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The Effect of Fungal Growth on Potential Phthalate Ester Exposures from Plasticized Polyvinyl Chloride

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by

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DEDICATION

This work is dedicated to my wife Teresa and my son William. This work would not have been possible without their love, encouragement, support, and inspiration.

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ABBREVIATIONS

***A. versicolor*:**	Aspergillus versicolor
ACGIH:	American Conference of Governmental Industrial Hygienists
ATSDR:	Agency for Toxic Substances and Disease Registry
BBP:	Butyl benzyl phthalate
°C:	Degrees centigrade
CCV:	Continuing calibration verification
CERHR:	Center for the Evaluation of Risks to Human Reproduction
cm:	Centimeter(s)
cm^2:	Square centimeters
%D:	Percent difference
DBP:	Dibutyl phthalate
DCM:	Dichloromethane
DEHP:	di-(2-ethylhexyl) phthalate
DEP:	Diethyl phthalate
DFTPP:	Decafluorotriphenylphospine
DNA:	Deoxyribonucleic acid
EPA:	Environmental Protection Agency
FSH:	Follicle stimulating hormone
ft:	Feet
fT:	Free testosterone
GC/MS:	Gas chromatograph/mass spectrometer

HVAC:	Heating, ventilation, and air conditioning
IARC:	International Agency for Research on Cancer
IPCS:	International Programme on Chemical Safety
Kg:	Kilogram(s)
LH:	Luteinizing hormone
m:	Meter(s)
m^2:	Square meters
m^3:	Cubic meters
MBP:	Monobutyl phthalate
MBzP:	Monobenzyl phthalate
MDL:	Method detection limit
MeHP:	Monoethylhexyl phthalate
MEP:	Monoethyl phthalate
mL:	Milliliter(s)
ND:	Non-detect
ng:	Nanogram(s)
NIOSH:	National Institute for Occupational Safety and Health
NTP:	National Toxicology Program
OSHA:	Occupational Safety and Health Administration
***P. lilacinum*:**	Penicillium lilacinum
PEL:	Permissible exposure limit
pPVC:	Plasticized polyvinyl chloride
PVC:	Polyvinyl chloride
REL:	Recommended exposure limit
RF:	Relative response factor

RSD:	Relative standard deviation
SD:	Standard deviation
SHBG:	Sex-hormone binding globulin
sp.:	Species
SVOC:	Semi-volatile organic compound
TLV®:	Threshold limit value
µg:	Microgram(s)
µL:	Microliter(s)
VOC:	Volatile organic compound

ABSTRACT

This study examined the effects of the presence of fungal growth on the phthalate ester content of plasticized polyvinyl chloride (pPVC) used as the vapor barrier component of metal building insulation and the impact the fungal growth may have on the resulting exposure potential to the workers in the building. Individual pieces of the pPVC material exhibiting fungal growth and individual pieces of the pPVC material exhibiting no fungal growth were collected from the building being investigated. Twenty-five pieces each of the pPVC material exhibiting fungal growth and of the pPVC material exhibiting no fungal growth were weighed, reduced to small pieces, and extracted with dichloromethane. The extracts were analyzed using a gas chromatograph coupled with a mass spectrometer. The phthalate content of four phthalate esters identified in the pPVC: diethyl phthalate (DEP), dibutyl phthalate (DBP), butyl benzyl phthalate (BBP), and di-(2-ethylhexyl) phthalate (DEHP), was quantified and the results extrapolated to concentration based on weight (µg/Kg) and area (µg/m^2). A paired samples *t* test and independent samples *t* test was used to identify where significant differences occurred ($\alpha = 0.05$) in the phthalate content between pieces. Using the mean concentration for each phthalate ester, a worst-case-scenario exposure was modeled. There was a statistically significant difference in the phthalate ester content between the pPVC exhibiting fungal growth and pPVC exhibiting no fungal growth for all four phthalate esters investigated. The mean phthalate ester concentration ranged from 13.74 µg/m^2-34.94 µg/m^2 in the pPVC exhibiting no fungal growth and 12.00 µg/m^2-30.63 µg/m^2 in the pPVC exhibiting fungal growth. The modeled exposure

concentration in the building ranged from 0.39 µg/m^3-0.98 µg/m^3 and was generally lower than published exposure concentrations in similar settings.

INTRODUCTION

Background

Phthalate esters are one of many organic compounds that can be used as a plasticizing agent. Phthalates are a class of organic compounds that includes numerous phthalate ester derivatives. As a class, they have high molecular weights, high boiling points, and low vapor pressures. These characteristics make them ideal for use as plasticizers as their stability allows them to be retained in the base material rather than be subject to evaporative losses (Rider & Sumner, 1945). Phthalate esters are manufactured by combining phthalic anhydride with an alcohol. The alcohol is dependent on the phthalate ester being produced (Graham, 1973). Phthalate esters are used as plasticizing agents to make the base material, in this study polyvinyl chloride (PVC), more pliable, flexible, and easier to work with (Cadogan & Howick, 2000). The resultant material is termed plasticized polyvinyl chloride (pPVC).

Plasticizers are ubiquitous in the indoor environment. Much research has been conducted on phthalate emissions from PVC flooring (Fujii et al., 2003), plastics, wall coverings (Uhde et al., 2001), and other types of sources as well as their related health effects (Okamoto et al., 2011) but extensive literature search has not identified any investigation of the interaction between fungal growth and pPVC used for the fronting of metal building insulation nor any evaluation of phthalate ester emissions that may result from this interaction.

Due to their low volatility, exposures to phthalate esters in the workplace are generally associated with sub-acute and chronic health effects rather than acute health effects (Autian,

1973). Within the past decade, more attention has been focused on the investigation into the endocrine-disrupting potential of phthalate esters. Investigations have mainly elucidated the effects on the development of the reproductive organs in infant males as well as the effects on adult males as those are the systems that appear to be the primary targets. Research has found that urinary levels of the metabolites of diethyl phthalate and di-(2-ethylhexyl) phthalate are associated with human sperm damage (Hauser et al., 2007). Prenatal exposures to phthalates esters have been shown to be associated with reduced penile width and incomplete testicular descent (Swan, 2008). Additionally, exposures to phthalate ester have been positively associated with lower gestational ages (Latini et al., 2003). However, exposures to phthalate esters have also been positively associated with thelarche (premature breast tissue development) in pre-pubescent girls (Colón et al., 2000).

Investigation Site

The Florida Department of Education Division of Blind Services' Talking Books Library is located in Daytona Beach, Florida. The facility houses the Division's library of audio and braille books. The facility consists of an administrative building containing offices, conference rooms, kitchen, etc. and four annexes each built in different years (in parentheses): Elizabeth Cope annex (1973 & 1978), braille annex (1981), cassette inspection annex (1993), and the broken tape annex (1997). See Figure 1.

The Braille annex and Cope annex were the primary focus of the investigation. The Braille annex comprises the northern side of the facility and runs lengthwise generally east-to-west. The Cope annex is attached to the eastern side of the administrative building and runs lengthwise generally north-to-south. The two annexes meet at the northwest corner of the

facility. The annexes are warehouse-type buildings and primarily contain rows and rows of metal shelving and motorized book cases to store the Division's catalog of materials.

Figure 1: Talking books library facility layout (Source: http://www.google.com)

The annexes are typical metal building structures; that is, they are conditioned, large, open buildings that are essentially a sheet metal shell supported by metal supports and trusses. The insulation used in these types of buildings is termed metal building insulation and consists of the fiberglass insulation material and a fronting material that faces the inside of the building. The fronting material is normally composed of either pPVC, polypropylene, or polyester and is typically white for aesthetic purposes but can be manufactured in a color of the customer's preference. When installed, the contractor can either purchase the metal building insulation already constructed as a single unit but more typically the contractor purchases the fronting material and insulation material separately and laminates the fronting material onto the insulation on site using a special machine. Several contiguous meters of material are installed as a single

piece, typically in the same direction as the long length of the building. The insulation is secured using the trusses of the building as the main support. Though there is some office space with drop-down tile ceilings, most of the annexes are open spaces where the metal building insulation is exposed.

Dr. David Krause was contacted by the facility's administrator to conduct a facility assessment due to complaints from the facility's occupants of symptoms of nausea, dizziness, sore throat, etc. Dr. Krause found several areas around the facility that showed significant fungal growth on the pPVC fronting of the metal building insulation. The fungal growth primarily occurred in areas around roof hatches designed to be opened in case of fire to allow smoke to escape the building, along the roof peaks, and along the edges of the ceiling where the ceiling meets the wall. Figures 2 and 3 illustrate the pPVC material with and without fungal growth, respectively. Evidence of water intrusion was visible around these hatches and in other areas of the facility. Initial microscopic inspection of the fungal growth indicated the primary fungal species present to be *Cladosporium cladosporiodes*.

Preliminary Investigation

Two approximately 71.1 cm X 71.1 cm sections of the metal building insulation, one section that exhibited fungal growth and one section that did not exhibit fungal growth, were collected for the purpose of conducting a preliminary screening of the pPVC fronting material by analyzing the material for the presence of metals, volatile organic compounds (VOCs), and semi-volatile organic compounds (SVOCs) and comparing the differences in results between the two section of pPVC with the intention of elucidating a potential path to pursue a more detailed investigation of the interaction between the fungal growth and the pPVC material. Both sample sections were taken from the northeastern corner of the braille annex.

Figure 2: pPVC material affected by fungal growth

Figure 3: pPVC material not affected by fungal growth

The section exhibiting fungal growth was collected from an area adjacent to a roof hatch and the section exhibiting no fungal growth was collected from the same run of material from an area adjacent to the fungal growth but separated by a metal truss. The section that exhibited fungal

growth was gray-brown in color and had obvious fungal colonization. This section also appeared to be more brittle and less pliable than the other section. The section that did not exhibit fungal growth was white in color and appeared intact.

Prior to analysis, the pPVC sheets were separated from the fiberglass material and a razor blade used to remove as much of the fiberglass material and glue still adhered to the pPVC as possible. To achieve randomization of the aliquots taken for analysis, the sections of pPVC were cut into small pieces of approximately 0.5 cm X 0.5 cm and the pieces composited. Each section was analyzed in duplicate for a total of four samples. The analytical results were then compared between the duplicates for analytical precision and between the mean analytical results of the section exhibiting fungal growth and the section exhibiting no fungal growth for differences between the two sections. The analytical results for the SVOC analyses indicated the presence of numerous compounds used in the manufacture of the pPVC material. These compounds included the plasticizing agents diethyl phthalate (DEP), dibutyl phthalate (DBP), butyl benzyl phthalate (BBP), di-(2-ethylhexyl) phthalate (DEHP), nonanoic acid, and Octicizer; a heat stabilizer, 2-ethylhexanoic acid; a fungicidal agent, o-hydroxybiphenyl; a waterproofing agent, palmitic acid; a lubricating/softening agent, stearic acid; an antioxidant, bisphenol A; and a fire retardant, triphenyl phosphate. The analytical results showed a difference of 24.6%-49.5% in the phthalate ester concentration between the two sections, with all phthalate ester concentrations being lower in the section exhibiting fungal growth.

Purpose

The purpose of this study is to determine if the presence of fungal growth on the pPVC used as the fronting material of metal building insulation significantly affects the phthalate ester content of the pPVC and, if there is a significant decrease in the phthalate ester content, how the

liberated phthalate esters affect the potential for airborne exposure and if the potential airborne exposure is comparable to exposures typically experienced in similar settings.

Chamber Sampling and Evaporation Study

It was intended that chamber sampling be used to collect phthalate ester emissions from the pPVC material. Two chambers were constructed using new metal paint cans purchased from a building supply retailer and fitting stainless steel inlet and outlet valves into the lid of the paint can. To test the ability to recover phthalate esters from the chamber, a known amount of the four phthalate esters of interest was added to an aluminum weigh boat placed at the bottom of the chamber. Air was pulled through the chamber for 18.5 hours and the exhaust air collected on a SKC Anasorb® charcoal sampling tube. The contents of the tube were desorbed using pesticide grade toluene and sonication. The toluene extract was analyzed using a gas chromatograph coupled with an mass spectrometer (GC/MS). The extract contained nearly none of the phthalate ester added to the chamber. The lone exception was a very low amount of DEP. The residue remaining in the aluminum weigh boat was reconstituted with toluene and analyzed by GC/MS and found to contain nearly the same amount of phthalate esters added before the sampling period started.

It did not appear that the low vapor pressures and corresponding evaporation rates of the phthalate esters would make it possible to conduct chamber sampling. To confirm this, an evaporation study was conducted to determine the evaporation rates of the four phthalate esters of interest. A known amount of the four phthalate esters was added to a pre-cleaned, pre-weighed borosilicate watch glass. The watch glass was weighed using an analytical balance over the course of a period of 30 days. The remaining residue was reconstituted with high-purity grade dichloromethane (DCM) and analyzed by GC/MS. The initial concentration was

compared to the final concentration to determine the evaporation rates of the 4 phthalate esters. All four had very slow evaporation rates.

In light of the chamber study and evaporation test results, it was determined that chamber testing of phthalate ester emissions from the pPVC would not be feasible. The decision was made to analyze the pPVC material for phthalate content and compare the results between the pPVC affected by fungal growth and pPVC not affected by fungal growth. These results were then used to model potential exposure concentrations and compare those concentrations to published concentrations from similar settings.

LITERATURE REVIEW

Polyvinyl Chloride

Polyvinyl chloride (PVC) is a polymer that is used in a wide variety of applications. PVC is versatile, durable, corrosion-resistant, easy to manufacture, and is less costly to produce. These qualities make PVC ideal for use in the production of construction materials such as vinyl flooring, window casings, plastic-wrapped wiring, and irrigation piping; automotive parts such as body moldings, audio and video components, and lighting components; medical supplies such as blood bags and tubing, drip chambers, goggles, and inhalation masks; containers; packaging materials such as shrink wrap, blister packaging and can coatings; and consumer products such as blinds and shades, table cloths, apparel, and toys (Whitfield and Associates, 2008).

In 2012, 15.3 billion pounds of PVC was manufactured in the United States (American Chemistry Council, 2013). PVC is manufactured by first producing the vinyl chloride monomer through an oxychlorination process of combining salt, which provides the chloride, and ethylene, which provides the organic base, and polymerizing the vinyl chloride monomer through one of a variety of industrial processes. This process results in a white powder resin, sometimes referred to as feedstock, to which one or more numerous additives can be added to provide stability, softness, color, lubrication, heat resistance, and flexibility (PVCplus, 2012).

The production and use of PVC has come under scrutiny and is the subject of major initiatives to limit or more closely monitor its production, use, and disposal. Beginning with the manufacturing of PVC, highly toxic dioxins and furans are by-products of the manufacturing

process. These chemicals are known to be extremely hazardous to human health. Concerns have arisen about the amount of consumption of PVC and its resultant disposition after use and disposal. Disposed PVC is subject to photodegradation, biodegradation, and leaching and the components of PVC, namely the additives, are potentially released into the environment and present the opportunity for human and animal exposure. Among the additives, phthalate esters are a focus of concern as there is still no consensus as to the health effects related to exposures.

Plasticized polyvinyl chloride (pPVC) is PVC to which a plasticizing agent has been added. Plasticizers are added to PVC to make the base material more flexible and more amenable to processing and formation (Cadogan & Howick, 2000). Plasticizers are not chemically bound to the base material but rather interact and remain attracted to the base material through Van der Waals forces (Liebhafsky et al., 1942). Plasticizers work by essentially filling in the gaps between PVC chains and preventing the PVC chains from forming bonds. This has the effect of preventing the PVC chains from cross-linking and becoming larger, more rigid chains. To be an effective plasticizer, the chemical chosen should be of low volatility and reactivity so as to remain in the base material and not chemically alter the material (Rider & Sumner, 1945). Low volatility is an important attribute of a plasticizer as loss of plasticizer affects the durability and service life of the material. Low reactivity of a plasticizer is desirable so as to not alter the structure or mechanical and chemical properties of the base material. A wide variety of plasticizers are available for use including cyclohexanoic diesters, phosphates, and adipates (Fink, 2010). The most commonly used plasticizers are phthalate esters.

Phthalate Ester Plasticizers

Phthalate esters are a class of organic compounds manufactured from the combination of phthalic acid and an appropriate alcohol. Phthalate esters structurally consist of a benzene ring

with carboxylic acid chains in the 1 and 2 positions of the benzene ring. When added to PVC for use as a plasticizer, the polar groups of the carboxylic acid chains interact with the positively charged areas of the PVC molecules. This polar interaction keeps the adjacent PVC chains separated. Phthalate esters are ideal for use as plasticizers because they have very high boiling points, very low vapor pressures, and are non-reactive with PVC.

Of the numerous phthalate esters used as plasticizers, four are of interest in this project: diethyl phthalate (DEP), dibutyl phthalate (DBP), butyl benzyl phthalate (BBP), di-(2-ethylhexyl) phthalate (DEHP).

Diethyl phthalate

DEP (CAS # 84-66-2) is colorless and is a liquid at room temperature. DEP is one of the more volatile phthalate esters. It has a relatively high vapor pressure of $2x10^{-3}$ mmHg. It has a boiling point of 295 °C and a molecular weight of 222.3 g/mol (NIOSH, 2007). DEP has a NIOSH recommended exposure limit (REL) and ACGIH threshold limit value (TLV®) of 5 mg/m^3 (AGGIH, 2012; NIOSH, 2007) and has no OSHA permissible exposure limit (PEL). DEP is manufactured by combining phthalic anhydride with ethanol in the presence of sulfuric acid (ATSDR, 1995). DEP may be introduced into the environment through evaporation, leaching into water, burning of plastic materials, from consumer products, and leaking landfills (ATSDR, 1995). Exposure to DEP typically occurs through inhalation or ingestion of foods or water contaminated with DEP but can also be absorbed through dermal contact (ATSDR, 1995). DEP is metabolized into its monoester form, monoethyl phthalate (MEP) (ATSDR, 1995). Occupational indoor airborne concentrations of DEP have been reported at levels of 1.60 to 2.03 $\mu g/m^3$ (Shields & Weschler, 1987) in a telephone switching office. Non-occupational indoor

airborne concentration of DEP have been reported at levels of 0.13 to 4.3 µg/m^3 (Rudel et al., 2003), 0.01 to 0.61 µg/m^3 (Otake et al., 2004), 0.05 to 0.19 µg/m^3 (Otake et al., 2001).

Dibutyl phthalate

DBP (CAS # 84-74-2) is slightly yellowish and is a liquid at room temperature. It has a vapor pressure of 7x10^{-5} mmHg, a boiling point of 340 °C, and a molecular weight of 278.3 g/mol (NIOSH, 2007). DBP has a PEL, REL, and TLV® of 5 mg/m^3 (AGGIH, 2012; NIOSH, 2007). DBP is manufactured by reacting phthalic anhydride with n-butyl alcohol in the presence of sulfuric acid or p-toluene sulfonic acid (ATSDR, 2001). DBP may be introduced into the environment through evaporation, leaching into water, and from consumer products (ATSDR, 2001). Exposure to DBP primarily occurs through inhalation though exposure may also occur through ingestion of foods or water contaminated with DBP (ATSDR, 2001). DBP is metabolized into its monoester form, monobutyl phthalate (MBP) (ATSDR, 2001). Background concentrations of DBP are often measured between 0.03-0.06 ppb in cities (ATSDR, 2001). Occupational indoor airborne concentrations of DBP have been reported at levels of 1.7-40 mg/m^3 in an artificial leather and PVC film manufacturer (Milkov et al., 1973) and 2.81 µg/m^3 in an office setting (Rudel et al., 2001) but typically DBP is not detected above reporting limits of 0.02 mg/m^3 (IPCS, 1997). Non-occupational indoor airborne concentrations of DBP have been reported at levels of 0.052 to 1.1 µg/m^3 (Rudel et al., 2003), 0.01 to 6.18 µg/m^3 (Otake et al., 2004), and 0.11 to 0.60 µg/m^3 (Otake et al., 2001).

Butyl benzyl phthalate

BBP (CAS # 85-68-7) is slightly yellowish and is a liquid at room temperature. It has a vapor pressure of 6x10^{-7} mmHg, a boiling point of 370 °C, and a molecular weight of 312.4 g/mol (NTP-CERHR, 2003). BBP has no published occupational exposure limit criteria. BBP is

manufactured by combining phthalic anhydride sequentially with n-butyl alcohol and benzyl chloride (NTP-CERHR, 2003). Exposure to BBP occurs almost exclusively through ingestion of foods or water contaminated with BBP (NTP-CERHR, 2003). BBP is metabolized into its monoester form, monobenzyl phthalate (MBzP) (NTP-CERHR, 2003). The International Programme on Chemical Safety (IPCS) considers exposure to airborne BBP to be negligible (IPCS, 1999). Occupational indoor airborne concentrations of BBP have been reported at levels of 0.001 to 0.020 $\mu g/m^3$ in offices (Weschler, 1984). Non-occupational indoor airborne concentrations of BBP have been reported at levels of 0.034 to 0.035 $\mu g/m^3$ (IPCS, 1999), non-detect (ND) to 0.48 $\mu g/m^3$ (Rudel et al., 2003), ND to 0.11 $\mu g/m^3$ (Otake et al., 2004), and ND to 0.10 $\mu g/m^3$ (Otake et al., 2001).

di-(2-Ethylhexyl) phthalate

DEHP (CAS # 117-81-7) is colorless and is a liquid at room temperature. It has a vapor pressure of $1x10^{-7}$ mmHg, a boiling point of 384 °C, and a molecular weight of 390.6 g/mol (ATSDR, 2002). DEHP has a PEL, REL, and TLV® of 5 mg/m^3 (AGGIH, 2012; NIOSH, 2007). DEHP is manufactured by combining phthalic anhydride with 2-ethylhexyl alcohol using an acid as a catalyst (ATSDR, 2002). DEHP accounts for 47% of the phthalate ester used in manufacturing (Cadogan & Howick, 2000). DEHP is considered to have the highest potential to be a health hazard of the phthalate esters investigated in this study and is the subject of numerous research projects attempting to elucidate the effects exposure to DEHP has on human health. Exposure to DEHP occurs primarily through the oral route (ATSDR, 2002). DEHP is primarily metabolized into its monoester form, monoethylhexyl phthalate (MEHP) (ATSDR, 2002). Occupational indoor airborne concentrations of DEHP have been reported at levels 11.5 $\mu g/m^3$ in a plastics melting facility (Rudel et al., 2001), 48 to 1214 $\mu g/m^3$ in a boot factory (Dirven et al.,

1993), 9 to 1266 µg/m^3 in a cable factory (Dirven et al., 1993), and 0.02 to 0.114 µg/m^3 (Rudel et al., 2001) and ND to 0.2 µg/m^3 (Toda et al., 2004) in an office setting. Non-occupational indoor airborne concentrations of DEHP have been reported at levels of ND to 1.0 µg/m^3 (Rudel et al., 2003), ND to 3.13 µg/m^3 (Otake et al., 2004), and 0.04 to 0.23 µg/m^3 (Otake et al., 2001).

Health Effects Related to Exposures to Phthalate Esters

Data is still being compiled on the health effects exposure to phthalate esters present to humans and most studies have focused on the potential of phthalate esters to be endocrine disruptors. To this point most information published has involved animal studies conducted with rats, mice, and rabbits. The endpoints of the animal studies are based on exposures to levels of phthalate esters that are much higher than those expected to be experienced by humans but are able to provide direction to researchers in regards to areas that can be focal points of human-related research studies. Epidemiological studies are beginning to elucidate human health effects. One limitation with published epidemiological studies is that most studies conducted only confirm their subjects have been exposed to phthalate esters by assessing the metabolic byproducts of exposure but do not attempt to correlate the concentrations to which the subjects were exposed nor how the exposures occurred. Another is that little is known about the interaction of phthalates when they exist in mixtures either combined with other phthalates or with other chemicals. Published research focuses on the health effects related to exposure to a single phthalate ester of interest even though real world exposures are concurrent with not only other phthalate esters but other potentially injurious chemicals as well (Lyche et al., 2009).

Because of the paucity of information, no consensus has been reached on the health effects related to phthalate ester exposure. The emergence of phthalate ester-related health effects seems to be dependent on not only the exposure route, but also the phthalate ester to

which a person is exposed. In this study, the primary routes of exposure are inhalation of aerosolized phthalate esters and inhalation or ingestion of dust particles on to which the phthalate esters have condensed.

Metabolism of Phthalate Esters

The pathways used by the human body to metabolize are well known. The biological half-life of phthalate esters ranges from several hours to approximately two days (ATSDR, 1995; Koch et al., 2005). In general, the lower molecular weight phthalate esters (i.e. DEP and DBP) are metabolized rapidly and have less of an effect on human health than do the higher molecular weight phthalate esters (i.e. BBP and DEHP) that have a multi-step metabolism pathway (Hauser & Calafat, 2005). Low molecular weight phthalate esters are metabolized by hydrolysis of an ester bond which converts the phthalate ester into its monoester form (Albro & Moore, 1974). Higher molecular weight phthalate esters undergo a multistep biotransformation. The first step is similar to the metabolism of the lower molecular weight phthalate esters in that high molecular weight phthalate esters are first converted into their monoester forms. After conversion, these monoesters are then enzymatically oxidized to a more hydrophilic oxidative metabolite (Albro & Moore, 1974). Once a phthalate ester has been metabolized, the metabolic byproducts can be detected in urine, breast milk, blood serum, feces, and saliva (Hauser & Calafat, 2005). The biotransformed byproducts are eliminated primarily through urine excretion but can also be eliminated through fecal excretion (Peck & Albro, 1982).

Fetal Developmental Effects

Most information on health effects related to phthalate ester exposure has been generated from animal studies and results from epidemiological studies have been rather inconclusive. Vrijheid and associates conducted a retrospective cohort study in an attempt to determine the

association between occupational exposures of pregnant women to phthalate esters and hypospadias (narrowing and/or incorrect placement of the urethra in newborn males) (Vrijheid et al., 2003). Their research found only a slight increase during a specific time period in the odds ratio between a job classified as having a "probable" exposure to phthalate esters and a job classified as "unlikely" to have exposures to phthalate esters. The authors concluded that overall there did not appear to be any significant correlation between exposure to phthalate esters and hypospadias but cautioned that the results are based on crude classifications of job exposures.

Swan and associates investigated the relationship of maternal phthalate ester exposure to reduced anogenital distance in male newborns (Swan et al., 2005). The authors were able to determine that there is a significant relationship between the presence of MEP, MBP, and MBzP and decreased anogenital distance. The presence of urinary metabolites indicates that an exposure to phthalate esters has occurred but does not offer any information regarding exposure level, setting, or route. The authors did not find a significant correlation between the presence of MEHP and decreased anogenital distance. The results of this study are noteworthy in that they are consistent with results obtained through animal studies.

Main and associates investigated perinatal exposures to phthalate esters through the ingestion of breast milk and the effects on the reproductive hormones of infant males (Main et al., 2006). The authors were able to find that the presence of MEP, MBP, and MBzP in breast milk was positively correlated with sex-hormone binding globulin (SHBG), luteinizing hormone (LH), and LH:free testosterone ratio. These hormones are involved with male sexual organ development and can have anti-androgenic effects. As with the Swan study, these results are consistent with those obtained from animal studies conducted with rats.

Reproductive Health Effects

Of the studies conducted attempting to discover the potential health effects of phthalate esters on the human reproductive system, most have been focused on the male reproductive system. Limited information has been generated in regards to the effects of phthalate ester exposure on the reproductive effects on women.

Duty and associates researched the effects of phthalate exposure on reproductive hormone levels in adult males (Duty et al., 2005). The authors were able to significantly associate a decrease in follicle-stimulating hormone (FSH) concentration with the presence of MBzP in urine. FSH is involved with spermatogenesis and low levels of FSH typically leads to low sperm count but can also be associated with hypogonadism (failure of gonadal function) (WebMD, 2011). The authors were also able to identify a relationship between the presence of MBP and an increase in inhibin B, though the authors noted the significance was marginal. Inhibin B is involved with regulating production of FSH (van Zonneveld et al., 2003).

Pan and associates conducted a cross-sectional study to investigate the relationship between occupational exposure to phthalate esters and the levels of free testosterone (fT) in the blood serum of male workers employed at a PVC flooring manufacturing facility in China (Pan et al., 2006). The authors were able to determine that the presence of MBP and MEHP were significantly associated with lower fT levels though the authors noted the association was only modest. The authors noted that the urinary levels of MBP and MEHP were 5-100 times greater in the group of exposed workers when compared to the control group of unexposed workers. Low fT levels are associated with hypogonadism and non-clinical effects such as erectile dysfunction and decreased libido (Pantalone K. M., Faiman C., 2012).

In another study conducted by Duty and associates, the authors were able to show a relationship between the presence of MEP and damage to human sperm cells (Duty et al., 2003b). The authors used the Comet assay technique to measure a variety of parameters related to sperm cell health and viability. The authors found a significant relationship between increasing concentration of urinary MEP and decreasing comet extent. Comet extent is the total length of the comet from the beginning of the head to the visible end of the tail and is used as an indicator of DNA damage. Shorter comets indicate more DNA damage than longer comets (Collins, 2004).

Jönsson and associates evaluated the urinary levels of MEP, MBP, MBzP, and MEHP and their effects on the reproductive hormones of Swedish military conscripts (Jönsson et al., 2005). The authors were able to associate the presence of MEP with increased testis volume, lower motility, and lower LH values but were not able to associate the metabolites of the other three phthalate esters with any health effects. The urinary levels of the metabolites detected were consistent with a study conducted by Blount and associates (Blount et al., 2000). However, these results are contradictory to another study conducted by Duty and associates who were able to find significant associations between the presence of MBP and MBzP and low sperm concentration and motility but not MEP (Duty et al., 2003a). And in contrast to the Duty study conducted in 2003, the authors were not able to correlate the presence of MEP with a significant effect on sperm integrity.

Latini and associates investigated the correlation between the presence of DEHP and its metabolite MEHP in the cord blood of newborn infants in Italy (Latini et al., 2003). The authors found a significant association between the presence of the MEHP in the cord blood of infants and a shorter gestational age at time of delivery and suggest that exposure to phthalate esters *in*

utero can lead to shorter pregnancies by inducing an inflammatory response, which is a risk factor for premature delivery. The authors found that newborns with MEHP detected in their cord blood had a mean gestational age of 38.16 weeks compared to 39.35 weeks for infants with no MEHP detected in their cord blood. The authors did not find a statistically significant correlation between the presence of DEHP and/or MEHP and birth weight. The authors noted that their study was limited by the low sensitivity and selectivity of the method used to detect DEHP and MEHP, whether DEHP was introduced via medical equipment during the delivery process, or whether the delivery occurred without complications.

Other Health Effects

While most scientific research has been focused on the endocrine disruption possibilities of phthalate esters, other studies have been conducted in regards to other health effects. The presence of MBP and MEP in urine has been associated with reduced pulmonary function (Hoppin et al., 2004).

Most studies on the carcinogenicity of phthalate esters have been conducted on lab animals and their extrapolation to human relevance is suspect. DEHP has been shown to be hepatocarcinogenic to lab rats though the pathway through which the cancer is developed is considered non-operative in humans (ATSDR, 2002). DEHP is classified as a probable human carcinogen by the EPA (ATSDR, 2002). In 2000 the IARC reclassified DEHP from "possible carcinogenic to humans" to "not classificable as to carcinogenicity in humans" (IARC, 2000). No data has been published to date regarding the carcinogenicity of DBP to humans or animals (ATSDR, 2001). DEP is not known to cause cancer in animals or humans and the EPA lists DEP as a Group D chemical (not classifiable as to its carcinogenicity) (ATSDR, 1995). There is

limited evidence that BBP is associated with increased multiple myelomas (NTP-CERHR, 2003).

Natural and Fungal Effects on pPVC

The loss of phthalate ester plasticizers will lead to the deterioration and failure of the pPVC material/product. Phthalate ester plasticizers will naturally be lost over long periods of time from the pPVC material in a process that is controlled by two mechanisms: the migration of the plasticizer to the surface of the material and the evaporation of the plasticizer into the atmosphere (Kovačić & Mrklić, 2002). Migration to the surface is controlled by Fick's law of diffusion and evaporation is dependent on the vapor pressure of the phthalate ester as well as ambient atmospheric conditions (Kovačić & Mrklić, 2002). Other factors contributing to natural phthalate ester loss are ambient temperature (Clausen et al., 2012), surface air flow (Shashoua, 2003), and removal via friction (i.e. wiping). Phthalate ester loss is not affected by the relative humidity of the surrounding atmosphere (Ekelund et al., 2010). Of the two loss mechanisms, the migration of the plasticizers through the PVC to the surface is considered to be much less of a significant contributor to plasticizer loss when compared to evaporation (Quackenbos, 1954). There is also a point to where plasticizer loss ceases due to the interaction of the PVC molecules with each other. As plasticizer is lost, the Van der Waals forces between the PVC chains pull the chains closer together and prevent migration of the plasticizer to the material surface (Liebhafsky et al., 1942).

Fungal colonization on the surface of the pPVC material will enhance deterioration (Gumargalieva et al., 1999). Materials that contain additives such as plasticizers are more easily colonized by fungal species as the plasticizers can serve as nutritional sources for the fungal species (Lugauskas, 2003). Other factors that affect biodegradation are environmental

conditions such as light, temperature, moisture, and the presence of other nutrients; the characteristics of the material being degraded such as surface area, molecular weight, and interaction with components; and the activity of the fungal intruder including the levels of spores, ability to produce degradation enzymes, and the nature of the fungal species (Lugauskas, 2003).

When subject to fungal attack, it is not the PVC polymer base material itself that is consumed but rather the plasticizer, biocidal, and flame retardant constituents added to the PVC. PVC has been shown to be unaffected when colonized by fungal species, including *Cladosporium* sp. (Roberts & Davidson, 1986). The presence of the additives enhances the susceptibility of the pPVC material to fungal invasion (Webb et al., 2000). When the pPVC material is first inoculated, it is thought that surface-bound pPVC constituents aid in retaining and supplying nutrients for the inoculum. Once the fungal colony has been established, the mechanism by which the fungal colony remains adhered to the pPVC has not been determined (Peciulyte, 2002). The fungus produces extracellular esterases that degrade the phthalate esters into forms that are more suitable for uptake (Webb et al., 2000). Removal of the phthalate ester plasticizers from the pPVC material causes the pPVC to become more brittle, results in weight loss, and loss of functionality as a vapor barrier (Flemming, 1998).

The fungal species present on the material is in large part determinative of how and if the pPVC is degraded. Some fungal species are able to utilize phthalate ester plasticizers as sources of carbon for nutrition while others cannot. Berk et al. showed that various species of *Aspergillus* and *Penicillium* could not use DBP as a carbon source (Berk et al., 1957). Stahl also showed that *A. versicolor* could not use DEHP as a carbon source (Stahl & Pessen, 1953). Engelhardt et al. showed that *P. lilacinum* was able to use DBP and DEHP as a carbon source

(Engelhardt et al., 1975). Nakamiya et al. and Webb et al. demonstrated that *Cladosporium herbarium* is able to utilize DEHP (Nakamiya et al., 2005; Webb et al., 2000). Roberts et al. were also able to show that *Cladosporium* sp. are able to utilize DEHP (Roberts & Davidson, 1986).

METHODS

Sample Collection

Samples of the pPVC material were collected from two of the building annexes. Prior to arriving on site to perform sample collection, 100 pieces of aluminum foil were cut to approximately 930 cm^2. The pieces were cleaned by submerging in high-purity grade dichloromethane (DCM) to remove manufacturing oils used as lubricants and then sterilized by wiping with a paper towel soaked in 70% isopropyl alcohol. The pieces were wrapped in larger lengths of aluminum foil that had been cleaned and sterilized in the same manner.

To collect samples, two 232 cm^2 ceramic tiles were purchased from a retail building supply store to be used as templates for removing sections of the insulation from the building. Both were sterilized with 70% isopropyl alcohol prior to use and one tile was marked "M" for use in collecting insulation that exhibited fungal growth and the other tile marked "C" for use in collecting insulation that did not exhibit fungal growth. Samples were removed from the ceiling by placing the tile template against the material and cutting around the edges of the tile template with a knife that had been sterilized by wiping with 70% isopropyl alcohol. Once the sample was removed from the ceiling, it was wrapped in one of the pieces of aluminum foil that had been previously prepared. The sample was labeled with the appropriate number and stored in a large tote bin.

Fifty samples each of pPVC that exhibited fungal growth and pPVC that did not exhibit fungal growth were collected from two of the annexes: the Cope annex and the Braille annex.

The other two annexes had already been remediated so no material was available for collection. Areas selected for sample collection were based on the presence, or lack thereof, of visible fungal growth fields. Of the 50 pairs of samples taken, 37 were collected from the Braille annex and 13 collected from the Cope annex. The reduced number of samples collected from the Cope annex was due to the lesser amount of fungal intrusion. Less total area in the Cope annex was subjected to fungal growth so the availability of areas to collect samples was limited.

It was intended that samples from the areas that exhibited fungal growth and the areas that did not exhibit fungal growth were to be collected and paired from continuous sections of insulation to ensure the same manufacturer and lot of material is being compared when analyzing results. This approach was partially used in the Cope annex but proved infeasible in the Braille annex. Nine of the 13 samples exhibiting fungal growth collected from the Cope annex were collected from around roof vents. The fungal growth areas in the Cope annex were less pervasive and samples that did not exhibit fungal growth were able to be collected from the same continuous section of insulation by moving approximately 15-20 ft. away from the areas of fungal growth. The remaining four samples that exhibited fungal growth were collected from along the east wall of the annex. This section of insulation material ran the entire length of the wall so no areas for collecting samples that did not exhibit fungal growth to pair with these four samples were available. The four samples that did not exhibit fungal growth that were to be paired with these four samples were collected from the section of insulation immediately adjacent to this section. In the Braille annex, fungal growth fields present on the insulation material in the loading dock were observed on the northern half of the area. Six samples that exhibited fungal growth were collected from these sections. No areas in these sections of insulation were available for collecting samples that did not exhibit fungal growth. The six

samples that did not exhibit fungal growth that were to be paired with these samples were collected from the southern half of the loading dock area. Thirty-one pairs of samples were collected from the main storage area of the Braille annex. Twenty-nine of the samples exhibiting fungal growth collected from this area were taken from the section of insulation that ran lengthwise along the centerline of the building as this section is where the fungal growth fields were primarily observed. The remaining two samples exhibiting fungal growth were collected from the northeast corner of the building. Samples exhibiting no fungal growth to be paired with those that did could not be collected from the same continuous sections as the areas that had no visible fungal growth were found to have fungal colonization on the insulation (non-observable) side of the pPVC. To overcome this, a test sample was collected from an area that had no visible fungal growth from the insulation sections immediately adjacent and on either side of the centerline section. These samples also exhibited fungal growth on the insulation side of the pPVC despite appearing to be clear of visible fungal growth. Test samples were collected from an area that had no visible fungal growth from the insulation sections immediately adjacent to these sections and again fungal colonization was observed on the insulation side of the pPVC. Upon this discovery, the remainder of the building was visually inspected and one large area in the northwest corner of the Braille annex was observed to be visibly clear of fungal growth. A test sample was taken from this area and found to be visibly clear of fungal growth on both sides of the pPVC material. This area is where 26 of the 31 samples that exhibited no fungal growth were collected. Of the remaining five samples, three were collected from the same general area but closer to the centerline of the building. These three samples will be paired with the three samples exhibiting fungal growth collected from the centerline area near this area. The remaining two samples were collected from an area clear of visible fungal growth from the same

section of insulation as the two samples with visible fungal growth collected from the northeast corner of the building and were paired with these samples.

Sample Preparation

Prior to sample preparation, the samples were inspected and the pPVC film collected from the Cope annex appeared to be a different material than that collected from the Braille annex and loading dock. The material collected from the Cope annex was a two-ply film, was thicker, and felt more rigid when compared to the material collected from the Braille annex and loading dock. The fungal growth on these pieces was limited to the layer that faced the inside of the building and the layer that faced the insulation appeared unaffected. Because the preliminary results were based on the material collected from the Braille annex and to avoid any confounding of the results, the material collected from the Cope annex was not used for the rest of the project.

The remaining samples were re-numbered from 1-37 and a random number generator was used to re-order the samples. The first 25 samples from the re-ordered list were selected for analysis. Table I lists the samples that were selected for analysis.

Samples were prepared by first removing as much of the remaining fiberglass insulation and adhesive as possible using a razor blade sterilized with 70% isopropyl

Table I: Samples selected for analysis

3	15	11	31	10
17	36	4	21	18
1	19	29	25	14
30	16	33	9	26
5	6	35	2	13

alcohol. The insulation and adhesive was removed by using the razor blade to scrape the material from the pPVC film. Each sample was weighed using a Mettler Toledo Model AB104-S analytical balance capable of weighing to 0.0001 g. Individual samples were prepared by cutting each piece into approximately 0.5 cm X 0.5 cm pieces. The pieces were transferred into a pre-cleaned and pre-rinsed 200 mL Pyrex® beaker. Approximately 85 mL of DCM was added to the beaker and 1 mL of a surrogate standard containing 25 µg/mL of nitrobenzene-d5, 2-fluorobiphenyl, and terphenyl-d14 was added to the beaker using a calibrated 1000 µL Eppendorf pipet. The purpose of the surrogate standard was to provide a reference point when assessing extraction efficiency. The surrogate standard selected is one typically used when monitoring for phthalate esters in environmental samples. The pPVC material was allowed to soak in the DCM for approximately 30 minutes with occasional agitation in the form of stirring with a pre-cleaned glass stir rod. The sample material and DCM were separated by filtering through an 18.5 cm filter paper into a pre-cleaned and pre-rinsed 150 mL concentrator tube. The beaker was rinsed once with approximately 20 mL of DCM and an additional aliquot of approximately 10 mL of DCM was used to rinse the material remaining in the filter paper. The DCM extract was concentrated using a heat and nitrogen blow-down technique on a LabConco RapidVap evaporation unit set at an operating temperature of 65°C and rotational speed set at 50%. The DCM extract was concentrated to a volume of approximately 2 mL. The initial 2 mL aliquot was transferred using a glass Pasteur transfer pipet into a pre-cleaned and pre-rinsed 5 mL Class A volumetric flask. Approximately 1 mL of fresh DCM was added to the concentrator tube and the sides of the concentrator tube rinsed to remove extract residue that remained adhered to the walls of the concentrator tube during concentration. This 1 mL aliquot was transferred to the same volumetric flask and the process repeated one more time with a fresh 1

mL aliquot of DCM. The second rinse was combined with the original aliquot and first rinse in the 5 mL volumetric flask and the concentrated extract brought up to the 5 mL mark with DCM. The concentrated extract was transferred using a glass Pasteur transfer pipet into a 4 mL amber borosilicate glass vial and labeled with the appropriate sample number.

The extraction process was performed over a period of five days with five samples each of pPVC exhibiting fungal growth and pPVC not exhibiting fungal growth extracted each day. Each day a method blank was extracted along with the pPVC samples for the purpose of ensuring the results obtained were not the result of contamination from the reagents, standards, or equipment used in the extraction procedure. The method blank was approximately 85 mL of the same lot of DCM used for extracting the pPVC samples and taken through the entire extraction process. The final volume of the method blank extracts was 2 mL rather than the 5 mL of the pPVC sample extracts.

Extract Analysis

Extracts were analyzed on an Agilent Technologies Model 7890 gas chromatograph coupled with an Agilent Technologies Model 5975 mass spectrometer (GC/MS) using an internal standard technique. Prior to introduction to the instrument, the DCM extracts required dilution due to the viscous nature of the extract. Dilution was performed by using a Hamilton 1 mL glass syringe to add 500 µL of the extract to a pre-rinsed 5 mL Class A volumetric flask and diluting to the mark with DCM for a dilution factor of 10. One mL of the diluted extract was transferred to a 2 mL clear borosilicate glass autosampler vial and 10 µL of a 4000 µg/mL internal standard containing naphthalene-d8, acenaphthene-d10, phenanthrene-d10, and chrysene-d12 was added. The purpose of the internal standard is to account for variability of the instrument between analytical injections. The internal standard selected is one typically used when monitoring for

phthalate esters in environmental samples. An automated sampling tower was used to inject 1 µL of the diluted extract into the inlet of the GC. The GC/MS instrument parameters are listed in Table II.

Prior to analysis of the DCM extracts, the GC/MS was calibrated by injecting seven standards ranging from 2.5 ng to 80 ng of the phthalate esters and surrogate analytes. The relative response factor (RF) for each analyte in each calibration level was calculated:

$$RF = (A_s \times C_{is}) \div (A_{is} \times C_s) \quad (1)$$

Where A_s is the area of the analyte peak, A_{is} is the area of the internal standard peak, C_s is the concentration of the analyte in ng, and C_{is} is the concentration of the internal standard in ng

Table II: GC/MS Instrument Parameters

Instrument Parameter	Setting
Oven Program	40 °C for 3 min., 20 °C/min to 120 °C for 0 min., 10 °C/min to 230 °C for 0 min, 15 °C/min to 320 °C for 5 min.
Injection Volume	1 µL
Inlet	280 °C, 10:1 split ratio
Column	Restek Rxi-5Sil MS 30m, 250 µm ID, 0.25 µm film thickness
Carrier Gas	UHP helium
Flow Rate	0.8 mL/min
Mass Range	50-550 amu
MS Interface Temperature	280 °C

. The mean response factor, standard deviation (SD), and percent relative standard deviation (RSD) was calculated for each analyte:

$$Mean\ RF = \overline{RF} = \frac{\sum_{i=1}^{n} RF_i}{n} \quad (2)$$

$$SD = \sqrt{\frac{\sum_{i=1}^{n} (RF_i - \overline{RF})^2}{n-1}} \quad (3)$$

$$RSD = \frac{SD}{\overline{RF}}\ x\ 100 \quad (4)$$

A criterion of ≤20 % RSD was used when evaluating the calibration curves for acceptability. This criterion is typically used in EPA GC/MS methodologies. Concentrations of the phthalate esters and surrogate analytes in the DCM extracts were calculated:

$$Concentration(ng) = \frac{R_s \times C_{is} \times DF}{R_{is} \times \overline{RF}} \quad (5)$$

Where R_s is the response in area counts of the analyte in the DCM extract and R_{is} is the response in area counts of the internal standard.

A 50 ng aliquot of decafluorotriphenylphospine (DFTPP) tuning standard was injected into the instrument prior to analyzing calibration standards and DCM extracts. The DFTPP is injected to ensure the MS is properly tuned and capable of separating and detecting the correct m/z ratios of the phthalate esters and surrogate analytes. The DFTPP standard was injected at a rate of once every 12 hours. This 12-hour block is defined as a tune window. The DFTPP was evaluated using the tune criterion published by the EPA and typically used when monitoring for phthalate esters in environmental samples. Table III lists the tuning criteria.

A 10 ng calibration verification standard (CCV) containing the four phthalate esters and three surrogate analytes was injected at the beginning of every 12 hour tune window immediately

after the DFTPP standard and prior to analyzing the DCM extracts. The purpose of the CCV was to ensure the instrument calibration was still valid. The percent difference (%D) between the CCV and calibration mean RF was calculated:

$$\%D = \frac{RF_v - \overline{RF}}{\overline{RF}}\ x\ 100 \quad (6)$$

Where RF_v is the response factor of the phthalate ester or surrogate analyte in the CCV. A criterion of ±20 %D was used to evaluate the acceptability of the CCV standards. This criterion is typically used in EPA GC/MS methodologies.

Table III: DFTPP Tune Acceptance Criteria

Mass	Ion Abundance Criteria
51	30-60% of mass 198
68	<2% of mass 69
69	Presence of mass 198
70	<2% of mass 69
127	40-60% of mass 198
197	<1% of mass 198
198	Base peak, 100% relative abundance
199	5-9% of mass 198
275	10-30% of mass 198
365	>1% of mass 198
441	Present, but less than mass 443
442	>40% of mass 198
443	17-23% of mass 442

Surrogate recovery percentages were calculated for the three surrogate analytes added during the extractions procedure:

$$\% \, Recovery = \frac{C_E}{C_A} \times 100 \tag{7}$$

Where C_E is the concentration in the extract and C_A is the concentration added.

Statistical Evaluation

The results obtained from the analysis of the DCM extracts were statistically analyzed using a one-tailed independent samples *t* test and paired samples *t* test. For calculation and comparison purposes α was set at 0.05. The null hypothesis (H_O) was that there is no difference in phthalate ester concentrations between the pPVC not exhibiting fungal growth (μ1) and the pPVC exhibiting fungal growth (μ2). The alternative hypothesis (H_A) was the phthalate ester concentrations in the pPVC not exhibiting fungal growth will be greater than in the pPVC exhibiting fungal growth.

The *t* value for the independent samples *t* test was calculated:

$$t = \frac{\tilde{x}_1 - \tilde{x}_2}{\sqrt{s_P^2\left(\frac{1}{n_1} + \frac{1}{n_2}\right)}} \tag{8}$$

Where x_1 is the mean of the phthalate ester concentration from pPVC not exhibiting fungal growth, x_2 is the mean of the phthalate ester concentration from pPVC exhibiting fungal growth, n_1 is the number of samples of pPVC not exhibiting fungal growth, n_2 is the number of samples pPVC exhibiting fungal growth, and s_P^2 is the pooled sample variance calculated as:

$$s_P^2 = \frac{\left(\sum x_1^2 - \frac{(\sum x_1)^2}{n_1}\right) + \left(\sum x_2^2 - \frac{(\sum x_2)^2}{n_2}\right)}{n_1 + n_2 - 2} \tag{9}$$

The *t* value for a one-tailed hypothesis test with α set at 0.05 and 48 degrees of freedom is 1.667.

The *t* value for the paired samples Student's *t* test was calculated:

$$t = \frac{\bar{d}}{\frac{SD_d}{\sqrt{n}}} \quad (10)$$

Where $\bar{d}$ is the mean of the sample difference scores, SD_d is the sample standard deviation of the difference scores, and n is the number of paired samples. The *t* value for a one-tailed hypothesis test with α set at 0.05 and 24 degrees of freedom is 1.711.

Exposure Modeling

A worst-case-scenario exposure model was calculated by taking the mean phthalate concentration per m^2 determined from the analytical results for each phthalate ester in the pPVC not exhibiting fungal growth and subtracting the mean phthalate concentration per m^2 of each phthalate ester in the pPVC exhibiting fungal growth. This calculated difference was used to calculate the potential exposure level if the entire mass of phthalate esters were to be released in a single moment. The potential total mass released was calculated:

$$Total\ mass = \frac{C_C - C_F}{A} \quad (11)$$

Where C_C is the mean concentration of the phthalate ester in the pPVC not exhibiting fungal growth, C_F is the mean concentration of the phthalate ester in the pPVC exhibiting fungal growth, and A is the total area of pPVC material.

The total potential mass released was divided by the interior volume of the building to obtain the potential airborne concentration of each phthalate ester:

$$C_{PE} = \frac{Total\ mass}{Volume} \quad (12)$$

The calculated results were compared to published exposure concentrations in similar exposure scenarios.

RESULTS

Method Detection Limits

All results obtained from the instrument were in ng. The method detection limits (MDL) for the blanks and four phthalate esters are listed in Table IV. The MDLs for the blanks are based on a final volume of 2 mL. The MDLs for the four phthalate esters are multiplied by a factor of 25 to account for the final volume of 5 mL and the dilution ratio of 1:10.

Table IV: Method Detection Limits (ng)

Analyte	Blank Detection Limit (ng)	pPVC Detection Limit (ng)
DEP	7.69	192
DBP	4.96	124
BBP	5.42	136
DEHP	3.90	97.5

Phthalate Ester Content

None of the five method blanks contained any of the four phthalate esters at or above the MDL, indicating that the extraction process was free of phthalate ester contamination. All four phthalate esters were detected at or above the MDL in all 25 samples of pPVC that did not exhibit fungal growth. Table V lists the phthalate ester content results of the individual pPVC samples with and without fungal growth.

Table V: Phthalate Ester Content (ng)

Sample #	No Fungal Growth (ng)				Fungal Growth (ng)			
	DEP	DBP	BBP	DEHP	DEP	DBP	BBP	DEHP
3	300	621	250	319	293	365	98.9	252
30	333	838	556	332	196	542	262	158
1	298	646	270	331	308	419	120	262
43	309	852	472	305	411	1148	500	381
5	296	576	265	335	319	529	290	299
28	346	899	656	362	272	714	376	234
50	436	916	485	332	482	789	691	331
32	356	860	635	344	269	1027	584	399
29	368	891	525	258	243	631	298	128
6	333	664	251	353	352	483	248	272
24	343	840	430	223	232	694	400	231
4	299	567	230	312	290	503	134	365
42	353	936	493	344	395	1048	471	363
47	314	858	547	265	127	429	589	240
49	367	779	439	288	332	675	382	135
44	356	953	570	354	219	879	495	383
34	319	748	537	335	330	1024	434	327
38	348	935	427	327	395	1012	469	357
22	312	873	573	324	181	543	437	256
2	272	482	181	290	258	337	104	232
23	334	930	596	349	174	589	385	240
31	331	836	627	337	300	719	483	298
27	345	902	550	268	296	783	388	234
39	368	987	474	341	379	997	412	346
26	333	878	651	342	295	884	453	239

All four phthalate esters were detected at or above the MDL in all 25 samples of pPVC that exhibited fungal growth with the following exceptions: DEP was detected below the MDL in samples 47, 22, and 23; and BBP in samples 3, 1, 4, and 2. The mean phthalate content for all four phthalate esters was above their respective MDLs.

Table VI: Mean Phthalate Ester Content (ng)

	DEP	DBP	BBP	DEHP
Mean No Fungal Growth:	335	811	468	319
Mean Fungal Growth:	294	711	380	278
Independent Samples t^A:	2.292	1.809	2.057	2.404
Paired Samples t^B:	2.677	2.713	3.856	2.961

A: *t* value 1.677
B: *t* value 1.711

The mean phthalate content for the pPVC exhibiting no fungal growth and pPVC exhibiting fungal growth is listed in Table VI. This table also lists the calculated *t* values for the independent samples *t* test and paired samples *t* test. The mean phthalate ester content was lower in the pPVC exhibiting fungal growth when compared to the pPVC that did not exhibit fungal growth for all four phthalate esters and the difference between the two was statistically significant when using both the independent samples *t* test and the paired samples *t* test. DEP had a mean content mass of 335 ng in the pPVC with no fungal growth and 294 ng in the pPVC with fungal growth; DBP 811 ng and 711 ng, respectively; BBP 468 ng and 380 ng, respectively; and DEHP 319 ng and 278 ng, respectively.

Phthalate Ester Concentration Based on Mass

Table VII: Mean Phthalate Ester Concentration Based on Mass (µg/Kg)

	Mass (g)	DEP	DBP	BBP	DEHP
Mean No Fungal Growth:	3.1953	105	253	147	100
Mean Fungal Growth:	3.1898	92.2	223	119	87.4
Independent Samples t^A:	0.120	2.321	1.766	2.047	2.393
Paired Samples t^B:	0.128	2.650	2.736	3.704	2.967

A: *t* value 1.677
B: *t* value 1.711

All samples were weighed prior to extraction and analysis. In general, there was little difference between the weights of the pPVC that exhibited fungal growth and the pPVC that did not exhibit fungal growth. Table VII lists the mean sample masses and phthalate ester concentrations in units of µg/Kg. This table also lists the calculated *t* values for the independent samples *t* test and paired samples *t* test. As with the mean phthalate ester content, the phthalate ester concentration per kilogram of pPVC material was lower in the pPVC exhibiting fungal growth when compared to the pPVC that did not exhibit fungal growth for all four phthalate esters and the difference between the two was statistically significant when using both the independent samples *t* test and the paired samples *t* test. There was no statistically significant difference in the masses of the pPVC exhibiting fungal growth and the pPVC not exhibiting fungal growth. DEP had a mean mass concentration of 105 µg/Kg in the pPVC with no fungal growth and 92.2 µg/Kg in the pPVC with fungal growth; DBP 253 µg/Kg and 223 µg/Kg, respectively; BBP 147 µg/Kg and 119 µg/Kg, respectively; and DEHP 100 µg/Kg and 87.4 µg/Kg, respectively.

Phthalate Ester Concentration Based on Area

Table VIII: Mean Phthalate Ester Concentration Based on Area (µg/m^2)

	DEP	DBP	BBP	DEHP
Mean No Fungal Growth:	14.4	34.9	20.2	13.7
Mean Fungal Growth:	12.7	30.6	16.4	12.0
Independent Samples t^A:	2.292	1.809	2.057	2.404
Paired Samples t^B:	2.677	2.713	3.856	2.961

A: *t* value 1.677
B: *t* value 1.711

All samples were collected using a template so all samples had a nominal area of 0.0232 m^2. Small variations in area existed in individual pieces but these variations, observed to be a maximum of ± 0.00025 m^2, would only account for approximately a ±1% change in total area. For ease of calculation these variations were considered minor and the standard area measurement of 0.0232 m^2 used. Table VIII lists the mean phthalate ester concentrations in units of µg/m^2. This table also lists the calculated *t* values for the independent samples *t* test and paired samples *t* test. As with the mean phthalate ester content, the phthalate ester concentration per square meter of pPVC material was lower in the pPVC exhibiting fungal growth when compared to the pPVC that did not exhibit fungal growth for all four phthalate esters and the difference between the two was statistically significant when using both the independent samples *t* test and the paired samples *t* test. DEP had a mean area concentration of 14.4 µg/m^2 in the pPVC with no fungal growth and 12.7 µg/m^2 in the pPVC with fungal growth; DBP 34.9 µg/m^2 and 30.6 µg/m^2, respectively; BBP 20.2 µg/m^2 and 16.4 µg/m^2, respectively; and DEHP 13.7 µg/m^2 and 12.0 µg/m^2, respectively.

Phthalate Ester Exposure Levels

The dimensions of the building being investigated were: 70.15 m in length, 21.35 m in width, 3.97 m from floor to ceiling, and 4.88 m from floor to the peak of the roof. The total volume of the building was 6624 m^3. The total area of material in the building was 1503 m^2. Using the mean concentration based on area for each individual phthalate ester from Table VIII the "worst-case-scenario" exposures were calculated to be 0.40 $\mu g/m^3$ for DEP, 0.98 $\mu g/m^3$ for DBP, 0.86 $\mu g/m^3$ for BBP, and 0.39 $\mu g/m^3$ for DEHP.

DISCUSSION

The purpose of this study is to determine if the presence of fungal growth on the pPVC used as the fronting material of metal building insulation significantly affects the phthalate ester content of the pPVC and, if there is a significant decrease in the phthalate ester content, how the liberated phthalate esters affect the potential for airborne exposure and if the potential airborne exposure is comparable to exposures typically experienced in similar settings.

The results of the chemical analysis showed that the mean content of all four phthalate esters was lower in the pieces that exhibited fungal growth when compared to the pieces that did not. DEP had a mean mass content of 335 ng in the pPVC with no fungal growth and 294 ng in the pPVC with fungal growth; DBP 811 ng and 711 ng, respectively; BBP 468 ng and 380 ng, respectively; and DEHP 319 ng and 278 ng, respectively. These results represented a range of a 12.2% to 18.7% decrease in phthalate ester mass between the pPVC with no fungal growth and pPVC with fungal growth.

DEP had a mean mass concentration of 105 µg/Kg in the pPVC with no fungal growth and 92.2 µg/Kg in the pPVC with fungal growth; DBP 253 µg/Kg and 223 µg/Kg, respectively; BBP 147 µg/Kg and 119 µg/Kg, respectively; and DEHP 100 µg/Kg and 87 µg/Kg, respectively. These results represented a range of a 12.0% to 18.7% decrease in phthalate ester mass concentration between the pPVC with no fungal growth and pPVC with fungal growth.

DEP had a mean area concentration of 14.4 $\mu g/m^2$ in the pPVC with no fungal growth and 12.7 $\mu g/ m^2$ in the pPVC with fungal growth; DBP 34.9 $\mu g/m^2$ and 30.6 $\mu g/m^2$, respectively;

BBP 20.2 µg/m^2 and 16.4 µg/m^2, respectively; and DEHP 13.7 µg/m^2 and 12.0 µg/m^2, respectively. These results represented a range of a 12.2% to 18.7% decrease in phthalate ester area concentration between the pPVC with no fungal growth and pPVC with fungal growth.

The results of the chemical analysis showed that there is a statistically significant difference between the clean pPVC film and pPVC film with fungal growth for all four phthalate esters investigated for phthalate ester content, phthalate ester concentration per unit mass, and phthalate ester concentration per unit area. The *t* value of 1.677 for the independent samples *t* test was exceeded for all parameters. The *t* value of 1.711 for the paired samples *t* test was also exceeded for all parameters.

The mean mass of the pieces selected was 3.1953 g for the pPVC with no fungal growth and 3.1898 g for the pPVC with fungal growth. This is a difference of 0.0055 g and the difference was not statistically significant, failing to exceed the *t* values for both the independent samples *t* test and the paired samples *t* test. While not statistically significant, this loss of mass was consistent with results reported by Peciulyte who observed mass loss of pPVC when colonized by fungal growth (Peciulyte, 2002). The pPVC material that exhibited fungal growth was observed to be more brittle, less pliable, and not as soft when compared to the pPVC that did not exhibit fungal growth. This observation is consistent with material that has been subjected to loss of phthalate ester plasticizers.

The potential worst case scenario concentrations were DEP 0.40 µg/m^3, DBP 0.98 µg/m^3, BBP 0.86 µg/m^3, and DEHP 0.39 µg/m^3. These concentrations represent those that would be experienced if the difference in the phthalate ester content based on area between the pPVC material exhibiting fungal growth and the pPVC material exhibiting no fungal growth were to be released all at once. This scenario is improbable due to the low volatility of the phthalate esters

and the slow rate of fungal growth in addition to the assumption that the phthalate esters would be released into a closed system with no ventilation. In reality the annex is both force and naturally ventilated via a HVAC system and ventilation fans. The ventilation fans are open to the outside and allow outside ambient air to be exchanged with the inside air. This scenario also does not account for air leakage through the roof vents, garage doors in the shipping area, or typical building leakage.

Even had this scenario been possible, the resultant concentrations do not appear likely to result in excessive exposure. The calculated concentrations for DEP and DBP are lower than published results found in similar occupational settings and similar to concentrations experienced in non-occupational settings. Of the four phthalate esters, BBP had the largest difference between calculated exposure concentration and published exposure concentration. The calculated exposure concentration was approximately 43X the lone published occupational exposure concentration identified and between 2-25X published non-occupational exposures. The calculated exposure concentration for DEHP was 2-4X published occupational exposure concentrations and was lower than published non-occupational exposures.

Limitations

The material analyzed was installed in 1981 and will have been subjected to natural phthalate ester loss. One consequence of this fact is that the initial phthalate ester concentrations present in the virgin material when it was produced could not be determined and as such no reference point was available to evaluate if the phthalate ester concentrations had already been significantly diminished. In this study the pPVC that exhibited no fungal growth was used as a control to assist in overcoming this limitation. Frissell determined that at room temperatures, significant plasticizer loss ceases after the first 2 ½ years. He studied two of the phthalate esters

of interest in this project and found 27% of DBP and 3.6% of DEHP was lost over this time period (Frissell, 1956). Visible fungal colonization was observed beginning three years ago so it is possible that if significant amounts of phthalate esters had already been lost naturally in the preceding 30 years there would be less for the fungal colony to liberate and would lead to the results observed being biased low. However, if the fungal colonization occurred earlier in the material's service life and more phthalate esters were still present in the material, the calculated airborne concentration results could be higher.

The mechanism for release of the phthalate esters is not fully known. Published reports indicate that the primary fungal species identified, *Cladosporium cladosporiodes*, is capable of utilizing DEHP as a carbon source (Roberts & Davidson, 1986) and metabolizing it into its monoester or other form so it is most likely that exposure to DEHP would normally be minimal. No information was able to be retrieved regarding how or if this fungal species is capable of utilizing DEP, DBP, and BBP so it is unclear if the lower observed amounts of these three phthalate esters in the pPVC material was the result of their being liberated by degradation of the material around them or if they were able to be utilized by the fungal colony and metabolized into other forms. Elucidating this mechanism may provide the subject for future investigation.

The indoor air environment contains a diversity of fungal species so it is unlikely that *Cladosporium cladosporiodes* was the sole fungal species present on the material. Phthalate ester utilization is species-dependent so it is possible that the presence of other fungal species could result in utilization of the phthalate esters rather than liberation which would result in the calculated airborne concentrations being biased high.

The pervasiveness of the fungal colonization prohibited collection of samples exhibiting fungal growth and no fungal growth from the same contiguous section of material. It was

intended to collect samples in this manner in order to be able to compare the phthalate ester content from areas on the same piece of material, which presumably would contain a uniform phthalate ester content throughout the piece. The pervasiveness of the fungal growth forced the collection of pPVC material exhibiting no fungal growth primarily from one area of the annex and on a different section of material. This material appeared to be similar in color, texture, and pliability to the material of the samples exhibiting fungal growth so it was assumed that the material was from the same manufacturer and installed at the same time.

Conclusion

The presence of fungal growth is related to lower phthalate ester content in the pPVC film used as the fronting material of metal building insulation. These results are similar to those obtained in studies conducted on pPVC employed in other applications. The loss of phthalate esters due to fungal degradation does not pose an increased health risk from exposure to phthalate esters for two main reasons. First, the calculated exposure concentrations are similar to or lower than published exposure concentrations workers in similar occupational settings, as well as in non-occupational settings, have been exposed to due to normal phthalate ester evaporative losses. Second, the fungal growth has the capability of metabolizing certain phthalate esters into their less hazardous monoester forms and through that process minimizes exposures to those phthalate esters.

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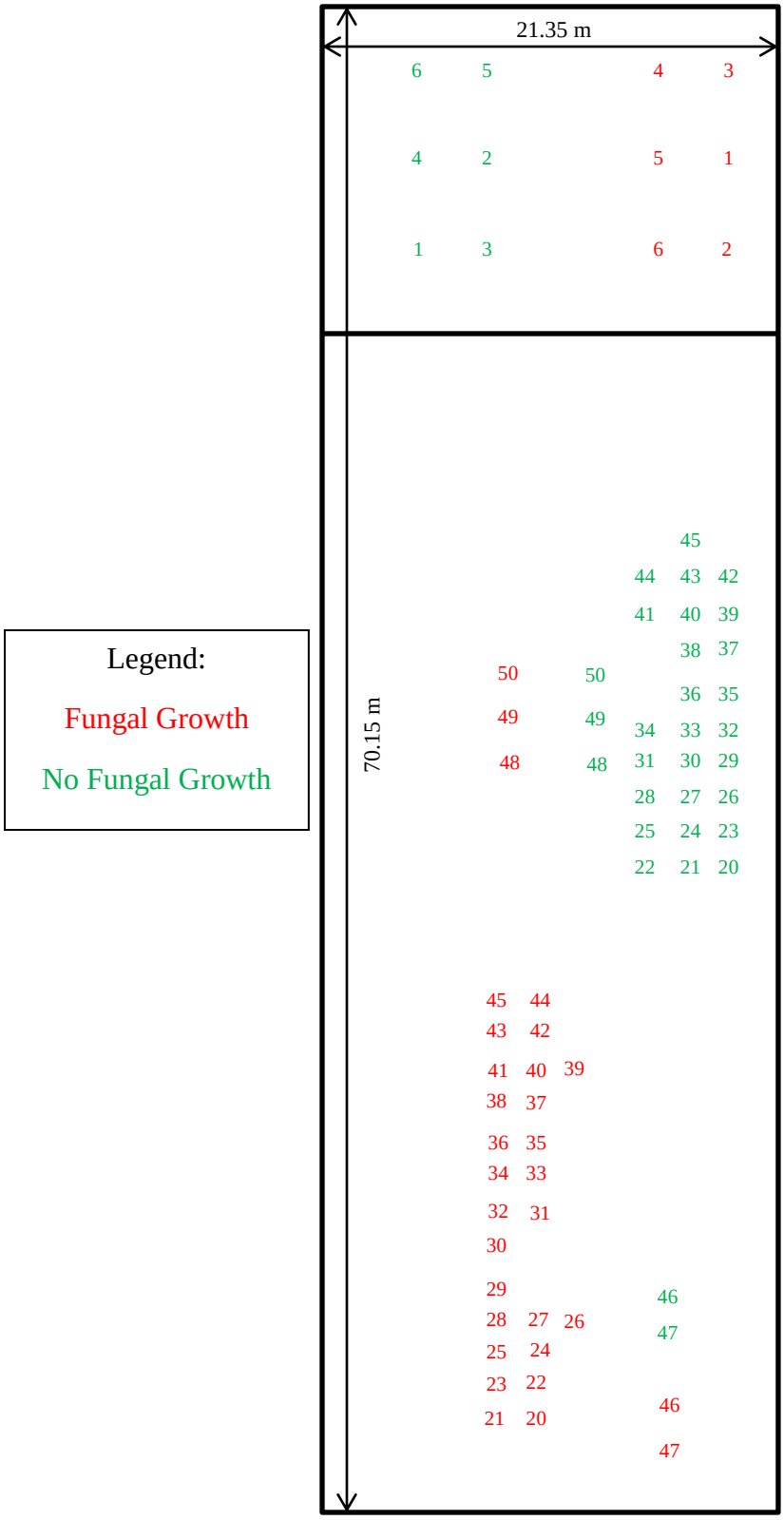
APPENDICES

Appendix A: Braille Annex and Loading Dock Layout and Sampling Locations



Appendix B: Analytical Run Sequence

```
    Sequence Name: C:\msdchem\1\sequence\140103A.s
          Comment: IS 1300915-4
         Operator: GKJ
        Data Path: C:\MSDCHEM\1\DATA\140103A\
Instrument Control  Pre-Seq Cmd:
Data Analysis       Pre-Seq Cmd:

Instrument Control Post-Seq Cmd:
Data Analysis      Post-Seq Cmd:

   Method Sections To Run      On A Barcode Mismatch
    (X) Full Method              (X) Inject Anyway
    ( ) Reprocessing Only        ( ) Don't Inject

   ------------------------------------------------------------------------
   Line                    Sample Name/Misc Info
 1) Sample          1  8270 IB
     Datafile          0103-01
     Method            PHTHALATE
 2)  Sample         2  DFTPP-01 8270FULL 50 ng DFTPP
 3) Sample          3  10 ug/mL 8270D CAL STD
     Datafile          0103-02
     Method            PHTHALATE
 4) Sample          4  BLANK #1
     Datafile          0103-03
     Method            PHTHALATE
 5) Sample          5  BLANK #2
     Datafile          0103-04
     Method            PHTHALATE
 6) Sample          6  BLANK #3
     Datafile          0103-05
     Method            PHTHALATE
 7) Sample          7  BLANK #4
     Datafile          0103-06
     Method            PHTHALATE
 8) Sample          8  BLANK #5
     Datafile          0103-07
     Method            PHTHALATE
 9) Sample          9  3
     Datafile          0103-08
     Method            PHTHALATE
10) Sample         10  30
     Datafile          0103-09
     Method            PHTHALATE
11) Sample         11  1
     Datafile          0103-10
     Method            PHTHALATE
12) Sample         12  43
     Datafile          0103-11
     Method            PHTHALATE
13) Sample         13  5
     Datafile          0103-12
     Method            PHTHALATE
14) Sample         14  28
     Datafile          0103-13
     Method            PHTHALATE
15) Sample         15  50
     Datafile          0103-14
     Method            PHTHALATE
16) Sample         16  32
     Datafile          0103-15
     Method            PHTHALATE
17) Sample         17  29
     Datafile          0103-16
     Method            PHTHALATE
18) Sample         18  6
     Datafile          0103-17
     Method            PHTHALATE
19) Sample         19  24
     Datafile          0103-18

   Last Modified: Fri Jan 03 11:27:45 2014                    Page: 1
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```
     Method          PHTHALATE
20) Sample       20  4
     Datafile        0103-19
     Method          PHTHALATE
21)  Sample      21  DFTPP-02 8270FULL 50 ng DFTPP
22) Sample       22  10 ug/mL 8270D CAL STD
     Datafile        0103-20
     Method          PHTHALATE
23) Sample       23  42
     Datafile        0103-21
     Method          PHTHALATE
24) Sample       24  47
     Datafile        0103-22
     Method          PHTHALATE
25) Sample       25  49
     Datafile        0103-23
     Method          PHTHALATE
26) Sample       26  44
     Datafile        0103-24
     Method          PHTHALATE
27) Sample       27  34
     Datafile        0103-25
     Method          PHTHALATE
28) Sample       28  38
     Datafile        0103-26
     Method          PHTHALATE
29) Sample       29  22
     Datafile        0103-27
     Method          PHTHALATE
30) Sample       30  2
     Datafile        0103-28
     Method          PHTHALATE
31) Sample       31  23
     Datafile        0103-29
     Method          PHTHALATE
32) Sample       32  31
     Datafile        0103-30
     Method          PHTHALATE
33) Sample       33  27
     Datafile        0103-31
     Method          PHTHALATE
34) Sample       34  39
     Datafile        0103-32
     Method          PHTHALATE
35) Sample       35  26
     Datafile        0103-33
     Method          PHTHALATE
36) Sample       36  3
     Datafile        0103-34
     Method          PHTHALATE
37) Sample       37  30
     Datafile        0103-35
     Method          PHTHALATE
38) Sample       38  1
     Datafile        0103-36
     Method          PHTHALATE
39) Sample       39  43
     Datafile        0103-37
     Method          PHTHALATE
40) Sample       40  5
     Datafile        0103-38
     Method          PHTHALATE
41)  Sample      41  DFTPP-03 8270FULL 50 ng DFTPP
42) Sample       42  10 ug/mL 8270D CAL STD
     Datafile        0103-39
     Method          PHTHALATE
43) Sample       43  28
     Datafile        0103-40
     Method          PHTHALATE

   Last Modified: Fri Jan 03 11:27:45 2014                    Page: 2
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Sequence Name: C:\msdchem\1\sequence\140103A.s

Line	Type	Vial	DataFile	Method	Sample Name
44)	Sample	44	0103-41	PHTHALATE	
45)	Sample	45	0103-42	PHTHALATE	
46)	Sample	46	0103-43	PHTHALATE	
47)	Sample	47	0103-44	PHTHALATE	
48)	Sample	48	0103-45	PHTHALATE	
49)	Sample	49	0103-46	PHTHALATE	
50)	Sample	50	0103-47	PHTHALATE	
51)	Sample	51	0103-48	PHTHALATE	
52)	Sample	52	0103-49	PHTHALATE	
53)	Sample	53	0103-50	PHTHALATE	
54)	Sample	54	0103-51	PHTHALATE	
55)	Sample	55	0103-52	PHTHALATE	
56)	Sample	56	0103-53	PHTHALATE	
57)	Sample	57	0103-54	PHTHALATE	
58)	Sample	58	0103-55	PHTHALATE	
59)	Sample	59	0103-56	PHTHALATE	
60)	Sample	60	0103-57	PHTHALATE	
61)	Sample	61			DFTPP-04 8270FULL 50 ng DFTPP
62)	Sample	62	0103-58	PHTHALATE	10 ug/mL 8270D CAL STD
63)	Sample	63	0103-59	PHTHALATE	
64)	Sample	64	0103-60	PHTHALATE	
65)	Sample	65	0103-61	PHTHALATE	2.5 ug/mL 8270D MRL STD

Appendix C: Initial Calibration and Continuing Calibration Reports

Response Factor Report GCMS3

Method Path : C:\msdchem\1\METHODS\
Method File : PHTHALATE.M
Title : Phthalates by Selective Ion Monitoring 9/14/13
Last Update : Fri Jan 03 09:09:09 2014
Response Via : Initial Calibration

Calibration Files
1 =1106-02.D 2 =1106-03.D 3 =1106-04.D 4 =1106-05.D 5 =1106-06.D 6 =1106-07.D 7 =1106-08.D

	Compound	1	2	3	4	5	6	7	Avg	%RSD
1) IA	Naphthalene-d8				ISTD					
2) SA	Nitrobenzene-d5	0.334	0.333	0.325	0.329	0.331	0.327	0.330	0.330	0.91
3) IA	Acenapthene-d10				ISTD					
4) SA	2-Fluorobiphenyl	1.246	1.238	1.221	1.219	1.231	1.226	1.223	1.229	0.80
5) TA	Diethyl phthalate	1.274	1.236	1.258	1.230	1.246	1.279	1.241	1.252	1.51
6) IA	Phenanthrene-d10				ISTD					
7) TA	Dibutyl phthalate	1.251	1.241	1.282	1.268	1.317	1.359	1.289	1.287	3.15
8) IA	Chrysene-d12				ISTD					
9) SA	p-Terphenyl-d14	0.817	0.852	0.821	0.821	0.843	0.816	0.819	0.827	1.77
10) TA	Butyl benzyl p...	0.527	0.538	0.559	0.532	0.564	0.583	0.565	0.553	3.74
11) TA	di-(2-Ethylhex...	0.762	0.764	0.796	0.786	0.801	0.809	0.762	0.783	2.56

(#) = Out of Range

PHTHALATE.M Fri Jan 03 09:11:57 2014 Page: 1

Evaluate Continuing Calibration Report

Data Path : C:\msdchem\1\DATA\140103A\
Data File : 0103-02.D
Acq On : 3 Jan 2014 12:44 pm
Operator : GKJ
Sample : 10 ug/mL 8270D CAL STD
Misc : 1300522 MIX [A]
ALS Vial : 3 Sample Multiplier: 1

Quant Time: Jan 03 13:10:31 2014
Quant Method : C:\MSDCHEM\1\METHODS\PHTHALATE.M
Quant Title : Phthalates by GC/MS (11/6/13)
QLast Update : Fri Jan 03 12:01:40 2014
Response via : Initial Calibration

Min. RRF : 0.000 Min. Rel. Area : 50% Max. R.T. Dev 0.50min
Max. RRF Dev : 20% Max. Rel. Area : 200%

	Compound	AvgRF	CCRF	%Dev	Area%	Dev(min)
1 IA	Naphthalene-d8	1.000	1.000	0.0	96	0.00
2 SA	Nitrobenzene-d5	0.330	0.290	12.1	86	0.00
3 IA	Acenapthene-d10	1.000	1.000	0.0	96	0.00
4 SA	2-Fluorobiphenyl	1.229	1.297	-5.5	102	0.00
5 TA	Diethyl phthalate	1.252	1.356	-8.3	104	0.00
6 IA	Phenanthrene-d10	1.000	1.000	0.0	108	0.00
7 TA	Dibutyl phthalate	1.287	1.311	-1.9	110	0.00
8 IA	Chrysene-d12	1.000	1.000	0.0	109	0.00
9 SA	p-Terphenyl-d14	0.827	0.831	-0.5	110	0.00
10 TA	Butyl benzyl phthalate	0.553	0.569	-2.9	111	0.00
11 TA	di-(2-Ethylhexyl) phthalate	0.783	0.813	-3.8	111	0.00

(#) = Out of Range SPCC's out = 0 CCC's out = 0

Evaluate Continuing Calibration Report

Data Path : C:\msdchem\1\DATA\140103A\
Data File : 0103-20.D
Acq On : 3 Jan 2014 11:55 pm
Operator : GKJ
Sample : 10 ug/mL 8270D CAL STD
Misc : 1300522 MIX [A]
ALS Vial : 22 Sample Multiplier: 1

Quant Time: Jan 04 00:20:47 2014
Quant Method : C:\MSDCHEM\1\METHODS\PHTHALATE.M
Quant Title : Phthalates by GC/MS (11/6/13)
QLast Update : Fri Jan 03 12:01:40 2014
Response via : Initial Calibration

Min. RRF : 0.000 Min. Rel. Area : 50% Max. R.T. Dev 0.50min
Max. RRF Dev : 20% Max. Rel. Area : 200%

	Compound	AvgRF	CCRF	%Dev	Area%	Dev(min)
1 IA	Naphthalene-d8	1.000	1.000	0.0	96	0.00
2 SA	Nitrobenzene-d5	0.330	0.314	4.8	92	0.00
3 IA	Acenapthene-d10	1.000	1.000	0.0	96	0.00
4 SA	2-Fluorobiphenyl	1.229	1.317	-7.2	104	0.00
5 TA	Diethyl phthalate	1.252	1.328	-6.1	101	0.00
6 IA	Phenanthrene-d10	1.000	1.000	0.0	110	0.00
7 TA	Dibutyl phthalate	1.287	1.253	2.6	107	0.00
8 IA	Chrysene-d12	1.000	1.000	0.0	108	0.00
9 SA	p-Terphenyl-d14	0.827	0.838	-1.3	111	0.00
10 TA	Butyl benzyl phthalate	0.553	0.508	8.1	98	0.00
11 TA	di-(2-Ethylhexyl) phthalate	0.783	0.802	-2.4	109	0.00

(#) = Out of Range SPCC's out = 0 CCC's out = 0

Evaluate Continuing Calibration Report

Data Path : C:\msdchem\1\DATA\140103A\
Data File : 0103-39.D
Acq On : 4 Jan 2014 11:41 am
Operator : GKJ
Sample : 10 ug/mL 8270D CAL STD
Misc : 1300522 MIX [A]
ALS Vial : 42 Sample Multiplier: 1

Quant Time: Jan 04 12:06:46 2014
Quant Method : C:\MSDCHEM\1\METHODS\PHTHALATE.M
Quant Title : Phthalates by GC/MS (11/6/13)
QLast Update : Fri Jan 03 12:01:40 2014
Response via : Initial Calibration

Min. RRF : 0.000 Min. Rel. Area : 50% Max. R.T. Dev 0.50min
Max. RRF Dev : 20% Max. Rel. Area : 200%

	Compound	AvgRF	CCRF	%Dev	Area%	Dev(min)
1 IA	Naphthalene-d8	1.000	1.000	0.0	100	0.00
2 SA	Nitrobenzene-d5	0.330	0.300	9.1	93	0.00
3 IA	Acenapthene-d10	1.000	1.000	0.0	102	0.00
4 SA	2-Fluorobiphenyl	1.229	1.307	-6.3	109	0.00
5 TA	Diethyl phthalate	1.252	1.323	-5.7	107	0.00
6 IA	Phenanthrene-d10	1.000	1.000	0.0	112	0.00
7 TA	Dibutyl phthalate	1.287	1.236	4.0	108	0.00
8 IA	Chrysene-d12	1.000	1.000	0.0	107	0.00
9 SA	p-Terphenyl-d14	0.827	0.851	-2.9	110	0.00
10 TA	Butyl benzyl phthalate	0.553	0.447	19.2	85	0.00
11 TA	di-(2-Ethylhexyl) phthalate	0.783	0.803	-2.6	108	0.00

(#) = Out of Range SPCC's out = 0 CCC's out = 0

Evaluate Continuing Calibration Report

Data Path : C:\msdchem\1\DATA\140103A\
Data File : 0103-58.D
Acq On : 4 Jan 2014 11:36 pm
Operator : GKJ
Sample : 10 ug/mL 8270D CAL STD
Misc : 1300522 MIX [A]
ALS Vial : 62 Sample Multiplier: 1

Quant Time: Jan 05 00:02:18 2014
Quant Method : C:\MSDCHEM\1\METHODS\PHTHALATE.M
Quant Title : Phthalates by GC/MS (11/6/13)
QLast Update : Fri Jan 03 12:01:40 2014
Response via : Initial Calibration

Min. RRF : 0.000 Min. Rel. Area : 50% Max. R.T. Dev 0.50min
Max. RRF Dev : 20% Max. Rel. Area : 200%

	Compound	AvgRF	CCRF	%Dev	Area%	Dev(min)
1 IA	Naphthalene-d8	1.000	1.000	0.0	111	0.00
2 SA	Nitrobenzene-d5	0.330	0.301	8.8	102	0.00
3 IA	Acenapthene-d10	1.000	1.000	0.0	111	0.00
4 SA	2-Fluorobiphenyl	1.229	1.305	-6.2	118	0.00
5 TA	Diethyl phthalate	1.252	1.323	-5.7	116	0.00
6 IA	Phenanthrene-d10	1.000	1.000	0.0	128	0.00
7 TA	Dibutyl phthalate	1.287	1.201	6.7	120	0.00
8 IA	Chrysene-d12	1.000	1.000	0.0	119	0.00
9 SA	p-Terphenyl-d14	0.827	0.861	-4.1	125	0.00
10 TA	Butyl benzyl phthalate	0.553	0.459	17.0	98	0.00
11 TA	di-(2-Ethylhexyl) phthalate	0.783	0.813	-3.8	121	0.00

(#) = Out of Range SPCC's out = 0 CCC's out = 0

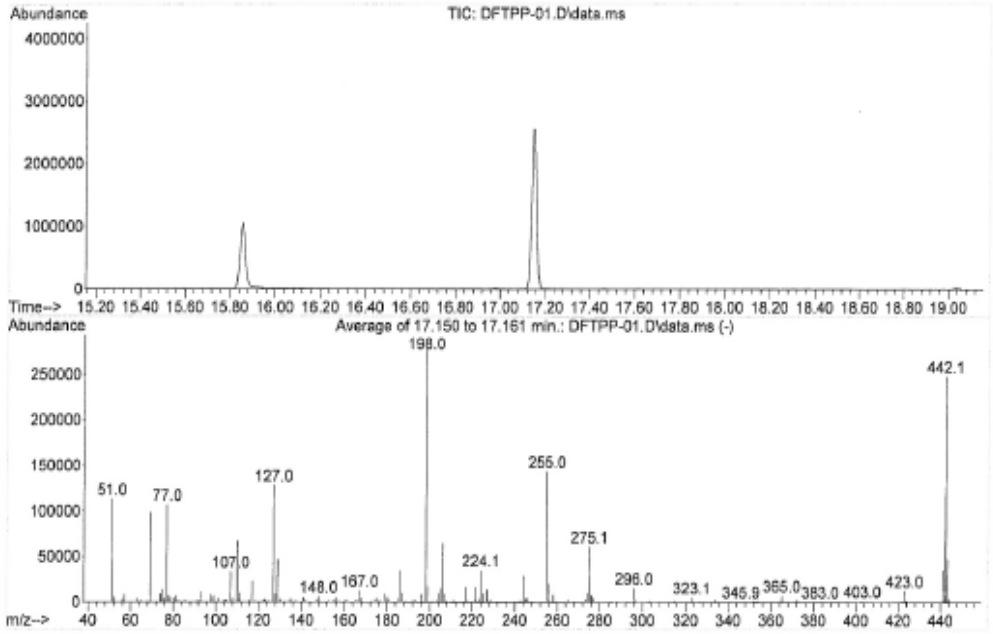
Appendix D: Tune Evaluation Reports

DFTPP

Data Path : C:\msdchem\1\DATA\140103A\
Data File : DFTPP-01.D
Acq On : 3 Jan 2014 12:09 pm
Operator : GKJ
Sample : 50 ng DFTPP
Misc : 1301207
ALS Vial : 2 Sample Multiplier: 1

Integration File: rteint.p

Method : C:\msdchem\1\METHODS\DFTPP625.M
Title : DFTPP Tune Check (GCMS3)
Last Update : Wed Nov 30 08:46:16 2011



AutoFind: Scans 2415, 2416, 2417; Background Corrected with Scan 2406

Target Mass	Rel. to Mass	Lower Limit%	Upper Limit%	Rel. Abn%	Raw Abn	Result Pass/Fail
51	198	30	60	40.5	113253	PASS
68	69	0.00	2	1.7	1723	PASS
69	198	0.00	100	35.2	98626	PASS
70	69	0.00	2	0.6	635	PASS
127	198	40	60	45.8	128328	PASS
197	198	0.00	1	0.5	1306	PASS
198	198	100	100	100.0	279893	PASS
199	198	5	9	6.4	17865	PASS
275	198	10	30	21.9	61301	PASS
365	198	1	100	2.7	7446	PASS
441	443	0.01	100	75.0	36176	PASS
442	198	40	100	88.9	248960	PASS
443	442	17	23	19.4	48232	PASS

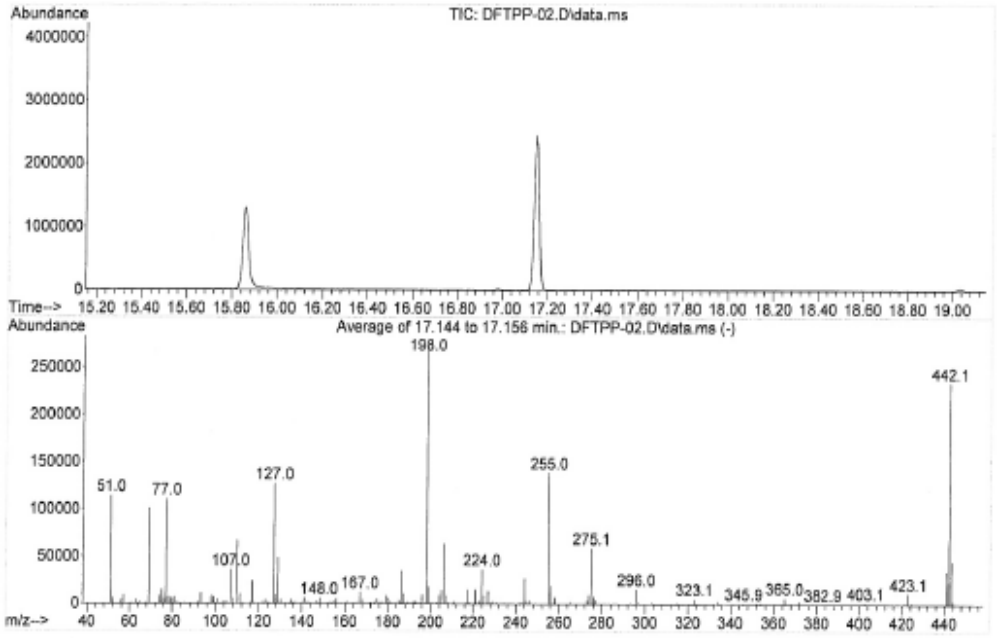
DFTPP625.M Mon Jan 06 12:40:46 2014 Page: 1

DFTPP

Data Path : C:\msdchem\1\DATA\140103A\
Data File : DFTPP-02.D
Acq On : 3 Jan 2014 11:19 pm
Operator : GKJ
Sample : 50 ng DFTPP
Misc : 1301207
ALS Vial : 21 Sample Multiplier: 1

Integration File: rteint.p

Method : C:\msdchem\1\METHODS\DFTPP625.M
Title : DFTPP Tune Check (GCMS3)
Last Update : Wed Nov 30 08:46:16 2011



AutoFind: Scans 2414, 2415, 2416; Background Corrected with Scan 2405

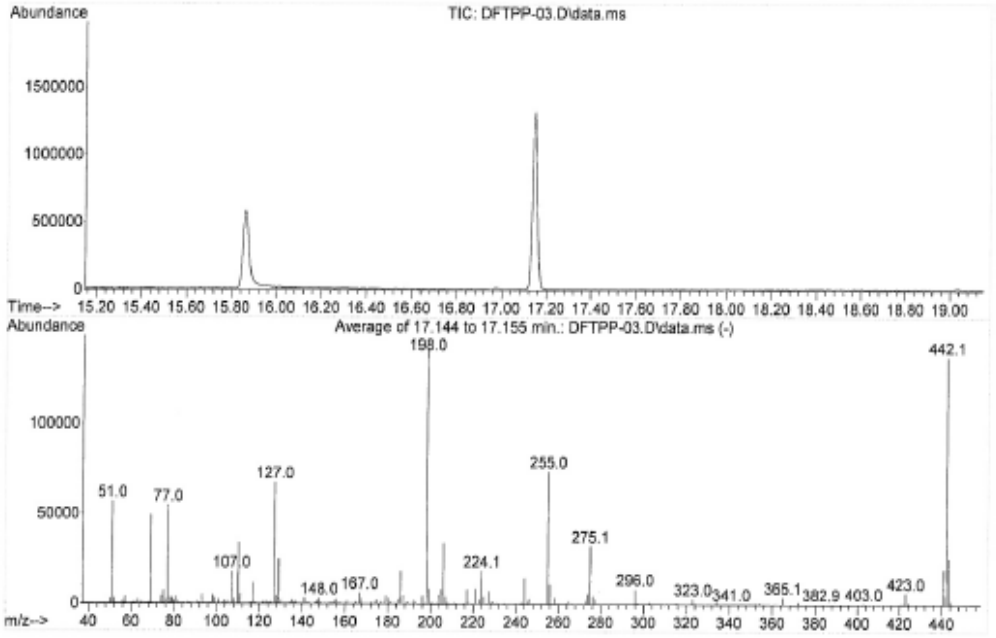
Target Mass	Rel. to Mass	Lower Limit%	Upper Limit%	Rel. Abn%	Raw Abn	Result Pass/Fail
51	198	30	60	42.3	114272	PASS
68	69	0.00	2	1.3	1324	PASS
69	198	0.00	100	37.3	100888	PASS
70	69	0.00	2	0.4	415	PASS
127	198	40	60	47.1	127360	PASS
197	198	0.00	1	0.5	1234	PASS
198	198	100	100	100.0	270144	PASS
199	198	5	9	6.8	18461	PASS
275	198	10	30	22.0	59464	PASS
365	198	1	100	2.6	6971	PASS
441	443	0.01	100	77.1	35320	PASS
442	198	40	100	87.0	234965	PASS
443	442	17	23	19.5	45787	PASS

DFTPP

Data Path : C:\msdchem\1\DATA\140103A\
Data File : DFTPP-03.D
Acq On : 4 Jan 2014 11:05 am
Operator : GKJ
Sample : 50 ng DFTPP
Misc : 1301207
ALS Vial : 41 Sample Multiplier: 1

Integration File: rteint.p

Method : C:\msdchem\1\METHODS\DFTPP625.M
Title : DFTPP Tune Check (GCMS3)
Last Update : Wed Nov 30 08:46:16 2011



AutoFind: Scans 2414, 2415, 2416; Background Corrected with Scan 2406

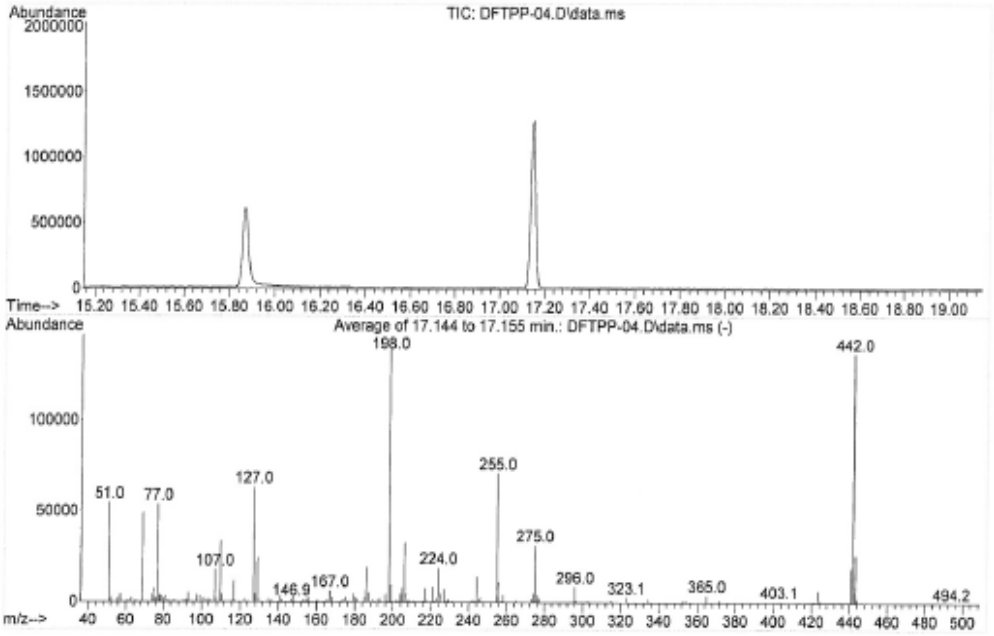
Target Mass	Rel. to Mass	Lower Limit%	Upper Limit%	Rel. Abn%	Raw Abn	Result Pass/Fail
51	198	30	60	40.1	56897	PASS
68	69	0.00	2	1.7	823	PASS
69	198	0.00	100	34.9	49543	PASS
70	69	0.00	2	0.7	362	PASS
127	198	40	60	47.5	67477	PASS
197	198	0.00	1	0.5	717	PASS
198	198	100	100	100.0	142000	PASS
199	198	5	9	6.0	8586	PASS
275	198	10	30	22.8	32328	PASS
365	198	1	100	2.8	3998	PASS
441	443	0.01	100	77.4	19800	PASS
442	198	40	100	97.0	137781	PASS
443	442	17	23	18.6	25597	PASS

DFTPP

Data Path : C:\msdchem\1\DATA\140103A\
Data File : DFTPP-04.D
Acq On : 4 Jan 2014 11:00 pm
Operator : GKJ
Sample : 50 ng DFTPP
Misc : 1301207
ALS Vial : 61 Sample Multiplier: 1

Integration File: rteint.p

Method : C:\msdchem\1\METHODS\DFTPP625.M
Title : DFTPP Tune Check (GCMS3)
Last Update : Wed Nov 30 08:46:16 2011



AutoFind: Scans 2414, 2415, 2416; Background Corrected with Scan 2405

Target Mass	Rel. to Mass	Lower Limit%	Upper Limit%	Rel. Abn%	Raw Abn	Result Pass/Fail
51	198	30	60	39.0	54628	PASS
68	69	0.00	2	1.4	683	PASS
69	198	0.00	100	35.0	49101	PASS
70	69	0.00	2	1.1	523	PASS
127	198	40	60	45.0	63147	PASS
197	198	0.00	1	0.4	602	PASS
198	198	100	100	100.0	140219	PASS
199	198	5	9	6.7	9350	PASS
275	198	10	30	22.4	31435	PASS
365	198	1	100	2.8	3988	PASS
441	443	0.01	100	73.3	19088	PASS
442	198	40	100	97.8	137141	PASS
443	442	17	23	19.0	26040	PASS

Appendix E: Surrogate Recovery and Internal Standard Reports

GC/MS QA-QC Check Report

Tune File : C:\msdchem\1\DATA\140103A\DFTPP-01.D
Tune Time : 3 Jan 2014 12:09 pm

Daily Calibration File : C:\msdchem\1\DATA\140103A\0103-02.D

1113810 627235 1182320

1274790

File	Sample	Surrogate Recovery %			Internal Standard Responses		
0103-03.D							
	BLANK #1	66	77	83	1133921	633995	1183478
			1234789				
0103-04.D							
	BLANK #2	66	77	80	1240459	684778	1251143
			1272911				
0103-05.D							
	BLANK #3	71	81	88	1276813	700428	1271783
			1267255				
0103-06.D							
	BLANK #4	63	74	82	1403623	799923	1460290
			1493705				
0103-07.D							
	BLANK #5	82	94	100	1096496	615731	1164674
			1224645				
0103-08.D							
	3	70	70	77	1143523	642345	1165729
			1182280				
0103-09.D							
	30	67	72	82	1213895	687706	1277134
			1302073				
0103-10.D							
	1	74	62	78	1326176	740582	1347324
			1345479				
0103-11.D							
	43	71	58	75	1286440	740381	1334912
			1332476				
0103-12.D							
	5	78	67	78	1259803	728184	1367514
			1366157				
0103-13.D							
	28	80	77	77	1269367	730703	1349232
			1338689				
0103-14.D							
	50	83	73	82	1243284	721488	1336216
			1351074				
0103-15.D							
	32	82	72	82	1291855	732417	1355261
			1338739				
0103-16.D							

29	58	69	75	1256799	701909	1299208
			1264466			
0103-17.D						
6	75	70	81	1330232	762688	1383024
			1366068			
0103-18.D						
24	74	69	74	1336652	770144	1433965
			1406258			
0103-19.D						
4	73	71	77	1400982	796193	1451842
			1408819			

(fails) - fails 12hr time check * - fails criteria

Created: Mon Jan 06 12:37:20 2014 GCMS3

GC/MS QA-QC Check Report

Tune File : C:\msdchem\1\DATA\140103A\DFTPP-02.D
Tune Time : 3 Jan 2014 11:19 pm

Daily Calibration File : C:\msdchem\1\DATA\140103A\0103-20.D

1106910 625190 1208470

1272860

File	Sample	Surrogate Recovery %			Internal Standard Responses		
0103-21.D							
	42	59	61	79	1362750	758465	1392581
			1355323				
0103-22.D							
	47	83	74	86	1303834	746124	1421810
			1379235				
0103-23.D							
	49	75	66	76	1372083	786051	1465152
			1445328				
0103-24.D							
	44	84	84	87	1333743	757721	1412161
			1393424				
0103-25.D							
	34	72	67	85	1313538	765934	1415730
			1398059				
0103-26.D							
	38	70	59	78	1266282	736496	1363489
			1329706				
0103-27.D							
	22	71	71	83	1284081	743858	1428030
			1407504				
0103-28.D							
	2	61	62	63	1349087	758141	1415200
			1368436				
0103-29.D							
	23	61	75	80	1374178	781407	1460545
			1421586				
0103-30.D							
	31	72	78	86	1328126	745943	1375160
			1353309				
0103-31.D							
	27	78	72	88	1287866	745805	1390421
			1334514				
0103-32.D							
	39	69	68	88	1245290	715670	1334874
			1300637				
0103-33.D							
	26	63	74	84	1116871	638158	1158881
			1128212				
0103-34.D							

	3	71	69	81	1176320	658953	1218052
				1163422			
0103-35.D							
	30	51	51	53*	1220323	685238	1263465
				1222366			
0103-36.D							
	1	72	76	79	1148601	649060	1217836
				1170705			
0103-37.D							
	43	77	66	90	1321314	755060	1387878
				1343078			
0103-38.D							
	5	78	70	75	1292356	732997	1344310
				1316080			

(fails) - fails 12hr time check * - fails criteria

Created: Mon Jan 06 12:38:33 2014 GCMS3

GC/MS QA-QC Check Report

Tune File : C:\msdchem\1\DATA\140103A\DFTPP-03.D
Tune Time : 4 Jan 2014 11:05 am

Daily Calibration File : C:\msdchem\1\DATA\140103A\0103-39.D

1160460 663135 1236020

1250380

File	Sample	Surrogate Recovery %			Internal Standard Responses		
0103-40.D							
	28	79	62	79	1283095	737233	1361085
			1325213				
0103-41.D							
	50	74	73	82	1281755	739425	1400403
			1405251				
0103-42.D							
	32	76	81	84	1217604	691873	1276952
			1261609				
0103-43.D							
	29	65	67	70	1336345	770582	1428258
			1408037				
0103-44.D							
	6	77	68	75	1335753	746099	1373367
			1342229				
0103-45.D							
	24	83	73	79	1308981	740960	1376999
			1308677				
0103-46.D							
	4	64	63	75	1354675	772514	1439426
			1383506				
0103-47.D							
	42	60	66	77	1377396	792324	1508305
			1447682				
0103-48.D							
	47	83	66	80	1397324	786215	1431401
			1387430				
0103-49.D							
	49	68	71	78	1410829	798433	1497811
			1427836				
0103-50.D							
	44	66	65	74	1267430	720846	1325923
			1269200				
0103-51.D							
	34	56	62	72	1286375	725469	1308847
			1279209				
0103-52.D							
	38	71	62	76	1497878	850675	1556686
			1464399				
0103-53.D							

	22	48	51 63	1451880	843156	1587612
			1524121			
0103-54.D						
	2	76	73 78	1419505	808037	1545972
			1511256			
0103-55.D						
	23	57	61 80	1727293	975470	1767437
			1687830			
0103-56.D						
	31	76	68 81	1422177	812990	1500783
			1454264			
0103-57.D						
	27	73	72 81	1452875	828215	1521502
			1440344			

(fails) - fails 12hr time check * - fails criteria

Created: Mon Jan 06 12:39:43 2014 GCMS3

GC/MS QA-QC Check Report

Tune File : C:\msdchem\1\DATA\140103A\DFTPP-04.D
Tune Time : 4 Jan 2014 11:00 pm

Daily Calibration File : C:\msdchem\1\DATA\140103A\0103-58.D

1282880 720954 1411500

1394140

File	Sample	Surrogate Recovery %			Internal Standard Responses		
0103-59.D							
	39	60	67	83	1456941	825240	1481647
			1436909				
0103-60.D							
	26	61	70	84	1375307	759403	1393842
			1328660				

(fails) - fails 12hr time check * - fails criteria

Created: Mon Jan 06 12:40:11 2014 GCMS3