central North Carolina. Figure 1 illustrates the foliar morphology of each species. Loblolly pines have needles that range from 12-22 cm in length and grow along branches in bundles of three, while short leaf pines have needles that range from 7-11 cm in length and grow in bundles of two. Cedars are morphologically different from loblolly and short leaf pines with needles that range from 2-4 mm in length and grow tightly appressed to twigs, presenting a scale-like appearance.

Figure 1. Botanical sketches of shoots of loblolly pine (left), short leaf pine (center) and cedar (right). *Not drawn to scale. [From: Gleason,1952]*

Design, construction and operation of exposure system

A 75-gallon glass aquarium (1.2m x 0.46m x 0.5m) was adapted as the exposure chamber. Four 0.6 cm ports were bored in the glass top panels for the aerosol inlet, two sampling lines, and an exhaust outlet. Ports for the aerosol inlet and sampling lines were equipped with Swagelok fittings. To support foliage samples, a mesh framework of stainless steel wire was constructed and secured to the inner walls of the chamber by hooks positioned 18 cm above the base panel.

Figure 2 is a schematic of the exposure system. A 1.26 g/L test solution of R6G in methanol was metered to the inlet of a Meinhard type TR-50-A2 glass concentric nebulizer (Meinhard, Golden, CO) using a programmable syringe pump (New Era Pump Systems, Inc., Farmingdale, NY) and 10-mL glass syringe with ID of 10 mm. Test aerosol was nebulized at 30 psig into a 4.0 L drying chamber where it was mixed with dried, HEPA filtered make-up air at 10 LPM. This arrangement allowed adequate time for the solvent to evaporate, leaving behind the residual solid, polydisperse test aerosol.

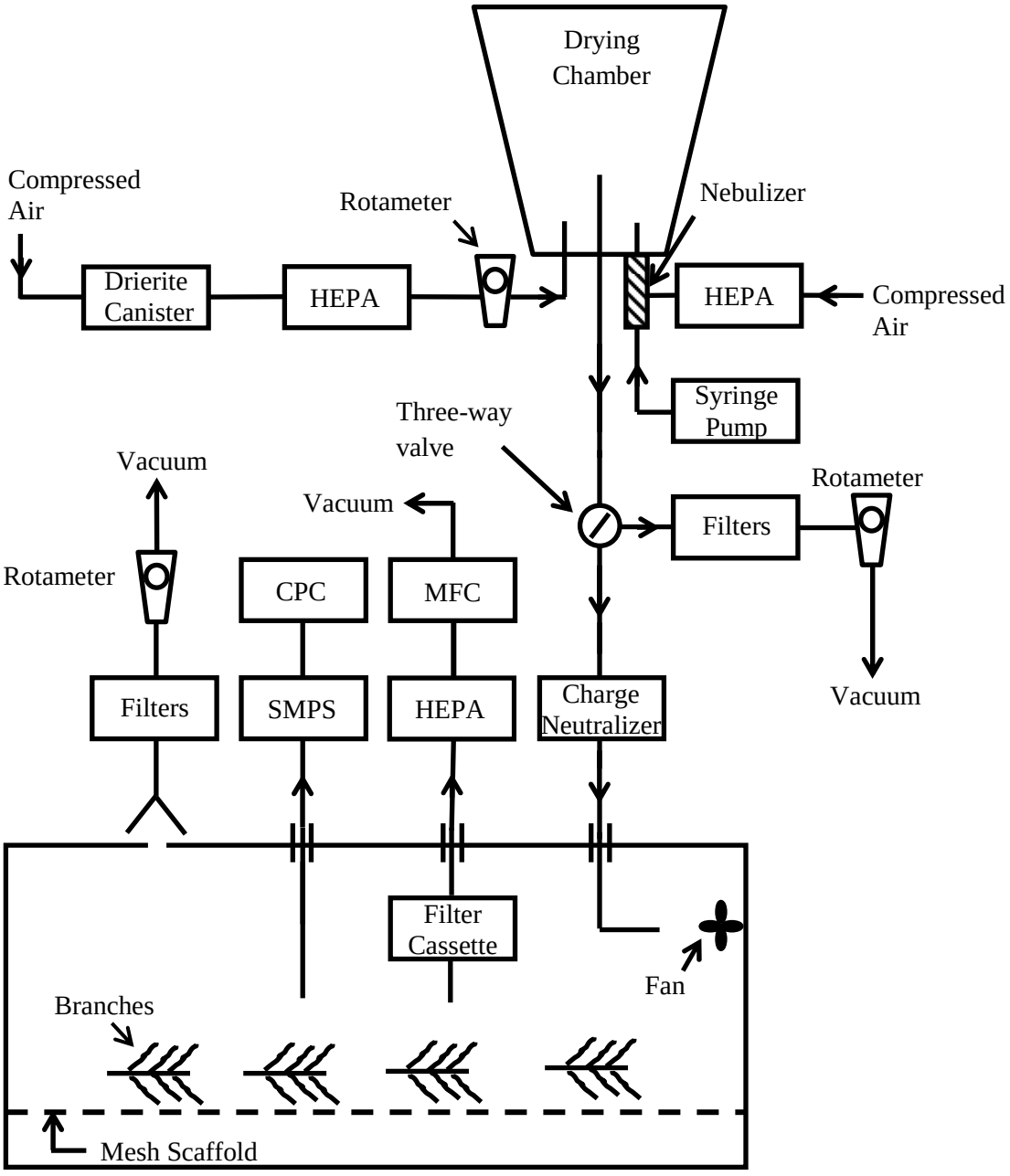


Figure 2. Schematic of the coniferous exposure system. *Not drawn to scale*.

Test aerosol was drawn from the drying chamber to a three-way diversion valve which, when in the open position, prevented the delivery of aerosol to the exposure chamber. Conversely, with the diversion valve in the closed position, test aerosol passed first through a Kr-85 charge neutralizer (TSI Inc., Shoreview, MN) and then to the exposure chamber. To improve mixing in the chamber, the test aerosol was directed toward the eye of a suspended muffin fan. To prevent the release of test aerosol to the laboratory, exhaust air passed through glass fiber filters.

The average mass concentration in the chamber was measured using a 47mm QMA quartz microfiber filter (Whatman, Piscataway, NJ) inside a closed cassette. Filter sampling was conducted at 0.5 LPM using a mass flow controller (MKS Instruments, Andover, MA). A second probe drew sample at 0.3 LPM to a scanning mobility particle sizer (SMPS, TSI Inc., Shoreview, MN) equipped with a condensation particle counter (CPC, TSI Inc., Shoreview, MN), that counted particles by light scattering. These instruments produced particle size distributions at 5-minute intervals. Additional sampling information is found in Appendix B.

Pumping program

The syringe pump delivered precise amounts of the aerosol test solution to the nebulizer using the pumping program shown in Table 1. This program established a 95-minute exposure cycle that included a 5-minute aerosol generation interval followed by a 90-minute dormancy interval. A 100 μL/min withdrawal phase prevented the aspiration of excess test solution from the nebulizer. The first minute of phase 5 brought the test solution back to the nebulizer tip by replacing the headspace created by the previous withdrawal

function, and the remaining 5-minutes corresponded to the aerosol generation interval. By executing a simple loop function from phases 5 to 7, the cycle could be repeated the desired number of times. For example, beginning the program at phase 1 and executing the specified loop three times would result in a 4-cycle exposure profile totaling 380 minutes.

Table 1. Phases and functions of the pumping program.

Phase	Command	Time (min)
1	Inject at 100 μL/min	10
2	Withdraw at 100 μL/min	1
3	Pause	88
4	Begin loop	-
5	Inject at 100 μL/min	6
6	Withdraw at 100 μL/min	1
7	Pause	88
8	End loop	-
9	Repeat loop	-

Exposure experiments

Five exposures were conducted in a logarithmic time progression as follows: 1-cycle, 2-cycle, 4-cycle, 8-cycle, and 16-cycle, where each cycle lasted 95 minutes as described above. Two additional exposures were also performed: a 4-cycle replicate exposure and a 4-cycle blank exposure, in which only solvent was nebulized. The purpose of the logarithmic progression was to generate a dataset favorable for statistical analysis while weighting the frequency of experiments for lower levels of exposure. The seven exposures were performed in random sequence.

On the morning of each exposure, samples of loblolly pine, short leaf pine, and cedar were taken from a remote site. Preliminary samples collected and analyzed from this site

suggested no prior contamination with PBDEs. For each species, 4 to 8 branches of approximately 30 cm were cut from three trees using hexane rinsed shears and placed in a cardboard box for transport.

At the laboratory, branches were sectioned into approximately 15-cm lengths and randomly distributed atop the wire scaffold in the chamber. The chamber was then purged for 10 minutes prior to aerosol generation. At the conclusion of the exposure, all flows were terminated, the filter cassette was removed, and all 4 ports were sealed. Exposed samples were equilibrated in the sealed chamber for approximately 24 hours before analysis. All internal surfaces of the chamber were then cleaned and dried prior to subsequent exposures.

R6G extraction/filtration

After equilibration, the exposed branches were placed on hexane-rinsed aluminum foil. Needles from each species were clipped using hexane-rinsed scissors while the woody stems and branches were discarded. A 5-g random sample of each species was then drawn from the population of exposed needles, cut into 1-cm sections, and placed in a 400-mL beaker. The needles were washed and agitated three times with 50-, 30-, and 15-ml methanol; after each rinse, the liquid was decanted into a 100-mL volumetric flask. After the final rinse, the flask was brought to volume with methanol and stored at 4°C until analysis.

For the R6G analysis, filters were triple-sonicated using consecutive 20-mL volumes of methanol, which were decanted to a 150-mL beaker between sonications. The combined fractions were then filtered to a 100-mL volumetric flask using a syringe filter. An additional 40 mL of pure solvent was delivered through the syringe filter to scavenge any R6G residues, and the resulting solution was stored at 4°C until analysis.

Quantification and data analysis

Quantification of all R6G extracts was carried out using a Turner Quantech digital filter fluorometer (Barnstead/Thermolyne, Dubuque, IA) operated in raw fluorescence mode. The instrument was equipped with light filters specific to the characteristic excitation/emission spectra of R6G. The excitation filter (NB540) permitted only light from the excitation source with a wavelength of 540 nm to irradiate the sample. The emission filter (SC585) permitted detection of only wavelengths emitted from the sample greater than 585 nm.

For quantification purposes, calibration standards of known R6G concentration were prepared in methanol and run before and after the unknown extracts. The calibration was constructed using a linear regression of the fluorescence response of all standards. The fluorescence contribution of the methanol solvent, albeit small, was accounted for in the calibration. By applying the linear calibration equation to the fluorescence response of the instrument and subtracting the fluorescence contribution of extraction blanks, the mass of R6G in the extract was determined. Further information on quantification procedures including calibration data can be found in Appendix C.

All data were analyzed using JMP 9.0 software (SAS Institute, Inc., Cary, NC). When appropriate, data were $\log_e$ transformed to satisfy assumptions of normality prior to statistical analysis.

Projected area measurement

Ten individual needle samples from each conifer species were weighed and placed on a standard light box. Loblolly and short leaf pine needles were positioned so that their largest cross-sectional dimension was parallel to the surface of a light box as shown in Figure 3. Cedar samples were positioned randomly. Transparent tape was used to secure loblolly and short leaf pine needles to the light box to eliminate twisting along the length of the needles. Images of their silhouettes were captured using a digital camera, and projected areas were calculated using ImageJ software (National Institute of Health, Bethesda, MD).

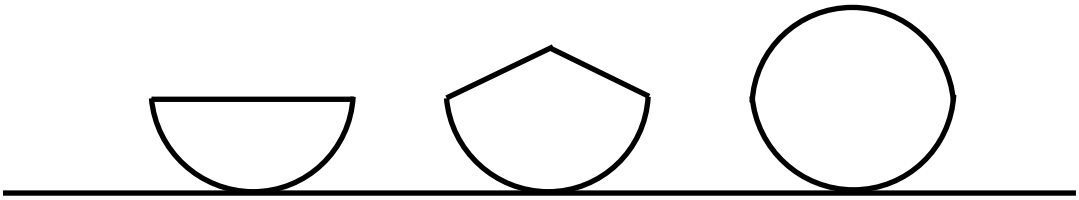


Figure 3. Cross-sectional orientation of needle samples on light box for short leaf pine (left), loblolly pine (center) and cedar (right). *Not drawn to scale.*

III. RESULTS

Figure 4 plots the mass concentration of the test aerosol during the 16-cycle exposure as determined by the SMPS/CPC. Although the amplitudes of each cycle vary slightly between 250 and 375 $\mu g/m^3$, the figure shows the effectiveness of the pumping program to generate a constant, 95-minute cyclic concentration of the test aerosol. The variations in amplitude are likely due to instabilities associated with the compressed air supply that provided the make-up air to the system. Data for the 1, 2, 4, and 8-cycle tests showed similar concentration profiles (Appendix A).

The SMPS also showed that the size distribution of the test aerosol did not fluctuate appreciably throughout the exposures. Figures 5a and 5b plot the progression of the mass median diameter (MMD) and geometric standard deviation (GSD), respectively, over the 1-cycle exposure as measured by the SMPS/CPC. To satisfy normality assumptions, MMD data were log-transformed. Regression analysis showed that the slope of the regression equation for MMD (Figure 5a) is not significantly different from zero ($p < 0.05$), suggesting that the MMD of the aerosol remained constant for each cycle, and particle agglomeration was minimal. Conversely, at the same significance level, the slope of the regression equation for GSD (Figure 5b) was significantly less than zero, indicating that as time progressed, the aerosol became slightly more monodisperse

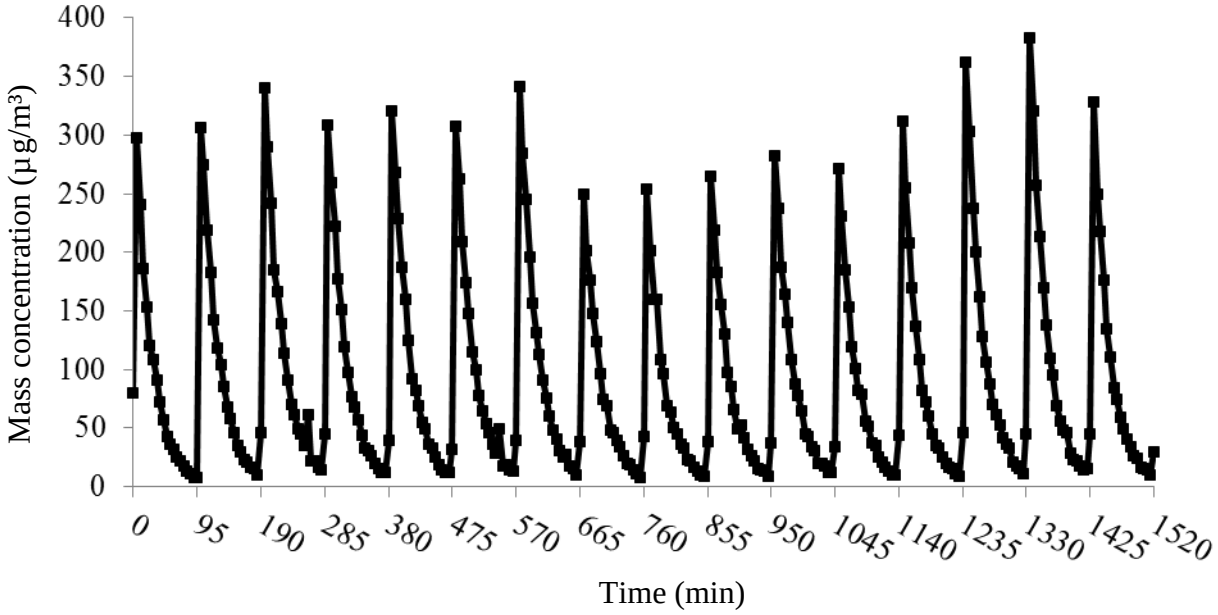


Figure 4. Mass concentrations of the test aerosol reported every 5 minutes by the SMPS for the 16-cycle exposure.

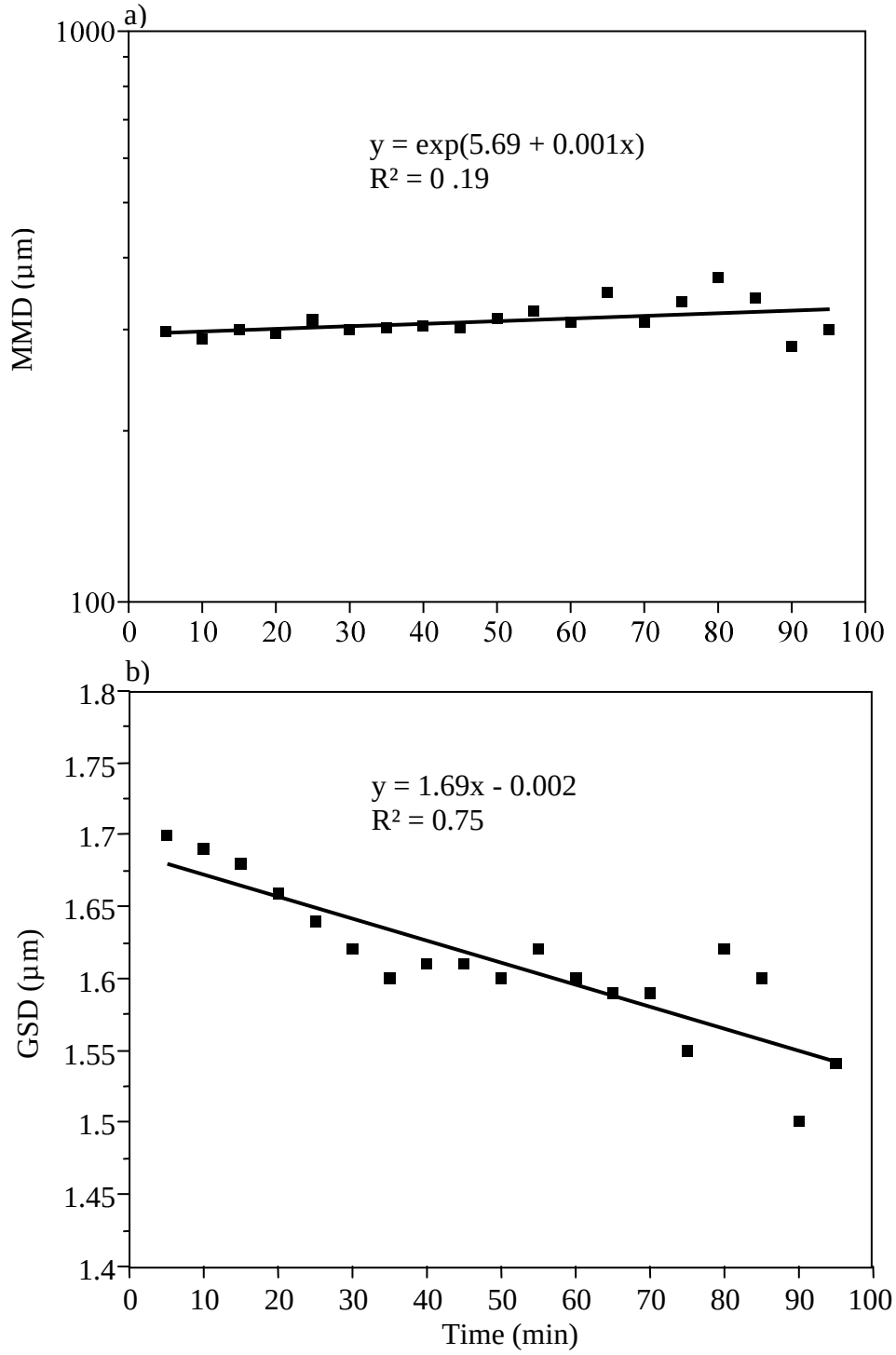


Figure 5. a) MMD and b) GSD of the test aerosol reported every 5 minutes by the SMPS for the 1-cycle exposure.

Table 2 lists time-weighted average mass concentrations and total exposures as determined by filter extraction results. R6G exposures spanned from 0.34 to 7.96 mg·hr /m³ and generally progressed logarithmically. The time-weighted mass concentration of R6G averaged over all experiments was 1.68 µg/m³ with a standard deviation of 0.42 µg/m³, and the coefficient of variation (C_v) was 0.26.

Table 2. Average mass concentrations and exposure estimates determined by filter extractions.

	1-cycle	2-cycle	4-cycle	4-cycle replicate	8-cycle	16-cycle
Average mass concentration (µg/m³)	217	264	398	205	255	314
Exposure (mg·hr/m³)	0.34	0.84	2.52	1.30	3.30	7.96

Figure 6 shows data and the best-fit linear models for measured values of R6G concentration from the needle extracts versus exposure for each species. R^2 values were greater than 0.96 for all species. A three-step covariance analysis was then used to determine statistical differences between the three regression lines. The analysis first investigated differences in slope by testing for interaction between the qualitative species variable and exposure. No significant interaction was found ($p = 0.47$) implying that the slopes of the three regression lines can be assumed equal.

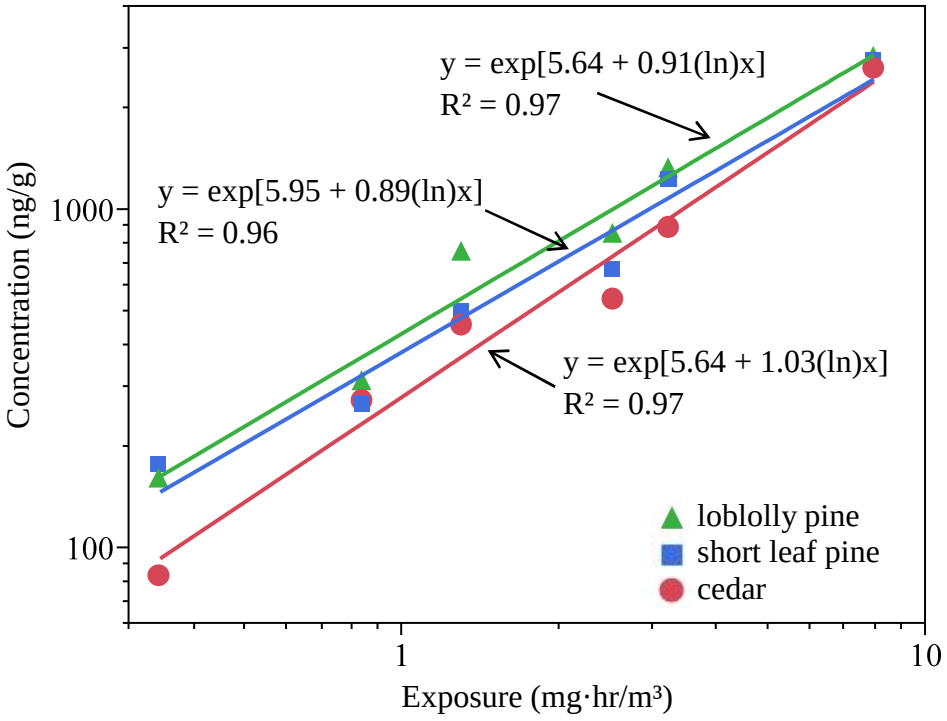


Figure 6. R6G concentration versus exposure for loblolly pine, short leaf pine and cedar.

Next a single, weighted average slope was calculated and an additive multiple regression model was fitted to the log data and qualitative species variable (Figure 7a). The model had an R^2 of 0.97 indicating that 97% of the variability in deposition can be attributed to differences in exposure and species, and that variability due to random experimental error was small. Finally, differences between intercepts were tested. Since the slopes in Figure 7a are assumed equal, differences between intercepts are analogous to differences between means. Therefore, the statistical software evaluates differences between means.

Table 3a gives ratios of mean concentrations, relative percent differences between means, and the state of statistical difference, as predicted by the statistical model. A significant difference was observed between mean R6G concentrations of cedar versus

loblolly pine samples. No significant differences were observed for either loblolly pine versus short leaf pine or cedar versus short leaf pine.

Combining the data for the two pine species and running a second covariance analysis also revealed a significant difference among mean values of the combined pine versus cedar, as shown in Figure 7b ($R^2 = 0.96$) and Table 3a. This model indicated that R6G concentrations of cedar samples were roughly 26% less than those of the combined pine species and that a normalization factor of 1/0.74 = 1.35 should be applied to cedar concentrations to normalize them for comparable exposures.

Results of the projected area measurements are displayed in Table 4. Loblolly pine had the greatest ratio of projected area to mass (2.79 mm^2/mg), followed by short leaf pine (2.32 mm^2/mg) and cedar (1.32 mm^2/mg). T-tests at the $\alpha = 0.05$ significance level revealed significant differences for all species comparisons.

The concentration data for each species were then normalized by their corresponding ratios of projected area to mass resulting in units of pg/mm^2, and log transformed. The effect of the normalization is illustrated in Figure 8 ($R^2 = 0.97$) and Table 3b. Results indicate that normalizing concentration data by the projected area to mass ratio eliminates interspecies variations in R6G deposition. This finding is illustrated in Figure 8 by the convergence of the lines, and in Table 3b by the larger p-values associated with the comparisons and the near unity ratios of the mean concentrations for each comparison. By measuring both the mass of R6G deposited per mass of needle sample, and the ratio of projected area to needle mass, the average exposure can be reasonably estimated for all three coniferous species.

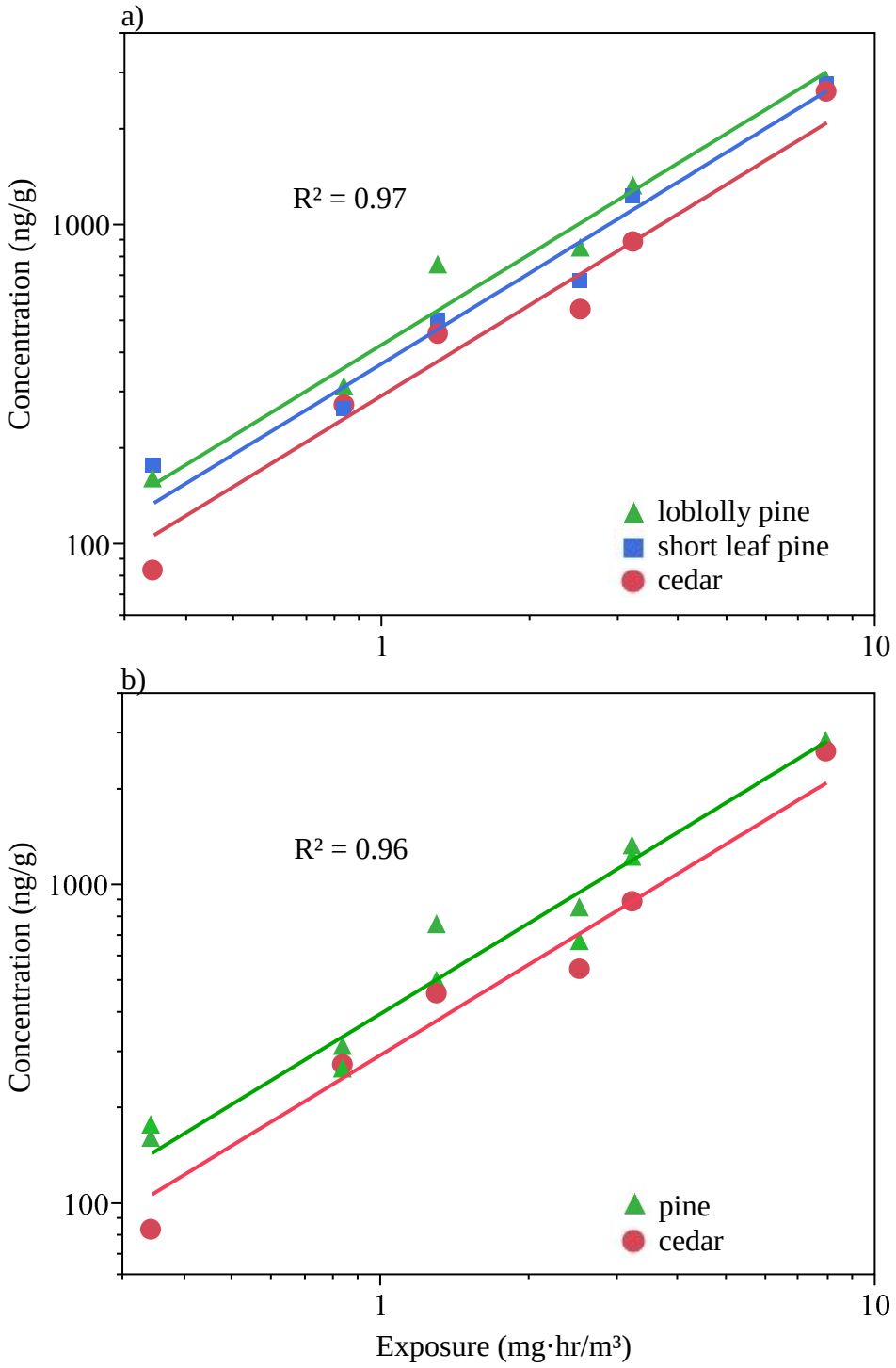


Figure 7. Best fit multiple regression model of R6G concentration on exposure for a) loblolly pine, short leaf pine and cedar and b) the combined pine species (loblolly pine and short leaf pine) and cedar.

Table 3. Ratios of mean R6G concentrations, relative percent differences between mean R6G concentrations, and p-values associated with these differences for: a) non-normalized concentration data (ng/g) and b) concentration data normalized by the ratio of projected area to mass (pg/mm²).

a)

Species comparison	Ratio of mean concentration	Relative % difference	p-value
loblolly pine vs. short leaf pine	1.14	13%	0.50
loblolly pine vs. cedar	1.44	36%	0.02
short leaf pine vs. cedar	1.26	23%	0.16
pine vs. cedar	1.35	30%	0.01

b)

Species comparison	Ratio of mean concentration	Relative % difference	p-value
loblolly pine vs. short leaf pine	0.95	4.6%	0.69
loblolly pine vs. cedar	0.94	5.7%	0.62
short leaf pine vs. cedar	0.99	1.1%	0.92

Table 4. Mean projected area to mass ratios, standard deviations, and 95% confidence intervals for each species (n=10).

Species	Mean projected area/mass (mm²/mg)	Standard deviation (mm²/mg)	Upper 95% confidence interval (mm²/mg)	Lower 95% confidence interval (mm²/mg)
loblolly pine	2.79	0.16	2.89	2.69
short leaf pine	2.32	0.16	2.42	2.22
cedar	1.82	0.07	1.86	1.77

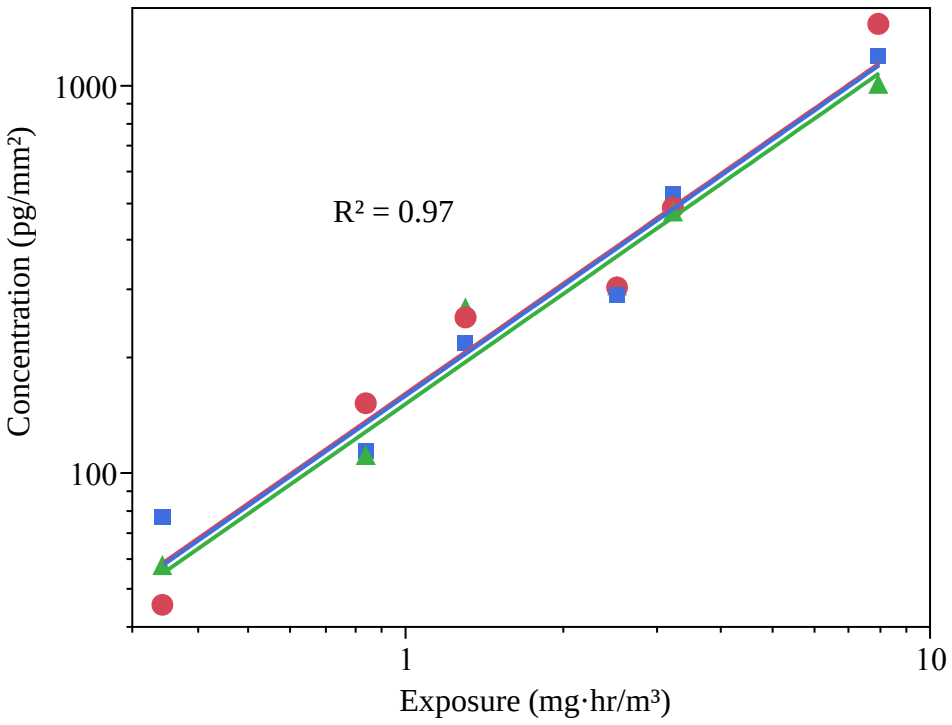


Figure 8. Best fit multiple regression model of R6G concentration on exposure after normalizing each species by its ratio of projected area to mass.

To validate assumptions related to the use of R6G as a surrogate for particle-bound BDE-209, the lipid content of each species was obtained from an external laboratory with advanced equipment and expertise. Table 5 shows the averaged lipid content measurements of the needles. The data show no differences among the three species at the $\alpha = 0.05$ significance level.

Table 5. Average lipids content of needles from each species with 95% confidence intervals (n=6).

Species	Mean % lipids	Standard deviation (%)	Upper 95% confidence interval (%)	Lower 95% confidence interval (%)
loblolly pine	6.84	0.21	7.70	5.99
short leaf pine	7.70	0.34	8.55	6.85
cedar	6.95	1.65	7.81	6.10

IV. DISCUSSION

Deposition of hydrophobic SVOCs with log K_{oa} >11 (*i.e.*, BDE-209) from the atmosphere to a plant surface is governed by the physical deposition mechanisms of the particle, such as gravitational settling, diffusion, impaction and phoretic processes (Horstmann and McLachlan, 1998; Sehmel, 1980). Furthermore, McLachlan (1999) determined that pollutant accumulation for SVOCs with log K_{oa} >11 is a function of the average particulate concentration of the pollutant (C_p), the deposition velocity of the particles (v_p), the surface area and volume of the vegetation, and a rate constant (K_e), related to the physical erosion of the contaminant from the plant surface. These model parameters have been validated with field measurements.

K_e may depend on atmospheric processes such as wind and rain, degradation pathways related to plant metabolism and photodegradation, and the affinity of the contaminant for the plant surface, which is related to surface characteristics of the particle/plant (*i.e.*, surface roughness) and its lipid content. For a given particle-bound contaminant, K_e is assumed constant if the contaminant remains associated with its carrier particle; however, if a contaminant diffuses from the particle to the lipid-rich cuticle of the plant, K_e may vary as a function of lipid content (McLachlan, 1999). The importance of these two mechanisms remains uncertain and likely depends on whether the contaminant is internally or externally mixed with the particle.

For this experiment, C_p and v_p can be considered constant for each exposure. Theoretically, if no significant differences in lipid content, surface roughness and plant metabolism exist among the three species, then there is no differential in persistence of a particle-bound SVOC that would cause K_e to vary between species, and results of the R6G experiments are more likely to explain the accumulation of particle-bound BDE-209 in field studies. The lipid content measurements (Table 5) revealed no significant differences between the three species. Furthermore, McLachlan and Horstmann (1998) suggested that chemical degradation due to plant metabolism can be neglected for SVOCs with log K_{oa} >10 and the surface roughness assumption is plausible given the similarities in genealogy that exist between the species. Therefore, under these assumptions, K_e can be considered constant in these three species regardless of particle mixing characteristics.

The data also lend strength to the suggestion that the normalization factor for the R6G marker can be extrapolated to infer foliar accumulation of BDE-209. Böhm et al. (1999) further validates this idea. They investigated interspecies variability in the accumulation of airborne SVOCs onto grassland species and concluded that, for compounds with log K_{oa} >11, differences in plant surface to volume ratios explained much of the variability. This finding, along with the theoretical considerations outlined above, suggests that the results of the present work can be integrated within a geographic information system to create maps of the spatial distribution of BDE-209 concentrations to estimate community exposures to airborne contaminants generated by land applications of sewage sludge.

The major limitation of the exposure system was its inability to generate particles larger than about 0.5 μm even when the concentration of R6G was increased in the aerosol test solution. Large particles were likely collected by inertial mechanisms in the neck of the

drying chamber, and in shelf-like elements created by tubing and Swagelok unions; visual inspection of these locations revealed considerable buildup of R6G residue. Additionally, the nebulizer did not perform well with high concentrations of the test solution because R6G deposits at the nozzle caused obstruction.

The SMPS data showed that the test aerosol had a MMD of approximately 300 nm and GSD of 1.6, indicating a relatively high degree of monodispersivity. In an environmental setting, a monodisperse aerosol is unlikely due to the myriad sources that contribute to atmospheric aerosols (*i.e.*, cars, agriculture, meteorology, industrial processes). Furthermore, considerable variation in atmospheric size distributions between different application locations is probable and may depend on the specified application method, local land-use characteristics, and meteorological conditions; however, no research was found on particle size distributions from land applications of sewage sludge.

Sludge applications that utilize a spraying device may produce smaller residual particles after solvent/liquid evaporation, whereas applications performed by slinging dehydrated sludge onto the fields are likely to produce larger particles due to the mechanical breakup of soil and sediment. Therefore, the investigation of accumulation to foliar surfaces over a range of particle sizes may provide a more comprehensive understanding of deposition processes and interspecies variation.

V. CONCLUSIONS

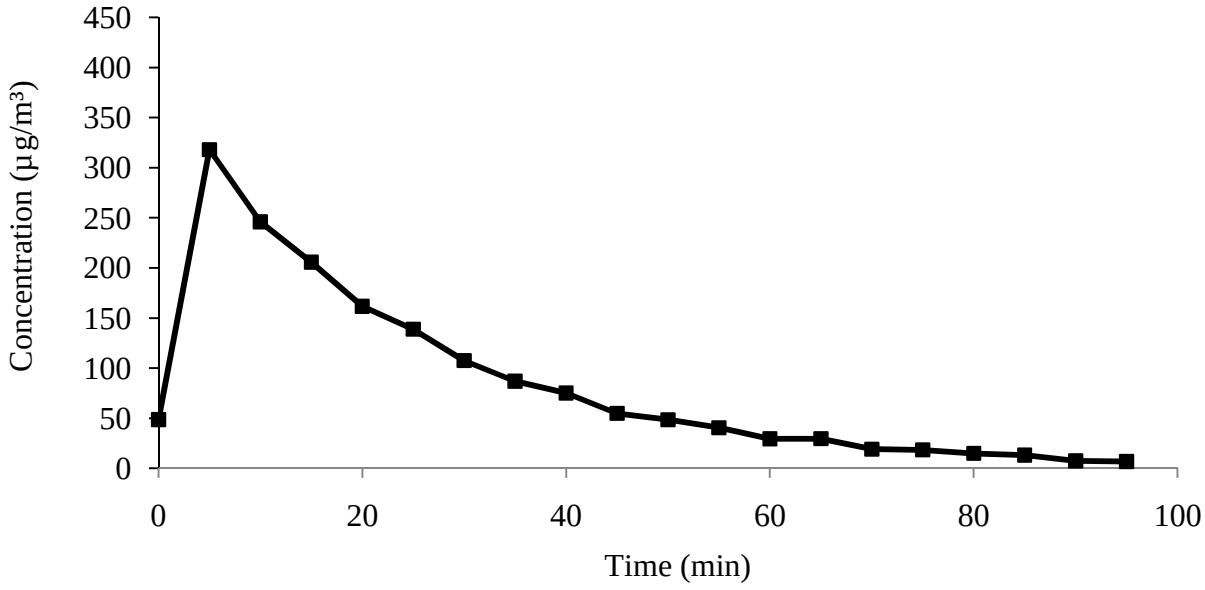
This work has demonstrated the use of a controlled exposure chamber to assess the dry deposition of a Rhodamine 6G aerosol to three coniferous species native to central North Carolina. Additionally, a method for normalizing concentration measurements using the ratio of needle projected area to mass is presented. Experimental results indicate that applying a normalization factor of 1.35 to cedar concentrations is effective in creating negligible differences in particulate accumulation between loblolly pine, short leaf pine, and cedar, and that this factor is largely due to differences in the projected area to mass ratios among these species.

These findings are consistent with the idea that conifers adjacent to permitted application sites may be used as passive samplers of BDE-209 to track the atmospheric transport of pollutants generated by land-applied sewage sludge. This analytical information could aid in producing data that correlate with community exposure and provides exposure estimates between geographic locations.

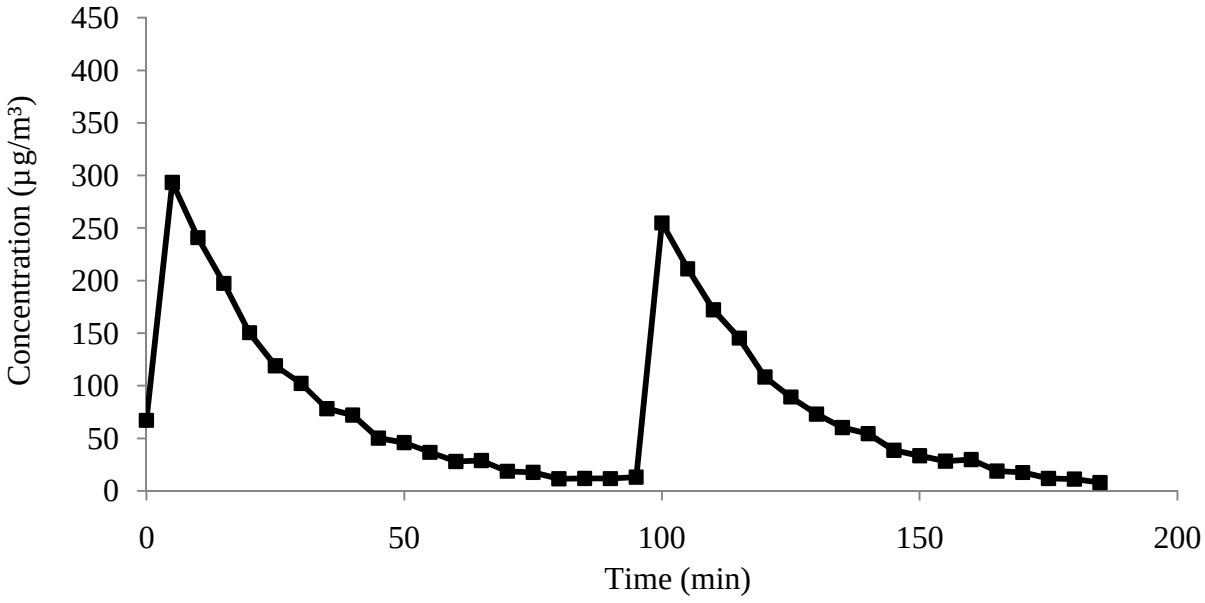
APPENDIX A

Exposure Profiles

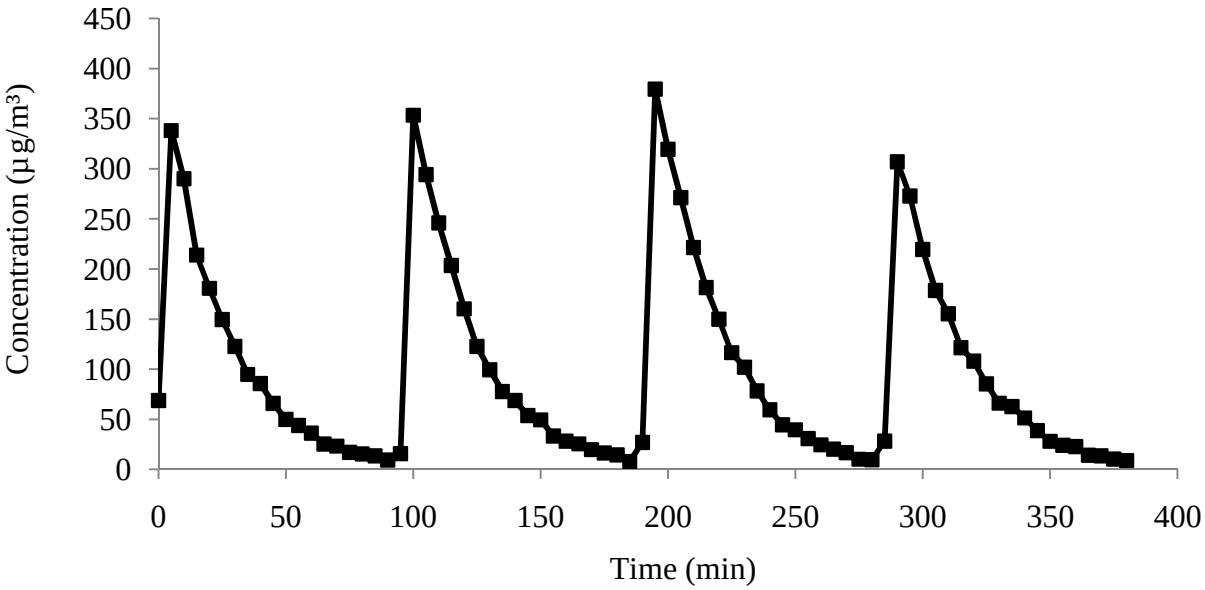
a)



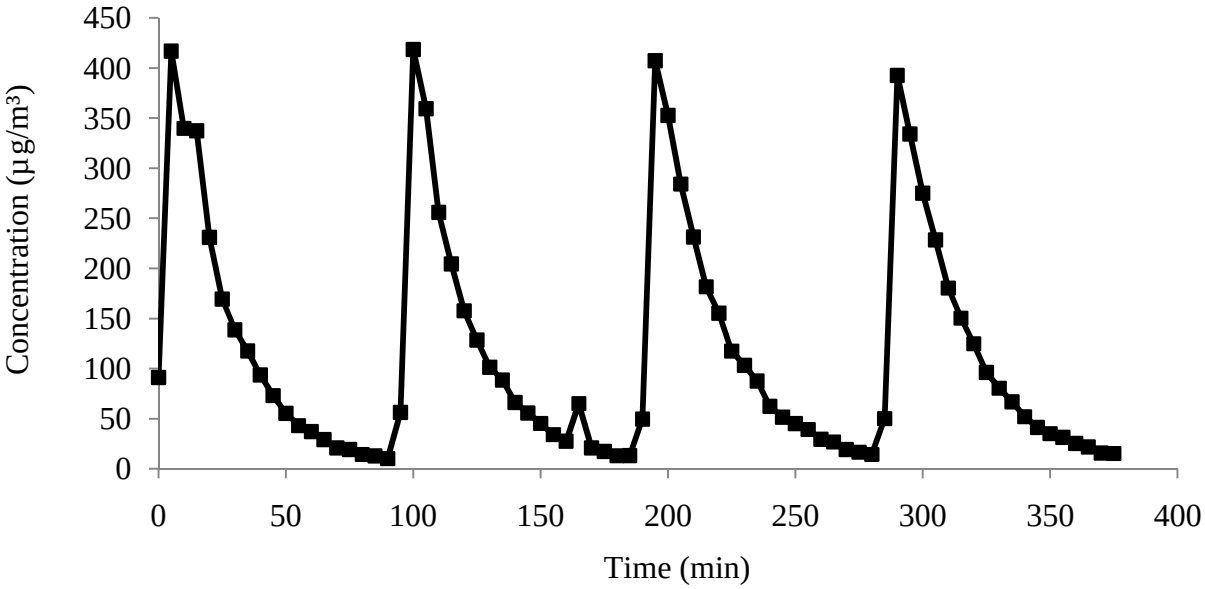
b)



c)


Concentration (µg/m³)
450
400
350
300
250
200
150
100
50
0
0
50
100
150
200
250
300
350
400
Time (min)

d)


Concentration (µg/m³)
450
400
350
300
250
200
150
100
50
0
0
50
100
150
200
250
300
350
400
Time (min)

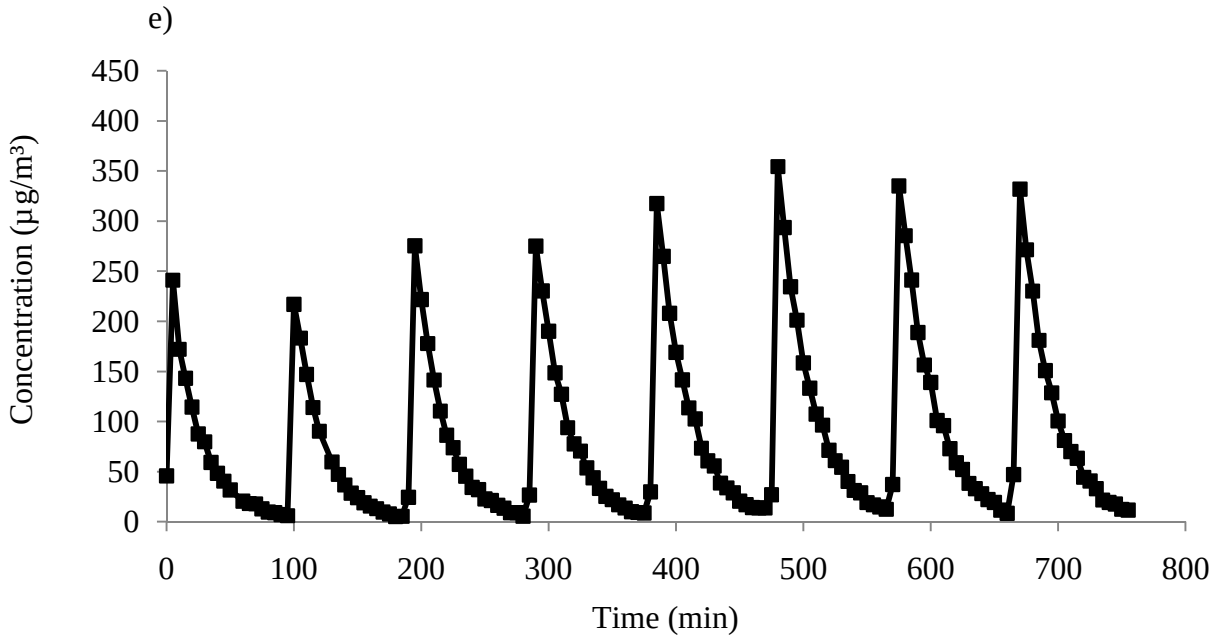


Figure A1. Mass concentrations of the test aerosol reported by the SMPS every 5-minutes for a) 1-cycle b) 2-cycle c) 4-cycle d) 4-cycle replicate and e) 8-cycle exposure.

APPENDIX B

Sampling Criteria

Davies (1968) determined that for negligible sampling bias due to particle settling for sampling probes in any orientation,

$$D_s \leq \frac{2}{5}\left(\frac{Q}{\pi\tau g}\right)^{\frac{1}{2}} \quad \text{(B1)}$$

where D_s is the inside diameter of the probe (cm), Q is the flow rate (cm^3/s), τ is the particle relaxation time (s), and g is the gravitational constant (cm/s^2). Sampling was done at 0.5 LPM $= 8.3 \times 10^{-6}$ m^3/s. Assuming standard density (1 g/cm^3), a 300 nm particle has $\tau = 4.29 \times 10^{-7}$ s. Using these values, equation (B1) indicates that, for negligible sampling bias due to particle setting, the ID of the sampling probe should be ≤ 32 cm.

Additionally, for negligible sampling bias due to particle inertia, Davies (1968) determined,

$$D_s \geq 10\left(\frac{Q\tau}{4\pi}\right)^{\frac{1}{3}} \quad \text{(B2)}$$

Again, assuming standard density, equation (B2) indicates that, for negligible sampling bias due to particle inertia, the ID of the sampling probe should be ≥ 0.7 mm.

Therefore, for the sampling conditions specified, the ID of the sampling probe should be between 0.7 mm and 32 cm. The actual probe chosen had an ID of 4.7 mm.

APPENDIX C

Quantification Procedure

Six calibration standards were prepared at concentrations listed in Table C1. Calibration standards were run before and after the unknown samples to reduce errors resulting from instrument drift. After accounting for the fluorescence contribution of the methanol solvent, a linear regression of ln(instrument reading) on ln(standard concentration) for the combined before and after measurements of the calibration standards was done. This procedure resulted in the final calibration equation displayed in Figure C1 ($R^2 = 0.99$).

Table C1. Standard concentrations and instrument readings used to construct calibration curve.

Standard concentration (ppb)	Natural log of standard concentration (ln ppb)	Instrument reading before/after (ppb)	Natural log of instrument reading before/after (ln ppb)
635.75	6.45	570.05 / 570.14	6.35 / 6.35
211.92	5.36	208.21 / 200.72	5.35 / 5.30
63.58	4.15	60.64 / 58.45	4.10 / 4.07
21.19	3.05	21.22 / 20.81	3.05 / 3.04
6.36	1.85	6.32 / 5.89	1.84 / 1.77
2.12	0.75	1.94 / 2.02	0.66 / 0.70

The instrument readings for all filter and needle extracts were recorded, log transformed and quantified using the calibration equation where,

$$\ln(\text{true concentration}) = \frac{\ln(\text{instrument reading}) + 0.034}{0.99} \qquad \text{(C1)}$$

Taking the exponential of this value gave the concentration of the extract. This concentration was then corrected for the fluorescence contribution of extraction blanks.

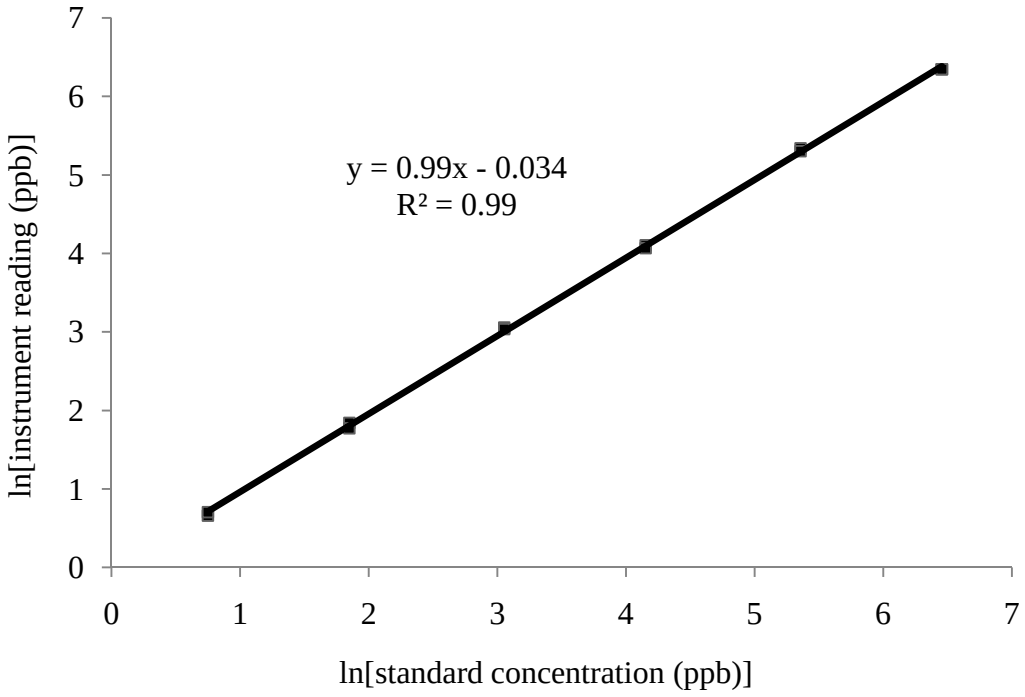


Figure C1. Calibration curve used for quantification

APPENDIX D

Photography

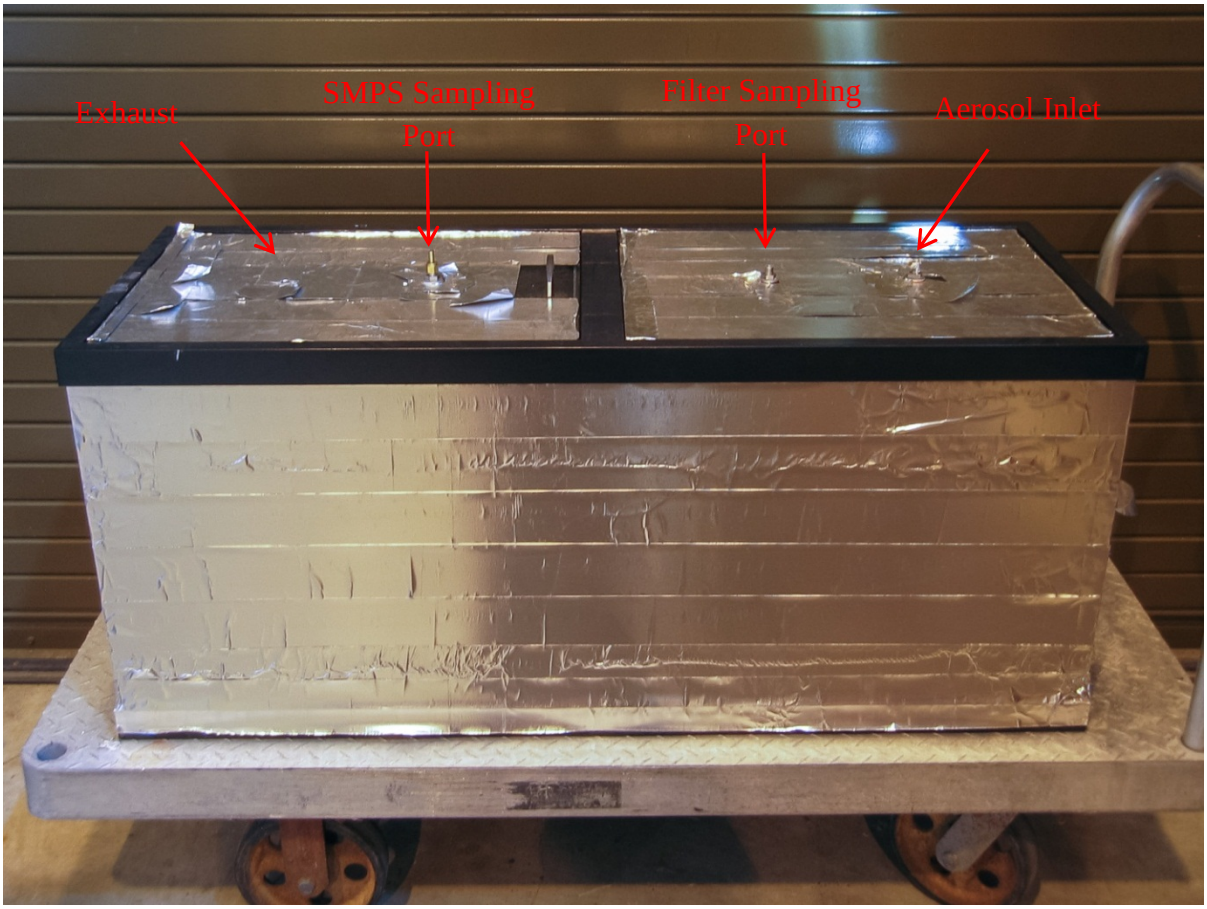


Figure D1. Exposure chamber

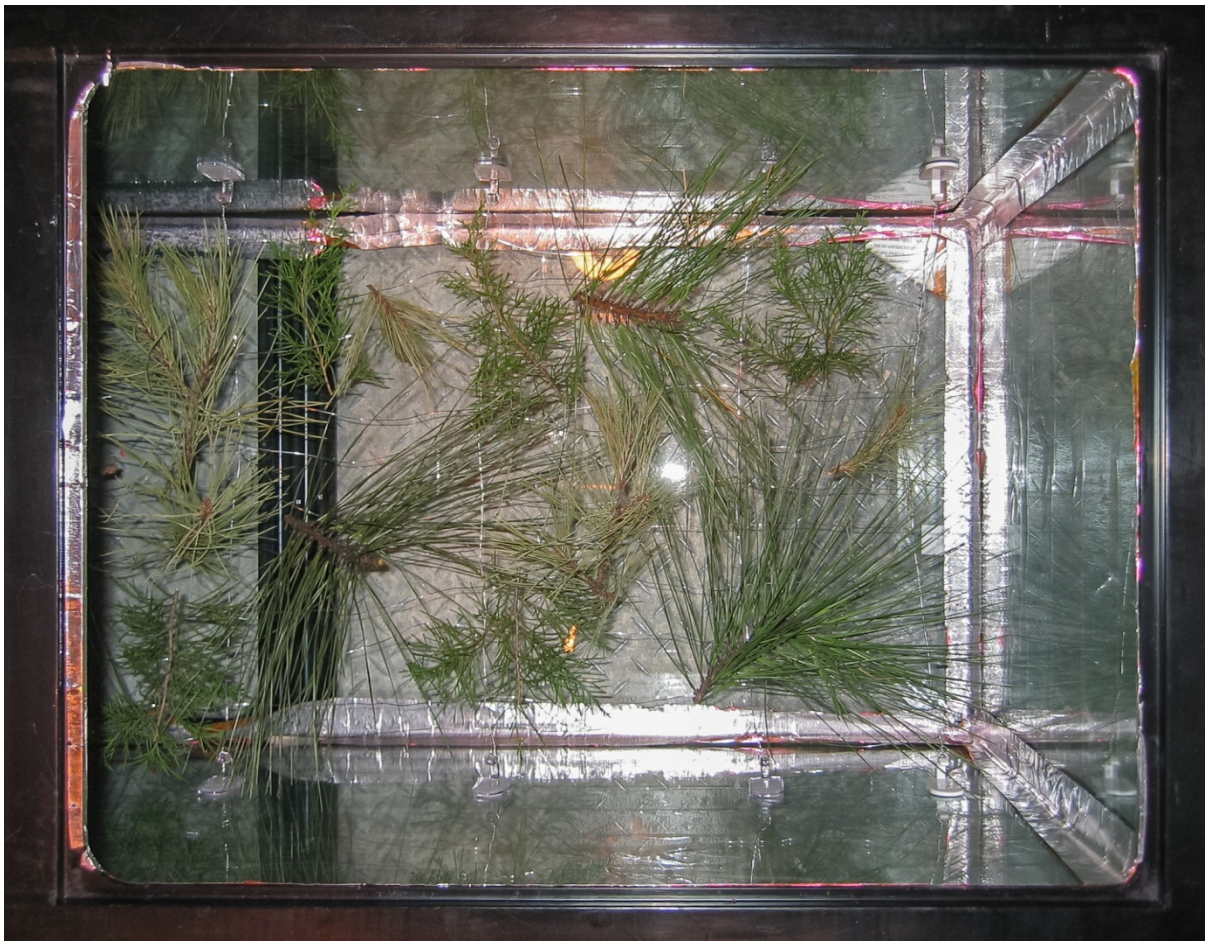


Figure D2. Needles samples arranged atop the metal scaffold in exposure chamber.

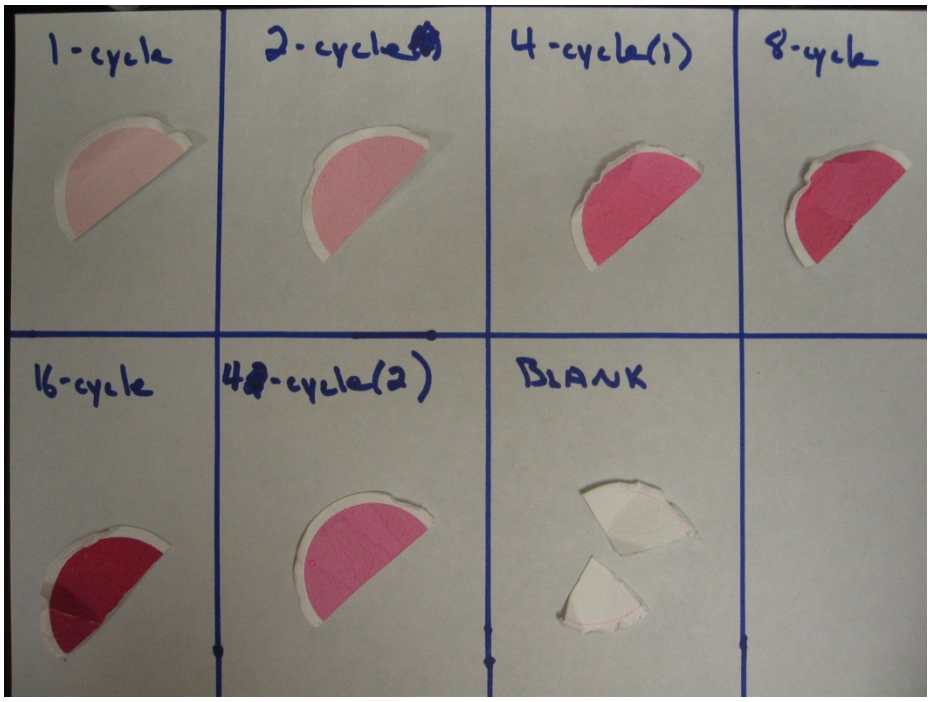


Figure D3. Filter samples obtained from all exposures prior to extraction.

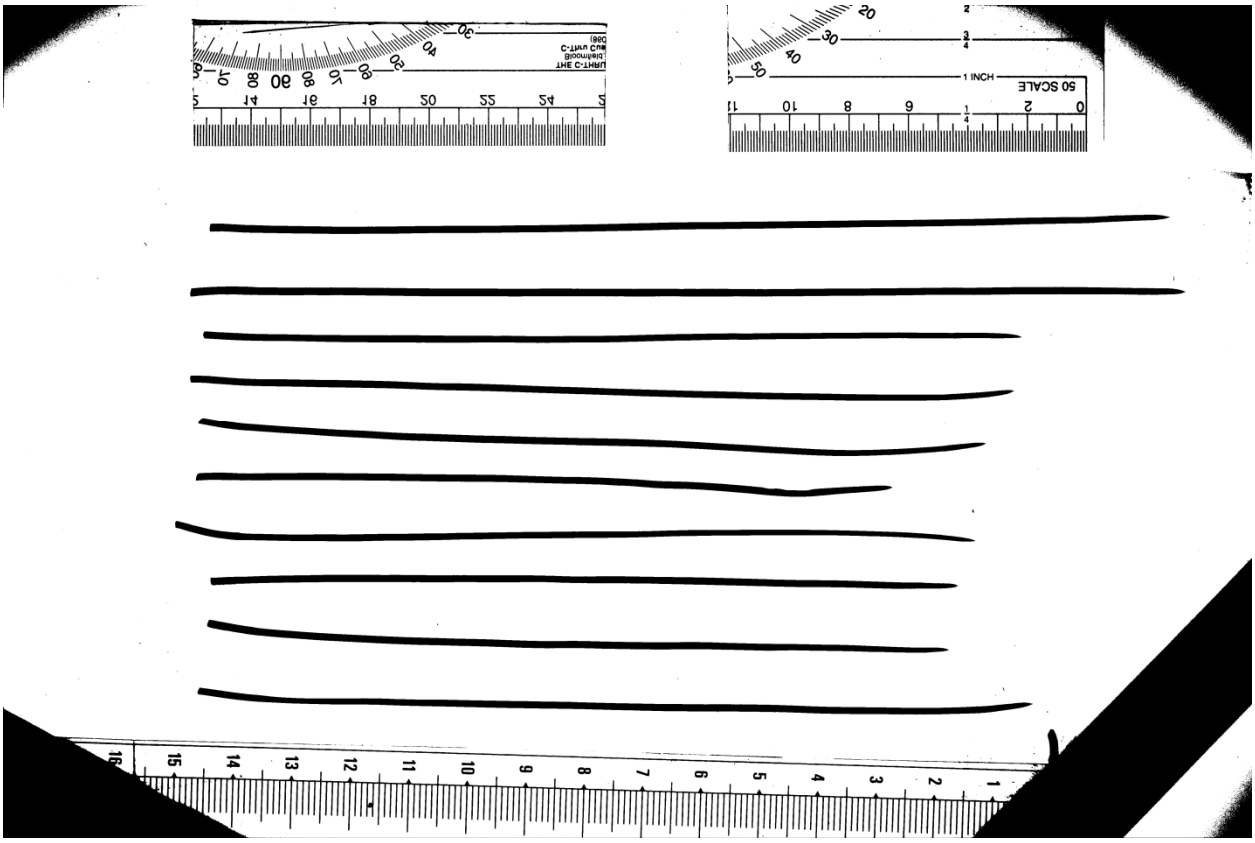


Figure D4. Raw image of loblolly pine silhouette. *Not to scale.*

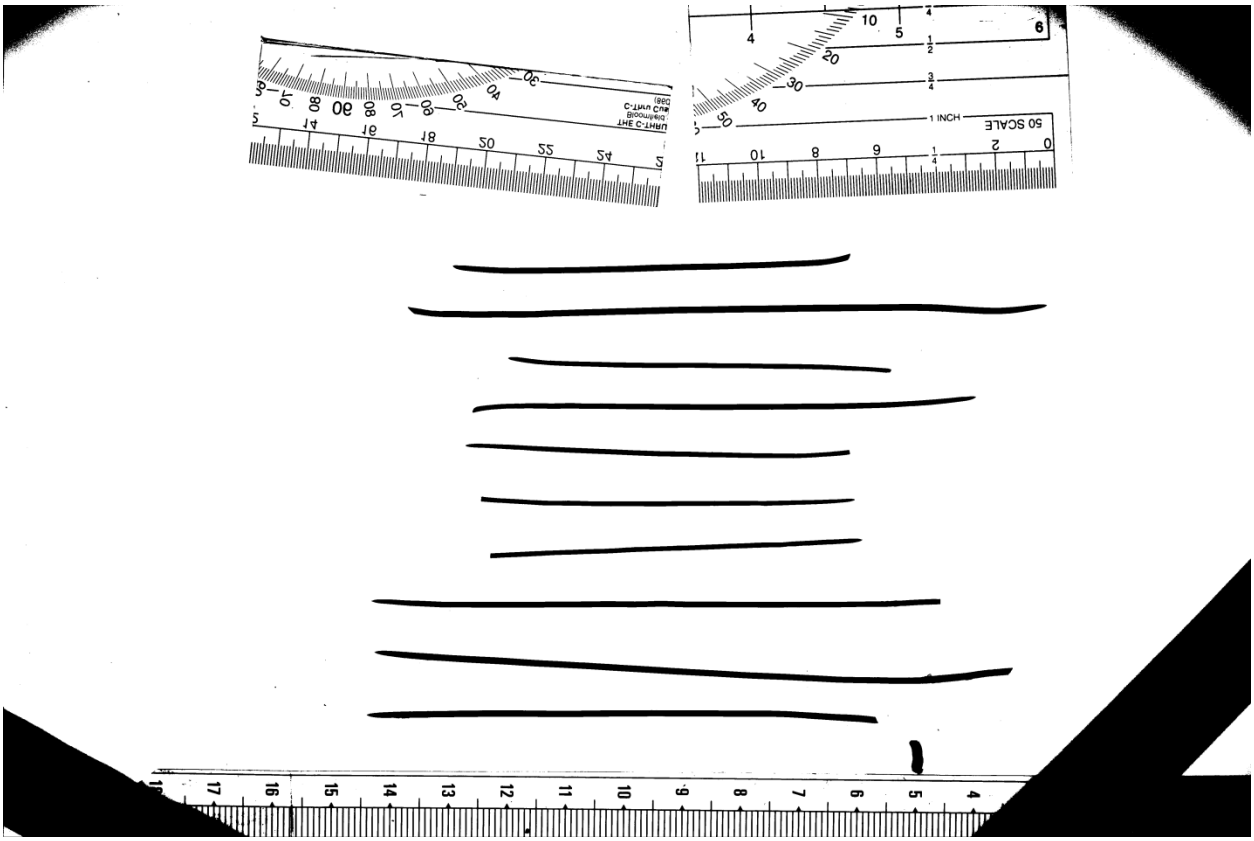


Figure D5. Raw image of short leaf pine silhouette. *Not to scale.*

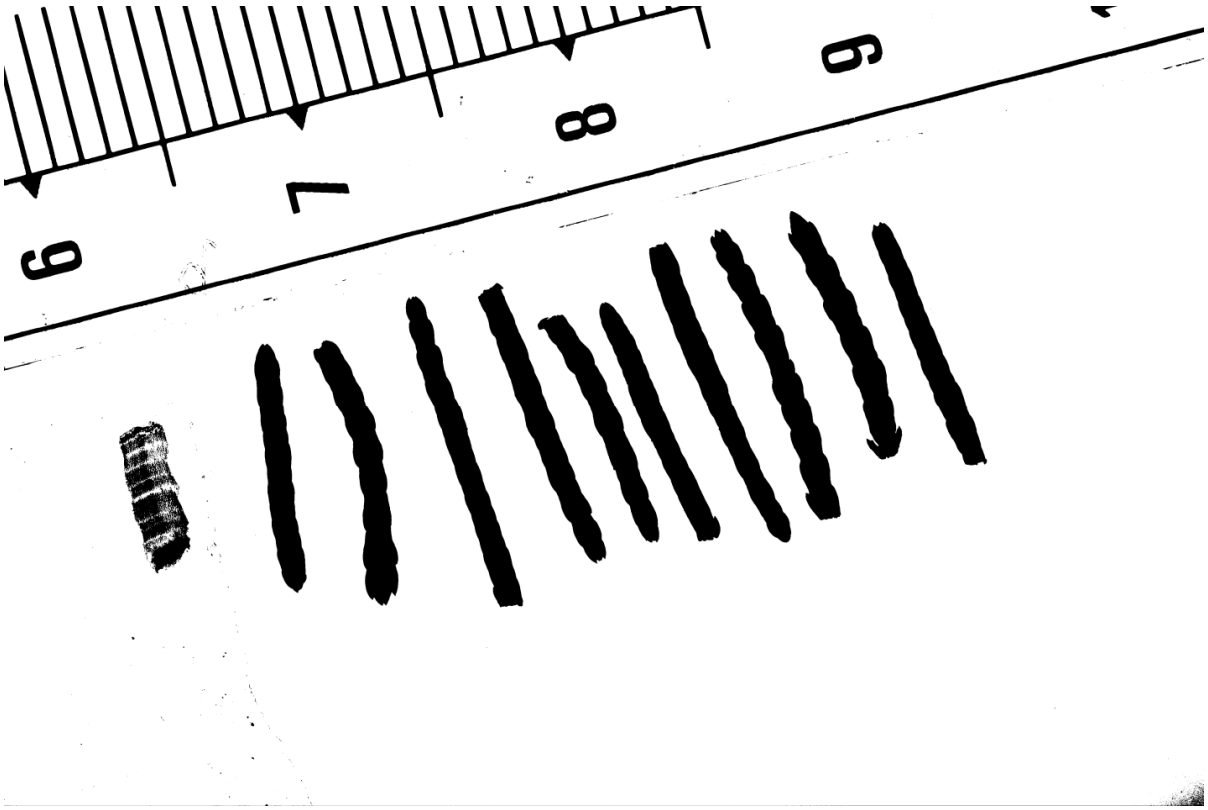


Figure D6. Raw image of cedar silhouette. *Not to scale.*

APPENDIX E

BDE-209 Results

To compare R6G accumulation to BDE-209 accumulation, the decaBDE technical mix (>98% BDE-209) was spiked into the aerosol test solution at a concentration of 7.55 mg/L. Filters were cut in half and sent along with needles not analyzed for R6G to an analytical chemistry laboratory directed by Dr. Rob Hale at the Virginia Institute for Marine Sciences. This laboratory is equipped with the advanced instrumentation required for trace analysis of PBDEs in various substrates.

The detection limit of the instrument was reported as 500 pg/g and the method detection limit (MDL) estimated from laboratory blanks was 1100 pg/g. Of the 18 samples analyzed for BDE-209, only 9 revealed concentrations above the MDL. The interpretation of these results is strongly dependent on how or if the value of the laboratory blank is subtracted from the data. For this reason, Figures E1a and E1b show best-fit regression models for both the raw results and the blank-corrected results on exposure.

Considerable scatter in the data is observed and no significant differences were revealed using either the raw or blank corrected data ($p > 0.40$). Results suggest that the analytical method cannot precisely quantify BDE-209 at these concentrations and that many of the true concentrations lie below the limit of quantitation.

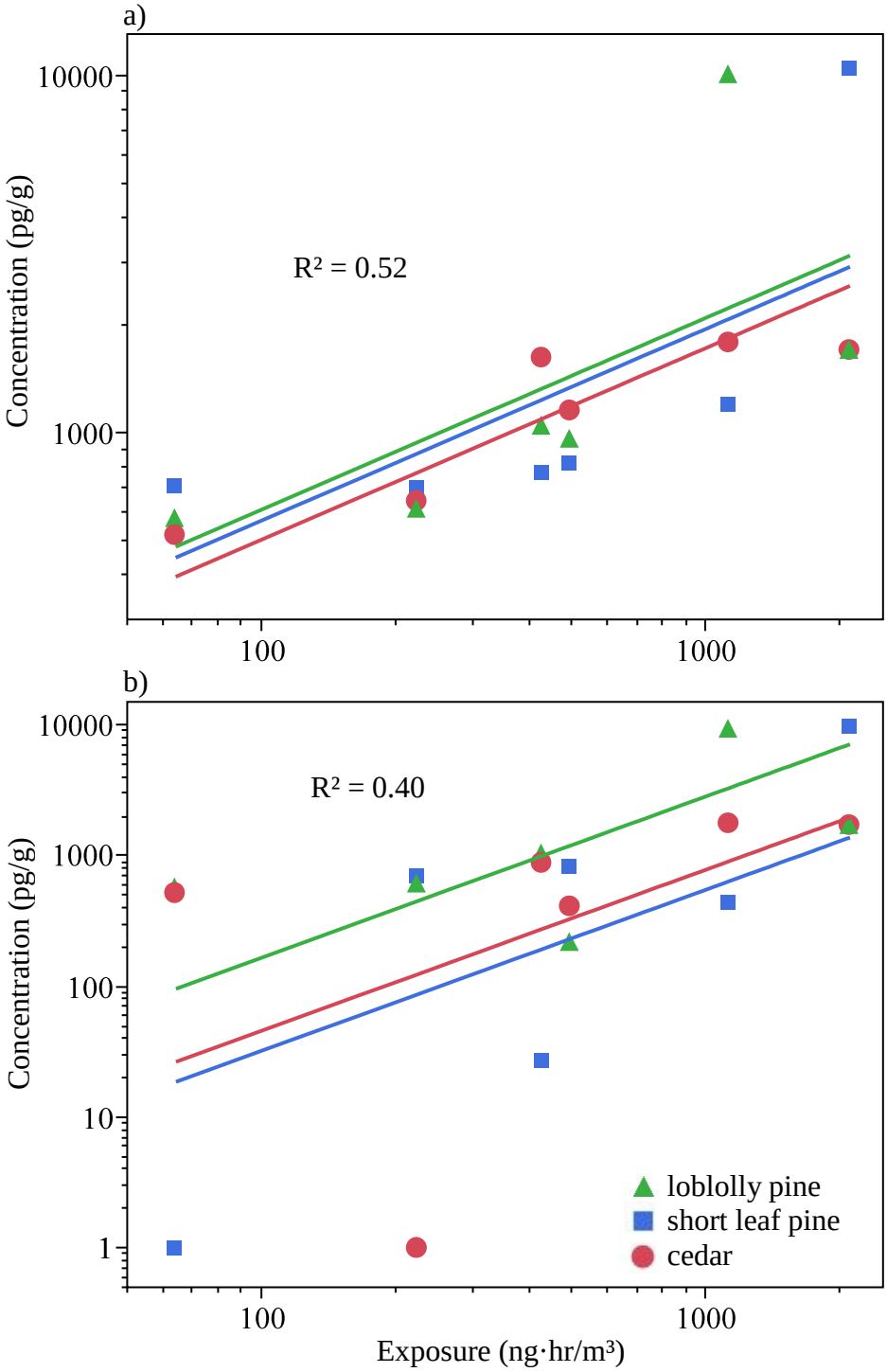


Figure E1. Best-fit multiple regression model of BDE-209 versus exposure for (a) raw data for each species and (b) blank-corrected data for each species

APPENDIX F

Model Parameters

Table F1. Parameter estimates of multiple regression models used for R6G analysis

Model	Parameter	Estimate	Standard error	Upper 95% confidence interval	Lower 95% Confidence interval
loblolly vs. short leaf vs. cedar (*not normalized*)	intercept (β_0)	5.89	0.05	6.01	5.77
	exposure (β_1)	0.95	0.05	1.05	0.84
	loblolly (β_2)	0.17	0.07	0.31	0.02
	short leaf (β_3)	0.03	0.07	0.18	-0.11
	cedar (β_4)	-0.20	0.07	-0.05	-0.35
pine vs. cedar (*not normalized*)	intercept (β_0)	5.84	0.06	5.96	5.72
	exposure (β_1)	0.95	0.05	1.05	0.84
	pine (β_2)	0.15	0.05	0.26	0.04
	cedar (β_3)	-0.15	0.05	-0.04	-0.26
loblolly vs. short leaf vs. cedar (*normalized*)	intercept (β_0)	5.07	0.05	5.18	4.95
	exposure (β_1)	0.95	0.05	1.05	0.84
	loblolly (β_2)	-0.04	0.07	0.11	-0.18
	short leaf (β_3)	0.01	0.07	0.16	-0.13
	cedar (β_4)	0.02	0.07	0.17	-0.12

**Note*

intercept units = ln(ng/g)

exposure units = ln(ng/g)/ln(mg·hr/m³)

species units = ln(ng/g)

Table F2. Parameter estimates of multiple regression models used for BDE-209 estimates

Model	Parameter	Estimate	Standard error	Upper 95% confidence interval	Lower 95% Confidence interval
loblolly vs. short leaf vs. cedar (*raw*)	intercept (β_0)	3.86	0.86	5.71	2.00
	exposure (β_1)	0.54	0.14	0.84	0.24
	loblolly (β_2)	0.09	0.22	0.56	-0.39
	short leaf (β_3)	0.02	0.22	0.49	-0.46
	cedar (β_4)	-0.11	0.22	0.37	-0.58
loblolly vs. short leaf vs. cedar (*blank-corrected*)	intercept (β_0)	-1.53	2.82	-7.58	4.52
	exposure (β_1)	1.23	0.46	0.26	2.21
	loblolly (β_2)	0.98	0.72	-0.57	2.52
	short leaf (β_3)	-0.67	0.72	-2.21	0.88
	cedar (β_4)	-0.31	0.72	-1.86	1.23

**Note*
intercept units = ln(pg/g)
exposure units = ln(pg/g)/ln(ng·hr/m³)
species units = ln(pg/g)

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