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A Novel Air Sampling and Analytical Method for Determination of Airborne Bronopol

by

John Charles Smyth

A dissertation submitted in partial fulfillment
of the requirements for the degree of
Doctor of Philosophy
Department of Environmental and Occupational Health
College of Public Health
University of South Florida

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Dedication

This work is dedicated to my wife, Mary; and my daughters, Kayti and Kelsey. I hope that my efforts serve as a model for my daughters as they prepare to finish high school. My family has been the motivation in my life.

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I would like to thank my father for his thoughtful review and cogent comments on this work. Without his contribution this publication would have been diminished.

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List of Acronyms, Abbreviations, and Symbols

a	In Beer's law, the extinction coefficient
A	In physical dimensions, area
A	In Beer's law, absorbance
α	Greek letter Alpha, the confidence level for a statistical test
b	In Beer's law, the sample cell pathlength
BRI	Building-related illness
ºC	Degrees Centigrade
ca.	Circa (approximately)
CASRN	Chemical Abstracts Service Registry Number
cm	Centimeter
CV	Coefficient of variation
d_a	Aerodynamic diameter of a particle
°	Degree
Σ	Greek letter Epsilon, sum of the values
η	Greek letter Eta, absolute viscosity
g	The acceleration of gravity on earth
GC	Gas chromatography
GFF	Glass fiber filters

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GLC	Gas-liquid chromatography
gm	Gram
>	Greater than
HEPA	High-efficiency particulate air (filter)
HPLC	High-pressure liquid chromatography
HVAC	Heating, ventilating, and air-conditioning
kg	Kilogram
L	Liter
LC50	Lethal concentration for fifty percent of the exposed population
LD50	Lethal dose for fifty percent of the exposed population
LOD	Limit of detection
$\log_{10}$	Logarithm to the base 10
LOQ	Limit of quantitation
LPM	Liters per minute
m	In linear regression, the slope of the line
MDI	Methylene diphenyl isocyanate
mg	Milligram
min	Minute
ml	Milliliter
mm	Millimeter
mmHg	Pressure expressed as millimeters of mercury
mol	Moles of a substance; 6.02×10^{23} molecules or atoms

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molar Concentration in solution expressed as moles per liter

P In Beer's law, the light power transmitted through a sample

% Percent

pH A measure of the acid or base concentration of a solution

P_o In Beer's law, the light power incident on a sample

ppm Parts per million

PVC Polyvinyl chloride

PVDF Polyvinylidene fluoride

1-2PP 1-(2-Pyridyl) piperazine

Q Volumetric gas flow rate

R^2 In linear regression, the coefficient of determination

ρ Greek letter Rho, density

ρ_p Greek letter Rho, sub p, density of a particle

Sr Pooled coefficient of variation

s_y Standard error of regression

UV-VIS Ultraviolet-visible

V_{avg} Average gas flow velocity of a gas stream

V_{cl} Gas flow velocity at the center line of the gas stream

V_{ts} Terminal settling velocity of a particle

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A Novel Air Sampling and Analytical Method for Determination of Airborne Bronopol

John Charles Smyth

ABSTRACT

Bronopol has been used as a preservative in drugs and cosmetics since 1964. Bronopol has low dermal irritancy at levels commonly used in cosmetics and pharmaceuticals but it is significantly irritating at higher concentrations. Laboratory testing of bronopol indicates a low potential for dermal sensitization; however, a number of case reports demonstrate human allergenic reactions. No reports were identified on the allergenic properties of bronopol for the inhalation route of exposure.

In 1983 approximately 5,200 people in the United States were occupationally exposed to bronopol. Current novel uses of bronopol include mold remediation and the sanitizing of ventilation system components. These new applications have the potential to expose vast new populations to the chemical. Since 89 million people in the United States work in indoor environments and 50 million Americans suffer from allergies, it is likely that a sizeable portion of these populations will be exposed to bronopol. This is significant since the dermal sensitizing properties of bronopol suggest that the material may also be a respiratory sensitizer, potentially resulting in chemically induced asthma. More people are being diagnosed with asthma today than at any time in the past; the

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causes of this increased prevalence are largely unknown.

In this work an existing ultraviolet spectrophotometric method for analysis of bronopol has been combined with conventional industrial hygiene air sampling techniques. No combined air sampling and analytical method for bronopol has previously been published in the literature.

A calibration curve has been developed with a linear range of 1 μg/ml to 25 μg/ml. The instrumental limit of detection is 1 μg/ml with an instrumental limit of quantitation of ca. 3 μg/ml.

During chamber sampling trials analytical recovery for treated glass fiber filters yielded a sampling recovery efficiency averaging 99.9 %. Bronopol concentration obtained during chamber sampling trials ranged from 10.80 mg/m^3 to 21.59 mg/m^3, with a pooled coefficient of variation of 4.33 % for all chamber sampling sets.

Treated glass fiber filters spiked with bronopol were found to be stable for a period of 48 hours; derivatized bronopol solutions were found to be stable for a period of fourteen days.

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Chapter One - Introduction

Public Health Implications of This Study

Bronopol is an important antimicrobial agent commonly used as a biocide in various products including cosmetics, medical products, and water treatment systems. Recent new uses include remediation of mold-affected building materials and sanitizing building ventilation systems. Bronopol has been shown to produce skin irritation (Frosch et al., 1990) and allergic dermatitis on dermal exposure (Wilson & Powell, 1990; Rudzki, Rebandel, & Grzywa, 1993) as well as irritation, dyspnea, and profuse mucous generation in inhalation studies on laboratory animals (Environmental Protection Agency [EPA], 1995).

Human occupational exposure to bronopol takes place during the manufacture of the compound, in preparing products containing the substance, and in the use and application of bronopol-containing formulations. Nonoccupational exposures occur when individuals use and consume bronopol-containing products, or when bronopol products are applied to building ventilation systems or mold-affected indoor building materials.

Indoor proliferation of microbiological agents can generate allergens and other byproducts in the indoor environment. It has been estimated that 50 million Americans suffer from allergic diseases and that 89 million Americans work in nonindustrial indoor environments. The new uses of bronopol to combat microbiological growth in the indoor

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environment have the potential to expose vast new populations to this compound, with the potential of subsequent adverse health effects in portions of the exposed groups (Mendell et al., 2002; American Academy of Allergy, Asthma & Immunology, Inc. [AAAAI], 2000).

While information is available on human reactions caused by dermal exposures to bronopol, currently no standard exists for permissible levels of human inhalation exposure of the compound. Airborne exposure levels in occupational and consumer applications are unknown. Currently, no sampling and analytical method for determination of airborne bronopol concentration is offered in the literature. Availability of an air sampling method for bronopol would permit determination of exposures to bronopol workers and of building occupants where bronopol is applied.

Purpose and Scope of the Study

The purpose of this work is to evaluate an existing bronopol analytical method for use in conjunction with industrial hygiene air sampling techniques, with the goal of developing a method to determine airborne concentrations of the compound. The analytical method utilized was published by Sanyal, Basu, and Banerjee (1996) and was originally intended for determination of bronopol in chemical formulations. The method utilizes a derivatizing reaction to form a stable chromophore suitable for analysis by ultraviolet spectrophotometry.

The scope of this work includes repetition of the Sanyal et al. (1996) laboratory procedures and development of a calibration curve for the analytical procedure. Filters treated with the derivatizing agent are spiked with known bronopol solutions to evaluate

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analytical recovery and consistency. Spiked filters and filter extract solutions are evaluated for stability upon storage. Finally, sampling of generated bronopol aerosols is conducted using the treated filters. No attempt is made to characterize the chemical reactions occurring during the development of the chromophore in the derivatizing and analytical procedures, as these are described in the Sanyal et al. paper.

Uses of Bronopol

Consumer and Industrial Applications

Bronopol reportedly exhibits biocidal activity against a variety of microorganisms including bacteria, fungi, and algae (EPA, 1995). A partial listing of reported uses is presented in Table 1.

Bronopol in Indoor Environmental Quality Applications

Modern indoor environments in developed countries, sometimes referred to as the "built environment", are generally considered to be safe and healthful places to work and live. However, the widespread introduction of air-conditioning coupled with energy conservation measures of the 1970s has led to reductions in the exchange rate of outdoor air. As a result, airborne contaminants released in the indoor environment may reach concentrations whereby building occupants complain of specific or nonspecific health and/or comfort symptoms (Occupational Safety and Health Administration [OSHA], n.d.).

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Table 1
A Partial Listing of Reported Uses of Bronopol

Adhesive preservative in food packaging	Mold remediation products
Antiseptics	Paper mill pulp and process water
Air-conditioning/ventilation systems	Paper slimicide
Coatings preservative	Potable water storage tanks, cleaning
Cooling towers	Preservatives
Cosmetics	Sanitizers
Disinfectants	Seed treatment against bacteria
Household products	Soil bacteriostat
Humidifiers	Toiletries
Metal working fluids	Water based inks

Note: *Information from BBJ Environmental Solutions, Inc. [BBJ], (2006); National Library of Medicine [NLM], (2004); & OSP Microcheck, Inc. 1 [OSP 1], (2006)*

In absolute terms the quantities of indoor air contaminants present may be very low in comparison with traditional industrial environments. The presence of airborne contaminants in the built environment may create actual or apparent perceptions of an unhealthy environment, especially when contrasted with the typical cleanliness of the built environment.

Most recently the potential adverse health effects of fungal (mold) growth in the indoor environment have resulted in a high level of public awareness. While mold spores are present in the air in most indoor and outdoor environments, adequate moisture must be available for the ubiquitous spores to germinate and grow. In a modern building

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control of relative humidity and water/moisture intrusion will minimize or eliminate the potential for mold growth to occur indoors. When water intrusion or elevated relative humidity does occur, conditions favorable for mold growth may be created.

Many building materials such as wood, drywall, and carpeting will sustain mold growth when in contact with water for periods of 24 to 48 or more hours, depending on ambient temperature and other factors (EPA, 2001). Prolonged conditions of elevated indoor relative humidity may also create conditions amenable to mold growth on various surfaces. Suitable conditions for the proliferation of mold growth are often found on the refrigeration coil and adjacent internal components of modern air-conditioning air-handling units. Airborne particulate matter impacting the refrigeration coil provides a nutrient source for the mold spores present and the cool surfaces of the refrigeration coil condense water from the moving air stream. Mold growth may occur as a result of conditions within the air-handling unit, resulting in likely distribution of mold fragments into the indoor environment through the ventilation system.

Commercial biocide products are available for minimizing mold growth on wetted building materials and on ventilation system components. At least one manufacturer utilizes bronopol as the active ingredient in a commercial line of mold remediation and ventilation system sanitizing products (BBJ, 2006). These products are applied as an aqueous solution to building material surfaces presenting with mold growth and on internal components of air-handling units and associated ductwork. This is significant because of the potential for airborne exposure to bronopol in a variety of new occupational and nonoccupational populations.

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Chemical and Physical Properties of Bronopol

The chemical name for bronopol is 2-bromo-2-nitro-1,3-propanediol, with a Chemical Abstracts Service Registry Number (CASRN) of 52-51-7 (BBJ, 1999). A representation of the molecular structure is presented in Figure 1. The empirical formula is $BrC_3H_6NO_4$ and the molecular weight is 199.99 grams per mole. The odorless material exists as white to pale yellow crystals or crystalline powder; the density for homogeneous solid bronopol is ca. 1.1 grams per cubic centimeter. Melting temperature is 131.5 ºC; at temperatures above 140 ºC exothermic decomposition is reported which releases hydrogen bromide and oxides of nitrogen.

The substance is stable for a minimum of one year under normal storage conditions and no photodecomposition is reported. Vapor pressure at 20 ºC is 1.26×10^{-5} mmHg.

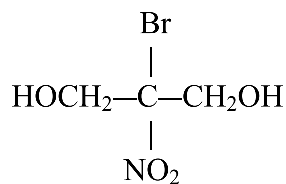

Figure 1. The Molecular Structure of Bronopol

At normal temperatures bronopol is readily soluble in water, low-molecular weight alcohols, low-molecular weight glycols, and n-methyl pyrrolidone. Dissolution in

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water is exothermic; solutions up to 28 weight percent in water are possible (National Library of Medicine [NLM], 2004; OSP 2, 2006).

Concentrated solutions of bronopol (20 weight percent) are corrosive to many metals with the exception of some grades of stainless steel. Concentrated solutions may degrade rubber and some grades of polyethylene. Dilute solutions (0.02 weight percent) are compatible with stainless steel, aluminum, brass, and copper and with most types of rubber and plastics (OSP 3, 2006).

Decomposition and Biocidal Activity of Bronopol

Bronopol decomposes more rapidly under conditions of elevated alkalinity and temperature. Decomposition products of bronopol under various conditions include formaldehyde, nitromethane, nitrous acid, glycolic acid, hydrogen bromide, oxides of nitrogen, formic acid, methanol, and other compounds (OSP 4, 2006). A listing of bronopol decomposition products is presented in Table 2.

Bronopol oxidizes thiol-containing amino acids with atmospheric oxygen as the final oxidizer. Products of this reaction include active oxygen species such as superoxide and peroxide. These oxygen species are directly responsible for the biocidal activity of the compound and for the suppression of microbial growth rates following application (Shepherd, Waigh, & Gilbert, 1988).

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Table 2
A Listing of Reported Bronopol Decomposition Products

Bromine ions	2-Bromo-2-nitroethanol
Carbon dioxide	Formaldehyde
Formic acid	Glycolic acid
Methanol	Nitroethanol
Nitromethane	Nitrous acid
Oxides of nitrogen	Tris(hydroxymethyl) nitromethane

Note: Information from OSP 4, (2006)

Importance of This Study

As long ago as 1983 it was estimated that 5,176 people in the United States were occupationally exposed to bronopol, both in locations where bronopol was produced and where it was incorporated into various formulations. This estimate includes both dermal and respiratory exposures (National Institute for Occupational Safety and Health [NIOSH], 1983) to workers and does not include exposures to consumers and end-users. Since that time the use of bronopol in various formulations and applications has increased. The recent use of bronopol in mold remediation and ventilation system applications has added significant new populations to those potentially exposed through the airborne route, including HVAC and mold remediation technicians and the occupants of the buildings in which the bronopol is applied.

The irritating and allergenic properties of bronopol upon dermal exposure have been demonstrated in animal and human studies. The potential sensitizing property of bronopol on dermal exposure suggests that the material may also be sensitizing for the

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inhalation route of exposure. Recently introduced indoor environmental quality applications of bronopol place biocide applicators and building occupants at risk of airborne exposures. Bronopol applied to HVAC components may become entrained in the ventilation system and distributed into the building interior. Bronopol used in mold remediation applications is applied to affected surfaces as an aqueous solution using a powered spray nebulizer, producing bronopol aerosols. Little information is available as to residual levels in building air following application of bronopol. Given the importance of bronopol as a preservative and biocide and its expanding uses in indoor environmental quality applications, it is important to develop an understanding of airborne exposure levels.

The reported vapor pressure of 1.26×10^{-5} mmHg is equivalent to an airborne concentration of ca. 0.14 mg/m^3. Given the reported stability of bronopol under normal conditions, the vapor pressure suggests that persons in building areas where bronopol has been applied may be subjected to continuous low-level exposures.

No air sampling method for bronopol currently exists in the scientific literature. Therefore airborne bronopol levels cannot be reliably measured. Without an air sampling methodology, airborne exposure levels for workers and building occupants cannot be characterized. Further, investigation of the dose-response relationship between airborne exposure levels and potential adverse health outcomes cannot be accomplished.

This paper presents the development of an air sampling method for bronopol. The existing Sanyal et al. (1996) analytical method and conventional industrial hygiene air sampling techniques are combined for the first time. This work produces a calibration curve using the Sanyal et al. analytical method and modifies the method to analyze

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bronopol collected on filters, both from filter spiking and from collection of aerosols generated in chamber studies.

Limitations of This Study

This paper utilizes an existing analytical method in combination with a limited number of analytical recovery studies from filter spiking and chamber experiments. Excluded from the study are large numbers of duplicate samplings from generated atmospheres and long-term sample and filter extract stability studies.

The chemical interactions taking place in the derivatizing reaction have already been described in the Sanyal et al. (1996) paper, and the decomposition pathways and products of bronopol are known. Although bronopol will decompose under certain conditions, the known decomposition products reportedly do not interfere with the analytical method. This study does not determine what, if any, compounds may interfere with the analytical method.

This study does not evaluate potential adverse health effects associated with dermal or inhalation exposures to bronopol. No field sampling activities are included in the study.

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Chapter Two - Review Of Pertinent Literature

Health Effects Associated with Exposure to Bronopol

Animal and Human Studies

Animal oral and subcutaneous toxicity. In 1964 bronopol was a new antimicrobial compound. Croshaw, Groves, and Lessel (1964) reported on the toxicity of bronopol in laboratory animals. LD50 for mice was reported as 350 mg/kg orally and 20 mg/kg intraperitoneally; for rats the reported LD50 was 400 mg/kg orally and 200 mg/kg subcutaneously. No further information was available on the age, variety, or sex of the rodents in the reported studies. Male and female albino rats (5 to 6 weeks old) were fed bronopol at 100 ppm and 1,000 ppm in food for twelve weeks; both dose amounts were tolerated with no adverse health effects noted.

Frear (1969) reported oral LD50 values of 180 mg/kg for rats and 270 mg/kg in mice. No further information was available on the age, variety, or sex of the rodents.

In animal studies of acute toxicity Bryce, Croshaw, Hall, Holland, and Lessel (1978) reported mouse oral LD50 values of 374 mg/kg for males and 327 mg/kg for females; and rat oral LD50 values of 307 mg/kg for males and 342 mg/kg for females. Intraperitoneal values were 34.7 mg/kg for male mice and 32.8 mg/kg for female mice; intraperitoneal values were 22.0 mg/kg for male rats and 30.2 mg/kg for female rats. No further information was available on the age or variety of the rodents.

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Animal inhalation toxicity. A four-hour LC50 of approximately 0.18 mg/L is reported for inhalation exposure on ten male and ten female rats of unspecified age or variety (Elder, 1980). Irritation is reported on the ears and paws of survivors.

Two acute inhalation studies were submitted to the Environmental Protection Agency (EPA, 1995) for pesticide registration approval of bronopol. In one study an LC50 (rats) of greater than 0.588 mg/L is reported with diffuse red lungs, sore eyelids, and severe dermatitis and ulceration on the head; the head effects are attributed to dermal exposure. In the second study a male rat LC50 of greater than 5 mg/L was determined. No further information was available on rodents used in the studies. Clinical signs noted during the study were eye irritation, dyspnea, profuse mucus production, and lethargy with chronic pneumonitis following cessation of exposure.

Animal dermal irritancy properties. A 2 % application to eye and skin of rabbits was irritating after one application (Croshaw et al., 1964).

A 0.4 ml application of 20 % aqueous bronopol solution on both abraded and unabraded rabbit skin produced severe irritation. In a study of acute inhalation toxicity irritation is reported on the ears and paws of male and female rate survivors (Elder, 1980).

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Animal eye irritancy properties. A 2 % application to the eyes of rabbits was irritating after one application (Croshaw et al., 1964). Application of 1.0 ml of 20 % aqueous bronopol solution applied to the eyes of rabbits produced severe damage (Elder, 1980).

Human dermal patch testing. Marzulli and Maibach (1974) conducted Draize patch testing of bronopol on human volunteers. Bronopol in petrolatum was applied to the upper lateral portion of the arm and covered; the patch was left in place 48 or 72 hours. Ten successive induction applications were made at the same site. Following a two-week rest period a challenge patch was applied and left in place for 72 hours. For 66 subjects no positive reactions were noted for induction with 2 % bronopol and subsequent challenge with 2.5 % bronopol. However, when induction was performed with 5 % bronopol an allergic reaction was observed in 11 of 93 subjects upon subsequent challenge with 2.5 % bronopol.

Following the prior Marzulli and Maibach (1974) positive patch testing for dermal sensitization, Maibach (1977) conducted further skin sensitization potential patch testing using bronopol in yellow soft paraffin. A 21-day preliminary assessment of eight normal subjects with patches of varying bronopol concentrations determined an irritancy threshold of approximately 0.5 to 1.0 %. Following this 120 subjects were fitted with 5 % bronopol patches over a period of 21 days; many subjects reported irritation from the patch. After a two-week rest period, a 0.25 % bronopol challenge patch was applied for 72 hours. Among the 93 subjects completing the challenge test no evidence of sensitization was reported.

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Work reported by Bryce et al. (1978) indicate that closed patch testing of bronopol in soft paraffin produced slight erythema in two of ten human subjects at a concentration of 1 % and moderate erythema in four of ten subjects at 2 % concentration. Allergenic sensitization of test subjects was not observed in this study.

Frosch et al. (1990) reported on dermal irritant and allergic testing of 8,194 European patients in seven dermatology clinics. Of these, 10 cases presented with irritation and 38 presented with allergic reactions. Clinically relevant reactions to bronopol were reported in 17 of the patients.

Case Reports

Occupational exposure data was developed for workers of the Boots Company, Limited and submitted to the Cosmetic, Toiletry & Fragrance Association. Boots is a manufacturer and distributor of bronopol. In the period from 1970 to 1978, 23 of 50 Boots workers reported rashes and/or superficial burns following dermal exposure to bronopol powder or concentrated aqueous solutions. Eight employees reported two occurrences, six employees reported three occurrences, and three employees reported a fourth occurrence. It was reported that the adverse reactions were irritant rather than allergenic in nature (Elder, 1980).

Eucerin is a retail nonprescription topical moisturizing skin cream; acute allergic dermatitis was experienced by seven patients on using Eucerin on previously dermatitic skin for periods of time from 5 weeks to 2 years. At that period in time Eucerin contained bronopol as a preservative. Bronopol and Eucerin patch testing on the patients was positive. As bronopol is a formaldehyde donor the patients were tested for

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formaldehyde allergy. None of the patients tested positive for formaldehyde or other preservatives that generate formaldehyde (Storrs & Bell, 1983).

In a case report a 35-year old large animal veterinarian presented with an erythematous dermatitis from the fingers to mid upper left arm. The man performed full arm rectal examinations on thoroughbred mares. In this procedure a glove was worn and a bronopol-containing lubricant was used; frequent soiling of the skin of the arm is reported in spite of the full-length surgical gloves used. As the cause of the condition was thought to be cellulitis the initial treatment was with antibiotics. Forced to use his right arm to perform the examinations he returned to the clinic four days later with an identical condition on that arm as well. The lubricant used during the equine examinations had been changed to a bronopol-containing formulation just prior to the onset of symptoms. Switching to another lubricant not containing bronopol resulted in a resolution of the condition. The man patch tested positive for allergic reaction to both bronopol and the bronopol-containing lubricant (Wilson & Powell, 1990).

Two additional case reports are made for cosmetics production workers. The first was a 25-year-old man that had been weighing cosmetic ingredients including bronopol for six months. The dermatitis developed on his hands and forearms and the patient patch-tested positive for bronopol. After a job change in which he did not handle bronopol the patient was symptom free after six months. The second case was a 54-year-old man that also handled bronopol in cosmetics production. After seven years in this job he developed dermatitis on both hands. Following a four-day sick leave his lesions were significantly reduced so he resumed work. The condition resumed after two weeks back on the job. The patient also patch-tested positive for bronopol. Following cessation of

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bronopol exposure the patient was symptom free in nine months of follow-up (Rudzki et al., 1993).

Summary of Health Effects

A summary of acute animal toxicological studies for bronopol is presented in Table 3. In general, no evidence of bronopol carcinogenic, embryotoxic, teratogenic, or mutagenic effects have been noted in the literature reviewed. Bronopol is moderately toxic orally in laboratory animals; intraperitoneally its toxicity is high in rodents (Elder, 1980). Reported acute LD50 toxicity by the oral route in laboratory rodents was in the range of 180 mg/kg for rats (Frear, 1969) to 400 mg/kg for rats (Croshaw et al., 1964). Chronic oral toxicity values reported are in the range of a well tolerated 20 mg/kg/day dose (rat) to daily doses of 80 to 160 mg/kg/day (rat) resulting in respiratory distress and some deaths. Intraperitoneal LD50s reported range from 20 mg/kg (mouse) to a 200 mg/kg rat value (Bryce et al., 1978). Inhalation LC50 values for rats of 0.18 mg/l (Elder) to greater than 5 mg/l (EPA, 1995) are reported. The cause(s) of the disparity in acute animal values is not known; little information on variables such as age, variety, and sex for the laboratory animals was presented in the studies available.

Skin irritancy in humans and laboratory animals at levels commonly used in cosmetics (0.01 to 0.1 %) is low. Bronopol in higher concentrations (1 % and greater) is significantly irritating in human skin patch studies (Maibach, 1977). The evidence for dermal sensitization and allergenic dermal reaction to bronopol is mixed. Laboratory testing of the allergenic properties of bronopol indicate a low potential for dermal sensitization; however, a number of case reports indicate isolated incidences of human

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allergenic reactions in various exposure situations (Bryce et al., 1978; Storrs & Bell, 1983; Frosch et al., 1990; Wilson & Powell, 1990; Rudzki et al., 1993).

No reports were identified on the allergenic properties of bronopol for the inhalation route of exposure.

Table 3

Reported Results for Acute Toxicological Studies of Bronopol

Evaluation Parameter	Minimum Value (Reference)	Maximum Value (Reference)
Oral LD50, Mouse	270 mg/kg (Frear, 1969)	350 mg/kg (Croshaw et al., 1964)
Oral LD50, Rat	180 mg/kg (Frear)	400 mg/kg (Croshaw et al.)
Intraperitoneal LD50, Mouse	20 mg/kg (Croshaw et al.)	32.8 mg/kg (female) (Bryce et al., 1978)
Intraperitoneal LD50, Rat	22.0 mg/kg (male) (Bryce et al.)	200 mg/kg (Croshaw et al.)
Eye Irritant, Rabbit	2 % in Solution (Croshaw et al.)	20 % in Solution (Elder, 1980)
Skin Irritant, Rabbit	2 % in Emulsion (Croshaw et al.)	20 % in Solution (Elder)
Inhalation LC50, Rat	0.18 mg/L (Elder)	> 5.0 mg/L (EPA, 1995)

LD50 = lethal dose for half of the study group
LC50 = lethal concentration for half of the study group
mg/kg = milligrams per kilogram of body weight
mg/L = milligrams per liter
% = percent
> = greater than

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Formaldehyde

Formaldehyde is a compound that is used as a sanitizing agent, as an embalming material, and in numerous chemical synthesis processes. Formaldehyde is a dermal sensitizer that can result in allergic contact dermatitis upon repeated skin contact (Marks & DeLeo, 1997). Although it is a respiratory irritant, formaldehyde is likely not a respiratory sensitizer (Rosenstock & Cullen, 1994).

Diphenymethane Diisocyanate (MDI)

The primary health effect associated with the isocyanate chemicals as a group is respiratory sensitization. Individuals sensitized to MDI may suffer from asthmatic symptoms even at very low exposure concentrations (NIOSH, 1986). MDI is also a potent skin sensitizer that produces allergic contact dermatitis in sensitized individuals, again at very low levels (Estlander, Keskinen, Jolanki, & Kanerva, 1992).

Bronopol

The evidence for dermal sensitization and allergenic dermal reaction to bronopol is mixed, with a number of case reports indicating incidences of human dermal allergenic reactions. The potential sensitizing property of bronopol on dermal exposure suggests the possibility that the material may be sensitizing for the inhalation route of exposure, although dermal sensitizers are not always respiratory sensitizers.

Given the wide spread use and the variety of new applications for the material, even low contact sensitization potential materials such as bronopol may produce

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sensitization in a large number of people when the exposure population is large. Continued exposure to a sensitizer may eventually sensitize susceptible individuals (Marzulli & Maibach, 1973).

Indoor Environmental Quality and Human Health

Indoor Air Quality

While many factors can affect the quality of the indoor environment, this discussion is focused on indoor air quality. People in the built environment may be subjected to building-related factors and agents that can degrade the actual or perceived quality of the air in the building. A partial listing of factors governing the perception of good indoor air quality is presented in Table 4. Good control of these factors should produce air quality that is acceptable to the majority of building occupants.

Table 4
A Partial Listing of Factors Affecting Perceived Building Air Quality

Sources of odors, contaminants, dusts, or allergens
Consistent temperature and relative humidity
Temperature and relative humidity at appropriate levels for the season of year, occupant clothing, and occupant activity
Circulation of air/drafts
Housekeeping and cleanliness
Facility lighting

Note: Information from EPA & NIOSH, (1991)

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There are four basic elements in the development of indoor air quality problems. A source of contaminants or discomfort must be present in the building. Potential indoor contaminant sources include chemicals, particulate sources, biological agents, and bioeffluent materials from the building occupants themselves. Then, a pathway must be present and available to facilitate transport of the contaminant from the source to the building occupants. The pathway is generally a moving body of air located in appropriate proximity to the source(s) and receiver(s). The pathway may exist as a result of differential air pressure between interior and exterior portions of the building, or the contaminant may be entrained and distributed by the building ventilation system. Next, the building must be occupied for any adverse perceptions and/or health effects to be manifested. If people are not present then air quality will not be an issue. Lastly, the heating, ventilating, and air-conditioning system may not be able to adequately control temperature and/or relative humidity, may not adequately dilute indoor contaminants with conditioned outdoor air, may not adequately filter airborne particulates, and may itself serve as a source of indoor contaminants. In this instance the source and pathway will function together to deliver the contaminant(s) to the building occupants.

Complaints of poor indoor air quality may be a result of comfort factors such as temperature and humidity control, stuffiness, or perception of moving air currents (drafts). Unpleasant odors are sometimes associated with poor indoor air quality, even if they are not associated with health complaints. Air quality complaints are often manifested as nonspecific health symptoms rather than a clearly defined illness. Certain subpopulations may have different tolerances for agents present in indoor air. The term 'sick building syndrome' refers to building occupants experiencing health or discomfort

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effects associated with their time in a building. A list of common indoor air quality health complaints is presented in Table 5. It is important to note that all of these symptoms may be present as a result of other factors not related to the building environment (EPA & NIOSH, 1991).

Table 5
Frequently Cited Indoor Air Quality Health Complaints

Headache	Fatigue
Shortness of breath	Sinus congestion
Cough	Sneezing
Eye, nose, and throat irritation	Skin irritation
Dizziness	Nausea

Note: Information from EPA & NIOSH, (1991)

Building related illness (BRI) refers to a clearly diagnosable illness brought on by an exposure to an agent present in indoor air. While these may be caused by chemicals or other agents of anthropogenic origin, BRIs are usually allergic reactions or specific infections associated with exposure to bioaerosols. For example, Legionnaire's disease is associated with proliferation of *Legionella* bacteria in ventilation system cooling water (EPA & NIOSH, 1991).

The impact of biological agents on indoor air quality is currently an active field of scientific and medical investigation. Indoor growth of fungi has generated attention from the mass media and has resulted in legislative activity to regulate the mold assessment and remediation industry. The three major health effects attributed to exposure to mold

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include infection, allergic reaction, and adverse reactions associated with generated mycotoxins. Humans with compromised immune systems are susceptible to opportunistic fungal infections that would not otherwise present a threat (American College of Occupational and Environmental Medicine [ACOEM], 2002). Nine percent of nosocomial infections are caused by fungi (Redd, 2002). Some pathogenic fungi such as *Blastomyces, Coccidioides, Cryptococcus, and Histoplasma* can infect people of normal health status (ACOEM, 2002).

The allergenic properties of mold are well known, generally resulting in hay fever-like symptoms in allergic individuals. Irritation of the eyes and upper respiratory tract as well as rhinitis is often reported and may be triggered by low-level exposures in sensitive individuals. People with no allergies may experience similar symptoms from high-level mold exposure. Those with allergies, asthma, or having preexisting respiratory diseases often report symptoms associated with exposure to mold (EPA & NIOSH, 2002). However, current scientific and medical inquiry generally does not support claims of adverse health effects as a result of mycotoxins from mold growth in water-contacted indoor environments (ACOEM, 2002).

Mold growth in the indoor environment is not acceptable from an indoor air quality standpoint. Mold growth results in unpleasant odors, damages materials on which it grows, and produces allergenic materials.

Mold spores are found in all indoor environments with the exception of special clean rooms or medical isolation rooms. The majority of indoor mold spores originate outdoors and drift indoor with moving air, are transported in on the person of the occupants, or are carried in on objects moved to the indoors. Readily available nutrient

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sources are available indoors in the form of building materials, furniture, and other contents. Mold colonization will generally not occur in the indoor environment unless a water leak or elevated relative humidity condition exists. Mold growth can occur on interior surfaces of air-conditioning air-handling units unless the nutrient source consisting of impacted particulate material is periodically removed.

Remediation of mold growth on indoor materials consists of four basic steps. First, since mold colonization has occurred as a result of water intrusion or elevated relative humidity, the source(s) of water intrusion or elevated humidity must be remedied. Then wetted materials should be dried as quickly as feasible. Thirdly, porous materials and materials that are impractical to effectively clean are removed and discarded. Lastly, remaining structural and finish materials are cleaned and sanitized and permitted to thoroughly dry (EPA, 2001). While biocides are not necessarily recommended in authoritative guidelines for mold remediation, these materials are often used in conjunction with cleaning solutions and high efficiency particulate air (HEPA) vacuuming to treat mold-affected areas.

Workers in Indoor Environments

Approximately 89 million people work in nonindustrial nonagricultural indoor environments in the United States. This represents about seventy percent of American workers. Workers may spend 2,000 hours or more per year in the occupational environment, a little less than one-fourth of every year of life in the working years. The occupational environment is unique in that most employees may have little direct control over their surroundings. In many cases the building will have mixed uses such as a

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having a laboratory or printing operation in the same building and sharing the same ventilation system with office occupants, allowing the possibility that airborne chemicals and particulates in these areas will enter the office areas of the building.

It is estimated that thirty-five to sixty million workers will experience one or more nonspecific building-related symptoms. The total financial impact of these symptoms including health care, lost time and productivity is estimated to be between twenty to seventy billion dollars annually (Mendell et al., 2002).

Nonoccupational Indoor Environments

In the United States about 50 million people suffer from allergic diseases each year. Allergies are the sixth leading cause of chronic disease, with health care costs estimated at over eighteen billion dollars annually. Americans spend more time indoors than ever before, making indoor air quality and exposure to indoor allergens important to a greater number of people. Nonoccupational indoor environments include homes, apartments, schools, day care, and shopping and entertainment venues. Exposures in nonoccupational settings include a wider range of occupant ages and health status than exposures associated with the workplace.

Asthma affects more than fifteen million people in the United States annually; of these approximately five million are children. Asthma accounts for more than five thousand deaths, eleven million doctor visits, ten million missed school days, and over one hundred million days of restricted activity. A portion of these statistics can be attributed to exposure to indoor allergens and other indoor air quality agents (AAAAI, 2000).

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Analytical Methods for Bronopol

A number of analytical methods have been published for quantitative determination of bronopol. Many of these are assay methods for quantifying bronopol in cosmetic and pharmaceutical products or purity verification of bronopol antimicrobial formulations. None of the published methods reviewed included analysis of bronopol from collected air samples.

Polarographic Assay

Bryce et al. (1978) published a treatise on bronopol analytical methods and other topics. The first method fully described in the paper used McIlvaine's buffer solution as a base electrolyte; this solution as described will maintain a constant pH of 4.0. Aqueous solutions of bronopol in the concentration range of 10^{-3} to 10^{-5} molar could be analyzed directly. Bronopol in fatty-base formulations such as cosmetic products could be analyzed following phase-transfer extraction of bronopol using the base electrolyte solution. Dissolved oxygen was removed by passing oxygen-free nitrogen through the analytical solution for ca. 10 to 15 minutes. Analysis was then performed using a suitable polarograph.

The polarographic method estimates the concentration of the nitro group in the analyte solution; therefore, any compound with a nitro group will potentially interfere with the method. Since some breakdown products of bronopol retain the nitro group, this analytical method was recommended for freshly prepared formulations. Given the lack of specificity for bronopol of the polarographic method, a calibration curve must be

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prepared for each formulation analyzed. Reported precision of the method in the absence of interfering compounds is ca. ± 2 %.

Gas-Liquid Chromatography

The Bryce et al. (1978) paper describes various GLC analytical methods for bronopol in aqueous solutions. One method involves an acetylation step whereby bronopol in chloroform is reacted with acetyl chloride. The solution is sealed in a vial and reacted in a steam bath for three hours. Using nitrogen as the carrier gas and injecting a sample into a packed column, the resultant reaction compound of bronopol diacetate is detected on a flame ionization detector. Another acetylation method described uses an electron capture detector.

In another Bryce et al. (1978) GLC method bronopol in chloroform is reacted with a silylating reagent containing trifluoroacetic acid and hexamethyldisilazane. The solution is sealed in a vial and reacted in a steam bath for one hour. Using nitrogen as the carrier gas and injecting a sample into a packed column, the resultant reaction compound of bronopol di(trimethylsilyl)ether is detected on a flame ionization detector.

The GLC methods described have been used to determine bronopol content in aqueous solution at concentrations as low as 5 to 50 ppm.

Titrimetric Assay

A titrimetric assay for bronopol is reported in a General Medical Council publication (1993). The assay combines bronopol, sodium hydroxide, and nickel-aluminum alloy; the mixture is refluxed for 1 hour. Following heating, nitric acid and

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silver nitrate are added; the mixture is titrated with ammonium thiocyanate using ammonium ferric sulfate as an indicator. The quantity of titration agent is proportional to the concentration of bronopol in the sample. The procedure must be repeated in the absence of bronopol to obtain a reference value for comparison.

Enzymic Assay

Sanyal, Chowdhury, and Banerjee (1993) described a method to assay liquid pharmaceutical formulations for bronopol based on the inhibition of a thiol protease, papain. An aliquot of the pharmaceutical formulation to be analyzed is added to 10 ml of a standard solution of 60 µg bronopol per milliliter concentration. The mixture is shaken for two hours at 40 ºC and the total volume is increased to 50 ml. The solution is then clarified by centrifugation at 3,000 g for 15 minutes.

Samples and calibration standards are cooled in an ice bath. A pH 5.5 enzyme solution containing papain, formic acid, and ethylenediaminetetraacetic acid (EDTA) is added to the samples and standards and these are incubated at 40 ºC for 15 minutes. Samples and standards are quickly cooled in an ice bath and a pH 8.0 buffered solution of azoalbumin, tris(hydroxymenthyl)methylamine, and hydrochloric acid is added. These are then incubated at 37 ºC for 15 minutes and cooled in an ice bath. A trichloroacetic acid mixture is then added to precipitate out residual solid material. Following centrifugation the absorbance is read at 390 nm for samples and standards and compared to a reaction blank. The method is linear in the range of 0.8 to 4.0 µg/ml.

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Ultraviolet Spectrophotometric Method

Sanyal et al. (1996) described a UV-VIS spectrophotometric method for bronopol analysis in raw materials using a derivatizing reaction step. The retroaldol reaction of aqueous bronopol with aqueous sodium hydroxide generates a bromonitroethanol chromophore. Experiments in the paper demonstrate complete conversion of bronopol to bromonitroethanol through the use of thin-layer chromatography (TLC) techniques. The bronopol spot with a retention factor (R_f) of 0.62 completely disappeared upon addition of sodium hydroxide and was replaced by a spot on the film with an R_f of 0.74. The compound with an R_f of 0.74 has been identified as bromonitroethanol.

The absorbance maximum of 244 nanometers is specific to bromonitroethanol; Sanyal et al. report that aqueous solutions of bronopol only (15 μg/ml) have practically no absorbance in the ultraviolet region of 220 to 350 nm. The reported molar absorption coefficient of bromonitroethanol at 244 nm is 8330; the method is reportedly linear in the concentration range of 5 to 25 μg/ml.

Spectrophotometric Analysis - Background

A spectrophotometer is a device for determining the amount of light that is transmitted through the sample being analyzed. A light passes through a series of slits and over a diffraction grating that serves to limit the light wavelength directed through the sample to a very narrow band. This portion of the spectrophotometer is called the monochrometer. The monochrometer may be motor driven to scan through a range of light wavelengths at a predetermined fixed rate. The light passes through the sample and is directed to a detector to determine the amount of light transmitted through the sample.

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The result is a spectral scan of light transmission through the sample (Skoog & West, 1980).

Under certain circumstances spectrophotometry can be used for the identification of unknown compounds. Different compounds will absorb light of different wavelengths in a unique pattern, which can be thought of as a "fingerprint". Identification depends on the presence or absence of spectral curve maxima and minima, the wavelength at which they occur, and sometimes the amplitude of transmission.

Spectrophotometry may also be used to determine the concentration of a particular compound in a sample, if the identities of the compounds in a sample are known. This is done by comparing the intensity of light transmitted through the sample of unknown concentration to the intensity of light transmitted through standards of known concentration (Stearns, 1969).

The term transmittance (T) can be thought of as the optical density of a substance at a particular wavelength of light and is defined as the fraction of the incident light power (P_o) transmitted through the sample (P). This is shown in Equation 1.

$$T = P/P_o \quad (1)$$

Transmittance is often expressed as a percentage. Absorbance (A) is defined as the negative logarithm to the base ten of the transmittance as shown in Equation 2.

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$$A = -\log_{10}T = \log_{10}P_o/P \quad (2)$$

In contrast to transmittance, the numerical magnitude of absorbance increases as the attenuation of the light beam through the sample becomes greater. It should be noted that absorbance cannot be measured directly as can transmittance; absorbance must be calculated from transmittance data. The relationship between absorbance and concentration of a species in a sample is described by Beer's law, which is expressed in Equation 3. In this equation a is the absorbtivity of the analyte species at a specific wavelength of light, b is the path length of light through the sample, and c is the concentration of the analyte in solution. The term “a” is sometimes called the extinction coefficient of the analyte and is unique for each particular compound.

$$A = abc \quad (3)$$

This relationship holds for dilute solutions; at high concentrations interaction between the absorbing species in solution alters their ability to absorb a given wavelength of light. It can be seen from this equation, that since a and b are fixed values, the absorbance varies linearly with concentration. Within the concentration limits described above, concentration can be determined by reading the absorbance and comparing to the absorbance for a known concentration of the same species (Skoog & West, 1980).

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Good spectrophotometric practices call for the sample cell orientation to be repeated consistently. Also, the use of a reference solution is called for to minimize handling and contamination variables. The reference solution must be handled in exactly the same manner as the sample(s) (Everett, 1981).

Air Sampling Methods Utilizing Treated Filters

OSHA Sampling and Analytical Method for MDI

OSHA (1989) Method 47 for diphenylmethane diisocyanate (MDI) utilizes glass fiber filters impregnated with 1-(2-pyridyl) piperazine (1-2PP). The spontaneous reaction of MDI with 1-2PP forms a stable derivative, which can be analyzed by high performance liquid chromatography using a fluorescence detector. This reaction takes place on the surface of the filter fibers. Citing difficulties in the generation of controlled MDI test atmospheres, method development utilized MDI derivative spiked onto coated filters. Analysis of samples collected during field trials indicates that MDI is collected and reacted on the 1-2PP treated filters under non-laboratory conditions.

Retention of the MDI derivative was verified by flowing 20 liters of humid air through spiked filters. A retention efficiency of 97 % is reported, accompanied by an extraction efficiency of 96.3 %. Detection limit of the overall sampling and analytical is 11.6 nanograms per sample.

OSHA Sampling and Analytical Method for Ozone

OSHA (1995) Method ID-214 for ozone utilized glass fiber filters impregnated with sodium nitrite, potassium carbonate, and glycerin. Ozone reacts with the nitrite to

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form nitrate via oxidation. The nitrate is analyzed by ion chromatography using a UV-VIS detector at 200 nanometers. The qualitative detection limit reported is 0.37 micrograms per milliliter of filter extract solution. Collection efficiency and desorption efficiency are each reported to be approximately 100 %.

The reaction of ozone with nitrite takes place in an aqueous film on the surface of the filter fibers. The presence of highly hydroscopic glycerin (Hawley, 1981) on the glass fiber filter surface sequesters ambient atmospheric water, providing an appropriate medium for the reaction.

Glass Fiber Filtration Media

Glass fiber filters used in industrial hygiene air sampling are generally constructed of multiple layers of randomly oriented fibers. Due to the nature of their construction the pore size is inconsistent and is by definition undefined; the pore size rating for glass fiber filters is usually assigned an approximate rating through testing. The thickness of glass fiber filters used for air sampling is usually in the hundreds of micrometers. Particles collected in glass fiber filters are captured within the fiber matrix as the air stream negotiates the tortuous pathway through the filter. For this reason these types of filters are often referred to as depth filters.

Glass fiber filters are of a random fiber composition and commonly have little mechanical strength. These commercially produced filters are available in two basic forms, with binder and binder-free. Those with binder are often used in applications where gravimetric analysis will be performed since the binder serves to minimize filter fiber loss during handling and sampling. Because the filter is comprised of glass, these

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filters are usually hydrophobic; therefore they perform efficiently under a wide ambient humidity range.

The high void volume of glass fiber filters results in a high loading capacity and the ability to flow high volumes of air. Glass fiber filters are useful for their high capture efficiency and the ability to trap very fine particulates (Hering, 1989; SKC Inc., 2005). These types of filters are also used where a pretreatment or derivatizing agent is desired at the point of sample collection (OSHA, 1989; OSHA, 1995).

Sampling and Analytical Method Validation

In 1995 NIOSH published a document providing a process for sampling and analytical method development. This document was part of the continuation of the Standards Completion Program. Generation of standard test atmospheres, evaluative methodologies, and statistical protocol and validation criteria for the air sampling methods and laboratory analysis are presented in this publication. Elements of this publication are utilized in this body of work.

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Chapter Three - Research Design

Overview

Investigations of bronopol determination using gas chromatography (GC) and high-pressure liquid chromatography (HPLC) were previously attempted in the current work. Analytical results obtained were inconsistent in nature; no suitable analytical method was developed using GC or HPLC. Based on the results of these prior investigations, it was felt that the irregular analytical results were due to the reactive nature of bronopol itself. As a result, the Sanyal et al. (1996) derivatizing procedure was evaluated as a method to form a stable compound suitable for analysis. The results of this line of investigation follow.

In general, the derivatizing and UV-VIS analytical techniques in the Sanyal et al. (1996) paper will comprise the analytical portion of this work. A calibration curve is generated and is used as a reference against which samples are compared. Glass fiber filters are treated with the derivatizing agent used in the Sanyal et al. work plus a humectant agent to sequester atmospheric moisture. Treated filters are spiked with aqueous bronopol solutions and are analyzed to gauge recovery efficiency from the filters. A chamber containing bronopol aerosol is sampled simultaneously with treated glass fiber filters and inert filters; the inert filters are used as a benchmark method. Finally, bronopol samples are generated and evaluated for sample storage stability.

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Reagents and Materials Used

Standard wet chemistry glassware was used. Glassware was washed and rinsed using distilled tap water and Aconox, Inc. laboratory detergent, part number 1104-1. Spectrophotometric analysis was performed on a Beckman Coulter, Inc. DU-64 single path UV-VIS Spectrophotometer. Cuvettes were 1.0-centimeter pathlength quartz with a Teflon stopper, Fisher Scientific International part number 14-385-904D. Cuvettes were rinsed with acetone. Filter treatment solution and filter spiking solution were applied using microsyringes from the Hamilton Company. Chemical reagents used are listed in Table 6.

The filters used for filter spiking and derivatizing air sampling were SKC Inc. brand glass fiber (GFF), 25 mm diameter, part number 225-702. These filters are binder free, have a nominal pore size of 1.0 µm, and have a cross-sectional thickness of 0.31 to 0.35 mm.

Inert reference filters used for air sampling were Millipore Corporation 25 mm diameter Durapore® brand polyvinylidene fluoride (PVDF) membrane filters, 0.45 µm pore size, part number HVHP02500. Syringe filters used to purify filter extract solutions were Millipore Corporation Millex®-LCR brand 25 mm PTFE, 0.45 µm pore size, part number SLCR 025 NS.

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Table 6
Chemical Reagents Used

Reagent	Use
Bronopol, 2-bromo-2-nitro-1,3-propanediol, Sigma-Aldrich Company, part number 13,470-8.	Used in all laboratory procedures.
Sodium hydroxide 50 weight percent solution (approximately 19 Molar), Fisher Scientific International, part number SS254-1.	Derivatizing agent for all laboratory procedures.
Glycerin, Certified/Spectroanalyzed, Fisher Scientific International, part number G153-1.	Humectant agent for treating glass fiber filters.
Water, spectrophotometric grade, Fisher Scientific International, part number W7-4 Optima.	Used in all laboratory procedures.
Nitrogen, Airgas, Inc., Ultra High Purity, product number NI UHP 300.	Used for chamber sampling trials.
Acetone, spectrophotometric grade, Fisher Scientific International, part number A19-1.	Used for rinsing cuvettes.

Bronopol as obtained from the supplier was in the form of small granular crystals. Bronopol used in air sampling experiments was ground for 10 minutes in a dry nitrogen atmosphere using a glass mortar and pestle. The quantity used for each chamber run was determined using a 5 ml volumetric flask.

Laboratory Equipment

Introduction

A schematic representation of the dust generator and sampling chamber utilized is presented in Figure 2.

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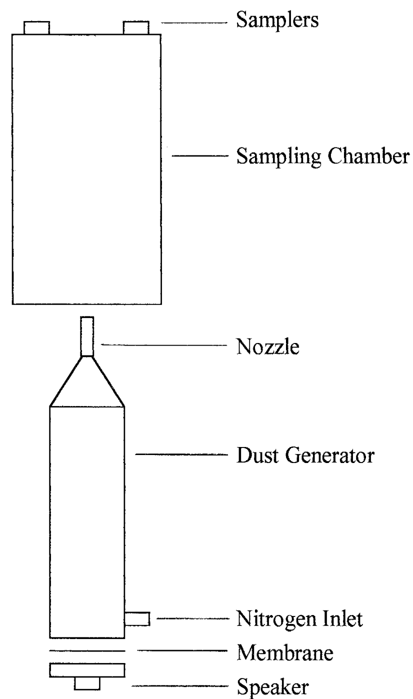

Figure 2. Schematic Diagram of the Dust Generator and Sampling Chamber.

Dust Generator

The dust generator was constructed of 3-inch nominal inner diameter PVC pipe and related components. The main body of the dust generator consisted of a 33 cm long segment of pipe; the dust generator was configured with the main body oriented vertically. Calculated dust generator internal volume is 1.5 liters. A membrane consisting of consumer grade Glad® polyethylene food cling wrap was stretched across the bottom end of the pipe; a loudspeaker was located directly beneath the polyethylene membrane. The loudspeaker was powered by a consumer type radio. Nitrogen gas was

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introduced at the bottom of the dust generator and flowed vertically out of the top of the dust generator into the sampling chamber. The top of the dust generator consisted of a series of tapered plumbing fixtures culminating in a nozzle of a nominal five eights inch inner diameter PVC pipe. Prior to the addition of pulverized bronopol the dust generator was purged with nitrogen gas for 10 minutes at 5 LPM.

In operation bronopol was placed directly on the membrane at the bottom of the dust generator and the loudspeaker was activated to vibrate the powder into the flowing nitrogen gas stream. Nitrogen gas was flowed at a nominal 2.0 liters per minute.

Aside from introducing a bronopol aerosol into the moving nitrogen stream, the dust generator also functions as a vertical elutriator. Subsequent to grinding with the mortar and pestle the bronopol is expected to be of a diverse particle size distribution. In order to introduce only fine particulates into the sampling chamber, size selection is performed by maintaining nitrogen gas flow at a specific velocity through the dust generator. With an inner diameter of 3 inches, the dust generator has an internal cross-sectional area of 45.6 cm^2. Burton (1991) provides a method to calculate the average gas velocity (V_{avg}) through the dust generator as follows:

$$V_{avg} = Q/A \quad (4)$$

In Equation 4 V is the average gas flow velocity, Q is the volumetric gas flow rate, and A is the cross-sectional area of the vessel through which the gas is flowing. At a

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nitrogen flow rate of 2 liters per minute the average vertical gas velocity through the dust generator is 0.365 cm/sec. A gas flowing through a pipe will experience friction as it moves against the interior surfaces. As a result gas flowing near the walls of the vessel will have a reduced velocity as compared with gas flowing nearer the center of the pipe (Burton, 1991). The relationship between average gas flow velocity through the pipe (V_{avg}) and the gas velocity at the centerline of the pipe (V_{cl}) is described as follows:

$$V_{avg} = 0.9 \times V_{cl} \tag{5}$$

The calculated maximum gas flow velocity at the centerline of the dust generator is 0.406 cm/sec.

In the dust generator, bronopol particles in the nitrogen gas stream will tend to move downward under the influence of gravity. This motion is counteracted by the upward direction of travel of the nitrogen gas stream. The settling velocity of a particle is determined by the physical size and density of the particle and the viscosity of the gas surrounding the particle. The extent to which a particle will travel upward or fall downward in the flowing nitrogen is determined by the settling velocity of the particle. In this experiment particles with a settling velocity slower than the velocity of the gas stream in the dust generator will travel upward and be carried into the sampling chamber. The terminal settling velocity (V_{ts}) of a particle is determined by the following relationship:

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$$V_{ts} = \rho_p d_a^2 g/18\eta \qquad (6)$$

In Equation 6 ρ_p is the density of the particle, d_a is the aerodynamic diameter of the particle, g is the acceleration of gravity, and η is the viscosity of the gas in which the material is traveling (Reist, 1993). Aerodynamic diameter is defined as the diameter of a sphere with a density of 1 gm/cm^3 having the same aerodynamic characteristics as the particle of interest. For bronopol (ρ of 1.1 gm/cm^3) particles with a settling velocity equivalent to the average nitrogen upward velocity of 0.365 cm/sec have an aerodynamic diameter of 10.4 µm. Particles with a settling velocity equivalent to the maximum nitrogen upward centerline velocity of 0.406 cm/sec have an aerodynamic diameter of 11.0 µm. Since the dust generator is tapered inward at the top, gas velocity will increase in this area. The result is that particles of increasing d_a will travel with the gas stream. Therefore it is likely that the bronopol particles with a d_a of up to 11 µm will be entrained in the moving stream of nitrogen and will be introduced into the sampling chamber.

The PVC pipe nozzle at the terminal end of the dust generator is 1.98 cm^2 in area. Using Equation 4 the nitrogen gas velocity as it exits the dust generator nozzle into the sampling chamber is 16.8 cm/sec (see Appendix A for all dust generator calculations).

Sampling Chamber

The sampling chamber was constructed of stainless steel residential chimney tubing fitted with flat caps for the top and bottom. The chamber has an internal diameter

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of 24.8 cm and a height of 48.9 cm. Calculated internal volume of the chamber is 23.6 liters.

Three sampling ports were located on the top of the sampling chamber in an equidistant fashion; the center of each sampler is 7.3 cm from the center of the chamber top. The sampling ports were of the same approximate diameter as the samplers used. Each port was fitted with a rubber o-ring that sealed against the body of the sampler. The bodies of the samplers were in contact with the sampling chamber, which provided an electrical bond. The sampling chamber and dust generator were electrically bonded to each other and connected to an earth ground to minimize the influence of static charges on the airborne bronopol particulate.

A small hole of approximately 1 cm diameter was centrally located on the top of the chamber to vent excess nitrogen flowing through the system. The bottom of the sampling chamber was fitted with a rubber grommet of a suitable size to accept the dust generator terminal nozzle.

Nitrogen gas and entrained bronopol particulate exit the circular dust generator nozzle in the form of a jet at 16.8 cm/sec. A jet will retain approximately 10 % of its initial velocity at 30 times the diameter of the jet exit (American Conference of Governmental Industrial Hygienists, Inc. [ACGIH], 1998). With a jet diameter of 1.59 cm the 10 % velocity boundary is at 30 x 1.59 cm = 47.7 cm away from the nozzle, very close to the 48.9 cm height of the chamber.

Nitrogen gas jet velocity at the top of the sampling chamber is 16.8 cm/sec x 0.1 = 1.7 cm/sec. The lowest sampler flow rate used in the experiments was approximately 300 ml/min. With a filter of 25 mm diameter the calculated filter face velocity at the

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lowest flow rate used is approximately 1 cm/sec. The nitrogen gas jet velocity at the top of the chamber is greater than the maximum velocity through the dust generator; therefore, the entire size range of bronopol particles introduced into the chamber should be available in the vicinity of the samplers. Ignoring the dynamics of the nitrogen jet flow within the chamber the average nitrogen gas velocity through the chamber is 0.069 cm/sec at 2 LPM flow. Particles with a settling velocity equivalent to the average nitrogen upward velocity of 0.069 cm/sec have an aerodynamic diameter of 4.5 μm. Bronopol particles with a d_a larger than 4.5 μm will likely settle out to the floor of the chamber interior unless captured by the samplers on the first pass.

For experimental sampling events nitrogen flow through the chamber was 2 LPM. Sampler flow rates ranged between 300 to 600 ml/min. Total flow volume for the three samplers ranged from 0.9 LPM to 1.8 LPM; consequently, excess nitrogen flow through the chamber ranged from 0.2 LPM to 1.1 LPM.

A NIOSH (1973) publication indicated that the spread angle of a circular unbounded jet gas stream is approximately 22 degrees. The nitrogen gas stream entering the sampling chamber from the dust generator enters the chamber at 16.8 cm/sec and flows upward toward the samplers; at the top of the chamber the jet has spread to a diameter of 19.8 cm based on the 22° expansion. The samplers are arranged equidistantly in a circle around the top of the chamber; the outer diameter of the circle formed by the samplers is 17.1 cm (see Appendix B for all sampling chamber calculations). Based on these calculations, the samplers are completely enclosed within the nitrogen gas stream from the dust generator. Other sources (Burton, 1999) reference spread angles for jets of upwards of 28°.

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Sampling Equipment

SKC Inc. brand Button Aerosol Samplers were used to hold the sampling filters, part number 225-360. These samplers are designed to sample inhalable particulate when used at 4 LPM; in these experiments the airflow rates used were lower than the rate specified for the sampler. The sampler is provided with a stainless steel back up screen for the filter. These samplers are of metal construction and provide electrical conductivity, ease of assembly/disassembly, and ease of cleaning.

Air sampling pumps were SKC Inc. brand AirChek Sampler model number 224-PCXR7. For low-flow sample collection at rates less than 0.4 liters per minute, a Gillian Instrument Corporation brand triple critical orifice manifold part number THK-TVM-200 was used with a single sampling pump.

Laboratory Methods

Examination of Chromophore Development

Analytical reference solution. All bronopol samples were analyzed using a sodium hydroxide/glycerin blank as a reference. The quantities of constituents in the blank solution are identical with the quantities in an analyte solution or with the quantities present on a treated glass fiber filter with the exception that no bronopol is added.

Verification of absorbance maximum. Sanyal et al. (1996) report an absorbance maximum for derivatized bronopol of 244 nm. To evaluate this, a 9.94 μg/ml bronopol solution was derivatized using the standard methods previously described. A second

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solution of 9.94 μg/ml bronopol was formulated in spectrophotometric grade water containing only glycerin in the same concentration as when the derivatizing agent is used. Appropriate blank solutions were prepared to provide analytical references. Spectrophotometric scans were obtained to verify the absorbance maximum and to evaluate any potentially interfering peaks.

A wavelength scan of the non-derivatized bronopol solution was obtained through the range of 900 to 200 nm at a rate of 500 nm/min; this represents the working limit of the spectrophotometer used. A second scan of the non-derivatized bronopol solution was made to focus on the region of interest from 320 to 230 nm, again at a rate of 500 nm/min. Finally, a scan of the derivatized bronopol solution was made from 320 to 230 nm at a rate of 500 nm/min. All scans were recorded graphically using an Epsom LX-800 dot-matrix printer.

Additionally, absorbance readings for a derivatized 10.11 μg/ml bronopol solution versus a blank solution were recorded manually in the range of 248 to 236 nm. Absorbance values were recorded in one-nanometer increments.

Chromophore development with time. The absorbance of a derivatized bronopol solution of 10.10 μg/ml bronopol was recorded at one minute after mixing; absorbance readings were then obtained every five minutes thereafter for a total of one hour. Sanyal et al. (1996) reported the reaction to be essentially instantaneous.

Concentration of sodium hydroxide. The effect of varying the sodium hydroxide concentration on the absorbance of the analyte was determined. One mole of sodium

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hydroxide is needed to react with one mole of bronopol. Bronopol was added to sodium hydroxide concentrations of 0.005, 0.01, 0.02, 0.04, 0.1, 0.2, 0.3, and 0.4 molar; final bronopol concentration in solution was 10.18 µg/ml. Absorbance at 244 nm was recorded for each solution.

Calibration Curve

A calibration curve for derivatized bronopol was developed. The range of bronopol concentrations in solution was 1 to 25 µg/ml in an approximation of the linear range described by Sanyal et al. (1996). Nine different concentrations of bronopol were targeted in formulating the calibration curve solutions: 1, 2, 4, 6, 8, 10, 15, 20, and 25 µg/ml. Six solutions of each concentration were developed and analyzed at 244 nm.

Preparation and Use of Glass Fiber Filters

Treatment of glass fiber filters with derivatizing agent. Glass fiber filters were treated with a derivatizing solution consisting of sodium hydroxide and glycerin in aqueous solution. Sodium hydroxide is the derivatizing agent as described in Sanyal et al. (1996); the reaction of sodium hydroxide with bronopol in an aqueous environment yields bromonitroethanol, which is a stable chromophore.

OSHA (1995) Method ID-214 for ozone utilizes glass fiber filters impregnated with derivatizing agents and glycerin. Glycerin is hygroscopic and functions as a humectant (Hawley, 1981) on the surface of the glass fibers comprising the filters in the ID-214 ozone sampling method.

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Glycerin was incorporated on the surface of the glass fiber filters in the current experiment; glycerin quantity used per filter (4 µl) was the same as the ID-214 method. The reaction of bronopol with sodium hydroxide takes place in an aqueous media. The highly hydroscopic glycerin on the glass fiber filter surface sequesters ambient atmospheric water, providing an appropriate medium for the reaction. Glycerin was applied to filters in this experiment in the same quantity as in the OSHA (1995) ozone method.

The reported linear range of the Sanyal et al. (1996) method is 5 to 25 µg/ml of bronopol. In this experiment filters are extracted in 5 ml of water; therefore, the maximum bronopol loading desired on each filter is 125 µg. Bronopol has a molecular weight of 199.99 gm/mol; 125 µg of bronopol is 6.25×10^{-7} mol.

The 50 weight percent sodium hydroxide reagent used in this experiment has a concentration of approximately 19 molar. The filter treatment solution used in this experiment consists of 2.0 ml of 50 weight percent NaOH and 1.0 ml of glycerin; final volume in aqueous solution is 50 ml. A 0.2 ml aliquot of the filter treatment solution is applied to each filter. This results in a 240-fold molar excess of sodium hydroxide to bronopol at the maximum desired filter loading. A large molar excess of sodium hydroxide is desirable to move the reaction equilibrium strongly toward the formation of bromonitroethanol (see Appendix C for all glass fiber filter treatment calculations).

Filters to be treated were placed on a clean glass surface. A 0.2 ml aliquot of the filter treatment solution was added to each filter while ensuring that the filter was thoroughly wetted over the entire surface. Filters were allowed to air dry under ambient laboratory conditions for approximately two hours prior to use.

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Filter spiking. Treated glass fiber filters were spiked with aqueous bronopol solutions of known concentration; the filters were manually spiked using a microsyringe while holding the filter with a pair of forceps. Each filter received 40 µl of bronopol solution in concentrations ranging from 0.48 to 2.8 mg/ml. The filter spike volume of 40 µl was chosen since it saturates only a portion of the filter surface; this allows the filter to be held at the dry edge of the filter without the spike solution contacting the forceps. This minimizes the loss of bronopol spiked onto the filters. Following spiking the filters were allowed to air-dry prior to extraction and analysis at 244 nm.

Spiked filters were grouped into low, medium, or high bronopol loading sets. Filters in the low group were spiked with approximately 19 µg bronopol, in the medium group approximately 44 µg bronopol, and in the high group approximately 113 µg bronopol. Ten filters were spiked in each of the three sets. Following extraction in 5.0 ml spectrophotometric grade water the final bronopol concentrations in solution were 3.8, 8.8, or 22.6 µg/ml.

Filter extraction procedures. Glass fiber filters containing bronopol were placed in glass screw cap 16 mm by 125 mm culture tubes and 5.0 ml of spectrophotometric grade water was added. Each tube and filter was placed in an ultrasonic bath for 5 minutes followed by gentle shaking. Since glass is strongly absorbing in the ultraviolet region, any glass fibers released from filters will be present in the filter extract solution and will create an interference with the spectrophotometric analytical method. Therefore, extract solutions were filtered through a syringe filter prior to placement into a cuvette for spectrophotometric analysis.

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PVDF filters containing bronopol were placed in a culture tube containing 4.8 ml of spectrophotometric grade water and 0.2 ml of filter treatment solution. From this point forward extraction procedures for PVDF filters were identical to that for glass fiber filters.

Filter blank count. Treated glass fiber filters with no bronopol were extracted and analyzed using the techniques previously described. GFF extract solutions were drawn through a syringe filter prior to analysis. Twelve blank values were obtained in this manner.

Chamber Verification and Chamber Sampling

Preparation of the sampling chamber for experimental trials. Prior to activation of the dust generator, nitrogen was allowed to flow through the chamber at 5 LPM for 50 minutes to purge water vapor and oxygen. Following this, nitrogen gas flow was reduced to 2 LPM and the dust generator was activated. The dust generator and sampling chamber were allowed to equilibrate for 2 hours prior to initiation of sampling.

Chamber sampling procedures. In general three chamber runs were performed sequentially with each chamber run generating 3 samples. Between sampling runs the nitrogen flow of 2 LPM was maintained and the dust generator loudspeaker was operating. Following the conclusion of sampling the dust generator and chamber were rinsed with tap water and air-dried.

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Distribution of bronopol particulate concentration in the sampling chamber. To verify consistent presentation of the bronopol aerosol to the chamber samplers, inert PVDF filters were used to collect samples from the chamber for several trials. A total of nine chamber particulate concentration distribution runs were performed, generating a total of twenty-seven samples. Each sampling event lasted 20 minutes. Sampling at different filter flow rates varied the quantity of bronopol collected on the filters; flow rates used were 300, 450, and 600 ml/min. The filters were extracted in a derivatizing solution and analyzed for bronopol.

Sampling of generated bronopol atmospheres. For sampling of bronopol particulate in the chamber, one inert PVDF filter and two treated glass fiber filters were used simultaneously. Each sampling event lasted 20 minutes. Sampling at different filter flow rates varied the quantity of bronopol collected on the filters.

One sampling trial was performed at 300 ml/min filter flow rate, one trial was performed at 450 ml/min, and one trial was performed at 600 ml/min; each sampling trial consisted of three chamber runs. In all this generated eighteen bronopol samples collected on treated glass fiber filters. The analytical results from the treated glass fiber filters were compared against the nine inert PVDF filters that were sampled concurrently.

Sample Storage Stability

Derivatized bronopol solutions. Aqueous bronopol was spiked onto 18 treated glass fiber filters; the filters were allowed to air dry. Six filters were spiked with 25 µg bronopol, six with 60 µg bronopol, and six with 125 µg bronopol. The filters were

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extracted and analyzed on the same day. The solutions from day one were stored and analyzed after fourteen days. All stored samples were kept in a laboratory cabinet under ambient laboratory conditions in glass screw cap 16 mm by 125 mm culture tubes.

Bronopol on treated glass fiber filters. Aqueous bronopol was spiked onto 36 treated glass fiber filters; the filters were allowed to air dry. Twelve filters were spiked with 25 μg bronopol, twelve with 60 μg bronopol, and twelve with 125 μg bronopol. Six of each set were extracted and analyzed on the same day; six of each set were extracted and analyzed after forty-eight hours.

Data Evaluation

Examination of Chromophore Development

Absorbance spectrum. Spectral curves of a 9.94 μg/ml non-derivatized bronopol solution was obtained in the range of 900 to 200 nm and 320 to 230 nm and recorded graphically. A derivatized bronopol solution of the same concentration was scanned from 320 to 230 nm. Absorbance in the 244 nm range was noted and compared against the absorbance maximum of 244 nm as reported by Sanyal et al. (1996). Observations were made of any potentially interfering peaks in the region of interest.

Verification of absorbance maximum. The absorbance for a derivatized 10.11 μg/ml bronopol solution was compared against the 244 nm maximum as reported by Sanyal et al. (1996).

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Chromophore development with time. The 244 nm absorbance of a 10.10 µg/ml derivatized bronopol solution was read at 1 minute and 5 minutes following addition of the derivatizing agent, and then every 5 minutes thereafter for a total of one hour. Absorbance read at each 5-minute interval was compared with the 1-minute value. Average, standard deviation, and percent coefficient of variation (CV %) analysis were obtained.

Concentration of sodium hydroxide. The 244 nm absorbance values of 10.18 µg/ml bronopol solutions were obtained at 0.005, 0.01, 0.02, 0.04, 0.2, 0.3, and 0.4 molar sodium hydroxide concentrations. These values were compared against the absorbance of bronopol in 0.1 molar sodium hydroxide. The sodium hydroxide concentration used in the Sanyal et al. (1996) work was 0.1 molar. Average, standard deviation, and percent coefficient of variation analysis were obtained.

Calibration Curve

Regression analysis. Regression analysis was performed using the Microsoft® Excel data analysis tools, including a least-squares graph of the regression line. The abscissa of the graph is the derivatized bronopol concentration in µg/ml of the solution analyzed. The ordinate is detector response in Absorbance units. The calculated coefficient of determination value R^2 for the regression line is reported.

The equation of the calibration curve as determined by regression analysis is used to predict absorbance values for comparison in subsequent experimental trials to include

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filter spiking, chamber particulate concentration distribution trials, chamber sampling trials, and sample storage stability studies.

Molar absorption coefficient. The molar absorption coefficient of derivatized bronopol was calculated for each calibration curve data point. Values obtained were compared against the value of 8330 liter/cm mol as reported by Sanyal et al. (1996). No data or statistical information was available in the Sanyal article for calculation of the reported molar absorbtivity. Lacking this, no statistical comparison was made of the molar absorption coefficient determined in this work versus the value reported in the literature.

For molar absorbtivity data generated by this work, average, standard deviation, and percent coefficient of variation analysis were obtained. To determine if the molar absorption coefficient varies with the concentration of derivatized bronopol in solution, analysis of variance was performed among the different concentration levels. If the molar absorption coefficient changes with concentration, then the analysis of variance test should show that the means of the data sets of the various concentrations are different. Analysis of variance was performed using the Microsoft® Excel ANOVA data analysis tool with $\alpha = 0.05$.

Limit of detection. NIOSH (1995) describes a method for calculating the instrumental limit of detection (LOD) using low-level calibration standards. The procedure uses calibration standard concentrations ranging from less than the anticipated

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limit of detection to no more than ten times the expected LOD. The standard error of the regression for these calibration standards is first calculated using Equation 7.

$$s_y = [\sum(\hat{y}_i - y_i)^2/(N - 2)]^{1/2} \tag{7}$$

In Equation 7 s_y is the standard error of the regression, $\hat{y}_i$ is the predicted detector response for a given analyte mass as calculated from the regression equation, y_i is the actual detector response at each given analyte mass, and N is the number of calibration points used in the s_y calculation. For this work, calibration standards with a concentration of 10 µg/ml or less bronopol were used in the calculation of s_y.

The LOD is then calculated using standard error of the regression s_y and the slope of the regression equation m as shown in Equation 8.

$$LOD = 3\ s_y/m \tag{8}$$

The instrumental LOD to be used for the analytical method should then be the highest of the following three alternatives: (a) the calculated LOD, (b) the lowest concentration calibration standard, or (c) the X-intercept if the regression equation has a negative Y-intercept.

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Limit of quantitation. The instrumental limit of quantitation (LOQ) is calculated using Equation 9 (NIOSH, 1995).

$$LOQ = 3.33 \times LOD \quad (9)$$

Analytical Recovery

Filter blank count. Treated glass fiber filters with no bronopol were extracted and analyzed. Filter extract solutions were drawn through a syringe filter prior to analysis. Twelve blank values were obtained in this manner. The average filter blank count was calculated.

All glass fiber filters and PVDF filters were extracted using the syringe filters. The adjusted absorbance reading obtained from a bronopol-bearing GFF or PVDF filter is the actual absorbance measurement read from the filter extract minus the filter blank count.

Filter spiking. Treated glass fiber filters were spiked with aqueous bronopol solutions then evaluated for analytical recovery by comparing the adjusted absorbance reading obtained (i.e., actual absorbance read minus filter blank count) versus that calculated from the bronopol spike quantity. Bronopol spike quantities were selected to approximate the linear range of the analytical method. Three sets of ten each treated filters were spiked with 19, 44, or 113 μg bronopol.

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Filters were evaluated for recovery efficiency as the adjusted absorbance value versus the quantity of bronopol spiked onto the filter. Intra-set data values were evaluated for outliers using the Q-test at a 95 % confidence level; the variances of the two data sets were then compared for consistency using the F-test. One-sided F-test analysis was performed with Microsoft® Excel using the data analysis tools with $\alpha = 0.05$.

A cumulative pooled CV was obtained for all filter-spiking trials. NIOSH (1995) specifies that spiked filter recovery should be at least 75 %. The Q-test for outliers was performed using Equation 10; pooled coefficient of variation for the filter spiking sets is calculated using Equation 11.

$$Q = \text{Gap}/\text{Range} \qquad (10)$$

A data set to be analyzed for outliers using the Q-test is arranged in increasing numerical order. In Equation 10, Q is the calculated test value and Range is the difference between the lowest and highest data point. Gap is the difference between the lowest value and its next lowest value, or the highest value and its next lower value; whichever difference is numerically greater. The calculated Q value from the data in question is then compared against a table of critical values; different critical Q values are indicated for data sets containing between 3 and 10 data points. Data points with a calculated Q value in excess of the critical Q value are rejected as outliers (Shoemaker, Garland, & Steinfield, 1974).

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$$S_r = [\sum f_i\{CV_i\}^2/f]^{1/2} \quad (11)$$

In Equation 11, S_r is the pooled coefficient of variation, f_i is the degrees of freedom for each spike set equal to n-1, CV_i is the coefficient of variation for each filter spiking set, and f is the sum of the degrees of freedom for all of the CVs included in the calculation.

Distribution of Bronopol Particulate Concentration in the Sampling Chamber

Pump flow calibration. Ten each pump flow measurements were made for both the pre- and post-calibration of sampling pumps. Pump calibration sets were analyzed for within set and between set variations for each calibration series. Pump calibration was deemed acceptable if all data points in both pre- and post-calibration sets were within 5 % of each other and if the averages of the pre-and post-calibration sets were within 5 % of each other (NIOSH, 1995).

Chamber flow calibration. Ten each chamber nitrogen flow measurements were made for both the pre- and post-calibration. Chamber flow calibration sets were analyzed for within set and between set variations for each calibration series. Chamber calibration was deemed acceptable if all data points in both pre- and post-calibration sets were within 5 % of each other and if the averages of the pre-and post-calibration sets were within 5 % of each other. Chamber flow calibration is important to assure proper particle size selection in the dust generator.

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Chamber particulate distribution runs. Bronopol collected on PVDF filters was extracted in a derivatizing solution and analyzed. Intra-set data values were evaluated for outliers using the Q-test at a 95 % confidence level. The three filters from each chamber run were compared for consistency via coefficient of variation analysis. Pooled CV was obtained for all chamber particulate concentration distribution trials.

Sampling of Generated Bronopol Atmospheres

Pump and chamber flow calibration. Pump and chamber flow calibration during bronopol sampling were accomplished in the same manner as for the chamber particulate concentration distribution trials.

Sampling and analytical recovery. For each individual chamber run two treated glass fiber filters and one PVDF filter were used. Intra-set data values were evaluated for outliers using the Q-test at a 95 % confidence level. Each individual GFF value was compared against the concurrent PVDF filter value and expressed as percent recovery, with the PVDF value as 100 % recovery. Intra-set values were compared for consistency via coefficient of variation analysis. Pooled CV was obtained for all sampling of generated bronopol atmosphere trials.

Overall Sampling and Analytical Method

Calculation of airborne bronopol concentration. The adjusted absorbance is obtained by subtracting the filter blank count from the absorbance reading obtained from a derivatized and extracted filter. The bronopol mass concentration in the filter extract

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solution may then be obtained using the linear regression equation. Once the mass of bronopol present on the filter is known, the airborne bronopol concentration may then be calculated in terms of mass per unit volume

Sample Storage Stability

Derivatized bronopol solutions. The value of each of the derivatized filter extract solutions on day fourteen was compared against the respective value read on day one. Intra-set data values were evaluated for outliers using the Q-test at a 95 % confidence level; the variances of the two data sets were then compared for consistency using the F-test. One-sided F-test analysis was performed with Microsoft® Excel using the data analysis tools with $\alpha = 0.05$. Coefficient of variation analysis was performed for filter sets at each spike loading level; a change of 10 % or less was considered acceptable (NIOSH, 1995).

Bronopol on treated glass fiber filters. Average values for spiked filters extracted and analyzed on the day of formulation were compared against average values for filters extracted and analyzed after forty-eight hours. Intra-set data values were evaluated for outliers using the Q-test at a 95 % confidence level; the variances of the two data sets were then compared for consistency using the F-test. One-sided F-test analysis was performed with Microsoft® Excel using the data analysis tools with $\alpha = 0.05$. Coefficient of variation analysis was performed for filter sets at each spike loading level; a change of 10 % or less was considered acceptable (NIOSH, 1995).

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Chapter Four - Results And Data Analysis

Examination of Chromophore Development

Absorbance Spectrum

The spectral curve of three 9.94 µg/ml bronopol solutions were obtained and recorded graphically. One curve for non-derivatized bronopol in water was obtained in the range of 900 to 200 nm; water was used as a reference for this scan. No strong absorbance bands are observed in the scanned region. This curve is presented in Figure 3.

Two curves were obtained in the range of 320 to 230 nm. Figure 4 shows the results of the scan of non-derivatized bronopol versus a water reference. No strong absorbance bands are observed in the scanned region. Figure 5 shows the result of derivatized bronopol versus a reference solution containing the derivatizing agent. The absorbance band centered at approximately 244 nm is in agreement with the absorbance maximum of 244 nm as reported by Sanyal et al. (1996).

Verification of Absorbance Maximum

Absorbance readings for a derivatized 10.11 µg/ml bronopol solution were obtained manually in one nm increments from 248 to 236 nm. Results of this analysis are presented in Table 7.

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The absorbance was compared against the 244 nm maximum as reported by Sanyal et al. (1996). The maximum absorbance value for the derivatized 10.11 µg/ml bronopol solution was obtained at 244 nm, in agreement with the published value.

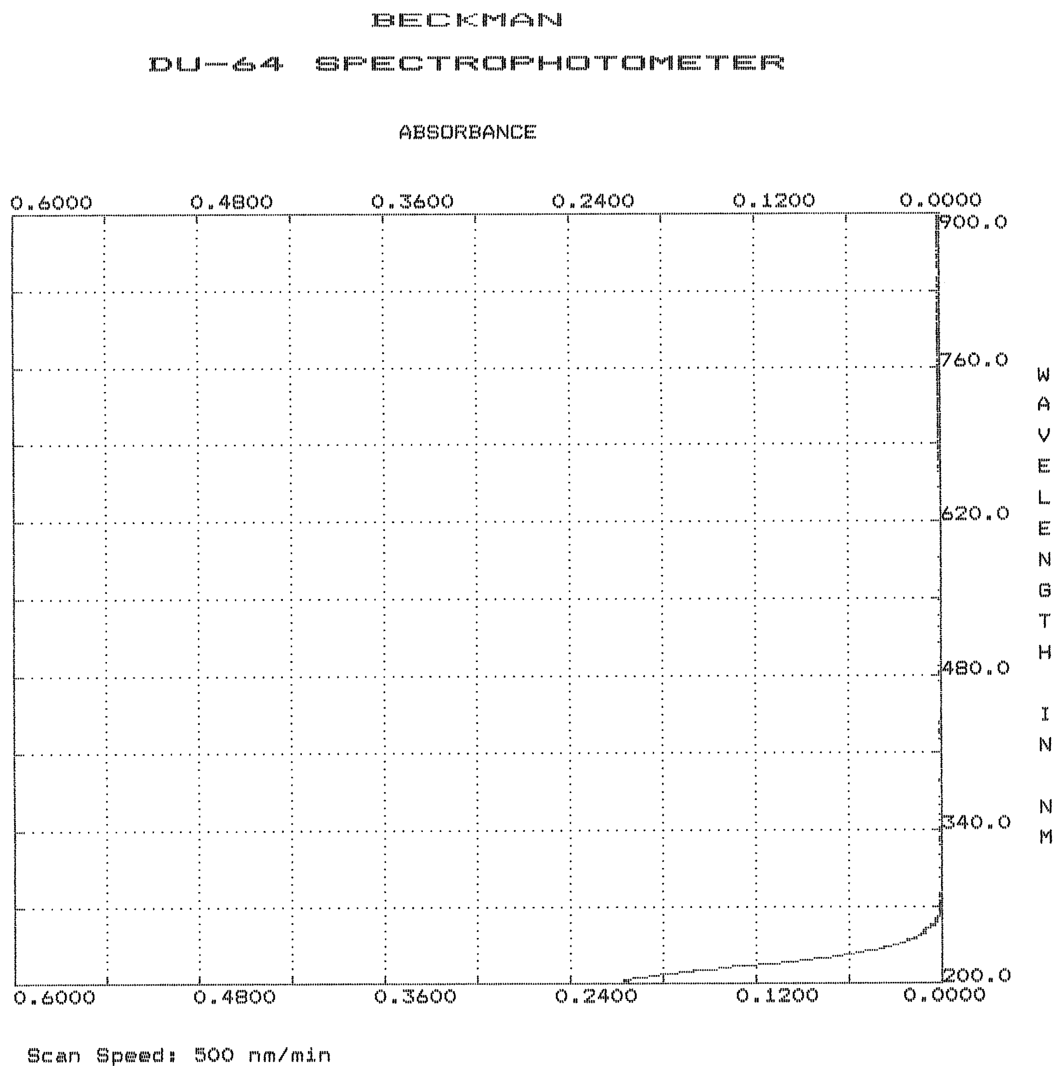

Figure 3. Spectrophotometric Scan of Non-Derivatized Bronopol, Full Range. Concentration 9.94 µg/ml, scan range 900 to 200 nm.

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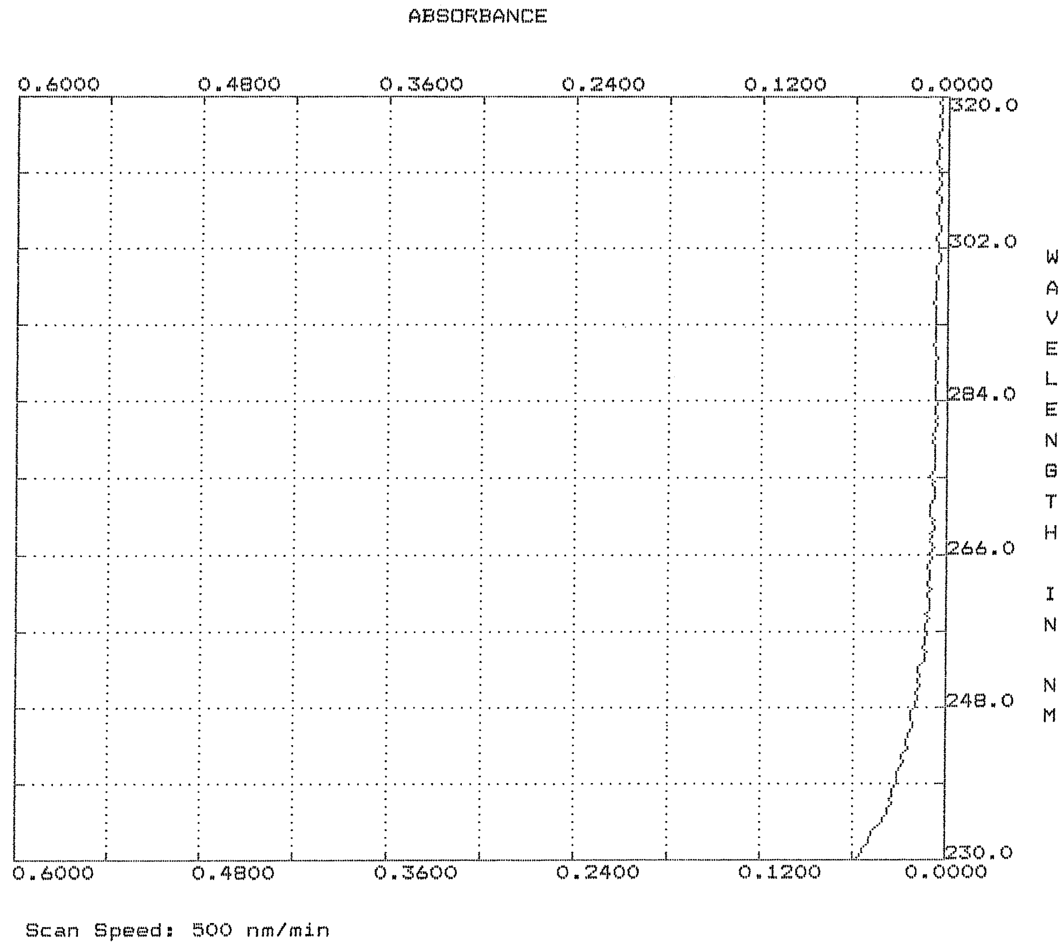

Figure 4. Spectrophotometric Scan of Non-Derivatized Bronopol, Limited Range. Concentration 9.94 µg/ml, scan range 320 to 230 nm.

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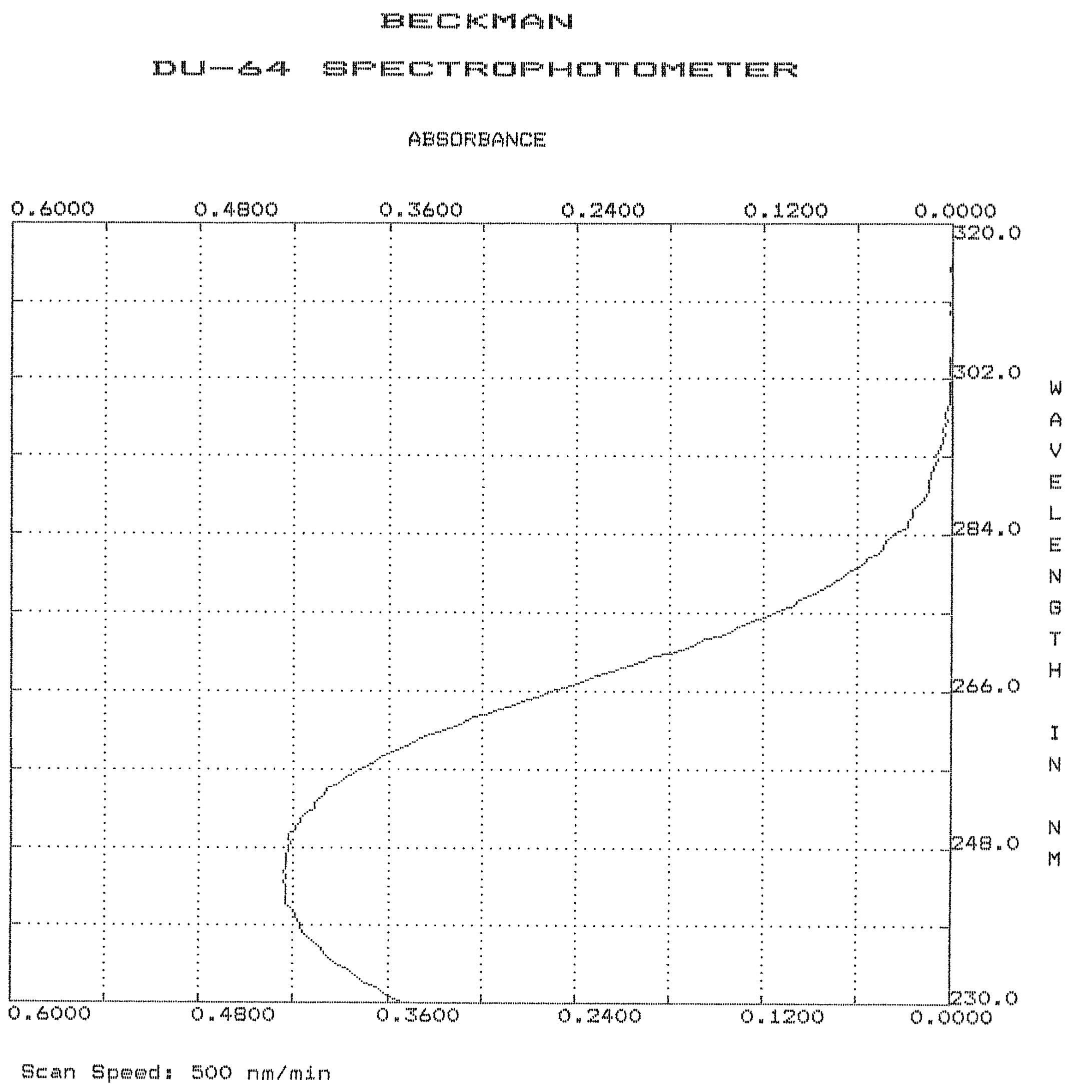

Figure 5. Spectrophotometric Scan of Derivatized Bronopol, Limited Range. Concentration 9.94 µg/ml, scan range 320 to 230 nm. Bronopol derivatized with sodium hydroxide at a final NaOH concentration of 0.03 molar.

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Table 7

Evaluation of the Absorbance Maximum of Derivatized Bronopol

Wavelength (nm)	Absorbance Value
248	0.421
247	0.424
246	0.426
245	0.428
<u>244</u>	<u>0.433</u>
243	0.431
242	0.431
241	0.430
240	0.430
239	0.429
238	0.428
237	0.425
236	0.421

<u>Note.</u> Bronopol concentration in solution 10.11 µg/ml. Underlined entries represent detected absorbance at the wavelength used by Sanyal et al. (1996).

Chromophore Development with Time

The 244 nm absorbance of a 10.10 µg/ml derivatized bronopol solution was read at 1 minute and 5 minutes following addition of the derivatizing agent, and then every 5 minutes thereafter for a total of one hour. Absorbance read at each 5-minute interval was compared with the 1-minute value. Results of this analysis are presented in Table 8.

Sanyal et al. (1996) reported the reaction to be essentially instantaneous. The data in Table 8 shows essentially no change in the absorbance value over the 60-minute period, with a calculated coefficient of variation of ca. 0.2 % for the data set.

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Table 8

Development of Derivatized Bronopol Chromophore with Time

Elapsed Time after Formulation (minutes)	Absorbance Value
1	0.434
5	0.433
10	0.433
15	0.432
20	0.431
25	0.433
30	0.433
35	0.432
40	0.432
45	0.431
50	0.432
55	0.432
60	0.431
Average	0.432
Standard Deviation	0.0009
Coefficient of Variation %	0.21 %

Note. Bronopol concentration in solution 10.11 μg/ml.

Concentration of Sodium Hydroxide

The 244 nm absorbance values of 10.18 μg/ml bronopol solutions were obtained for 0.005, 0.01, 0.02, 0.04, 0.2, 0.3, and 0.4 molar sodium hydroxide concentrations. These values were compared against the absorbance of bronopol in 0.1 molar sodium hydroxide as used in the Sanyal et al. (1996) work. Results are presented in Table 9.

The data in Table 9 shows little change in the absorbance value over the range of sodium hydroxide concentrations, with a calculated coefficient of variation of ca. 0.6 % for the data set.

The filter treatment process deposits 0.00015 mole of sodium hydroxide on each glass fiber filter; following the extraction of the filter in 5.0 ml water the sodium

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hydroxide concentration in solution is 0.03 molar. Although this is only 30 % of the sodium hydroxide concentration used in Sanyal et al. (1996), the molar quantity of sodium hydroxide on each filter is still 240 times the desired molar quantity of bronopol per filter; this should be well within the concentration needed to drive the derivatizing reaction equilibrium towards completion.

Table 9
Development of Bronopol Chromophore with Change in Sodium Hydroxide Concentration

Sodium Hydroxide Concentration (Molar)	Absorbance Value
0.005	0.441
0.01	0.439
0.02	0.444
0.04	0.443
<u>0.1</u>	<u>0.442</u>
0.2	0.438
0.3	0.437
0.4	0.439
Average	0.440
Standard Deviation	0.003
Coefficient of Variation %	0.57 %

<u>Note.</u> Bronopol concentration in solution 10.18 µg/ml. Underlined entries represent detected absorbance at the sodium hydroxide concentration used by Sanyal et al. (1996).

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Calibration Curve

Regression Analysis

The calculated equation of the least-squares regression line is shown in Equation 12, where Y is the absorbance of the derivatized bronopol solution and X is the derivatized bronopol concentration in μg/ml solution.

$$Y = 0.0435X - 0.0015 \quad (12)$$

A representation of the calibration curve for derivatized bronopol is presented in Figure 5. The bronopol mass portion (μg) as part of the concentration (μg/ml) in solution represents the bronopol mass prior to the derivitization reaction with sodium hydroxide. The calculated coefficient of determination value R^2 for the regression line is 0.9996. Calibration curve data and regression analysis information are presented in Appendix D.

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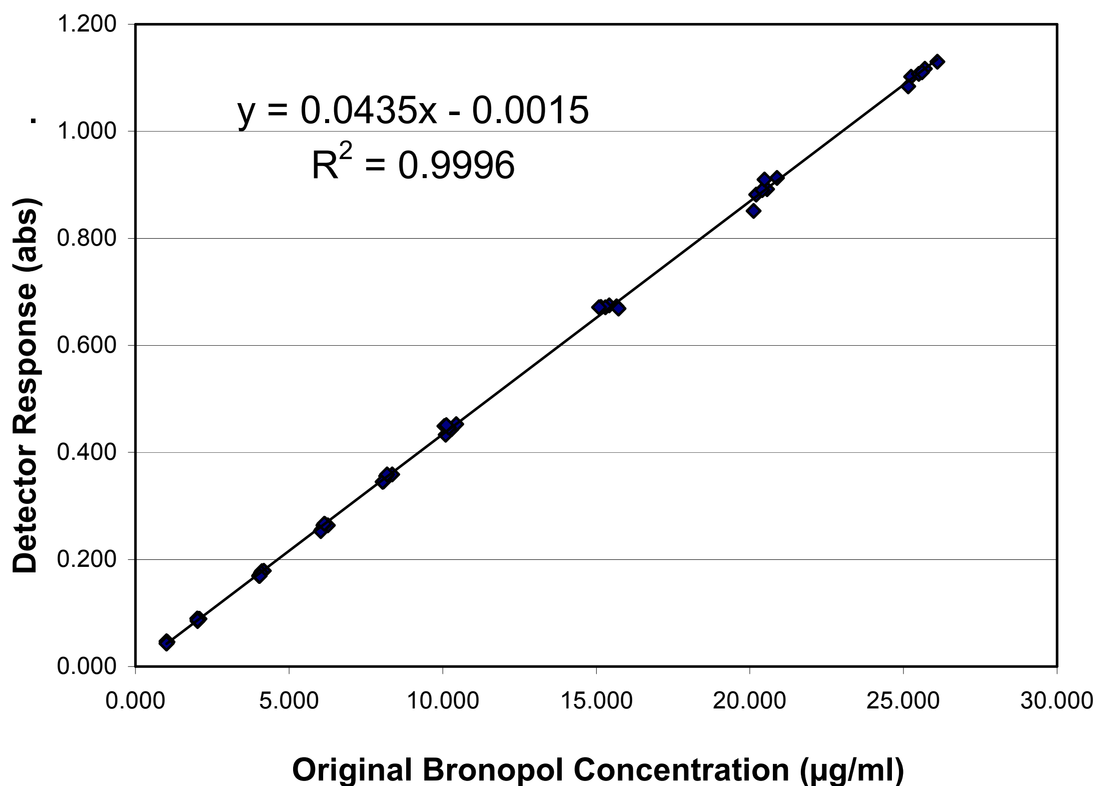

Figure 5. Calibration Curve for Derivatized Bronopol. N = 6 at each concentration. Bronopol derivatized with sodium hydroxide at a final NaOH concentration of 0.03 molar; concentration indicated is for bronopol prior to derivitization.

Molar Absorption Coefficient

The molar absorption coefficient of derivatized bronopol was calculated for each calibration curve data point. The average molar absorption coefficient obtained from the calibration curve data is 8,660 liter/cm mol with a calculated coefficient of variation of

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ca. 2.0 % for the data set. A value of 8,330 liter/cm mol was reported by Sanyal et al. (1996).

The ANOVA analysis of the molar absorption coefficient information does not indicate a different mean for each bronopol concentration level. This demonstrates that the conversion rate of bronopol to bromonitroethanol upon addition of sodium hydroxide is not dependent on bronopol concentration in the concentration range studied. Molar absorption coefficient data and calculations are presented in Appendix D.

Limit of Detection

The most appropriate instrumental limit of detection (LOD) is the highest of the following three procedures: (a) the LOD calculated using Equation 7 and Equation 8, (b) the lowest concentration calibration standard, or (c) the X-intercept if the equation of the least-squares regression line for an analytical method has a negative Y-intercept (NIOSH, 1995).

For option (a), the limit of detection calculated using the low-level calibration standards is 0.31 μg/ml. For option (b), the lowest calibration curve standards used in this work were 1 μg/ml. For option (c), the regression line does have a negative Y intercept of -0.0015. The X intercept of the regression line is calculated using the regression line by setting Y = 0; the calculated X intercept is then 0.034 μg/ml.

The highest value of the three methods is the low concentration calibration standard used at 1 μg/ml; this option (b) result is the appropriate limit of detection to use for this work. Limit of detection data and calculations are presented in Appendix D.

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Limit of Quantitation

The instrumental limit of quantitation (LOQ) is calculated using Equation 9 (NIOSH, 1995). Since the LOD for this work was determined to be 1 µg/ml, the calculated instrumental LOQ is then 3.33 µg/ml. The limit of quantitation calculation is presented in Appendix D.

Analytical Recovery

Filter Blank Count

Treated glass fiber filters with no bronopol were extracted and analyzed. Filter extract solutions were drawn through a syringe filter prior to analysis. Twelve blank values were obtained in this manner. The average filter blank count obtained was 0.015 absorbance units. Filter blank count data and calculations are presented in Appendix E.

Filter Spiking

Treated glass fiber filters were spiked with aqueous bronopol solutions then evaluated for analytical recovery by comparing the actual absorbance reading obtained (minus filter blank count) versus that calculated from the bronopol spike quantity. Three sets of ten each treated filters were spiked with 19, 44, or 113 µg bronopol.

No filter spiking data points were rejected as outliers. F-test analysis of intra-set data values did not indicate a different mean for the filter spiking sets, demonstrating that same-concentration filters spiked on different days yielded similar recoveries.

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Recovery data is summarized in Table 10. Recovery efficiency for the low, medium, and high range filter spiking sets ranged from 94.5 % to 96.8 %, with an overall recovery of 95.7 %. NIOSH (1995) specifies that spiked filter recovery should be at least 75 %.

Pooled coefficient of variation for the low, medium, and high range filter spiking sets was 2.02 %, 1.05 %, and 1.31 %, respectively, with an overall pooled coefficient of variation for the spiked filter sets of 1.52 %. Filter spiking data and calculations are presented in Appendix E.

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Table 10

Summary of Bronopol Recovery from Treated Glass Fiber Filters

LOW RANGE FILTERS	
Set No. 1, filter spike quantity 19.04 µg bronopol.	
Filter Recovery Average	95.4 %
Standard Deviation	2.18
Coefficient of Variation %	2.28 %
Set No. 2, filter spike quantity 19.00 µg bronopol.	
Filter Recovery Average	98.2 %
Standard Deviation	1.69
Coefficient of Variation %	1.73 %
Combined Average Filter Recovery - Low	96.8 %

MEDIUM RANGE FILTERS	
Set No. 1, filter spike quantity 43.04 µg bronopol.	
Filter Recovery Average	92.1 %
Standard Deviation	1.12
Coefficient of Variation %	1.21 %
Set No. 2, filter spike quantity 44.16 µg bronopol.	
Filter Recovery Average	96.8 %
Standard Deviation	0.84
Coefficient of Variation %	0.87 %
Combined Average Filter Recovery - Medium	94.5 %

HIGH RANGE FILTERS	
Set No. 1, filter spike quantity 113.24 µg bronopol.	
Filter Recovery Average	94.0 %
Standard Deviation	1.31
Coefficient of Variation %	1.39 %
Set No. 2, filter spike quantity 113.12 µg bronopol.	
Filter Recovery Average	97.3 %
Standard Deviation	1.20
Coefficient of Variation %	1.23 %
Combined Average Filter Recovery - High	95.7 %

Note: *N = 5 for each for each filter spike set, a total of 30 filters spiked and analyzed. Each filter extracted in 5.0 ml spectrophotometric grade water.*

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Distribution of Bronopol Particulate Concentration in the Sampling Chamber

Inert PVDF filters were used to collect samples from the chamber for several trials. A total of nine chamber particulate concentration distribution runs were performed, generating a total of twenty-seven samples. The quantity of bronopol collected on the filters was varied by sampling at different filter flow rates. Flow rates used were 300, 450, and 600 ml/min and were designated as the Low, Medium, and High Ranges, with three sets per filter flow rate. The filters were extracted in a derivatizing solution and analyzed for bronopol at 244 nm.

Chamber particulate distribution runs. Chamber particulate concentration distribution data is summarized in Table 11. Overall airborne bronopol concentration for the low, medium, and high range chamber particulate distribution sets ranged from 12.31 mg/m^3 to 19.67 mg/m^3. No chamber particulate concentration distribution data points were rejected as outliers. Pooled coefficients of variation for the low, medium, and high range chamber particulate distribution sets were 3.32 %, 3.16 %, and 3.01 % respectively, with an overall pooled coefficient of variation of 3.17 % for all nine chamber particulate concentration distribution sets.

Pump and chamber flow calibration. Pre- and post-calibration of sampling pumps agreed within the 5 % variance allowed by NIOSH (1995), as did the chamber flow calibration sets. Chamber particulate concentration distribution data and calculations are presented in Appendix F.

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Table 11

Summary of Chamber Distribution of Bronopol Particulate Concentration Results

LOW RANGE SETS – 300 ML/MIN FILTER FLOW RATE		
Set No. 1.	Average Bronopol Concentration (mg/m^3)	19.67
	Standard Deviation	0.63
	Coefficient of Variation %	3.21 %
Set No. 2.	Average Bronopol Concentration (mg/m^3)	14.68
	Standard Deviation	0.14
	Coefficient of Variation %	0.95 %
Set No. 3.	Average Bronopol Concentration (mg/m^3)	12.31
	Standard Deviation	0.57
	Coefficient of Variation %	4.68 %

MEDIUM RANGE SETS – 450 ML/MIN FILTER FLOW RATE		
Set No. 1.	Average Bronopol Concentration (mg/m^3)	19.11
	Standard Deviation	0.36
	Coefficient of Variation %	1.90 %
Set No. 2.	Average Bronopol Concentration (mg/m^3)	18.17
	Standard Deviation	0.72
	Coefficient of Variation %	3.98 %
Set No. 3.	Average Bronopol Concentration (mg/m^3)	14.42
	Standard Deviation	0.46
	Coefficient of Variation %	3.23 %

HIGH RANGE SETS – 600 ML/MIN FILTER FLOW RATE		
Set No. 1.	Average Bronopol Concentration (mg/m^3)	18.74
	Standard Deviation	0.13
	Coefficient of Variation %	0.71 %
Set No. 2.	Average Bronopol Concentration (mg/m^3)	16.65
	Standard Deviation	0.79
	Coefficient of Variation %	4.75 %
Set No. 3.	Average Bronopol Concentration (mg/m^3)	12.80
	Standard Deviation	0.26
	Coefficient of Variation %	2.03 %

Note: *N = 3 for each for each chamber particulate concentration distribution set, total of 27 PVDF filters sampled and analyzed. Mg/m^3 is the calculated bronopol concentration expressed as milligrams per cubic meter of air.*

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Sampling of Generated Bronopol Atmospheres

For sampling of bronopol particulate in the chamber, one inert PVDF filter and two treated glass fiber filters were used simultaneously during each chamber run. The quantity of bronopol collected on the filters was varied by sampling at different filter flow rates. Flow rates used were 300, 450, and 600 ml/min and were designated as the Low, Medium, and High Ranges, with three sets per filter flow rate. PVDF filters were extracted in a derivatizing solution; glass fiber filters were extracted in spectrophotometric grade water. All were analyzed at 244 nm. In all this produced eighteen bronopol samples collected on treated glass fiber filters. The analytical results from the treated glass fiber filters are compared against the nine inert PVDF filters that were sampled concurrently and expressed as percent recovery, with the PVDF value assumed to have 100 % recovery.

Chamber sampling analytical recovery. Chamber sampling recovery data is summarized in Table 12. Average bronopol recovery from glass fiber filters for the individual low, medium, and high range chamber sampling sets ranged from 92.9 % to 110.7 %, with n = 2 GFF per set. Overall average GFF bronopol recovery from all nine chamber sampling runs was 99.9 %. No chamber sampling data points were rejected as outliers. Pooled coefficients of variation for the low, medium, and high range chamber sampling sets including only GFF filters were 4.43 %, 3.82 %, and 3.72 % respectively, with an overall pooled coefficient of variation of 4.00 % for all nine chamber sampling sets.

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Chamber sampling bronopol concentration. Chamber sampling concentration data is summarized in Table 13. Calculated bronopol concentration in the sampling chamber ranged from 10.80 mg/m^3 to 21.59 mg/m^3 for the nine chamber sampling sets. Pooled coefficients of variation for the low, medium, and high range chamber sampling sets including PVDF and GFF filters were 3.91 %, 4.50 %, and 4.57 % respectively, with an overall pooled coefficient of variation of 4.33 % for all nine chamber sampling sets.

Pump and chamber flow calibration. Pre- and post-calibration of sampling pumps agreed within the 5 % variance allowed by NIOSH (1995), as did the chamber flow calibration sets. All chamber sampling data and calculations are presented in Appendix G.

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Table 12
Summary of Bronopol Chamber Sampling Recovery Results

LOW RANGE SETS – 300 ML/MIN FILTER FLOW RATE		
Set No. 1.	Average Recovery for Treated GFF	96.2 %
	Standard Deviation	4.13
	Coefficient of Variation %	4.30 %
Set No. 2.	Average Recovery for Treated GFF	96.4 %
	Standard Deviation	5.63
	Coefficient of Variation %	5.83 %
Set No. 3.	Average Recovery for Treated GFF	104.8 %
	Standard Deviation	2.66
	Coefficient of Variation %	2.54 %
	Combined Average GFF Recovery - Low	99.1 %

MEDIUM RANGE SETS – 450 ML/MIN FILTER FLOW RATE		
Set No. 1.	Average Recovery for Treated GFF	94.1 %
	Standard Deviation	4.61
	Coefficient of Variation %	4.90 %
Set No. 2.	Average Recovery for Treated GFF	105.1 %
	Standard Deviation	3.04
	Coefficient of Variation %	2.89 %
Set No. 3.	Average Recovery for Treated GFF	92.9 %
	Standard Deviation	3.14
	Coefficient of Variation %	3.38 %
	Combined Average GFF Recovery - Medium	97.4 %

HIGH RANGE SETS – 600 ML/MIN FILTER FLOW RATE		
Set No. 1.	Average Recovery for Treated GFF	95.8 %
	Standard Deviation	2.94
	Coefficient of Variation %	3.06 %
Set No. 2.	Average Recovery for Treated GFF	110.7 %
	Standard Deviation	0.56
	Coefficient of Variation %	0.50 %
Set No. 3.	Average Recovery for Treated GFF	102.8 %
	Standard Deviation	5.81
	Coefficient of Variation %	5.65 %
	Combined Average GFF Recovery - High	103.1 %

Note: N = 2 GFF for each for each chamber sampling set, total of 18 GFF filters sampled and analyzed. Average Recovery for Treated GFF is the average concentration for both GFF filters as a percent of the concentration for the concurrently sampled PVDF filter.

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Table 13

Summary of Bronopol Chamber Sampling Concentration Results

LOW RANGE SETS – 300 ML/MIN FILTER FLOW RATE		
Set No. 1.	Average Bronopol Concentration (mg/m^3)	20.46
	Standard Deviation	0.77
	Coefficient of Variation %	3.76 %
Set No. 2.	Average Bronopol Concentration (mg/m^3)	15.70
	Standard Deviation	0.72
	Coefficient of Variation %	4.59 %
Set No. 3.	Average Bronopol Concentration (mg/m^3)	13.68
	Standard Deviation	0.45
	Coefficient of Variation %	3.26 %

MEDIUM RANGE SETS – 450 ML/MIN FILTER FLOW RATE		
Set No. 1.	Average Bronopol Concentration (mg/m^3)	21.59
	Standard Deviation	1.06
	Coefficient of Variation %	4.91 %
Set No. 2.	Average Bronopol Concentration (mg/m^3)	21.38
	Standard Deviation	0.75
	Coefficient of Variation %	3.52 %
Set No. 3.	Average Bronopol Concentration (mg/m^3)	18.23
	Standard Deviation	0.90
	Coefficient of Variation %	4.92 %

HIGH RANGE SETS – 600 ML/MIN FILTER FLOW RATE		
Set No. 1.	Average Bronopol Concentration (mg/m^3)	18.12
	Standard Deviation	0.60
	Coefficient of Variation %	3.29 %
Set No. 2.	Average Bronopol Concentration (mg/m^3)	16.50
	Standard Deviation	0.95
	Coefficient of Variation %	5.74 %
Set No. 3.	Average Bronopol Concentration (mg/m^3)	10.80
	Standard Deviation	0.47
	Coefficient of Variation %	4.33 %

Note: N = 3 for each for each chamber sampling set, all PVDF and GFF filters included in all chamber sampling concentration calculations. Mg/m^3 is the calculated bronopol concentration expressed as milligrams per cubic meter of air.

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Sample Storage Stability

Derivatized Bronopol Solutions

Aqueous bronopol was spiked onto 18 treated glass fiber filters; the filters were allowed to air dry. Six filters were spiked with 25 µg bronopol, six with 60 µg bronopol, and six with 125 µg bronopol. The filters were extracted and analyzed on the same day. The solutions from day one were stored and analyzed after fourteen days. All stored samples were kept in a laboratory cabinet under ambient laboratory conditions in glass screw cap 16 mm by 125 mm culture tubes.

No derivatized bronopol solution data points were rejected as outliers. F-test analysis of intra-set data values did not indicate a different mean for the solution storage sets on different days, demonstrating that no significant change had taken place in the samples over the 14-day storage period.

Derivatized bronopol solution storage stability data is summarized in Table 14. Adjusted absorbance was obtained by subtracting the filter blank count of 0.015 absorbance units from the actual absorbance read at 244 nm. The calculated difference in average adjusted absorbance values from day one to day fourteen ranged from 0.58 % to 0.95 %, with a cumulative average change of 0.71 % for all stored bronopol solutions. A change of 10 % or less is considered acceptable (NIOSH, 1995). Sample storage stability data and calculations for derivatized bronopol solutions are presented in Appendix H.

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Table 14

Summary of Derivatized Bronopol Solution Storage Stability Results

	Adjusted Absorbance First Day	Recovery (%)	Adjusted Absorbance Day Fourteen	Recovery (%)
LOW RANGE SET – Each filter spiked with 25.2 µg bronopol, predicted absorbance is 0.218.				
AVG	0.210	96.6	0.212	97.1
SD	0.004	2.03	0.003	1.47
CV %	2.10 %	2.10 %	1.52 %	1.52 %
	Change in average absorbance values from first day to day fourteen is 0.95 %			
MEDIUM RANGE SET – Each filter spiked with 59.76 µg bronopol, predicted absorbance is 0.518.				
AVG	0.495	95.4	0.492	94.8
SD	0.008	1.47	0.010	1.88
CV %	1.54 %	1.54 %	1.98 %	1.98 %
	Change in average absorbance values from first day to day fourteen is 0.61 %			
HIGH RANGE SET – Each filter spiked with 126.12 µg bronopol, predicted absorbance is 1.096.				
AVG	1.043	95.2	1.037	94.6
SD	0.022	2.02	0.024	2.18
CV %	2.12 %	2.12 %	2.30 %	2.30 %
	Change in average absorbance values from first day to day fourteen is 0.58 %			

Note. *N = 6 for each filter spike set. Treated glass fiber filters were spiked, extracted and analyzed the first day; the filter extract solutions were then stored under ambient laboratory conditions and analyzed again after fourteen days. The adjusted absorbance is calculated as the absorbance read from each sample minus the filter blank count of 0.015 absorbance units; Recovery is then the adjusted absorbance divided by the predicted absorbance expressed as percent.*

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Bronopol on Treated Glass Fiber Filters

A preliminary trial of spiked filters revealed a significant change in bronopol recovery after storage for fourteen days; therefore a 48-hour storage period was selected for the spiked filter stability study. Industrial hygiene samples taken in the field are typically sent to a laboratory for analysis via a commercial overnight carrier; based on this it was felt that a 48-hour storage period would be more than adequate for many foreseeable sampling and shipment situations.

Aqueous bronopol was spiked onto 36 treated glass fiber filters; the filters were allowed to air dry. Twelve filters were spiked with 25 μg bronopol, twelve with 60 μg bronopol, and twelve with 125 μg bronopol. Six of each set were extracted and analyzed on the same day; six of each set were extracted and analyzed after forty-eight hours. All stored samples were kept in a laboratory cabinet under ambient laboratory conditions in glass screw cap 16 mm by 125 mm culture tubes.

No spiked filter data points were rejected as outliers. F-test analysis of intra-set data values did not indicate a different mean for the spiked filter sets on different days, demonstrating that no significant change had taken place in the samples over the 48-hour storage period.

Spiked treated glass fiber filter storage stability data is summarized in Table 15. Adjusted absorbance was obtained by subtracting the filter blank count of 0.015 absorbance units from the actual absorbance read at 244 nm. The calculated difference in average adjusted absorbance values from day one to 48 hours later ranged from 0.48 % to 1.01 %, with a cumulative average change of 0.73 % for all stored spiked filters. A change of 10 % or less is considered acceptable (NIOSH, 1995). Sample

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storage stability data and calculations for spiked treated glass fiber filters are presented in Appendix H.

Table 15

Summary of Treated and Spiked Glass Fiber Filter Storage Stability Results

	Adjusted Absorbance First Day	Recovery (%)	Adjusted Absorbance 48 Hours	Recovery (%)
LOW RANGE SET – Each filter spiked with 25.88 µg bronopol, predicted absorbance is 0.224.				
AVG	0.210	93.9	0.209	93.6
SD	0.007	3.15	0.006	2.78
CV %	3.35 %	3.35 %	2.97 %	2.97 %
Change in average absorbance values from first day to 48 hours later is 0.48 %				
MEDIUM RANGE SET – Each filter spiked with 59.36 µg bronopol, predicted absorbance is 0.515.				
AVG	0.492	95.6	0.497	96.6
SD	0.011	2.15	0.006	1.07
CV %	2.25 %	2.25 %	1.11 %	1.11 %
Change in average absorbance values from first day to 48 hours later is 1.01 %				
HIGH RANGE SET – Each filter spiked with 124.08 µg bronopol, predicted absorbance is 1.078.				
AVG	1.016	94.2	1.023	94.9
SD	0.026	2.41	0.021	1.95
CV %	2.56 %	2.56 %	2.05 %	2.05 %
Change in average absorbance values from first day to 48 hours later is 0.69 %				

Note. *N = 12 for each filter spike set. Six of each set of spiked treated glass fiber filters were extracted and analyzed the first day; the remaining six spiked filters from each set were stored under ambient laboratory conditions then extracted and analyzed after 48 hours. The adjusted absorbance is calculated as the absorbance read from each sample minus the filter blank count of 0.015 absorbance units; Recovery is then the adjusted absorbance divided by the predicted absorbance expressed as percent.*

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Chapter Five - Conclusions And Recommendations

Findings of the Study

Summary

An existing ultraviolet spectrophotometric method for analysis of derivatized bronopol has been combined with conventional industrial hygiene air sampling techniques. The derivatizing characteristic of this new air sampling method will serve to minimize loss of the reactive bronopol during sampling and analysis. No air sampling method for bronopol has previously been published in the literature.

A calibration curve has been developed for the analytical method with a linear range of 1 µg/ml to 25 µg/ml. The instrumental limit of detection is 1 µg/ml with an instrumental limit of quantitation of ca. 3 µg/ml.

Sampling of airborne bronopol from generated atmospheres has been conducted under laboratory conditions. Analytical recovery for treated glass fiber filters versus the benchmark method yielded a sampling recovery efficiency averaging 99.9 %. Calculated bronopol concentration obtained during chamber sampling trials ranged from 10.80 mg/m^3 to 21.59 mg/m^3, with a pooled coefficient of variation of 4.33 % for all chamber sampling sets.

Treated glass fiber filters spiked with bronopol were found to be stable for a period of 48 hours; derivatized bronopol solutions were found to be stable for a period of fourteen days.

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Ultraviolet Spectrophotometric Method for Bronopol Analysis

Analytical wavelength. The Sanyal et al. (1996) spectrophotometric method for reacting bronopol with sodium hydroxide and analyzing for the derivatized chromophore at 244 nm is used in this body of work. A spectrophotometric scan of derivatized bronopol as shown in Figure 5 confirms the absorbance maximum at 244 nm, as does a manual wavelength scan as shown in Table 7.

Chromophore development. Development of the chromophore upon addition of sodium hydroxide was found to be essentially instantaneous as reported by Sanyal et al. (1996). Repeated absorbance measurements of a derivatized bronopol solution over a period of 60 minutes showed very little change of the absorbance value with time as shown in Table 8.

Variations in the sodium hydroxide concentration of the derivatizing solution showed little change in detected absorbance within the range evaluated. Sanyal et al. (1996) used a 0.1 molar solution, but sodium hydroxide concentrations in the range of 0.005 molar to 0.4 molar were equally effective as shown in Table 9. The concentration in solution used in this work is 0.03 molar. Given the known linear range of the analytical method, 0.00015 mol of sodium hydroxide applied to a sampling filter will have at least a 240-fold molar excess when compared to the desired molar concentration of bronopol on a sampling filter.

Calibration curve. Derivatized bronopol solutions of known concentration yielded a linear calibration curve in the concentration range from 1 μg/ml to 25 μg/ml,

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which is beyond the reported Sanyal et al. (1996) range of 5 to 25 µg/ml. The resulting linear regression line as shown in Equation 12 has a calculated coefficient of determination (R^2) value of 0.9996. In all the calibration curve data provides 54 measurements comprising of six measurements at each of nine bronopol concentrations. The calculated average molar absorption coefficient obtained was 8,660 liter/cm mol versus the published Sanyal et al. value of 8,330 liter/cm mol. No difference was found in molar absorption coefficient for low versus high bronopol concentrations, indicating that the conversion rate of bronopol to bromonitroethanol upon addition of sodium hydroxide is not dependent on bronopol concentration in the concentration range studied.

Limits of detection and quantitation. The instrumental limit of detection of 1 µg/ml was equal to the lowest calibration curve concentration used. The calculated instrumental limit of quantitation was 3.33 µg/ml.

Sampling and Analysis of Bronopol on Filters

Filter spiking. The Sanyal et al. (1996) spectrophotometric method for analysis of bronopol in cosmetic materials has been adapted to analyze bronopol collected on air sampling filters. The sodium hydroxide derivatizing agent, along with a humectant material, has been applied to glass fiber filters.

Bronopol spiked onto treated glass fiber filters demonstrated average recovery efficiency of 95.7 %, with a pooled coefficient of variation of 1.52 % for analytical recovery from all filter spiking sets.

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Distribution of bronopol particulate concentration in the sampling chamber. A dust generator and sampling chamber were constructed to introduce pulverized bronopol aerosol to three sampling filters simultaneously. Nitrogen gas flow through the dust generator was regulated in a manner to limit the maximum particle size of the bronopol particulate introduced into the sampling chamber. Bronopol particles no greater than 11.0 μm aerodynamic diameter pass through the nozzle of the dust generator into the sampling chamber. Due to a gas velocity of ca. 16.8 cm/sec at the dust generator nozzle a jet of nitrogen gas and bronopol particles is transmitted to the sampling filters at the top of the sampling chamber.

To demonstrate consistent presentation of bronopol particles to the samplers, chamber particulate concentration distribution runs were performed using inert PVDF filters. PVDF filters were extracted in a derivatizing solution and analyzed at 244 nm. A total of nine chamber particulate distribution runs were performed consisting of three runs at each of three filter sampling flow rates. Calculated bronopol concentration obtained during the nine chamber particulate concentration distribution runs ranged from 12.31 mg/m^3 to 19.67 mg/m^3, with a pooled coefficient of variation of 3.17 % for all sets.

Sampling of generated bronopol atmospheres. For sampling of bronopol particulate in the chamber, one inert PVDF filter and two treated glass fiber filters were used simultaneously during each chamber run. PVDF filters were extracted in a derivatizing solution; glass fiber filters were extracted in spectrophotometric grade water. All filters were analyzed at 244 nm.

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A total of nine chamber sampling runs were performed consisting of three runs at each of three filter sampling flow rates. Calculated bronopol concentration obtained during the nine chamber sampling sets ranged from 10.80 mg/m^3 to 21.59 mg/m^3, with a pooled coefficient of variation of 4.33 % for all chamber sampling sets including all GFF and PVDF filters. Calculated recovery as measured by average GFF concentration versus the benchmark PVDF filter sampled concurrently averaged 99.9 %.

Sample Storage Stability

Aqueous bronopol was spiked onto treated glass fiber filters, the filters were allowed to air dry. Filters were stored, extracted, and analyzed over varying periods of time to evaluate stability of derivatized bronopol in solution and on filters.

Storage stability of derivatized bronopol solutions. Bronopol of three different quantities was spiked onto 18 treated glass fiber filters. The filters were extracted and analyzed on the same day. The solutions from day one were stored and analyzed after fourteen days. The average change for all stored sample solutions was 0.71 %. NIOSH (1995) considers a change of 10 % or less to be acceptable.

Storage stability of bronopol on treated GFF. Bronopol of three different quantities was spiked onto 36 treated glass fiber filters. Six of each set were extracted and analyzed on the same day; six of each set were stored then extracted and analyzed after forty-eight hours. The average change for all stored spiked filters was 0.73 %, against the reference NIOSH (1995) value of 10 % or less.

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Limitations of This Study

Sampling and Analytical Method

Derivitization reaction. The Sanyal et al. (1996) method describes a complete conversion of bronopol to bromonitroethanol upon addition of sodium hydroxide, however; only thin-layer chromatography analysis is offered as evidence of this assertion.

In the current work supporting evidence is developed which supports the Sanyal et al. claim of complete conversion of bronopol to bromonitroethanol. First, ANOVA analysis was performed on the molar absorbtivity data. If the molar absorption coefficient changes with concentration, then the analysis of variance test should show that the means of the data sets of the various concentrations are different. This would indicate that the derivitization conversion of bronopol differed with varying concentrations. This was not found to be the case, indicating that the conversion of bronopol to bromonitroethanol upon addition of sodium hydroxide takes place at a constant rate in the concentration ranges studied.

Secondly, in all cases the sodium hydroxide molar concentration was at least 240 times the bronopol concentration. While this is not a guarantee of complete derivitization of bronopol, a large molar excess would be expected to drive the reaction towards completion.

While these factors are supporting evidence of a significant conversion rate, the current study did not offer a definitive analysis to verify the conversion rate of bronopol to bromonitroethanol upon addition of sodium hydroxide.

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Calibration curve. The calibration curve range studied was limited to the range of 1 μg/ml to 25 μg/ml. While this is beyond the reported Sanyal et al. (1996) range of 5 to 25 μg/ml, further studies may expand the known linear range of the analytical method.

Limits of detection and quantitation. The instrumental limit of detection of 1 μg/ml was limited to the lowest concentration calibration curve standard used. Similarly, the calculated instrumental limit of quantitation of 3.33 μg/ml is based on the lowest calibration curve analytical standard used. No attempt was made to evaluate calibration curve standards in the concentration range less than 1 μg/ml.

Filter spiking. Spiking of treated glass fiber filters was limited to the range of 19 μg to 113 μg of bronopol per filter. The quantities of bronopol loading on a treated filter were limited by the bounds of the known linear range of the calibration curve. Spike quantities at higher or lower ranges were not evaluated.

Sampling of generated bronopol atmospheres. The sampling chamber used was limited by design to using three samplers simultaneously. This was based on the maximum total flow rate of 1,800 ml/min through the samplers versus the total chamber flow rate of 2 LPM. The 2 LPM flow rate through the dust generator and subsequently through the sampling chamber was necessary to achieve appropriate size selection in the vertical elutriator portion of the dust generator. The flow rate through the sampling chamber could not be altered from the 2 LPM flow rate used.

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Filter sampling flow rates used in the study were limited to 300, 450, and 600 ml/min. Traditional industrial hygiene sampling flow rates for airborne particulates are often upwards of one and even as much as four LPM.

Control of bronopol aerosol concentration collected on the sampling filters was attained through changing the sampler flow rates, by selecting a fixed sampling time, and by selecting an appropriate sampling chamber equilibration time prior to activation of the samplers. With the laboratory apparatus used it was not possible to directly control the airborne bronopol concentration. Within these limitations calculated airborne bronopol concentrations obtained were in the range of 10.80 mg/m^3 to 21.59 mg/m^3.

The dust generator and sampling chamber used in this study were designed to eliminate specific variables that would be present under actual field sampling conditions. Oxygen and water vapor were excluded from the dust generator and the sampling chamber through the use of a dry nitrogen gas atmosphere. While the temperature of the laboratory apparatus was not controlled per se, the ambient laboratory temperature was maintained within a normal range for the interior of an air-conditioned building. Sampling under actual field conditions would draw atmospheric oxygen and water vapor through the treated glass fiber filters containing collected bronopol aerosol. Temperature under actual field conditions would at times likely be outside the range normally maintained inside air-conditioned buildings. Of course, the airborne bronopol present in the atmosphere to be sampled would be subjected to uncontrolled environmental conditions; understanding the implications of these variables is beyond the scope of this study.

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Sample storage stability. Industrial hygiene media that has been used to collect samples may be subjected to environmental conditions that can alter the stability or recovery of the analyte on the media. Several variables may come into play such as storage temperature, relative humidity, transit time, exposure to oxygen, exposure to light, vibration, and potential interactions of the analyte with the media. In addition, the laboratory analyzing the samples may not process the samples immediately upon receipt.

In this work derivatized bronopol in solution was determined to be stable for at least 14 days. No stability studies were performed on derivatized bronopol solutions in excess of this time period.

Preliminary studies for stability of derivatized bronopol on treated glass fiber filters indicated that this combination was not stable for 14 days. The 48-hour period selected for bronopol on treated GFFs in this study is a very minimal storage period and would require close coordination between field sampling personnel and the receiving laboratory to assure that the 48-hour time frame was met. At the very minimum the received samples would need to be extracted upon receipt; the analysis could then be performed at a later date.

Field Trials

No field trials of bronopol workplaces or bronopol application areas were conducted in this work.

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Health Effects of Bronopol

Animal or human studies to determine the potential health effects associated with bronopol exposure were not within the scope of this work

Public Health Importance of This Study

Populations with Potential Exposure to Bronopol

While bronopol has been used as a preservative in drugs and cosmetics since at least 1964, recent formulations for mold remediation and the sanitizing of ventilation system components have the potential to expose vast new populations to the chemical. The most recent statistic available for United States workers occupationally exposed to bronopol specifies exposure of 5,176 individuals (NIOSH, 1983). This is prior to the recent novel applications of this biocidal compound for mold and ventilation systems. Given that 89 million workers in the United States work in nonindustrial nonagricultural indoor environments and 50 million Americans suffer from allergic diseases each year (Mendell et al., 2002), it is likely that a sizeable portion of these populations will be exposed to bronopol used to control indoor microbiological growth.

Health Effects Associated with Exposure to Bronopol

Skin irritancy in humans and laboratory animals at levels commonly used in cosmetics is low. Bronopol in higher concentrations (1 % and greater) is significantly irritating in human skin patch studies (Maibach, 1977). The evidence for dermal sensitization and allergenic dermal reaction to bronopol is mixed. Laboratory testing of the allergenic properties of bronopol indicate a low potential for dermal sensitization;

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however, a number of case reports demonstrate incidences of human allergenic reactions in various exposure situations (Bryce et al., 1978; Storrs & Bell, 1983; Frosch et al., 1990; Wilson & Powell, 1990; Rudzki et al., 1993).

Given the widespread use of bronopol and the variety of uses for the material, bronopol may produce sensitization in a large number of people when the exposure population is large. Continued exposure to a sensitizing agent may eventually sensitize susceptible individuals (Marzulli & Maibach, 1973). The potential sensitizing property of bronopol on dermal exposure suggests that the material may be sensitizing for the inhalation route of exposure.

Importance of the Study

Currently this is the only air sampling and analytical method for bronopol described in the scientific literature. Based on the potential health effects of bronopol exposure and the large and diverse populations that may be exposed to bronopol in the workplace and in their place of residence, understanding the duration and intensity of exposure to these populations is critical.

Recommendations for Further Research Efforts

Sampling and Analytical Method for Bronopol

Derivitization reaction. While Sanyal et al. (1996) described the conversion of bronopol to bromonitroethanol upon addition of sodium hydroxide, only thin-layer chromatography analysis was offered as evidence of this conversion. A definitive quantitation of the conversion rate would be beneficial to the analytical method.

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Calibration curve. It is not known over what concentration range the regression line will remain linear beyond the 1 to 25 µg/ml range demonstrated in this work. It would be useful to determine the full extent of the linear range of the analytical method in order to allow a wider range of effective air sampling rates, volumes, and concentrations for the sampling and analytical method. The linearity of the regression line at the upper concentration range may allow for extension of the calibration curve data in this direction, although absorbance values are in the range of 1.1 absorbance units at 25 µg/ml bronopol concentration. Good UV-VIS spectrophotometric technique may limit the desirability of absorbance values much in excess of one, since at this level only 10 % of the incident light is passing through the sample.

Limit of detection and quantitation. Extending the range at the low end of the calibration curve would permit a more accurate determination of the instrumental limit of detection and limit of quantitation. The calculated instrumental limit of detection of 1 µg/ml was equal to the lowest calibration curve concentration used. Further experimentation may result in a smaller LOD by developing additional calibration curve data in the concentration range below 1 µg/ml. The calculated instrumental limit of quantitation of 3.33 µg/ml may also be reduced in value by developing additional calibration curve data in the lower concentration regime.

The filter extraction procedure used in this work results in a five ml filter extract volume. Filter extraction trials using a smaller volume of water such as two to three milliliters may demonstrate adequate analytical recovery from the filters. A smaller filter

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extract volume would serve to reduce the dilution factor of the bronopol collected on the filter, resulting in a lowered overall method limit of detection and quantitation.

Sampling of generated bronopol atmospheres. The sampling chamber used in this study presented a very controlled atmosphere to the samplers. To more thoroughly evaluate the efficacy of the treated glass fiber filters for collection of airborne bronopol the environmental parameters of the sampled atmosphere should be closer to the conditions found under actual field conditions. For example, in subsequent work the sampled atmosphere used should consist of air instead of nitrogen. The effects, if any, of moisture content of the sampled atmospheres should be evaluated. Although exclusion of oxygen and water vapor in sampled atmospheres was essential to this current work, field sampling using this method will be subjected to atmospheric oxygen and variable temperatures and relative humidity levels.

The concentration range of bronopol atmospheres sampled in this work was limited; no means were available to directly control the bronopol concentration. These limitations restricted the sampled bronopol concentration range to 10.80 mg/m^3 to 21.59 mg/m^3. Further work is needed to determine the effective range of treated glass fiber filters in sampling airborne bronopol particulate.

Sample storage stability. While the 14 day storage stability of derivatized bronopol solutions is generally adequate, it would not be unreasonable to evaluate the storage stability of bronopol solutions over a longer period of time. Additionally, it is not unreasonable to expect samples in transit to be subjected to a wide range of

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environmental conditions. Based on this, it is recommended that derivatized bronopol solutions be subjected to stability studies under an expanded range of storage conditions.

Further study of the stability of derivatized bronopol on treated glass fiber filters would potentially allow an industrial hygienist or laboratory using the method to relax the 48-hour extraction timeframe. This short time frame was selected as a minimum necessary time frame for the stability study. As discussed previously the 48-hour threshold would require close coordination between field sampling personnel and the receiving laboratory to assure that the 48-hour time frame was met. As with the derivatized bronopol solutions it is recommended that bronopol collected on treated GFFs be subjected to stability studies under an expanded range of storage conditions.

It may be possible to develop a method that would allow storage of the filters in the extraction solution without the benefit of the complete extraction procedure. This would permit field personnel to place the sampled filter in a vial for transport and storage at the laboratory prior to extraction and analysis.

Field testing trials. Field testing will be necessary to develop an understanding of workplace exposures to bronopol during formulation and application of bronopol-containing products. Field testing will also serve to quantify the exposures, if any, of building occupants during bronopol application to ventilation system components or to indoor materials that have developed microbiological growth.

Use of the analytical method to determine bronopol concentration in other media. Bronopol applied during mold remediation and in ventilation system components has the

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potential to be present in other environmental media in the build environment, including on surfaces and in carpeting. In combination with sampling techniques appropriate for the individual media, the analytical method from the current work could be used to determine residual bronopol concentrations.

Children playing on carpeting with bronopol residuals have a potential for exposure through both dermal contact and through inhalation from re-entrainment of bronopol particulate into the air. Sampling of the carpeting using a vacuum source and a glass fiber filter treated with the derivatizing agent should produce results similar to those obtained in this publication. The filter extract solution would need to be filtered to exclude not only glass fibers from the filter but also any fine particulates captured during sampling. Quantitative results in terms of bronopol mass per unit of area would be obtained if the sampling were confined to a measured area.

Similar results could be obtained from sampling of impervious indoor horizontal surfaces. Sampling would be conducted using a wetted filter or wipe media. The sample would be immersed in the derivatizing solution and then the extracted solution would be filtered and analyzed. Again, quantitative results would be obtained if the sampled region were confined to a measured area.

Health effects of bronopol exposure. The skin irritancy of bronopol in humans and laboratory animals at levels commonly used in cosmetics (0.01 to 0.1 %) is low; however, at higher concentrations (1 % and greater) it is significantly irritating in human skin patch studies (Maibach, 1977). The evidence for dermal sensitization and allergenic dermal reaction to bronopol is mixed.

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Inhalation LC50 values for rats of 0.18 mg/l (Elder, 1980) to greater than 5 mg/l (EPA, 1995) are reported.

The potential sensitizing property of bronopol on dermal exposure suggests the possibility that the material may be sensitizing for the inhalation route of exposure, although dermal sensitizers are not always respiratory sensitizers.

Further research is needed to identify the possible health effects from airborne and dermal exposures.

Summary

The results from this research are favorable enough to recommend continued development of this sampling and analytical method, with the ultimate aim of providing a fully validated method to evaluate airborne exposures to bronopol under a wide range of environmental conditions.

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Appendices

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Appendix A: Dust Generator Calculations

Calculation of Dust Generator Cross Sectional Area

Inner diameter of the dust generator is nominally 3 inches; radius (r) is 1.5 inches.

$$r = \frac{1.5\ \text{inch}}{} \Big| \frac{2.54\ \text{cm}}{1\ \text{inch}} = 3.81\ \text{cm}$$

The internal area of the dust generator ($AREA_{DG}$) is then calculated:

$$AREA_{DG} = \frac{(3.81\ \text{cm})^2}{} \Big| \frac{\pi}{} = 45.6\ \text{cm}^2$$

Calculation of Average Nitrogen Gas Velocity Inside the Dust Generator

Average gas velocity (V_{avg}) through the dust generator is calculated using Equation 4:

$$V_{avg} = Q/A \tag{4}$$

$$V_{avg} = \frac{2\ \text{liter}}{\text{min}} \Big| \frac{}{45.6\ \text{cm}^2} \Big| \frac{1{,}000\ \text{cm}^3}{1\ \text{liter}} \Big| \frac{1\ \text{min}}{60\ \text{sec}} = 0.365\ \text{cm/sec}$$

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Calculation of Maximum Nitrogen Gas Velocity Inside the Dust Generator

Maximum or centerline gas velocity (V_{cl}) through the dust generator is calculated by rearranging Equation 5:

$$V_{avg} = 0.9 \times V_{cl} \qquad (5)$$

$$V_{cl} = \frac{0.365 \text{ cm}}{\text{sec}} \bigg| \frac{}{0.9} = 0.406 \text{ cm/sec}$$

Calculation of Particle Aerodynamic Diameter using Average Nitrogen Gas Velocity

Factors used in these calculations are shown in Table A1:

Table A1
Factors Used in Calculation of Aerodynamic Diameter

Factor Name	Symbol	Factor and Units	Reference Source
Absolute Viscosity (Nitrogen)	η	178.1×10^{-6} gm/cm sec (poise) (at 27.4 °C)	Weast, 1982
Particle Density (Bronopol)	ρ_p	1.1 gm/cm^3	OSP 2, 2006
Acceleration of Gravity	g	981 cm/sec^2	Weast, 1982

The aerodynamic diameter (d_a) of a bronopol particle with a terminal settling velocity (V_{ts}) equal to the average upward gas velocity of 0.365 cm/sec is calculated by rearranging Equation 6 as follows:

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$V_{ts} = \rho_p d_a^2 g / 18\eta$ (6)

$$d_a = \left(\frac{V_{ts}\, 18\, \eta}{\rho_p\, g} \right)^{1/2}$$

$$d_a = \left(\frac{0.365 \text{ cm}}{\text{sec}} \cdot 18 \cdot \frac{178.1 \times 10^{-6} \text{ gm}}{\text{cm sec}} \cdot \frac{\text{cm}^3}{1.1 \text{ gm}} \cdot \frac{\text{sec}^2}{981 \text{ cm}} \right)^{1/2} = 1.04 \times 10^{-3} \text{ cm}$$

$$d_a = 1.04 \times 10^{-3} \text{ cm} \cdot \frac{10 \text{ mm}}{1 \text{ cm}} \cdot \frac{1{,}000\ \mu\text{m}}{1 \text{ mm}} = 10.4\ \mu\text{m}$$

Calculation of Particle Aerodynamic Diameter using Maximum Nitrogen Gas Velocity

The aerodynamic diameter of a bronopol particle with a terminal settling velocity equal to the maximum upward gas velocity of 0.406 cm/sec is calculated using the rearranged Equation 6 as follows:

$$d_a = \left(\frac{0.406 \text{ cm}}{\text{sec}} \cdot 18 \cdot \frac{178.1 \times 10^{-6} \text{ gm}}{\text{cm sec}} \cdot \frac{\text{cm}^3}{1.1 \text{ gm}} \cdot \frac{\text{sec}^2}{981 \text{ cm}} \right)^{1/2} = 11.0\ \mu\text{m}$$

Calculation of Dust Generator Nozzle Cross Sectional Area

Inner diameter (d) of the dust generator nozzle is nominally 0.625 inches; radius (r) is 0.3125 inches.

$$r = 0.3125 \text{ inch} \cdot \frac{2.54 \text{ cm}}{1 \text{ inch}} = 0.794 \text{ cm}$$

$$\text{AREA}_{\text{Nozzle}} = (0.794 \text{ cm})^2 \cdot \pi = 1.98 \text{ cm}^2$$

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Calculation of Dust Generator Nozzle Nitrogen Gas Velocity

Gas velocity through the dust generator nozzle (V_{Nozzle}) is calculated using Equation 4:

$$V_{Nozzle} = \frac{2 \text{ liters}}{\text{min}} \left| \frac{}{1.98 \text{ cm}^2} \right| \frac{1{,}000 \text{ cm}^3}{1 \text{ liter}} \left| \frac{1 \text{ min}}{60 \text{ sec}} \right. = 16.8 \text{ cm/sec}$$

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Appendix B: Sampling Chamber Calculations

Calculation of Sampling Chamber Cross Sectional Area

The inner diameter of the sampling chamber is 24.8 cm; radius is 12.4 cm.

$$AREA_{Chamber} = \frac{(12.4\ cm)^2 \mid \pi}{\quad} = 483\ cm^2$$

Calculation of Average Nitrogen Gas Velocity through the Sampling Chamber

Nitrogen gas flow through the chamber is 2 LPM. Ignoring the dynamics of the nitrogen jet flow within the chamber the average nitrogen gas velocity through the chamber is calculated using Equation 4:

$$V_{avg} = \frac{2\ liter}{min} \cdot \frac{1}{483 cm^2} \cdot \frac{1{,}000\ cm^3}{1\ liter} \cdot \frac{1\ min}{60\ sec} = 0.069\ cm/sec$$

Calculation of Particle Aerodynamic Diameter using Average Nitrogen Gas Velocity

The d_a of a bronopol particle with a settling velocity V_{ts} equal to the average upward gas velocity of 0.069 cm/sec is calculated by using the rearranged Equation 6 as follows:

$$d_a = \left(\frac{0.069\ cm}{sec} \cdot 18 \cdot \frac{178.1 \times 10^{-6}\ gm}{cm\ sec} \cdot \frac{cm^3}{1.1\ gm} \cdot \frac{sec^2}{981\ cm} \right)^{1/2} = 4.53 \times 10^{-4}\ cm$$

$$d_a = 4.53 \times 10^{-4}\ cm \cdot \frac{10\ mm}{1\ cm} \cdot \frac{1{,}000\ \mu m}{1\ mm} = 4.5\ \mu m$$

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Calculation of Dust Generator Nozzle Jet Diameter at Top of the Sampling Chamber

In NIOSH (1973) the spread angle of an air jet is reported to be approximately 22 degrees. This is used to calculate the diameter of the jet stream at the top of the sampling chamber as follows. The chamber height is 48.9 cm. Ignoring the width of the jet nozzle, the diameter of the nitrogen gas jet in the vicinity of the samplers will be:

$$WIDTH_{Jet} = 48.9 \text{ cm} \times \tan(22°) = 19.8 \text{ cm}$$

Calculation of Outer Diameter of Sampler Region

The samplers are configured on the top of the sampling chamber in an equidistant manner with the center of each sampler 7.3 cm from the centerline of the chamber. Each sampler is 2.5 cm in diameter; one-half of each sampler diameter extends beyond the sampler center.

$$D_{Samplers} = (7.3 \text{ cm} \times 2) + (1.25 \text{ cm} \times 2) = 17.1 \text{ cm}$$

Calculation of Sampling Filter Face Velocity

Filter diameter is 2.5 cm. The lowest sampling flow rate used is approximately 0.3 LPM. The area of the filter is calculated first, and then minimum filter face velocity is obtained by solving Equation 4 as follows:

$$AREA_{Filter} = (1.25 \text{ cm})^2 \cdot \pi = 4.91 \text{ cm}^2$$

$$V_{Filter} = \frac{300 \text{ cm}^3}{\text{min}} \cdot \frac{1}{4.91 \text{ cm}^2} \cdot \frac{1 \text{ min}}{60 \text{ sec}} = 1.02 \text{ cm/sec}$$

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Appendix C: Glass Fiber Filter Treatment Calculations

Maximum Desired Bronopol Loading per Filter

Based on Sanyal et al. (1996) the linear range of the analytical method is 5 to 25 µg/ml. In this experiment filters are extracted in 5 ml of water; therefore, the maximum bronopol (BNPD) loading desired on each filter is:

$$MASS_{BNPD} = \frac{25\ \mu g}{ml} \cdot \frac{5\ ml}{} = 125\ \mu g$$

$$MOL_{BNPD} = 125\ \mu g \cdot \frac{mol}{199.99\ gm} \cdot \frac{1\ mg}{1000\ \mu g} \cdot \frac{1\ gm}{1000\ mg} = 6.25 \times 10^{-7}\ mol$$

6.25×10^{-7} mol is the maximum bronopol quantity desired on each filter.

Molar Concentration of Reagent Sodium Hydroxide

The specific gravity of 50 weight percent sodium hydroxide is 1.5253 (Perry, 1950). Fifty percent of 1.5253 is approximately 0.763 gm/ml; the molecular weight of sodium hydroxide is 40 grams per mole. The concentration of the sodium hydroxide reagent is calculated to be approximately 19 molar as follows:

$$CONCENTRATION_{NaOH} = \frac{0.763\ gm}{ml} \cdot \frac{1\ mol}{40\ gm} \cdot \frac{1000\ ml}{1\ liter} = 19.01\ molar$$

Quantity of Sodium Hydroxide on Treated Glass Fiber Filters

Each glass fiber filter is treated with 0.2 ml of filter treatment solution. The solution is formulated from 2.0 ml of 50 weight percent NaOH and 1.0 ml of glycerin; final volume in aqueous solution is 50 ml.

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The quantity of sodium hydroxide on each filter can be calculated as follows:

$$MOL_{NaOH} = \frac{2\ ml}{50\ ml} \cdot \frac{19.01\ mol}{liter} \cdot \frac{0.2\ ml}{} \cdot \frac{1\ liter}{1000\ ml} = 0.00015\ mol$$

1.5×10^{-4} mol of sodium hydroxide is present on each treated glass fiber filter.

Sodium Hydroxide Concentration in Filter Extract Solutions

In the 5 ml filter extract solution 1.5×10^{-4} mol of sodium hydroxide is present. The molar concentration of sodium hydroxide in solution is calculated as follows:

$$CONCENTRATION_{NaOH} = \frac{1.5 \times 10^{-4}\ mol}{5.0\ ml} \cdot \frac{1000\ ml}{1\ liter} = 0.03\ molar$$

The concentration of sodium hydroxide in filter extract solutions is 0.03 molar.

Sodium Hydroxide Molar Excess on Treated Filters

A large molar excess of sodium hydroxide on the derivatizing filters is desirable to quickly drive the reaction with bronopol to completion. The ratio of the amount of sodium hydroxide per filter to the maximum desired bronopol per filter is as follows:

$$RATIO_{NaOH/BNPD} = \frac{0.00015\ mol\ NaOH}{0.000000625\ mol\ BNPD} = 240\text{-fold molar excess of NaOH}$$

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Appendix D: Calibration Curve

Calibration Curve Data

A calibration curve for derivatized bronopol was developed. The range of bronopol concentrations in solution was 1 to 25 μg/ml in an approximation of the linear range described by Sanyal et al. (1996); bronopol mass as part of the concentration represents the bronopol mass prior to the derivitization reaction with sodium hydroxide. Sodium hydroxide concentration in solution was 0.03 molar. Nine different concentrations of bronopol were targeted in formulating the calibration curve solutions: 1, 2, 4, 6, 8, 10, 15, 20, and 25 μg/ml. Six solutions of each concentration were developed and analyzed at 244 nm. Calibration curve data is presented in Table D1.

Regression Analysis

Regression analysis was performed with Microsoft® Excel using the regression data analysis tool. The results of the regression analysis are presented in Table D2.

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Table D1

Calibration Curve Data

Calibration Set Number	Bronopol Conc. (μg/ml)	Abs. at 244 nm	Calibration Set Number	Bronopol Conc. (μg/ml)	Abs. at 244 nm
1	1.010	0.043	4	1.020	0.045
	2.020	0.085		2.040	0.087
	4.040	0.171		4.080	0.175
	6.060	0.256		6.120	0.264
	8.080	0.346		8.160	0.356
	10.100	0.433		10.200	0.446
	15.150	0.672		15.300	0.671
	20.200	0.882		20.400	0.890
	25.250	1.102		25.500	1.107
2	1.028	0.045	5	1.006	0.047
	2.056	0.089		2.012	0.090
	4.112	0.178		4.024	0.169
	6.168	0.266		6.036	0.253
	8.224	0.355		8.048	0.345
	10.280	0.443		10.060	0.449
	15.420	0.675		15.090	0.671
	20.560	0.892		20.120	0.851
	25.700	1.117		25.150	1.084
3	1.044	0.046	6	1.012	0.043
	2.088	0.089		2.024	0.087
	4.176	0.179		4.048	0.169
	6.264	0.264		6.144	0.267
	8.352	0.359		8.192	0.359
	10.440	0.453		10.120	0.451
	15.660	0.673		15.729	0.669
	20.880	0.913		20.480	0.910
	26.100	1.130		25.600	1.110

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Table D2

Calibration Curve Regression Analysis

SUMMARY OUTPUT

Regression Statistics	
Multiple R	0.999801373
R Square	0.999602785
Adjusted R Square	0.999595146
Standard Error	0.007078597
Observations	54

ANOVA

	df	SS	MS	F	Significance F
Regression	1	6.556917886	6.556917886	130859.5418	4.13613E-90
Residual	52	0.00260554	5.01065E-05		
Total	53	6.559523426			

	Coefficients	Standard Error	t Stat	P-value	Lower 95%	Upper 95%	Lower 95.0%	Upper 95.0%
Intercept	-0.001515224	0.001573442	-0.962999627	0.340004743	-0.004672564	0.001642116	-0.004672564	0.001642116
X Variable 1	0.043539634	0.00012036	361.7451338	4.13613E-90	0.043298114	0.043781153	0.043298114	0.043781153

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Molar Absorption Coefficient

The molar absorption coefficient of derivatized bronopol was calculated for each calibration curve data point. Values obtained were compared against the value of 8330 liter/cm mol as reported by Sanyal et al. (1996). Calculated results are presented in Table D3. To determine if the molar absorption coefficient varies with the concentration of derivatized bronopol in solution, analysis of variance was performed among the different concentration levels. Results of this analysis are presented in Table D4.

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Table D3
Molar Absorption Coefficient Data

Calibration Set Number	Bronopol Conc. (µg/ml)	Absorbance at 244 nm	Molar Concentration (mol/liter)	Molar Absorbtivity (liter/cm mol)
1	1.010	0.043	0.00000505	8515
	2.020	0.085	0.0000101	8416
	4.040	0.171	0.0000202	8465
	6.060	0.256	0.0000303	8449
	8.080	0.346	0.0000404	8564
	10.100	0.433	0.0000505	8574
	15.150	0.672	0.00007575	8871
	20.200	0.882	0.000101	8733
	25.250	1.102	0.00012625	8729
2	1.028	0.045	0.00000514	8755
	2.056	0.089	0.00001028	8658
	4.112	0.178	0.00002056	8658
	6.168	0.266	0.00003084	8625
	8.224	0.355	0.00004112	8633
	10.280	0.443	0.0000514	8619
	15.420	0.675	0.0000771	8755
	20.560	0.892	0.0001028	8677
	25.700	1.117	0.0001285	8693
3	1.044	0.046	0.00000522	8812
	2.088	0.089	0.00001044	8525
	4.176	0.179	0.00002088	8573
	6.264	0.264	0.00003132	8429
	8.352	0.359	0.00004176	8597
	10.440	0.453	0.0000522	8678
	15.660	0.673	0.0000783	8595
	20.880	0.913	0.0001044	8745
	26.100	1.130	0.0001305	8659

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Table D3 (continued)
Molar Absorption Coefficient Data

Calibration Set Number	Bronopol Conc. (μg/ml)	Absorbance at 244 nm	Molar Concentration (mol/liter)	Molar Absorbtivity (liter/cm mol)
4	1.020	0.045	0.0000051	8824
	2.040	0.087	0.0000102	8529
	4.080	0.175	0.0000204	8578
	6.120	0.264	0.0000306	8627
	8.160	0.356	0.0000408	8725
	10.200	0.446	0.000051	8745
	15.300	0.671	0.0000765	8771
	20.400	0.890	0.000102	8725
	25.500	1.107	0.0001275	8682
5	1.006	0.047	0.00000503	9344
	2.012	0.090	0.00001006	8946
	4.024	0.169	0.00002012	8400
	6.036	0.253	0.00003018	8383
	8.048	0.345	0.00004024	8574
	10.060	0.449	0.0000503	8926
	15.090	0.671	0.00007545	8893
	20.120	0.851	0.0001006	8459
	25.150	1.084	0.00012575	8620
6	1.012	0.043	0.00000506	8498
	2.024	0.087	0.00001012	8597
	4.048	0.169	0.00002024	8350
	6.144	0.267	0.00003072	8691
	8.192	0.359	0.00004096	8765
	10.120	0.451	0.0000506	8913
	15.729	0.669	0.000078645	8507
	20.480	0.910	0.0001024	8887
	25.600	1.110	0.000128	8672
			Average	8660
			SD	173.9
			CV %	2.01 %

Note. *Molar Concentration is the bronopol concentration in solution calculated using the bronopol molecular weight of 199.99 grams/mol. Molar Absorbtivity is calculated by rearranging Equation 3 and solving for the extinction coefficient; cuvette path length is 1 cm.*

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Table D4

Analysis of Variance Results for Molar Absorption Coefficient

Anova: Single Factor

SUMMARY

Bronopol Conc. (µg/ml)	Count	Sum	Average	Variance
1	6	52748	8791	94131.9
2	6	51671	8612	33354.2
4	6	51024	8504	14001.2
6	6	51204	8534	16526.0
8	6	52858	8810	155231.9
10	6	52455	8743	22110.7
15	6	52392	8732	23337.2
20	6	52226	8704	19441.1
25	6	52055	8676	1315.0

ANOVA

Source of Variation	SS	df	MS	F	P-value	F crit
Between Groups	560061.7	8	70007.7	1.6604849	0.13481	2.1521345
Within Groups	1897245.2	45	42161.0			
Total	2457306.8	53				

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Calculated Instrument Limit of Detection

The procedure uses calibration standard concentrations ranging from less than the anticipated limit of detection to no more than ten times the expected LOD. The standard error of the regression for these calibration standards is first calculated using Equation 7. Calculations for the standard error of regression for bronopol calibration standards ranging in concentration from 1 µg/ml to 10 µg/ml are presented in Table D5. The calculated instrumental limit of detection is then calculated using Equation 8.

Table D5
Calculation of the Standard Error of Regression for Low Level Calibration Standards

Calibration Set Number	Bronopol Conc. (µg/ml)	Absorbance at 244 nm	Predicted Absorbance	(Pred. Abs. – Abs.)^2
1	1.010	0.043	0.042435	0.000000319225
	2.020	0.085	0.08637	0.000001876900
	4.040	0.171	0.17424	0.000010497600
	6.060	0.256	0.26211	0.000037332100
	8.080	0.346	0.34998	0.000015840400
	10.100	0.433	0.43785	0.000023522500
2	1.028	0.045	0.043218	0.000003175524
	2.056	0.089	0.087936	0.000001132096
	4.112	0.178	0.177372	0.000000394384
	6.168	0.266	0.266808	0.000000652864
	8.224	0.355	0.356244	0.000001547536
	10.280	0.443	0.44568	0.000007182400
3	1.044	0.046	0.043914	0.000004351396
	2.088	0.089	0.089328	0.000000107584
	4.176	0.179	0.180156	0.000001336336
	6.264	0.264	0.270984	0.000048776256
	8.352	0.359	0.361812	0.000007907344
	10.440	0.453	0.45264	0.000000129600
4	1.020	0.045	0.04287	0.000004536900
	2.040	0.087	0.08724	0.000000057600
	4.080	0.175	0.17598	0.000000960400
	6.120	0.264	0.26472	0.000000518400
	8.160	0.356	0.35346	0.000006451600
	10.200	0.446	0.4422	0.000014440000

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Table D5 (continued)

Calculation of the Standard Error of Regression for Low Level Calibration Standards

Calibration Set Number	Bronopol Conc. (µg/ml)	Absorbance at 244 nm	Predicted Absorbance	(Pred. Abs. – Abs.)^2
5	1.006	0.047	0.042261	0.000022458121
	2.012	0.090	0.086022	0.000015824484
	4.024	0.169	0.173544	0.000020647936
	6.036	0.253	0.261066	0.000065060356
	8.048	0.345	0.348588	0.000012873744
	10.060	0.449	0.43611	0.000166152100
6	1.012	0.043	0.042522	0.000000228484
	2.024	0.087	0.086544	0.000000207936
	4.048	0.169	0.174588	0.000031225744
	6.144	0.267	0.265764	0.000001527696
	8.192	0.359	0.354852	0.000017205904
	10.120	0.451	0.43872	0.000150798400
			Sum	0.000711856296

Note. *Predicted absorbance is calculated using Equation 12, the least-squares regression calibration line. N = 36.*

Standard Error of Regression for Calibration Standards

$$s_y = [\sum(\hat{y}_i - y_i)^2/(N - 2)]^{1/2} \quad (7)$$

$$s_y = [0.000711856296/(36 - 2)]^{1/2}$$

$$s_y = 0.00452853$$

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Calculated Instrument Limit of Detection

From Equation 12, the least-squares regression calibration line, the slope m is 0.0435.

$$LOD = 3\ s_y/m \quad (8)$$

LOD = (3 x 0.00452853)/0.0435

LOD = 0.31

Calculated Instrument Limit of Quantitation

The instrumental limit of quantitation (LOQ) is calculated using Equation 9. The most appropriate value for the LOD is 1 µg/ml, which the lowest concentration for calibration curve standards used in this work.

$$LOQ = 3.33 \times LOD \quad (9)$$

LOQ = 3.33 x 1 µg/ml

LOQ = 3.33 µg/ml

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Appendix E: Analytical Recovery of Bronopol from Spiked Filters

Filter Blank Count

Twelve treated glass fiber filters with no bronopol were extracted and analyzed. Sodium hydroxide concentration in solution was 0.03 molar. Filter extract solutions were drawn through a syringe filter prior to analysis. Data and the calculated average filter blank count are presented in Table E1.

Table E1
Blank Treated Glass Fiber Filter
Extracts Filtered through Syringe Filters

Filter No.	Absorbance Value
1	0.023
2	0.015
3	0.024
4	0.021
5	0.004
6	0.003
7	0.024
8	0.019
9	0.004
10	0.005
11	0.018
12	0.016
Average	0.015

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Filter Spiking Data

Treated glass fiber filters were spiked with aqueous bronopol solutions then evaluated for analytical recovery by comparing the adjusted absorbance reading obtained (i.e., actual absorbance read minus filter blank count) versus that calculated from the bronopol spike quantity. Three sets of ten each treated filters were spiked with 19, 44, or 113 μg bronopol and were designated as the Low, Medium, and High Ranges.

Filter spiking data and calculations are presented in Tables E2, E5, and E8. The Q-test for outliers is performed on intra-set values in Tables E3, E6, and E9. The F-test for variances is performed on inter-set values in Tables E4, E7, and E10. Filter spiking average analytical recovery and pooled coefficient of variation are presented at the end of the appendix.

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Table E2

Filter Spiking Data and Calculations – Low Range

Filter Number	Actual Absorbance	Adjusted Absorbance	Percent Recovery
Low Range Set No. 1, filter spike quantity 19.04 µg bronopol, predicted absorbance value 0.164			
1	0.173	0.158	96.3
2	0.168	0.153	93.2
3	0.177	0.162	98.7
4	0.171	0.156	95.0
5	0.169	0.154	93.8
		AVG	95.4
		SD	2.18
		CV %	2.28 %
Low Range Set No. 2, filter spike quantity 19.00 µg bronopol, predicted absorbance value 0.164			
1	0.180	0.165	100.7
2	0.177	0.162	98.9
3	0.173	0.158	96.5
4	0.175	0.160	97.7
5	0.174	0.159	97.1
		AVG	98.2
		SD	1.69
		CV %	1.73 %

Note. *Adjusted absorbance is the actual absorbance read for the sample minus the filter blank count of 0.015 absorbance units.*

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Table E3

Low Range Filter Spiking Q-Test For Outliers

	Ranked Recovery (%)	Gap
Low Range Set No. 1		
	93.2	
	93.8	0.6
	95.0	1.2
	96.3	1.3
	98.7	2.4
Range	5.5	
	Calculated Q for highest data point	0.436
	Critical Q Value	0.710
	Result	Accept Value
Low Range Set No. 2		
	96.5	
	97.1	0.6
	97.7	0.6
	98.9	1.2
	100.7	1.8
Range	4.2	
	Calculated Q for highest data point	0.429
	Critical Q Value	0.710
	Result	Accept Value

Table E4

Low Range Filter Spiking F-Test For Variances

	Low Range Set No. 1	Low Range Set No. 2
Mean	95.402	98.168
Variance	4.750	2.870
Observations	5	5
df	4	4
F Calculated	1.655	
F Critical one-tail	6.388	
Result	Variance is consistent between data sets	

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Calculated average analytical recovery for Low Range filter spiking sets is:

$$\frac{95.4\,\% + 98.2\,\%}{2} = 96.8\,\%$$

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Table E5

Filter Spiking Data and Calculations – Medium Range

Filter Number	Actual Absorbance	Adjusted Absorbance	Percent Recovery
Medium Range Set No. 1, filter spike quantity 43.04 µg bronopol, predicted absorbance value 0.373			
1	0.363	0.348	93.3
2	0.354	0.339	90.9
3	0.363	0.348	93.3
4	0.356	0.341	91.4
5	0.357	0.342	91.7
		AVG	92.1
		SD	1.12
		CV %	1.21 %
Medium Range Set No. 2, filter spike quantity 44.16 µg bronopol, predicted absorbance value 0.383			
1	0.385	0.370	96.7
2	0.388	0.373	97.5
3	0.381	0.366	95.6
4	0.389	0.374	97.7
5	0.384	0.369	96.4
		AVG	96.8
		SD	0.84
		CV %	0.87 %

Note. *Adjusted absorbance is the actual absorbance read for the sample minus the filter blank count of 0.015 absorbance units.*

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Table E6

Medium Range Filter Spiking Q-Test For Outliers

	Ranked Recovery (%)	Gap
Medium Range Set No. 1		
	90.9	
	91.4	0.5
	91.7	0.3
	93.3	1.6
	93.3	0
Range	2.4	
	Calculated Q for highest data point	0.667
	Critical Q Value	0.710
	Result	Accept Value
Medium Range Set No. 2		
	95.6	
	96.4	0.8
	96.7	0.3
	97.5	0.8
	97.7	0.2
Range	2.1	
	Calculated Q for lowest data point	0.381
	Critical Q Value	0.710
	Result	Accept Value

Table E7

Medium Range Filter Spiking F-Test For Variances

	Medium Range Set No. 1	Medium Range Set No. 2
Mean	92.131	96.788
Variance	1.244	0.703
Observations	5	5
df	4	4
F Calculated	1.769	
F Critical one-tail	6.388	
Result	Variance is consistent between data sets	

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Calculated average analytical recovery for Medium Range filter spiking sets is:

$$\frac{92.1\ \% + 96.8\ \%}{2} = 94.5\ \%$$

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Table E8

Filter Spiking Data and Calculations – High Range

Filter Number	Actual Absorbance	Adjusted Absorbance	Percent Recovery
High Range Set No. 1, filter spike quantity 113.24 µg bronopol, predicted absorbance value 0.984			
1	0.943	0.928	94.3
2	0.942	0.927	94.2
3	0.918	0.903	91.8
4	0.945	0.930	94.5
5	0.952	0.937	95.3
		AVG	94.0
		SD	1.31
		CV %	1.39 %
High Range Set No. 2, filter spike quantity 113.12 µg bronopol, predicted absorbance value 0.983			
1	0.988	0.973	99.0
2	0.961	0.946	96.3
3	0.960	0.945	96.2
4	0.968	0.953	97.0
5	0.977	0.962	97.9
		AVG	97.3
		SD	1.20
		CV %	1.23 %

Note. *Adjusted absorbance is the actual absorbance read for the sample minus the filter blank count of 0.015 absorbance units.*

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Table E9

High Range Filter Spiking Q-Test For Outliers

	Ranked Recovery (%)	Gap
High Range Set No. 1		
	91.8	
	94.2	2.4
	94.3	0.1
	94.5	0.2
	95.3	0.8
Range	3.5	
	Calculated Q for lowest data point	0.686
	Critical Q Value	0.710
	Result	Accept Value
High Range Set No. 2		
	96.2	
	96.3	0.1
	97.0	0.7
	97.9	0.9
	99.0	1.1
Range	2.8	
	Calculated Q for highest data point	0.393
	Critical Q Value	0.710
	Result	Accept Value

Table E10

High Range Filter Spiking F-Test For Variances

	High Range Set No. 1	High Range Set No. 2
Mean	94.034	97.268
Variance	1.721	1.436
Observations	5	5
df	4	4
F Calculated	1.198	
F Critical one-tail	6.388	
Result	Variance is consistent between data sets	

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Calculated average analytical recovery for High Range filter spiking sets is:

$$\frac{94.0\ \% + 97.3\ \%}{2} = 95.7\ \%$$

Filter Spiking Average Analytical Recovery

Calculated average analytical recovery for all filter spiking sets is:

$$\frac{96.8\ \% + 94.5\ \% + 95.7\ \%}{3} = 95.7\ \%$$

Filter Spiking Pooled Coefficient of Variation

The pooled coefficient of variation for all six filter spike sets at the three different spiking concentrations is calculated as follows. Coefficient of variation is expressed as percent.

$$S_r = \left(\frac{4(2.28)^2 + 4(1.73)^2 + 4(1.21)^2 + 4(0.87)^2 + 4(1.39)^2 + 4(1.23)^2}{24} \right)^{1/2} = 1.52$$

$S_r = 1.52\ \%$

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Appendix F: Sampling Chamber Particulate Concentration Distribution

Distribution of Bronopol Particulate Concentration in the Sampling Chamber

To verify consistent presentation of the bronopol aerosol to the chamber samplers, inert PVDF filters were used to collect samples from the chamber for several trials. A total of nine chamber particulate concentration distribution runs were performed, generating a total of twenty-seven samples. Each sampling event lasted 20 minutes. The quantity of bronopol collected on the filters was varied by sampling at different filter flow rates. Flow rates used were 300, 450, and 600 ml/min and were designated as the Low, Medium, and High Ranges. The filters were extracted in a derivatizing solution and analyzed for bronopol; sodium hydroxide concentration in solution was 0.03 molar. Chamber particulate distribution data and calculations are presented in Tables F1, F5, and F9; the Q-test for outliers is performed on intra-set values in Tables F2, F6, and F10. Camber flow calibration data and calculations are presented in Tables F3, F7, and F11; sampling pump flow calibration data and calculations are presented in Tables F4, F8, and F12.

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Table F1

Chamber Particulate Concentration Data and Calculations – Low Range

Filter Number	Pump Flow (ml/min)	Sample Absorbance	Bronopol Concentration (abs/m^3)	Bronopol Concentration (mg/m^3)
Low Range Set No. 1				
1	290.8	0.993	170.7	19.65
2	291.0	1.027	176.5	20.31
3	300.6	0.995	165.5	19.05
		AVG	170.9	19.67
		SD	5.48	0.63
		CV %	3.21 %	3.21 %
Low Range Set No. 2				
4	290.8	0.733	126.0	14.52
5	291.0	0.746	128.2	14.76
6	300.6	0.770	128.1	14.75
		AVG	127.4	14.68
		SD	1.21	0.14
		CV %	0.95 %	0.95 %
Low Range Set No. 3				
7	290.8	0.592	101.8	11.73
8	291.0	0.622	106.9	12.31
9	300.6	0.672	111.8	12.88
		AVG	106.8	12.31
		SD	4.99	0.57
		CV %	4.68 %	4.68 %

Note. Sampling time 20 minutes for each chamber particulate concentration distribution set. Bronopol Concentration in mg/m^3 is obtained using Equation 12 from the bronopol calibration curve.

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Table F2

Low Range Chamber Particulate Concentration Q-Test For Outliers

	Ranked Recovery (%)	Gap
Low Range Set No. 1		
	19.05	
	19.65	0.60
	20.31	0.66
Range	1.26	
	Calculated Q for highest data point	0.524
	Critical Q Value	0.970
	Result	Accept Value
Low Range Set No. 2		
	14.52	
	14.75	0.23
	14.76	0.01
Range	0.24	
	Calculated Q for lowest data point	0.958
	Critical Q Value	0.970
	Result	Accept Value
Low Range Set No. 3		
	11.73	
	12.31	0.58
	12.88	0.57
Range	1.15	
	Calculated Q for lowest data point	0.504
	Critical Q Value	0.970
	Result	Accept Value

Pooled CV for Low Range chamber particulate concentration distribution sets is:

$$S_r = \left(\frac{2(3.21)^2 + 2(0.95)^2 + 2(4.68)^2}{6}\right)^{1/2} = 3.32$$

$S_r = 3.32$ %

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Table F3

Chamber Flow Calibration – Low Range

	Pre-Calibration Flow (ml/min)	Post-Calibration Flow (ml/min)
	2012	2008
	2013	2008
	2012	2008
	2014	2007
	2012	2008
	2012	2007
	2013	2006
	2012	2007
	2012	2006
	2012	2006
AVG	2012	2007
SD	0.70	0.88
CV %	0.035 %	0.044 %
Chamber Average Flow 2010 ml/min		

Table F4

Pump Flow Calibration – Low Range

	Pump 1		Pump 2		Pump 3	
	Pre (ml/min)	Post (ml/min)	Pre (ml/min)	Post (ml/min)	Pre (ml/min)	Post (ml/min)
	287.7	295.5	294.4	287.3	296.4	303.3
	287.7	295.5	294.3	288.1	296.7	303.7
	287.6	294.9	294.5	288.7	296.9	303.9
	287.6	294.7	294.4	287.9	296.9	304.1
	287.6	294.2	294.3	288.1	297.1	303.7
	287.3	293.9	294.4	288.0	297.3	304.0
	287.4	293.9	294.4	287.5	297.2	304.2
	286.9	293.9	292.2	287.3	297.0	304.5
	286.5	293.2	294.7	287.1	297.3	304.9
	286.7	293.2	294.9	287.7	297.2	305.6
AVG	287.3	294.3	294.3	287.8	297.0	304.2
SD	0.44	0.84	0.74	0.49	0.29	0.67
CV %	0.15 %	0.28 %	0.25 %	0.17 %	0.10 %	0.22 %
Pump Avg.	*290.8 ml/min*		*291.0 ml/min*		*300.6 ml/min*	

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Table F5

Chamber Particulate Concentration Data and Calculations – Medium Range

Filter Number	Pump Flow (ml/min)	Sample Absorbance	Bronopol Concentration (abs/m^3)	Bronopol Concentration (mg/m^3)
Medium Range Set No. 1				
1	464.6	1.577	169.7	19.53
2	463.0	1.523	164.5	18.92
3	456.5	1.498	164.1	18.88
		AVG	166.1	19.11
		SD	3.15	0.36
		CV %	1.90 %	1.90 %
Medium Range Set No. 2				
4	464.6	1.470	158.2	18.20
5	463.0	1.403	151.5	17.43
6	456.5	1.498	164.1	18.88
		AVG	157.9	18.17
		SD	6.29	0.72
		CV %	3.98 %	3.98 %
Medium Range Set No. 3				
7	464.6	1.126	121.2	13.95
8	463.0	1.197	129.3	14.88
9	456.5	1.144	125.3	14.42
		AVG	125.2	14.42
		SD	4.04	0.46
		CV %	3.23 %	3.23 %

Note. *Sampling time 20 minutes for each chamber particulate concentration distribution set. Bronopol Concentration in mg/m^3 is obtained using Equation 12 from the bronopol calibration curve.*

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Table F6
Medium Range Chamber Particulate Concentration Q-Test For Outliers

	Ranked Recovery (%)	Gap
Medium Range Set No. 1		
	18.88	
	18.92	0.04
	19.53	0.61
Range	0.65	
	Calculated Q for highest data point	0.938
	Critical Q Value	0.970
	Result	Accept Value
Medium Range Set No. 2		
	17.43	
	18.20	0.77
	18.88	0.68
Range	1.45	
	Calculated Q for lowest data point	0.531
	Critical Q Value	0.970
	Result	Accept Value
Medium Range Set No. 3		
	13.95	
	14.42	0.47
	14.88	0.46
Range	0.93	
	Calculated Q for lowest data point	0.505
	Critical Q Value	0.970
	Result	Accept Value

Pooled CV for Medium Range chamber particulate concentration distribution sets is:

$$S_r = \left(\frac{2(1.90)^2 + 2(3.98)^2 + 2(3.23)^2}{6} \right)^{1/2} = 3.16$$

$S_r = 3.16\ \%$

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Table F7

Chamber Flow Calibration – Medium Range

	Pre-Calibration Flow (ml/min)	Post-Calibration Flow (ml/min)
	2025	2005
	2025	2006
	2026	2005
	2026	2005
	2025	2004
	2024	2005
	2024	2005
	2024	2005
	2025	2004
	2025	2004
AVG	2025	2005
SD	0.74	0.63
CV %	0.036 %	0.032 %
Chamber Average Flow 2015 ml/min		

Table F8

Pump Flow Calibration – Medium Range

	Pump 1		Pump 2		Pump 3	
	Pre (ml/min)	Post (ml/min)	Pre (ml/min)	Post (ml/min)	Pre (ml/min)	Post (ml/min)
	456.8	473.3	464.1	462.2	448.7	462.5
	456.9	473.7	464.7	463.1	448.6	462.6
	457.9	473.6	464.5	463.0	449.6	463.7
	457.6	473.1	464.3	463.0	449.9	463.5
	458.7	468.9	464.4	463.0	449.9	463.5
	458.3	468.8	464.5	463.5	449.6	463.5
	459.1	468.9	464.0	463.3	449.0	463.2
	459.9	469.2	464.0	463.2	449.2	463.3
	460.0	468.5	457.9	463.0	449.9	463.2
	460.5	468.3	458.1	462.4	451.3	464.3
AVG	458.6	470.6	463.1	463.0	449.6	463.3
SD	1.30	2.42	2.67	0.39	0.78	0.52
CV %	0.28 %	0.51 %	0.58 %	0.08 %	0.17 %	0.11 %
Pump Avg.	*464.6 ml/min*		*463.0 ml/min*		*456.5 ml/min*	

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Table F9

Chamber Particulate Concentration Data and Calculations – High Range

Filter Number	Pump Flow (ml/min)	Sample Absorbance	Bronopol Concentration (abs/m^3)	Bronopol Concentration (mg/m^3)
High Range Set No. 1				
1	604.8	1.968	162.7	18.72
2	585.3	1.921	164.1	18.88
3	610.9	1.977	161.8	18.61
		AVG	162.9	18.74
		SD	1.16	0.13
		CV %	0.71 %	0.71 %
High Range Set No. 2				
4	604.8	1.732	143.2	16.47
5	585.3	1.782	152.2	17.51
6	610.9	1.695	138.7	15.96
		AVG	144.7	16.65
		SD	6.88	0.79
		CV %	4.75 %	4.75 %
High Range Set No. 3				
7	604.8	1.373	113.5	13.06
8	585.3	1.276	109.0	12.54
9	610.9	1.357	111.1	12.78
		AVG	111.2	12.80
		SD	2.26	0.26
		CV %	2.03 %	2.03 %

Note. *Sampling time 20 minutes for each chamber particulate concentration distribution set. Bronopol Concentration in mg/m^3 is obtained using Equation 12 from the bronopol calibration curve.*

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Table F10

High Range Chamber Particulate Concentration Q-Test For Outliers

	Ranked Recovery (%)	Gap
High Range Set No. 1		
	18.61	
	18.72	0.11
	18.88	0.16
Range	0.27	
	Calculated Q for highest data point	0.593
	Critical Q Value	0.970
	Result	Accept Value
High Range Set No. 2		
	15.96	
	16.47	0.51
	17.51	1.04
Range	1.55	
	Calculated Q for highest data point	0.671
	Critical Q Value	0.970
	Result	Accept Value
High Range Set No. 3		
	12.54	
	12.78	0.24
	13.06	0.28
Range	0.52	
	Calculated Q for highest data point	0.538
	Critical Q Value	0.970
	Result	Accept Value

Pooled CV for High Range chamber particulate concentration distribution sets is:

$$S_r = \left(\frac{2(0.71)^2 + 2(4.75)^2 + 2(2.03)^2}{6} \right)^{1/2} = 3.01$$

$S_r = 3.01$ %

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Table F11
Chamber Flow Calibration – High Range

	Pre-Calibration Flow (ml/min)	Post-Calibration Flow (ml/min)
	2016	2007
	2016	2006
	2015	2008
	2020	2007
	2015	2006
	2015	2006
	2017	2005
	2016	2006
	2012	2005
	2015	2002
AVG	2016	2006
SD	2.00	1.62
CV %	0.10 %	0.08 %
Chamber Average Flow 2011 ml/min		

Table F12
Pump Flow Calibration – High Range

	Pump 1		Pump 2		Pump 3	
	Pre (ml/min)	Post (ml/min)	Pre (ml/min)	Post (ml/min)	Pre (ml/min)	Post (ml/min)
	597.4	612.1	587.0	577.1	611.6	609.9
	597.0	612.0	587.9	577.1	611.8	610.0
	596.6	612.0	588.9	577.0	611.8	609.8
	597.0	612.0	595.5	577.1	612.0	609.8
	596.9	612.2	595.7	577.2	612.2	609.6
	597.3	612.2	595.7	577.0	612.2	609.5
	597.3	612.6	595.6	578.3	612.0	609.6
	598.1	612.5	595.0	578.0	612.2	609.6
	597.9	612.5	595.0	578.1	612.2	609.5
	597.7	612.5	594.4	578.1	612.3	609.4
AVG	597.3	612.3	593.1	577.5	612.0	609.7
SD	0.47	0.24	3.60	0.55	0.23	0.19
CV %	0.08 %	0.04 %	0.61 %	0.09 %	0.04 %	0.03 %
Pump Avg.	*604.8 ml/min*		*585.3 ml/min*		*610.9 ml/min*	

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Sampling Chamber Bronopol Particulate Concentration Pooled Coefficient of Variation

The pooled coefficient of variation for all nine chamber particulate concentration distribution sets at the three different sampling flow rates is calculated as follows. Coefficient of variation is expressed as percent.

$$S_r = \left(\frac{2(3.21)^2 + 2(0.95)^2 + 2(4.68)^2 + 2(1.90)^2 + 2(3.98)^2 + 2(3.23)^2 + 2(0.71)^2 + 2(4.75)^2 + 2(2.03)^2}{18} \right)^{1/2} = 3.17$$

$S_r = 3.17$ %

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Appendix G: Sampling and Analytical Recovery

Sampling of Generated Bronopol Atmospheres

For sampling of bronopol particulate in the chamber, one inert PVDF filter and two treated glass fiber filters were used simultaneously. Each sampling event lasted 20 minutes. The quantity of bronopol collected on the filters was varied by sampling at different filter flow rates. Flow rates used were 300, 450, and 600 ml/min and were designated as the Low, Medium, and High Ranges.

In all this produced eighteen bronopol samples collected on treated glass fiber filters. The analytical results from the treated glass fiber filters are compared against the nine inert PVDF filters that were sampled concurrently. PVDF filters were extracted in a derivatizing solution; glass fiber filters were extracted in spectrophotometric grade water. Sodium hydroxide concentration in solution for all filter types was 0.03 molar. Chamber sampling data and bronopol concentration calculations are presented in Tables G1, G6, and G11; the Q-test for outliers is performed on intra-set values in Tables G2, G7, and G12. Recovery data and calculations are presented in Tables G3, G8, and G13. Chamber flow calibration data and calculations are presented in Tables G4, G9, and G14. Sampling pump flow calibration data and calculations are presented in Tables G5, G10, and G15.

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Table G1

Chamber Sampling Data and Concentration Calculations – Low Range

Filter Number	Pump Flow (ml/min)	Sample Absorbance	Bronopol Concentration (abs/m^3)	Bronopol Concentration (mg/m^3)
Low Range Set No. 1				
1 (PVDF)	316.8	1.156	182.4	21.00
2 (GFF)	303.4	1.097	180.8	20.81
3 (GFF)	328.0	1.116	170.1	19.58
		AVG	177.8	20.46
		SD	6.69	0.77
		CV %	3.76 %	3.76 %
Low Range Set No. 2				
4 (PVDF)	316.8	0.885	139.7	16.08
5 (GFF)	303.4	0.851	140.2	16.15
6 (GFF)	328.0	0.847	129.1	14.87
		AVG	136.4	15.70
		SD	6.26	0.72
		CV %	4.59 %	4.59 %
Low Range Set No. 3				
7 (PVDF)	316.8	0.729	115.1	13.25
8 (GFF)	303.4	0.745	122.8	14.14
9 (GFF)	328.0	0.777	118.4	13.64
		AVG	118.8	13.68
		SD	3.87	0.45
		CV %	3.26 %	3.26 %

Note. Sampling time 20 minutes for each chamber sampling set. Bronopol Concentration in mg/m^3 is obtained using Equation 12 from the bronopol calibration curve.

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Table G2
Low Range Chamber Sampling Q-Test For Outliers

	Ranked Recovery (%)	Gap
Low Range Set No. 1		
	19.05	
	19.65	0.60
	20.31	0.66
Range	1.26	
	Calculated Q for highest data point	0.524
	Critical Q Value	0.970
	Result	Accept Value
Low Range Set No. 2		
	14.52	
	14.75	0.23
	14.76	0.01
Range	0.24	
	Calculated Q for lowest data point	0.958
	Critical Q Value	0.970
	Result	Accept Value
Low Range Set No. 3		
	11.73	
	12.31	0.58
	12.88	0.57
Range	1.15	
	Calculated Q for lowest data point	0.504
	Critical Q Value	0.970
	Result	Accept Value

Pooled CV for concentration in Low Range chamber sampling sets including all GFF and PVDF filters is:

$$S_r = \left(\frac{2(3.76)^2 + 2(4.59)^2 + 2(3.26)^2}{6} \right)^{1/2} = 3.91$$

$S_r = 3.91$ %

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Table G3

Chamber Sampling Data and Recovery Calculations – Low Range

Filter Number (Type)	Pump Flow (ml/min)	Sample Absorbance	Concentration (abs/m^3)	Recovery Versus Standard
Low Range Set No. 1				
1 (PVDF)	316.8	1.156	182.4	-
2 (GFF)	303.4	1.097	180.8	99.1 %
3 (GFF)	328.0	1.116	170.1	93.3 %
		AVG	177.8	96.2 %
		SD	6.69	4.13
		CV %	3.76 %	4.30 %
Low Range Set No. 2				
4 (PVDF)	316.8	0.885	139.7	-
5 (GFF)	303.4	0.851	140.2	100.4 %
6 (GFF)	328.0	0.847	129.1	92.4 %
		AVG	136.4	96.4 %
		SD	6.26	5.63
		CV %	4.59 %	5.83 %
Low Range Set No. 3				
7 (PVDF)	316.8	0.729	115.1	-
8 (GFF)	303.4	0.745	122.8	106.7 %
9 (GFF)	328.0	0.777	118.4	102.9 %
		AVG	118.8	104.8 %
		SD	3.87	2.66
		CV %	3.26 %	2.54 %

Note. Sampling time 20 minutes for each chamber sampling set. Recovery Versus Standard is the concentration for each GFF filter as a percent of the concentration for the concurrently sampled PVDF filter.

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Calculated average analytical recovery for the Low Range chamber sampling sets is:

$$\frac{96.2\ \% + 96.4\ \% + 104.8\ \%}{3} = 99.1\ \%$$

Pooled CV for analytical recovery in Low Range chamber sampling sets with glass fiber filter (GFF) data exclusively is:

$$S_r = \left(\frac{1(4.30)^2 + 1(5.83)^2 + 1(2.54)^2}{3} \right)^{1/2} = 4.43$$

$S_r = 4.43\ \%$

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Table G4

Chamber Flow Calibration – Low Range

	Pre-Calibration Flow (ml/min)	Post-Calibration Flow (ml/min)
	2010	2002
	2010	2001
	2010	2003
	2009	2002
	2008	2002
	2010	1998
	2009	2000
	2009	2000
	2009	2001
	2008	2000
AVG	2009	2001
SD	0.79	1.45
CV %	0.039 %	0.072 %
Chamber Average Flow 2005 ml/min		

Table G5

Pump Flow Calibration – Low Range

	Pump 1		Pump 2		Pump 3	
	Pre (ml/min)	Post (ml/min)	Pre (ml/min)	Post (ml/min)	Pre (ml/min)	Post (ml/min)
	323.6	309.2	299.2	307.3	331.8	322.9
	323.9	309.1	299.2	307.1	330.9	323.5
	323.7	309.0	299.1	306.9	331.7	323.6
	323.7	310.2	299.1	306.7	331.9	323.7
	323.6	310.7	299.4	309.9	331.9	323.8
	323.7	310.6	299.3	306.9	332.1	324.4
	324.4	310.2	299.7	306.7	332.4	324.5
	323.9	310.1	302.1	306.4	332.1	324.2
	323.6	309.6	299.9	306.5	332.2	324.8
	323.8	309.1	300.4	306.2	332.6	325.1
AVG	323.8	309.8	299.7	307.1	332.0	324.1
SD	0.24	0.66	0.93	1.05	0.46	0.67
CV %	0.07 %	0.21 %	0.31 %	0.34 %	0.14 %	0.21%
Pump Avg.	*316.8 ml/min*		*303.4 ml/min*		*328.0 ml/min*	

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Table G6

Chamber Sampling Data and Concentration Calculations – Medium Range

Filter Number	Pump Flow (ml/min)	Sample Absorbance	Bronopol Concentration (abs/m^3)	Bronopol Concentration (mg/m^3)
Medium Range Set No. 1				
1 (PVDF)	463.7	1.812	195.4	22.48
2 (GFF)	456.1	1.619	177.5	20.42
3 (GFF)	452.6	1.722	190.2	21.89
		AVG	187.7	21.59
		SD	9.22	1.06
		CV %	4.91 %	4.91 %
Medium Range Set No. 2				
4 (PVDF)	463.7	1.667	179.8	20.68
5 (GFF)	456.1	1.688	185.0	21.29
6 (GFF)	452.6	1.745	192.8	22.18
		AVG	185.9	21.38
		SD	6.55	0.75
		CV %	3.52 %	3.52 %
Medium Range Set No. 3				
7 (PVDF)	463.7	1.543	166.4	19.14
8 (GFF)	456.1	1.443	158.2	18.20
9 (GFF)	452.6	1.365	150.8	17.35
		AVG	158.5	18.23
		SD	7.80	0.90
		CV %	4.92 %	4.92 %

Note. Sampling time 20 minutes for each chamber sampling set. Bronopol Concentration in mg/m^3 is obtained using Equation 12 from the bronopol calibration curve.

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Table G7

Medium Range Chamber Sampling Q-Test For Outliers

	Ranked Recovery (%)		Gap
Medium Range Set No. 1			
	20.42		
	21.89		1.47
	22.48		0.59
Range	2.06		
		Calculated Q for highest data point	0.714
		Critical Q Value	0.970
		Result	Accept Value
Medium Range Set No. 2			
	20.68		
	21.29		0.61
	22.18		0.89
Range	1.50		
		Calculated Q for lowest data point	0.593
		Critical Q Value	0.970
		Result	Accept Value
Medium Range Set No. 3			
	17.35		
	18.20		0.85
	19.14		0.94
Range	1.79		
		Calculated Q for lowest data point	0.525
		Critical Q Value	0.970
		Result	Accept Value

Pooled CV for concentration in Medium Range chamber sampling sets including all GFF and PVDF filters is:

$$S_r = \left(\frac{2(4.91)^2 + 2(3.52)^2 + 2(4.92)^2}{6} \right)^{1/2} = 4.50$$

$S_r = 4.50$ %

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Table G8

Chamber Sampling Data and Recovery Calculations – Medium Range

Filter Number (Type)	Pump Flow (ml/min)	Sample Absorbance	Concentration (abs/m^3)	Recovery Versus Standard
Medium Range Set No. 1				
1 (PVDF)	463.7	1.812	195.4	-
2 (GFF)	456.1	1.619	177.5	90.8 %
3 (GFF)	452.6	1.722	190.2	97.4 %
		AVG	187.7	94.1 %
		SD	9.22	4.61
		CV %	4.91 %	4.90 %
Medium Range Set No. 2				
4 (PVDF)	463.7	1.667	179.8	-
5 (GFF)	456.1	1.688	185.0	103.0 %
6 (GFF)	452.6	1.745	192.8	107.3 %
		AVG	185.9	105.1 %
		SD	6.55	3.04
		CV %	3.52 %	2.89 %
Medium Range Set No. 3				
7 (PVDF)	463.7	1.543	166.4	-
8 (GFF)	456.1	1.443	158.2	95.1 %
9 (GFF)	452.6	1.365	150.8	90.6 %
		AVG	158.5	92.9 %
		SD	7.80	3.14
		CV %	4.92 %	3.38 %

Note. *Sampling time 20 minutes for each chamber sampling set. Recovery Versus Standard is the concentration for each GFF filter as a percent of the concentration for the concurrently sampled PVDF filter.*

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Calculated average analytical recovery for the Medium Range chamber sampling sets is:

$$\frac{94.1\ \% + 105.1\ \% + 92.9\ \%}{3} = 97.4\ \%$$

Pooled CV for analytical recovery in Medium Range chamber sampling sets with glass fiber filter (GFF) data exclusively is:

$$S_r = \left(\frac{1(4.90)^2 + 1(2.89)^2 + 1(3.38)^2}{3} \right)^{1/2} = 3.82$$

$S_r = 3.82\ \%$

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Table G9

Chamber Flow Calibration – Medium Range

	Pre-Calibration Flow (ml/min)	Post-Calibration Flow (ml/min)
	2022	2012
	2020	2013
	2021	2010
	2019	2012
	2017	2011
	2018	2011
	2020	2013
	2020	2012
	2019	2009
	2020	2010
AVG	2020	2011
SD	1.43	1.34
CV %	0.071 %	0.066 %
Chamber Average Flow 2015 ml/min		

Table G10

Pump Flow Calibration – Medium Range

	Pump 1		Pump 2		Pump 3	
	Pre (ml/min)	Post (ml/min)	Pre (ml/min)	Post (ml/min)	Pre (ml/min)	Post (ml/min)
	455.9	472.4	461.0	449.7	457.7	449.9
	454.7	472.5	461.2	449.3	457.0	450.4
	455.4	472.0	461.9	448.6	457.5	449.1
	455.1	472.3	462.2	448.9	457.6	449.5
	454.9	472.7	462.3	449.4	451.4	449.1
	454.5	472.6	462.6	451.3	456.9	448.3
	454.7	472.5	462.5	450.8	456.9	448.1
	454.6	472.7	462.9	450.7	456.7	447.8
	454.0	473.0	463.5	450.1	456.5	447.9
	453.8	473.3	464.0	449.7	456.6	447.7
AVG	454.8	472.6	462.4	449.9	456.5	448.8
SD	0.62	0.36	0.93	0.87	1.83	0.95
CV %	0.14 %	0.08 %	0.20 %	0.19 %	0.40 %	0.21 %
Pump Avg.	*463.7 ml/min*		*456.1 ml/min*		*452.6 ml/min*	

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Table G11

Chamber Sampling Data and Concentration Calculations – High Range

Filter Number	Pump Flow (ml/min)	Sample Absorbance	Bronopol Concentration (abs/m^3)	Bronopol Concentration (mg/m^3)
High Range Set No. 1				
1 (PVDF)	604.4	1.959	162.1	18.64
2 (GFF)	602.5	1.911	158.6	18.24
3 (GFF)	598.9	1.819	151.9	17.47
		AVG	157.5	18.12
		SD	5.19	0.60
		CV %	3.29 %	3.29 %
High Range Set No. 2				
4 (PVDF)	604.4	1.619	133.9	15.41
5 (GFF)	602.5	1.779	147.6	16.98
6 (GFF)	598.9	1.781	148.7	17.11
		AVG	143.4	16.50
		SD	8.23	0.95
		CV %	5.74 %	5.74 %
High Range Set No. 3				
7 (PVDF)	604.4	1.113	92.07	10.60
8 (GFF)	602.5	1.095	90.87	10.46
9 (GFF)	598.9	1.179	98.43	11.33
		AVG	93.79	10.80
		SD	4.06	0.47
		CV %	4.33 %	4.33 %

Note. Sampling time 20 minutes for each chamber sampling set. Bronopol Concentration in mg/m^3 is obtained using Equation 12 from the bronopol calibration curve.

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Table G12

High Range Chamber Sampling Q-Test For Outliers

	Ranked Recovery (%)	Gap
High Range Set No. 1		
	17.47	
	18.24	0.77
	18.64	0.40
Range	1.17	
	Calculated Q for highest data point	0.658
	Critical Q Value	0.970
	Result	Accept Value
High Range Set No. 2		
	15.41	
	16.98	1.57
	17.11	0.13
Range	1.70	
	Calculated Q for lowest data point	0.924
	Critical Q Value	0.970
	Result	Accept Value
High Range Set No. 3		
	10.46	
	10.60	0.14
	11.33	0.73
Range	0.89	
	Calculated Q for lowest data point	0.839
	Critical Q Value	0.970
	Result	Accept Value

Pooled CV for concentration in High Range chamber sampling sets including all GFF and PVDF filters is:

$$S_r = \left(\frac{2(3.29)^2 + 2(5.74)^2 + 2(4.33)^2}{6} \right)^{1/2} = 4.57$$

$S_r = 4.57$ %

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Table G13

Chamber Sampling Data and Recovery Calculations – High Range

Filter Number (Type)	Pump Flow (ml/min)	Sample Absorbance	Concentration (abs/m^3)	Recovery Versus Standard
High Range Set No. 1				
1 (PVDF)	604.4	1.959	162.1	-
2 (GFF)	602.5	1.911	158.6	97.9 %
3 (GFF)	598.9	1.819	151.9	93.7 %
		AVG	157.5	95.8%
		SD	5.19	2.94
		CV %	3.29 %	3.06 %
High Range Set No. 2				
4 (PVDF)	604.4	1.619	133.9	-
5 (GFF)	602.5	1.779	147.6	110.2 %
6 (GFF)	598.9	1.781	148.7	111.1 %
		AVG	143.4	110.7 %
		SD	8.23	0.56
		CV %	5.74 %	0.50 %
High Range Set No. 3				
7 (PVDF)	604.4	1.113	92.07	-
8 (GFF)	602.5	1.095	90.87	98.7 %
9 (GFF)	598.9	1.179	98.43	106.9 %
		AVG	93.79	102.8 %
		SD	4.06	5.81
		CV %	4.33 %	5.65 %

Note. *Sampling time 20 minutes for each chamber sampling set. Recovery Versus Standard is the concentration for each GFF filter as a percent of the concentration for the concurrently sampled PVDF filter.*

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Calculated average analytical recovery for the High Range chamber sampling sets is:

$$\frac{95.8\ \% + 110.7\ \% + 102.8\ \%}{3} = 103.1\ \%$$

Pooled CV for analytical recovery in High Range chamber sampling sets with glass fiber filter (GFF) data exclusively is:

$$S_r = \left(\frac{1(3.06)^2 + 1(0.50)^2 + 1(5.65)^2}{3}\right)^{1/2} = 3.72$$

$S_r = 3.72\ \%$

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Table G14
Chamber Flow Calibration – High Range

	Pre-Calibration Flow (ml/min)	Post-Calibration Flow (ml/min)
	2012	1993
	2012	1994
	2012	1997
	2011	1999
	2010	1997
	2009	1998
	2009	2002
	2010	2001
	2009	1999
	2010	2000
AVG	2010	1998
SD	1.26	2.87
CV %	0.063 %	0.144 %
Chamber Average Flow 2004 ml/min		

Table G15
Pump Flow Calibration – High Range

	Pump 1		Pump 2		Pump 3	
	Pre (ml/min)	Post (ml/min)	Pre (ml/min)	Post (ml/min)	Pre (ml/min)	Post (ml/min)
	612.2	595.8	605.8	598.7	593.9	603.5
	612.5	595.8	605.7	598.9	594.1	605.7
	614.5	595.7	605.5	599.3	594.3	603.0
	615.5	595.3	605.4	599.9	594.3	603.4
	614.4	595.3	605.0	600.0	594.2	603.3
	613.8	595.3	604.9	600.0	594.4	603.0
	613.6	595.7	604.8	599.8	594.0	603.2
	613.1	595.5	605.2	599.9	594.1	603.3
	612.4	595.3	605.0	600.4	593.6	603.7
	611.7	595.3	605.4	601.1	593.7	605.4
AVG	613.4	595.5	605.3	599.8	594.1	603.8
SD	1.20	0.23	0.34	0.70	0.26	0.97
CV %	0.20 %	0.04 %	0.06 %	0.12 %	0.04 %	0.16 %
Pump Avg.	*604.4 ml/min*		*602.5 ml/min*		*598.9 ml/min*	

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Sampling of Airborne Bronopol Pooled Coefficient of Variation

The pooled coefficient of variation for all nine chamber sampling sets at the three different sampling flow rates is calculated as follows. PVDF and GFF filter data are included in the calculation. Coefficient of variation is expressed as percent.

$$S_r = \left(\frac{2(3.76)^2 + 2(4.59)^2 + 2(3.26)^2 + 2(4.91)^2 + 2(3.52)^2 + 2(4.92)^2 + 2(3.29)^2 + 2(5.74)^2 + 2(4.33)^2}{18} \right)^{1/2} = 4.33$$

S_r = 4.33 % (Including all PVDF and GFF filters)

The pooled coefficient of variation for all nine chamber sampling sets at the three different sampling flow rates is calculated as follows. Only GFF filter data are included in the calculation. Coefficient of variation is expressed as percent.

$$S_r = \left(\frac{1(4.30)^2 + 1(5.83)^2 + 1(2.54)^2 + 1(4.90)^2 + 1(2.89)^2 + 1(3.38)^2 + 1(3.06)^2 + 1(0.50)^2 + 2(5.65)^2}{9} \right)^{1/2} = 4.00$$

S_r = 4.00 % (Including only GFF filters)

Sampling of Airborne Bronopol Average Recovery

Calculated average recovery for all of the glass fiber filters used in chamber sampling sets is:

$$\frac{99.1\ \% + 97.4\ \% + 103.1\ \%}{3} = 99.9\ \%$$

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Appendix H: Sample Storage Stability

Derivatized Bronopol Solutions

Aqueous bronopol was spiked onto 18 treated glass fiber filters; the filters were allowed to air dry. Six filters were spiked with 25 µg bronopol, six with 60 µg bronopol, and six with 125 µg bronopol; these were designated as the Low, Medium, and High Ranges. Filters were extracted and analyzed on the same day. The solutions from day one were stored and analyzed again after fourteen days. Coefficient of variation analysis was performed for filter sets at each spike loading level; a change of 10% or less was considered acceptable (NIOSH, 1995).

Bronopol solution sample storage stability data and calculations are presented in Tables H1, H4, and H7. The Q-test for outliers is performed on intra-set values in Tables H2, H5, and H8. The F-test for variances is performed on inter-set values in Tables H3, H6, and H9. The calculated average change in absorbance values from the first day to day fourteen is presented at the end of the appendix.

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Table H1

Bronopol Solution Storage Stability Data and Calculations – Low Range

Solution Number	Adjusted Absorbance First Day	Recovery (%)	Adjusted Absorbance Day Fourteen	Recovery (%)
1	0.217	99.7	0.217	99.7
2	0.206	94.6	0.211	96.9
3	0.206	94.6	0.208	95.5
4	0.212	97.4	0.209	96.0
5	0.208	95.5	0.211	96.9
6	0.213	97.8	0.213	97.8
AVG	0.210	96.6	0.212	97.1
SD	0.004	2.03	0.003	1.47
CV %	2.10 %	2.10 %	1.52 %	1.52 %

Note. *Each treated GFF filter spiked with 25.2 µg bronopol; predicted absorbance for the derivatized bronopol is 0.218. The adjusted absorbance is calculated as the absorbance read from each sample minus the filter blank count of 0.015 absorbance units; Recovery is then the adjusted absorbance divided by the predicted absorbance expressed as percent.*

The calculated percent difference in average absorbance values from the initial analysis to day fourteen is:

$$\frac{|0.210 - 0.212|}{(0.210 + 0.212)/2} \times 100\,\% = 0.95\,\%$$

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Table H2

Low Range Solution Storage Stability Q-Test For Outliers

	Ranked Recovery (%)	Gap
Low Range Set First Day		
	94.6	
	94.6	0
	95.5	0.9
	97.4	1.9
	97.6	0.2
	99.7	2.1
Range	5.1	
	Calculated Q for highest data point	0.412
	Critical Q Value	0.625
	Result	Accept Value
Low Range Set Day Fourteen		
	95.5	
	96.0	0.5
	96.9	0.9
	96.9	0
	97.8	0.9
	99.7	1.9
Range	4.2	
	Calculated Q for highest data point	0.452
	Critical Q Value	0.625
	Result	Accept Value

Table H3

Low Range Solution Storage Stability F-Test For Variances

	First Day	Day Fourteen
Mean	96.6	94.9
Variance	4.09	3.55
Observations	6	6
df	5	5
F Calculated	1.15	
F Critical one-tail	5.05	
Result	Variance is consistent between data sets	

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Table H4

Bronopol Solution Storage Stability Data and Calculations – Medium Range

Solution Number	Adjusted Absorbance First Day	Recovery (%)	Adjusted Absorbance Day Fourteen	Recovery (%)
1	0.482	93.0	0.482	93.0
2	0.503	97.0	0.499	96.3
3	0.492	94.9	0.502	96.8
4	0.502	96.8	0.499	96.3
5	0.494	95.3	0.479	92.4
6	0.494	95.3	0.489	94.3
AVG	0.495	95.4	0.492	94.8
SD	0.008	1.47	0.010	1.88
CV %	1.54 %	1.54 %	1.98 %	1.98 %

Note. *Each treated GFF filter spiked with 59.76 µg bronopol; predicted absorbance for the derivatized bronopol is 0.518. The adjusted absorbance is calculated as the absorbance read from each sample minus the filter blank count of 0.015 absorbance units; Recovery is then the adjusted absorbance divided by the predicted absorbance expressed as percent.*

The calculated percent difference in average absorbance values from the initial analysis to day fourteen is:

$$\frac{|0.495 - 0.492|}{(0.495 + 0.492)/2} \cdot 100\ \% = 0.61\ \%$$

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Table H5

Medium Range Solution Storage Stability Q-Test For Outliers

	Ranked Recovery (%)	Gap
Medium Range Set First Day		
	93.0	
	94.9	1.9
	95.3	0.4
	95.3	0
	96.8	1.5
	97.0	0.2
Range	4.0	
	Calculated Q for lowest data point	0.475
	Critical Q Value	0.625
	Result	Accept Value
Medium Range Set Day Fourteen		
	92.4	
	93.0	0.6
	94.3	1.3
	96.3	2
	96.3	0
	96.8	0.5
Range	4.4	
	Calculated Q for lowest data point	0.136
	Critical Q Value	0.625
	Result	Accept Value

Table H6

Medium Range Solution Storage Stability F-Test For Variances

	First Day	Day Fourteen
Mean	95.4	94.9
Variance	2.11	3.55
Observations	6	6
df	5	5
F Calculated	0.595	
F Critical one-tail	5.05	
Result	Variance is consistent between data sets	

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Table H7

Bronopol Solution Storage Stability Data and Calculations – High Range

Solution Number	Adjusted Absorbance First Day	Recovery (%)	Adjusted Absorbance Day Fourteen	Recovery (%)
1	1.077	98.3	1.066	97.3
2	1.058	96.6	1.041	95.0
3	1.019	93.0	1.006	91.8
4	1.043	95.2	1.028	93.8
5	1.021	93.2	1.019	93.0
6	1.042	95.1	1.062	96.9
AVG	1.043	95.2	1.037	94.6
SD	0.022	2.02	0.024	2.18
CV %	2.12 %	2.12 %	2.30 %	2.30 %

Note. *Each treated GFF filter spiked with 126.12 µg bronopol; predicted absorbance for the derivatized bronopol is 1.096. The adjusted absorbance is calculated as the absorbance read from each sample minus the filter blank count of 0.015 absorbance units; Recovery is then the adjusted absorbance divided by the predicted absorbance expressed as percent.*

The calculated percent difference in average absorbance values from the initial analysis to day fourteen is:

$$\frac{|1.043 - 1.037|}{(1.043 + 1.037)/2} \cdot 100\ \% = 0.58\ \%$$

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Table H8
High Range Solution Storage Stability Q-Test For Outliers

	Ranked Recovery (%)	Gap
High Range Set First Day		
	93.0	
	93.2	0.2
	95.1	1.9
	95.2	0.1
	96.6	1.4
	98.3	1.7
Range	5.3	
	Calculated Q for highest data point	0.321
	Critical Q Value	0.625
	Result	Accept Value
High Range Set Day Fourteen		
	91.8	
	93.0	1.2
	93.8	0.8
	95.0	1.2
	96.9	1.9
	97.3	0.4
Range	5.5	
	Calculated Q for lowest data point	0.218
	Critical Q Value	0.625
	Result	Accept Value

Table H9
High Range Solution Storage Stability F-Test For Variances

	First Day	Day Fourteen
Mean	95.2	94.6
Variance	4.08	4.75
Observations	6	6
df	5	5
F Calculated	0.858	
F Critical one-tail	5.05	
Result	Variance is consistent between data sets	

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The calculated average change in absorbance values for all derivatized bronopol solutions from the initial analysis to day fourteen is:

$$\frac{0.95\ \% + 0.61\ \% + 0.58\ \%}{3} = 0.71\ \%$$

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Bronopol on Treated Glass Fiber Filters

Aqueous bronopol was spiked onto 36 treated glass fiber filters; the filters were allowed to air dry. Twelve filters were spiked with 25 µg bronopol, twelve with 60 µg bronopol, and twelve with 125 µg bronopol. Six of each set were extracted and analyzed on the same day; six of each set were extracted and analyzed after 48 hours. Coefficient of variation analysis was performed for filter sets at each spike loading level; a change of 10 % or less was considered acceptable (NIOSH, 1995).

Bronopol spiked sample storage stability data and calculations are presented in Tables H10, H13, and H16. The Q-test for outliers is performed on intra-set values in Tables H11, H14, and H17. The F-test for variances is performed on inter-set values in Tables H12, H15, and H18. The calculated average change in absorbance values from the same day to 48 hours later is presented at the end of the appendix.

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Table H10

Spiked Filter Storage Stability Data and Calculations – Low Range

Filter Numbers	Adjusted Absorbance First Day	Recovery (%)	Adjusted Absorbance 48 Hours	Recovery (%)
1 / 7	0.216	96.6	0.211	94.3
2 / 8	0.214	95.7	0.217	97.0
3 / 9	0.206	92.1	0.215	96.1
4 / 10	0.218	97.5	0.204	91.2
5 / 11	0.206	92.1	0.201	89.9
6 / 12	0.200	89.4	0.208	93.0
AVG	0.210	93.9	0.209	93.6
SD	0.007	3.15	0.006	2.78
CV %	3.35 %	3.35 %	2.97 %	2.97 %

Note. Each treated GFF filter spiked with 25.88 µg bronopol; predicted absorbance for the derivatized bronopol is 0.224. Filter Numbers reflect the filter number extracted and analyzed on the first day and the filter number extracted and analyzed after 48 hours. The adjusted absorbance is calculated as the absorbance read from each sample minus the filter blank count of 0.015 absorbance units; Recovery is then the adjusted absorbance divided by the predicted absorbance expressed as percent.

The calculated percent difference in average absorbance values from the initial analysis to 48 hours later is:

$$\frac{|0.210 - 0.209|}{(0.210 + 0.209)/2} \times 100\ \% = 0.48\ \%$$

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Table H11

Low Range Spiked Filter Storage Stability Q-Test For Outliers

	Ranked Recovery (%)	Gap
Low Range Set First Day		
	89.4	
	92.1	2.7
	92.1	0
	95.7	3.6
	96.6	0.9
	97.5	0.9
Range	8.1	
	Calculated Q for lowest data point	0.444
	Critical Q Value	0.625
	Result	Accept Value
Low Range Set After 48 Hours		
	89.9	
	91.2	1.3
	93.0	1.8
	94.3	1.3
	96.1	1.8
	97.0	0.9
Range	7.1	
	Calculated Q for lowest data point	0.254
	Critical Q Value	0.625
	Result	Accept Value

Table H12

Low Range Spiked Filter Storage Stability F-Test For Variances

	First Day	After 48 Hours
Mean	93.9	93.6
Variance	10.0	7.62
Observations	6	6
df	5	5
F Calculated	1.32	
F Critical one-tail	5.05	
Result	Variance is consistent between data sets	

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Table H13

Spiked Filter Storage Stability Data and Calculations – Medium Range

Filter Numbers	Adjusted Absorbance First Day	Recovery (%)	Adjusted Absorbance 48 Hours	Recovery (%)
1 / 7	0.478	92.8	0.497	96.5
2 / 8	0.496	96.3	0.498	96.7
3 / 9	0.508	98.7	0.506	98.3
4 / 10	0.488	94.8	0.492	95.5
5 / 11	0.500	97.1	0.500	97.1
6 / 12	0.484	94.0	0.491	95.4
AVG	0.492	95.6	0.497	96.6
SD	0.011	2.15	0.006	1.07
CV %	2.25 %	2.25 %	1.11 %	1.11 %

Note. Each treated GFF filter spiked with 59.36 µg bronopol; predicted absorbance for the derivatized bronopol is 0.515. Filter Numbers reflect the filter number extracted and analyzed on the first day and the filter number extracted and analyzed after 48 hours. The adjusted absorbance is calculated as the absorbance read from each sample minus the filter blank count of 0.015 absorbance units; Recovery is then the adjusted absorbance divided by the predicted absorbance expressed as percent.

The calculated percent difference in average absorbance values from the initial analysis to 48 hours later is:

$$\frac{|0.492 - 0.497|}{(0.492 + 0.497)/2} \cdot 100\ \% = 1.01\ \%$$

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Table H14

Medium Range Spiked Filter Storage Stability Q-Test For Outliers

	Ranked Recovery (%)	Gap
Medium Range Set First Day		
	92.8	
	94.0	1.2
	94.8	0.8
	96.3	1.5
	97.1	0.8
	98.7	1.6
Range	5.9	
	Calculated Q for lowest data point	0.458
	Critical Q Value	0.625
	Result	Accept Value
Medium Range Set After 48 Hours		
	95.4	
	95.5	0.1
	96.5	1
	96.7	0.2
	97.1	0.4
	98.3	1.2
Range	2.9	
	Calculated Q for lowest data point	0.207
	Critical Q Value	0.625
	Result	Accept Value

Table H15

Medium Range Spiked Filter Storage Stability F-Test For Variances

	First Day	After 48 Hours
Mean	95.6	96.6
Variance	4.68	1.16
Observations	6	6
df	5	5
F Calculated	4.03	
F Critical one-tail	5.05	
Result	Variance is consistent between data sets	

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Table H16

Spiked Filter Storage Stability Data and Calculations – High Range

Filter Numbers	Adjusted Absorbance First Day	Recovery (%)	Adjusted Absorbance 48 Hours	Recovery (%)
1 / 7	0.993	92.1	1.041	96.6
2 / 8	1.036	96.1	0.996	92.4
3 / 9	0.986	91.5	1.015	94.2
4 / 10	1.055	97.9	1.051	97.5
5 / 11	1.010	93.7	1.007	93.4
6 / 12	1.016	94.2	1.030	95.5
AVG	1.016	94.2	1.023	94.9
SD	0.026	2.41	0.021	1.95
CV %	2.56 %	2.56 %	2.05 %	2.05 %

Note. *Each treated GFF filter spiked with 124.08 µg bronopol; predicted absorbance for the derivatized bronopol is 1.078. Filter Numbers reflect the filter number extracted and analyzed on the first day and the filter number extracted and analyzed after 48 hours. The adjusted absorbance is calculated as the absorbance read from each sample minus the filter blank count of 0.015 absorbance units; Recovery is then the adjusted absorbance divided by the predicted absorbance expressed as percent.*

The calculated percent difference in average absorbance values from the initial analysis to 48 hours later is:

$$\frac{|1.016 - 1.023|}{(1.016 + 1.023)/2} \; 100\,\% = 0.69\,\%$$

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Table H17

High Range Spiked Filter Storage Stability Q-Test For Outliers

	Ranked Recovery (%)	Gap
High Range Set First Day		
	91.5	
	92.1	0.6
	93.7	1.6
	94.2	0.5
	96.1	1.9
	97.9	1.8
Range	6.4	
	Calculated Q for lowest data point	0.266
	Critical Q Value	0.625
	Result	Accept Value
High Range Set After 48 Hours		
	92.4	
	93.4	1
	94.2	0.8
	95.5	1.3
	96.6	1.1
	97.5	0.9
Range	5.1	
	Calculated Q for lowest data point	0.235
	Critical Q Value	0.625
	Result	Accept Value

Table H18

High Range Spiked Filter Storage Stability F-Test For Variances

	First Day	After 48 Hours
Mean	94.3	94.9
Variance	5.85	3.80
Observations	6	6
df	5	5
F Calculated	1.54	
F Critical one-tail	5.05	
Result	Variance is consistent between data sets	

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The calculated average change in absorbance values for all treated/spiked filters from the initial analysis to 48 hours later is:

$$\frac{0.48\ \% + 1.01\ \% + 0.69\ \%}{3} = 0.73\ \%$$

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Appendix I: Outline of Method Development

Analytical Method

- Review Sanyal et al. (1996) publication.
- Conduct spectrophotometric scan of non-derivatized bronopol in aqueous solution.
    - No derivatizing agent present.
    - Scan through entire working range of UV-VIS spectrophotometer.
    - Examine absorbance spectrum for peaks that may potentially interfere with absorbance maximum reported in Sanyal et al.
- Verify absorbance maximum of bronopol derivatized with sodium hydroxide.
    - Derivatizing sodium hydroxide agent present.
    - Scan through wavelength region of interest.
    - Examine spectrophotometric scan for absorbance maximum reported in Sanyal et al.
    - Examine spectrophotometric scan for peaks that may potentially interfere with absorbance maximum reported in Sanyal et al.
    - Manually scan a derivatized bronopol solution in the region of the absorbance maximum reported in Sanyal et al.
- Evaluate potential time dependence of chromophore development upon addition of sodium hydroxide to bronopol
    - Add derivatizing sodium hydroxide agent.
    - Manually scan the derivatized solution at 244 nm at specific time intervals for a period of one hour.
    - Evaluate absorbance data for potential time dependence of chromophore development.
- Evaluate potential sodium hydroxide concentration dependence on chromophore development.
    - Formulate solutions containing derivatizing sodium hydroxide agent in varying concentrations; include sodium hydroxide
    - Include sodium hydroxide concentration specified in Sanyal et al.
    - Evaluate absorbance data for potential sodium hydroxide concentration dependence.
- Develop calibration curve for derivatized bronopol.
    - Minimum calibration curve range initially includes range from Sanyal et al. publication.
    - Nine different concentrations of derivatized bronopol.
    - Include dilutions from different bronopol stock solutions to minimize effects from dilution errors.
    - Include calibration curve data from different time periods to minimize effects of instrument drift.

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Sampling Filters

- Review literature for filter types used for derivatizing air sampling methods.
- Develop filter treatment procedures.
    - Review literature for hydroscopic filter treatments.
    - Evaluate quantity of filter treatment solution needed to saturate filter to assure complete filter coverage.
    - Develop filter drying procedures.
- Develop filter spiking procedures.
    - Determine quantity of filter spiking solution needed to saturate approximately half of filter area.
- Conduct filter spiking trials.
    - Determine bronopol spike solution concentrations needed to span calibration curve range.
    - Spike treated filters while holding treated filter by edge; prohibit spike solution from contacting forceps.
    - Allow filter to dry completely prior to placement in extraction vessel.
    - Extract filters with 5 ml spectrophotometric grade water.
    - Sonicate filters for 5 minutes prior to removal of filter extract.
    - Evaluate efficiency of recovery of derivatized bronopol from spiked filters.
    - Evaluate consistency of recovery from spiked filters.

Sampling Chamber and Dust Generator

- Select dust generator and sampling chamber materials of construction.
- Measure internal dimensions of dust generator and sampling chamber.
    - Calculate nitrogen gas flow rate necessary to achieve appropriate particle size selection in dust generator.
    - Calculate nitrogen gas flow rate and flow duration needed to purge dust generator and sampling chamber of oxygen and water vapor.
- Conduct sampling of bronopol aerosols to verify even distribution of particulate in sampling chamber.
    - Sample using non-treated HVHP filters; extract filters in derivatizing solution.
    - Determine appropriate time to initiate sampling after activation of dust generator.
    - Conduct chamber particulate concentration distribution trials.
    - Conduct sampling at differing filter flow rates to vary bronopol loading on filters.
    - Evaluate filter-to-filter variation for each chamber run to assure even bronopol concentration in chamber during individual runs.

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Chamber Sampling

- Conduct chamber sampling using treated GFF
    - Chamber sampling using two treated GFF and one HVHP filters simultaneously.
    - Conduct sampling at differing filter flow rates to vary bronopol loading on filters.
    - Compare treated GFF results against concurrently sampled PVDF results to evaluate efficacy of derivatizing sampling process.
    - Evaluate filter-to-filter variation for each chamber run to assure even bronopol concentration in chamber during individual runs.

Sample Storage Stability

- Storage stability of filter extract solutions.
    - Spike treated filters with varying bronopol concentrations.
    - Follow procedures developed in filter spiking trials.
    - Extract and analyze spiked filters on the same day.
    - Store filter extract solutions and analyze after an appropriate storage period.
    - Evaluate efficiency of recovery of derivatized bronopol from spiked filters.
    - Evaluate consistency of recovery from spiked filters.
    - Compare filter recovery from day one versus after storage period.

- Storage stability of spiked filters.
    - Spike treated filters with varying bronopol concentrations.
    - Follow procedures developed in filter spiking trials.
    - Extract and analyze half of the spiked filters on the same day.
    - Store the second half of the spiked filters and extract and analyze after an appropriate storage period.
    - Evaluate efficiency of recovery of derivatized bronopol from spiked filters.
    - Evaluate consistency of recovery from spiked filters.
    - Compare filter recovery from day one versus after storage period.

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About the Author

John Smyth, Certified Industrial Hygienist (CIH), obtained an undergraduate degree in chemistry and mathematics from the University of Pittsburgh. He obtained his Master of Science in Public Health degree specializing in Industrial Hygiene and Safety Management from the University of South Florida.

He has over eighteen years of experience in management of environmental health and safety in a manufacturing facility, in a materials testing laboratory, and in consulting. He is a Diplomate of the Academy of Industrial Hygiene, a member of the American Chemical Society, and a member of the American Industrial Hygiene Association, National and Florida Sections. He is a past President of the Florida Section of the AIHA. He has served on the Suncoast Safety Council (former National Safety Council chapter) Board of Directors, including a term as Vice-President, and on the Florida Region 8 Local Emergency Planning Committee.

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