PREVALENCE OF ENDOCRINE DISRUPTING PHTHALATE ESTERS IN SELECTED FOODS AND FOOD WRAPPERS FROM SOME SUPERMARKETS AROUND PRETORIA, SOUTH AFRICA.

by

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DECLARATION

I, Ntsako Dellas Baloyi, hereby declare that this research project entitled "Prevalence of Endocrine Disrupting Phthalate Esters in Selected Foods and Food Wrappers from some supermarkets around Pretoria" was carried out by me as part of the funded research project by the South African National Research Foundation (NRF). I also declare that all the sources that I have used or quoted have been indicated and acknowledged by means of complete references. The dissertation has not been submitted or will not be submitted to a university or any institution for the award of a degree.

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Date

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ABSTRACT

Food is one of the main routes by which xenobiotic (synthetic) chemicals enter the body of man and wildlife. The routes could be from wrappers in which the foods are presented with possible transfer of the compounds to consumers, hence need for regular screening. The research work is aimed at investigating possible prevalence of phthalate esters in selected foods (cheese, polony and vienna) and their plastic wrappers from commercial stores in Tshwane metropolis. Food samples were purchased from selected stores, taken to the laboratory and stored at $4^{o}C$ until analysed. Analysis was done by soxhlet extraction while determination and quantification of phthalates was carried out using Gas Chromatography-Flame Ionization Detection (GC-FID). Quality assurance of the process was by standard addition of the phthalate ester standards.

Results obtained revealed good chromatographic separation of the analysed esters which ranged from 5.55 min for Dimethyl phthalate (DMP) to 8.96 min for Benzylbutyl phthalate (BBP). Instrumental detection limit of the esters varied from 0.03 - 0.05 µg/kg. The percentage recovery of the phthalate esters ranged from 75 – 90% from spiked cheese samples; 33 – 66% from spiked polony samples and 69 – 99% from spiked vienna samples. These recoveries are quite acceptable and applicable to the analysis and quantification of the compounds in the samples with the exception of Dibutyl phthalate (DBP) (33%); DMP (34%) and BBP (46 %) in polony samples. Results from chromatographic quantification revealed the absence of or non-detection of most of the analysed phthalate esters in the selected food samples. However, level of 0.031 µg/kg of BBP - 0.816 µg/kg of DMP were obtained in some of the analysed samples.

Keywords: Prevalence, Endocrine disrupting, Phthalate esters, Food wrappers, Foods, Pretoria, South Africa

ABBREVIATIONS AND ACRONYMS

ADHD	Attention Deficit/Hyperactivity Disorder
ADI	Allowed Daily Intake
ATSDR	Agency for Toxic Substances and Disease Registry
BBP	Benzyl butyl phthalate
BDL	Below detection limit
BPA	Bisphenol-A
CEDI	Cumulative Estimated Daily Intake
CERHR	Center for the Evaluation of Risks to Human Reproduction
CHLORO	Tetrachlorodecane
DBP	Dibutyl phthalate
DEHA	Di-2-ethylhexyl adipate
DEHP	Di-2-ethylhexyl phthalate
DMP	Dimethyl phthalate
DnBP	Di-n-butyl phthalate
ECPI	European Council for Plasticisers and Intermediates
EDs	Endocrine Disruptors
DEPA	Danish Environmental Protection Agency
EU	European Union
GC-FID	Gas Chromatography-Flame Ionization Detection
IDL	Instrument detection limit
IPCS	International Programme on Chemical Safety
MBP	Monobutyl Phthalate
NRF	National Research Foundations
PPARs	Peroxisome Proliferator-activated Receptors
PVC	Polyvinyl Chloride
RF	Response factor
RT	Retention time
SCHER	Scientific Committee on Health and Environmental Risks
SML	Specific Migration Limit
SRM	Standards Reference Materials

STDev	Standard deviation
TDI	Tolerable Daily Intake
US	United States
USNRC	United States Nuclear Regulatory Commission
WHO	World Health Organization

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CHAPTER 1

BACKGROUND INFORMATION

1.1 Introduction

Endocrine disruptors (EDs), sometimes also referred to as hormonally active agents are exogenous substances that act like hormones in the endocrine system and disrupt the physiologic function of endogenous hormones. Endocrine systems are found in most varieties of animal life. The endocrine system is made up of glands, which secrete hormones, and receptors which detect and react to the hormones. Studies have linked endocrine disruptors to adverse biological effects in animals, giving rise to concerns that low-level exposure might cause similar effects in human beings (Krimsky, 2001).

Hormones are released by glands and travel throughout the body, acting as chemical messengers. They interface with cells that contain matching receptors in or on their surfaces. The hormone binds with the receptor, much like a key would fit into a lock. Sex steroids such as estrogens and androgens, as well as thyroid hormones, are subject to feedback regulation, which tends to limit the effects of environmental chemicals.

The theory of endocrine disruption posits that low-dose exposure to chemicals that interact with hormone receptors can interfere with reproduction, development, and other hormonally mediated processes. Furthermore, since endogenous hormones are typically present in the body in relatively tiny concentrations, the theory holds that exposure to relatively small amounts of exogenous hormonally active substances can disrupt the proper functioning of the body's endocrine system. Thus, an endocrine disruptor might be able to elicit adverse effects at much lower doses than a toxicant acting through a different

mechanism. The timing of exposure is also presumed to be critical, since different hormone pathways are active during different stages of development.

While a few sources of human exposure to EDs are from natural sources, the overwhelming majority are from thousands of manmade synthetic products. It is inaccurate to place blame on one chemical since exposure is through multiple paths and substances, each contributing to the cumulative total. In spite of present regulations being aimed at each chemical individually, real world combinations are infinite and have unpredictable effects.

A significant exposure to EDs is from plastic, which is displacing natural products at an ever increasing pace. Less than 50 years ago plastic products were considered inferior and people lived healthy, productive lives without them. Polyvinyl chloride (PVC) probably contributes the greatest exposure to EDs of all plastics. It is toxic during production, use and when it is disposed of. World production capacity of PVC in 1998 was 27 million tons (Rogan and Rogan 2003). It was made into residential and municipal water pipes, toys, food wrap, clothing, raincoats, shoes, building products such as windows, siding, roofing, flooring, and medical equipment such as hospital blood bags, tubing and many other devices.

Food and bodily contact with polyvinylchloride is hazardous because of the various plasticizers and additives utilized in it. Bisphenol-A (BPA) is the most common plasticizer in PVC. BPA leaches into liquid and fatty products packaged in it. Flexible PVC products can be more than half plasticizers by weight, but the constituent chemicals vary between products and manufacturers. Plasticizers account for more than half the weight of some flexible PVC products. About 95% of phthalates are used in PVC (Latini et al., 2004a).

Polyvinyl chloride is generally not recycled. Since a great deal of PVC is disposed of by incineration, dioxin will be created again. Ironically, this was called

recycling by the plastics industry, and was included in official recycling statistics (McEwen et al., 2008).The incinerated PVC creates dioxin. Again, industry readily accepted the cancers, endocrine system dysfunction, and environmental pollution because the costs had been externalized. Phthalate esters are one of the major endocrine disruptors.

Phthalates esters belong to a group of industrial compounds with a common chemical structure, dialkyl or alkyl/aryl esters of 1,2-benzenedicarboxylic acid. Since about the 1930s phthalates have been used for a variety of purposes, including personal-care products (e.g. perfumes, lotions, and cosmetics), paints, industrial plastics, and certain medical devices and pharmaceuticals (Blount et al., 2000; ATSDR, 2001). Some phthalates are commonly added to these commercial products to hold color or fragrance, to provide a film or gloss, or, in the case of some pharmaceuticals, to provide timed releasing. However, phthalates are primarily used as plasticizers to impart flexibility to an otherwise rigid polyvinylchloride (Kirkpatrick et al., 1989).

Phthalates are not bound chemically in the plastics and can consequently, penetrate these materials and migrate into food that comes into contact with it. The presence of phthalates in packaging materials and their migration into wrapped foods have been confirmed by a number of authors (Castle et al., 1988; Page and Lacroix, 1992; Nerin et al., 1993).

The amount of phthalates in wrapped foods depends on many factors including the concentration of phthalates in the wrapping material or printing ink, storage period, storage temperature, fat content in the food and the contact area. A few of the possible health effects include; birth defects; alterations in sexual and functional development (Safe, 2000), immunologic disorders, early puberty in young girls (Hayes et al., 2006), breast (USNRC, 2005). colon, vaginal, cervix and testicular cancers, sexual differentiation of the brain and other estrogen target tissues, structural abnormalities of the oviduct, uterus, cervix and vagina, a

contributing factor to subfertility, reduced physical stamina (Erickson et al., 2008), genital birth defects: hypospadias and cryptorchidism (Bets, 2008) altered anogenital distance in male (Fisher, 2004), reduced sperm counts (Swan et al., 2005), and enlargement/reduction of prostate, developmental, behavioral and mental disorders, anger, inattention, decreased mental capacity, learning disabilities, dyslexia, attention deficit/hyperactivity disorder (ADHD), autism, propensity to violence, reduced motor skills, and gross and fine eye-hand coordination (Chou et al., 2002).

High doses of many phthalates have shown hormonal activity in rodent studies, these studies on rodents involving large amounts of phthalates have shown damage to the liver and testes and cause birth defects. In addition, a British study showed that the phthalate di (n-butyl) phthalate (DBP) and its metabolite monobutyl phthalate (MBP) suppresses steroid genesis by fetal-type Leydig cells in primates as in rodents (Hallmark et al., 2007)

All people are exposed to chemicals with estrogenic effects in their everyday life, because endocrine disrupting chemicals are found in low doses in literally thousands of products. Chemicals commonly detected in people include Bisphenol A, Polybrominated diphenyl ethers, and a variety of Phthalates (EU Restrictions on the use of phthalates in toys, European Council for Plasticisers and Intermediates (ECPI). There is some dispute in the scientific community surrounding the claim that these chemical actually disrupt the endocrine system. Many believe that there is little evidence that the degree of exposure in humans is enough to warrant concern, while many others believe there is evidence that these chemicals pose some risk to human health (Bornehag et al., 2004; Swan et al., 2005; Hallmark et al., 2007).

1.2 Problem statement

- Phthalate esters are compounds that are known to be toxic to human; some have been found to be endocrine disruptors.
- They are primarily used as plasticizers in wrappers which have been found to have contaminated foods in which they are wrapped in. Once these foods are consumed by human, the compounds are also transferred into the human body.
- They are also responsible for many ailments.

1.3 Rationale/justification of the study

Food is one of the basic substances required for the sustenance of life (Stubbs et al., 2006). Incidentally, it is one of the major modes by extraneous and unwanted substances including chemicals are acquired by man and wildlife. The consumption of safe and uncontaminated food is one of the major priorities of the South African government. This is handled and ensured through governmental agencies such as the Departments of Health, agriculture, Water Affairs and Forestry and the Medical Research Council.

The use of packaging materials for foods which might cause serious acute and possibly chronic health problems to citizens should be a matter of priority. Superficially, it does not appear that there could be serious health risks associated with wrapping of consumed foods with plastic films and papers, especially those that are in direct contact with the foods. However, chronic problems that are known to be associated with additives that are present in wrapping materials such as platicisers e.g. phthalates esters in adhesives and printing inks deserve utmost attention.

Plasticisers have been shown to elute at a constant rate from plastic products to the environment. Increasing chemical use needs a better understanding of how these pollutants may affect human health. In particular, certain members of this chemical class, such as di-[2-ethylhexyl]-phthalate (DEHP), have been shown to cause reproductive and developmental toxicity and are suspected to be endocrine disruptors (Latini et al., 2004b). Phthalate plasticizers, in particular dibutyl phthalate (DBP) and di (2-ethylhexyl) phthalate (DEHP) are known to have serious health effects in human. Reproductive effects such as chronic renal disease have been observed in experimental animals treated with di (n-butyl) phthalate (DBP), a phthalate esters used in soft plastics and a variety of consumer products (Takeuchi et al., 2004).

Phthalate esters have been detected in several materials and media such as in tap and bottled mineral water, in baby food and infant formulae (Petersen et al., 2000), in women (Adibi et al., 2003), soil, river sediment (Chan et al., 1994) and in air (Toda et al., 2004).

Research into the presence of Phthalate Esters in packaging materials in South Africa is very limited. Fatoki et al. (2001) reported the gross pollution of river and marine water samples in the Eastern Cape region by several phthalate esters such as dimethyl phthalate (DMP), diethyl phthalate (DEP), dibutyl phthalate (DBP) and diethylhexyl phthalate (DEHP). This study, which was reported in the year 2001, showed that these compounds are prevalent in our environment. However, there has been limited investigation into their possible presence in several other media such as food, air, soil and even human in the country. This necessitates the need to conduct a comprehensive survey of phthalates in packaging materials in South Africa. The outcome of this research might shed more light and provide a new dimension into probable causes of some health problems in our society.

Several phthalates and their metabolic products have been shown to be developmental and reproductive toxicants affecting particularly male reproductive development (Parks et al., 2000; Ema et al., 2001) and are suspected of having endocrine disrupting or modulating effects (Latini et al., 2004). An endocrine disruptor is a chemical with the potential to alter hormone action within the body (Sharpe et al., 2004). Thus, these chemicals have been found to interfere with the function of the endocrine system, which is responsible for growth, sexual development and many other essential physiological functions both in males and females (Lovekamp et al., 2003).

1.4 Research questions

Based on the above mentioned possible health implications associated with usage of plastic wrappers for food, the following research questions arose:

- What are the types/kinds of foods that are presented to consumers in these plastic containers/wrappers?
- Are these endocrine disrupting phthalate esters present in the plastic containers used to wrap the sampled foods?
- If present, at what levels do they exist in the samples?
- Are the levels within or above acceptable limits?
- What are the health implications of the presence of these compounds in wrappers and foods?
- Are there alternative wrappers that could be used for wrapping foods which do not contain these toxic plasticizers?

1.5 Research aims and Objectives

The main thrust of this research is to carry out a survey of the prevalence of endocrine disrupting phthalate esters in selected consumed foods and food wrappers around Tshwane metropolis. In order to achieve this, the following specific investigations would be performed:

- To evaluate and optimize soxhlet technique for the extraction of phthalates esters in samples.
- To detect phthalates esters in food samples (cheese, polony and vienna) and food wrappers
- To quantify and compare obtained data/results with data from developed and undeveloped countries with reference to National and EU regulations.
- To make applicable recommendations to the relevant South African government departments such as the Department of Agriculture and Department of Science and Technology with regards to the results obtained.

CHAPTER 2

LITERATURE REVIEW

2.1 Introduction

This chapter discusses the arguments and findings of a number of researchers with regards to PEs found in selected foods (cheese, polony and vienna) and their plastic wrappers. Issues that will be addressed in this chapter include the history, properties as well as health and environmental effects of PEs. It also examines the various methods used by other researchers worldwide to determine PEs on those selected food samples.

2.2 Properties of phthalate esters

Phthalate esters are the dialkyl or alkyl aryl esters of phthalic acid (also called 1,2-benzenedicarboxylic acid); the name phthalate derives from phthalic acid, which itself is derived from word "naphthalene". When added to plastics, phthalate esters allow the long polyvinyl molecules to slide against each another. The phthalates have low water solubility, high oil solubility, and low volatility. The polar carboxyl group contributes little to the physical properties of the phthalates, except when R and R' are very small (such as ethyl and methyl groups). They are colourless, odourless liquids produced by reacting phthalic anhydride with an appropriate alcohol. Kembra et al., 2008) below is a general structure of phthalate ester.

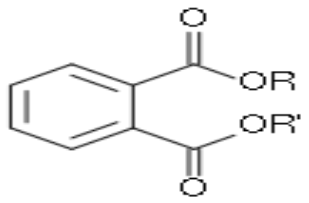

Figure 2.1: General structure of phthalate ester

Table 2.1: List of the most common phthalates esters

Name	Structural formula	CAS No.
Dimethyl phthalate	$C_6H_4(COOCH_3)_2$	131-11-3
Diethyl phthalate	$C_6H_4(COOC_2H_5)_2$	84-66-2
Diallyl phthalate	$C_6H_4(COOCH_2CH=CH_2)_2$	131-17-9
Di-n-propyl phthalate	$C_6H_4[COO(CH_2)_2CH_3]_2$	131-16-8
Di-n-butyl phthalate	$C_6H_4[COO(CH_2)_3CH_3]_2$	84-74-2
Diisobutyl phthalate	$C_6H_4[COOCH_2CH(CH_3)_2]_2$	84-69-5
Butylcyclohexyl phthalate	$CH_3(CH_2)_3OOCC_6H_4COOC_6H_{11}$	84-64-0
Di-n-pentyl phthalate	$C_6H_4[COO(CH_2)_4CH_3]_2$	131-18-0
Dicyclohexyl phthalate	$C_6H_4[COOC_6H_{11}]_2$	84-61-7
Butylbenzyl phthalate	$CH_3(CH_2)_3OOCC_6H_4COOCH_2C_6H_5$	85-68-7
Di-n-hexyl phthalate	$C_6H_4[COO(CH_2)_5CH_3]_2$	84-75-3
Diisohexyl phthalate	$C_6H_4[COO(CH_2)_3CH(CH_3)_2]_2$	146-50-9
Diisoheptyl phthalate	$C_6H_4[COO(CH_2)_4CH(CH_3)_2]_2$	41451-28-9
Butyldecyl phthalate	$CH_3(CH_2)_3OOCC_6H_4COO(CH_2)_9CH_3$	89-19-0
Di(2-ethylhexyl) phthalate	$C_6H_4[COOCH_2CH(C_2H_5)(CH_2)_3CH_3]_2$	117-81-7
Di(n-octyl) phthalate	$C_6H_4[COO(CH_2)_7CH_3]_2$	117-84-0
Diisooctyl phthalate	$C_6H_4[COO(CH_2)_5CH(CH_3)_2]_2$	27554-26-3
n-Octyl-n-decyl phthalate	$CH_3(CH_2)_7OOCC_6H_4COO(CH_2)_9CH_3$	119-07-3
Diisononyl phthalate	$C_6H_4[COO(CH_2)_6CH(CH_3)_2]_2$	28553-12-0
Diisodecyl phthalate	$C_6H_4[COO(CH_2)_7CH(CH_3)_2]_2$	26761-40-0
Diundecyl phthalate	$C_6H_4[COO(CH_2)_{10}CH_3]_2$	3648-20-2
Diisoundecyl phthalate	$C_6H_4[COO(CH_2)_8CH(CH_3)_2]_2$	85507-79-5
Ditridecyl phthalate	$C_6H_4[COO(CH_2)_{12}CH_3]_2$	119-06-2
Diisotridecyl phthalate	$C_6H_4[COO(CH_2)_{10}CH(CH_3)_2]_2$	68515-47-9
Polyethylene terephthalate	$[C_{10}H_80_4]_n$	25038-59-9

(Source: www.wikipedia.com/phthalate esters; 2008/06/08)

2.3 Uses of phthalate esters

Phthalates are used in a large variety of products, from enteric coatings of pharmaceutical pills and nutritional supplements to viscosity control agents, gelling agents, film formers, stabilizers, dispersants, lubricants, binders, emulsifying agents, and suspending agents (Kirkpatrick et al., 1989). End applications include adhesives and glues, agricultural adjuvants, building materials, personal care products, medical devices, detergents and surfactants, packaging, children's toys, modelling clay, waxes, paints, printing inks and coatings, pharmaceuticals, food products and textiles (Shanker et al., 1985; Ling et al., 2007; Huang et al 2008). Phthalates are also frequently used in soft plastic fishing lures, caulk, paint pigments, and sex toys made of so-called "jelly rubber." They are used in a variety of household applications such as shower curtains, vinyl upholstery, adhesives, floor tiles, food containers and wrappers, and cleaning materials. Personal care items containing phthalates include perfume, eye shadow, moisturizer, nail polish, liquid soap, and hair spray (Rudel et al., 2008). They are also found in modern electronics and medical applications such as catheters and blood transfusion devices.

The most widely-used phthalates are the di-2-ethyl hexyl phthalate (DEHP), the diisodecyl phthalate (DIDP) and the diisononyl phthalate (DINP). DEHP is the dominant plasticizer used in PVC, due to its low cost. Benzylbutyl phthalate (BBzP) is used in the manufacture of foamed PVC, which is mostly used as a flooring material (Rudel et al., 2008).

2.4 Migration of Phthalates from packaging materials into food

Food packaging can interact with the packaged foodstuff by diffusion-controlled process which mainly depends on the chemical properties of the food contact materials and the foodstuff, temperatures at packaging, during heat treatment

and storage, exposure to UV light, and storage time of the product (Arvanitoyanis and Bosnea, 2004). This interaction can lead to food contact materials leaching from the packaging to the food. Compounds that leach from the packaging materials are substances used in the polymerisation step, like monomers or catalysts, and additives that are included during the manufacturing process to achieve special material properties (e.g. plasticisers for material softening) (Muncke, 2009).

2.5 Human exposure to phthalate esters

Human exposure is found in all individuals and can occur via ingestion, inhalation, dermal skin absorption, as well as through intravenous injections and placental routes. Phthalates are easily released into the environment because there is no covalent bond between them and plastics in which they are mixed. As plastics age and break down, the release of phthalates accelerates. Phthalates in the environment are subject to biodegradation, photo degradation, and anaerobic degradation therefore they do not generally persist in the outdoor environment. Outdoor air concentrations are higher in urban and suburban areas than in rural and remote areas (Rudel et al., 2008). Indoor air concentrations are generally higher than outdoor air concentrations due to the nature of the sources. Because of their volatility, DEP and DMP are present in higher concentrations in air in comparison with the heavier and less volatile DEHP. Higher air temperatures result in higher concentrations of phthalates in the air. PVC flooring leads to higher concentrations of BBP and DEHP which are more prevalent in dust (Rudel et al., 2008).

People are commonly exposed to phthalates, and the majority of Americans tested by the Centres for Disease Control and Prevention had metabolites of multiple phthalates in their urine (Heudorf et al., 2007). Because phthalate plasticizers are not chemically bound to PVC, they can easily leach and

evaporate into food or the atmosphere. Phthalate exposure can be through direct use or indirectly through leaching and general environmental contamination. Diet is believed to be the main source of DEHP and other phthalates in the general population. Fatty foods such as milk, butter, cheese, polony, vienna and meats are a major source. Low molecular weight phthalates such as DEP, DBP, BBzP may be dermally absorbed. Inhalational exposure is also significant with the more volatile phthalates (Heudorf et al., 2007).

A study conducted by Kolarik et al. (2008) in Bulgaria, revealed that higher dust concentrations of DEHP were found in homes of children with asthma and allergies, compared with healthy children's homes. The same study also found that DEHP, BBzP, and DnOP were in significantly higher concentrations in dust samples collected in homes where polishing agents were used. Data on flooring materials was collected, but there was no significant difference in concentrations between homes where no polish was used that have balatum (PVC or linoleum) flooring verses homes with wood. High frequency of dusting did decrease the concentrations of DEHP, BBzP and DnOP.

Children's exposure to phthalates generally is greater than adults. In a 1990's Canadian study that modelled ambient exposures, it was estimated that daily exposure to DEHP was 9 mcg/kg bodyweight per day in infants, 19 mcg/kg bodyweight per day in toddlers, 14 mcg/kg bodyweight per day in children, and 6 mcg/kg bodyweight per day in adults (Heudorf et al., 2007). Infants and toddlers are at the greatest risk of exposure due to their mouthing behaviour. Body care products containing phthalates are a source of exposure for infants. The authors of a 2008 study observed that reported use of infant lotion, infant powder, and infant shampoo were associated with increased infant urine concentrations of phthalate metabolites, and this association is strongest in younger infants. These findings suggest that dermal exposures may contribute significantly to phthalate body burden in this population. Though they did not examine health outcomes, they noted that young infants are more vulnerable to the potential adverse effects

of phthalates given their increased dosage per unit body surface area, metabolic capabilities, and developing endocrine and reproductive systems (Sathyanarayana et al., 2008).

In 2008, the Danish Environmental Protection Agency (DEPA) found a variety of phthalates in erasers and warned of health risks when children regularly suck and chew on them. However, the European Commission Scientific Committee on Health and Environmental Risks (SCHER) considers that, even in the case when children bite off pieces from erasers and swallow them, it is unlikely that this exposure leads to health consequences (http://copublications.greenfacts.org/en/phthalates-school-supplies)

Phthalates are also found in medications, where they are used as inactive ingredients in producing enteric coatings. It is not known how many medications are made using phthalates, but some include omeprazole, didanosine, mesalamine, and theophylline. A recent study found that urinary concentrations of monobutyl phthalate, the DBP metabolite, of mesalamine users was 50 times higher than the mean of nonusers (some formulations of mesalamine do not contain phthalates) (Hernández-Díaz et al., 2009). The study showed that exposures from phthalate containing medications can far exceed population levels from other sources (Hernández-Díaz et al., 2009). DBP in medications raises concern about health risks due to the high level of exposures associated with taking these medications especially in vulnerable segments of the population, including pregnant women and children (Hernández-Díaz et al., 2009).

Also in 2008, the United States National Research Council recommended that the cumulative effects of phthalates and other antiandrogens be investigated. It criticized US EPA guidance, which stipulated that when examining cumulative effects, the chemicals examined should have similar mechanisms of action and/or similar structures, as too restrictive. Instead, it recommended that the

effects of chemicals which cause similar adverse outcomes should be examined cumulatively (Hallmark et al., 2007). Thus the effect of phthalates should be examined together with other antiandrogens, which otherwise may have been excluded because their mechanisms or structure were different.

2.6 Health effects associated with phthalate esters

In studies of rodents exposed to certain phthalates, high doses have been shown to change hormone levels and cause birth defects. A British study showed that the phthalate di (n-butyl) phthalate (DBP) or its metabolite monobutyl phthalate (MBP) suppresses steroidogenesis by fetal-type Leydig cells in primates as in rodents (Hallmark et al., 2007).

A study published in 2005 reported that human phthalate exposure during pregnancy resulted in decreased anogenital distance among baby boys. In this study phthalate metabolites were measured in urine samples collected from the pregnant women who gave birth to the infants. After birth, the genital features and anogenital distance of those women's babies were measured and correlated with the residue levels in their mother's urine. Boys born to mothers with the highest levels of phthalates were 7 times more likely to have a shortened anogenital distance (Swan et al., 2005). An editorial concerning this paper in the same volume stated that the study population was small, and needs to be investigated more thoroughly in a larger, more diverse population (Barrett et al., 2005). While anogenital distance was routinely used as a measure of foetal exposure to endocrine disruptors in animals (Salazar-Martinez et al., 2004), this parameter was rarely assessed in humans, and its significance was doubtful (Postellon et al., 2008).

The Swan study is thought by some to suggest that male reproductive development in humans could be affected by prenatal exposure to

environmentally relevant levels of phthalates (Tilson, 2008). Some authors of a study of boys with undescended testis hypothesized that exposure to a combination of phthalates and anti-androgenic pesticides may have contributed to that condition (Toppari et al., 2006). In contrast to the Swan study, an earlier study found that adolescents exposed to significant quantities of DEHP as neonates showed no significant adverse effects on their physical growth and pubertal maturity (Rais-Bahrami et al., 2004). This study, however, examined children exposed intravenously to phthalate diesters, and intravenous exposure results in little metabolic conversion of the relatively nontoxic phthalate diester to its more toxic monoester metabolite (Huber et al., 1996). There may be link between the obesity epidemic and endocrine disruption and metabolic interference. Studies conducted on mice exposed to phthalates in utero did not result in metabolic disorder in adults (Desvergne et al., 2009), Although, in a national cross-section of U.S. men, concentrations of several prevalent phthalate metabolites showed statistically significant correlations with abnormal obesity and insulin resistance (Rais-Bahrami et al., 2004).

Mono-ethyl-hexyl-phthalate, a metabolite of DEHP, has been found to interact with all three peroxisome proliferator-activated receptors (PPARs). PPARs are members of the nuclear receptor superfamily. The author states that the roles of PPARs in lipid and carbohydrate metabolism raised the question of their activation by a sub-class of pollutants tentatively named metabolic disrupters. Phthalates belong to this class of metabolic disruptors. It is a possibility that over many years of exposure to these metabolic disruptors living cells are able to deregulate complex metabolic pathways in a subtle manner (Desvergne et al., 2009).

In 2004, a joint Swedish-Danish epidemiologic team found a link between allergies in children and the phthalates DEHP and BBzP. Their review article and meta-analysis of published data relating to phthalates and asthma found a correlation between phthalates in the home and asthma especially in children,

but this evidence was limited by imprecise data on exact levels of exposure (Bornehag et al., 2004). In 2007, a cross-sectional study of U.S. males concluded that urine concentrations of four phthalate metabolites correlate with waist size and three phthalate metabolites correlate with the cellular resistance to insulin, a precursor to Type II diabetes. The authors noted the need for follow-up longitudinal studies, as waist size is known to correlate with insulin resistance to diabetes (Stahlhut et al., 2007).

2.7 Regulation of food contact materials and compounds

European Union (EU) regulation focuses on substance migration from the packing materials into foodstuff while the United States (US) regulation is based on estimated consumer exposure.

Table 2.2: Conversions for migration, exposure and risk assessment of food contact material migrants in United States and European Union.

	US	EU
Food/packaging ratio	1kg food/6.45 dm^2(10 g/in^2	1 kg food/6 dm^2 (11 g/in^2)
Weight per person	60 kg	60 kg
Food consumption per day	3 kg all foods (solid + liquid)	1 kg of any given food
Risk management tool	ADI based on CEDI	SML based on TDI

NB: kg/dm^2 refers to surface of the packaging in contact with foodstuff.

According to the US food contact regulation, the cumulative estimated daily intake of 1.5 µg/person/day or below is recommended. In the EU, a 60 mg/kg or less is recommended in terms of leaching from packaging into foodstuff. (Muncke, 2009).

According to the EU Scientific Committee for Food (SCF) the tolerable daily intakes of 0.1mg/kg bodyweight/day for DBP and 0.05mg/kg bodyweight/day for BBP and DEHP have been recommended. By convention, these TDI-values are equivalent to specific migration limits of 6mg/kg for DBP, 3mg/kg for BBP and DEHP and 18mg/kg for DEHA in foods. These calculations of specific migration limits are, by convention, based on a daily intake of 1 kg food by an adult person weighing 60 kg (Jens and Torben., 2000).

CHAPTER 3

METHODOLOGY

3.1 Introduction

This chapter presents the study area, methodology, the experimental procedures used in the study as well as the sampling procedure. Selected packaged food (cheese, polony and vienna) samples were purchased for phthalate esters analysis. The methodology for the determination of phthalate esters from purchased samples involved a four-step procedure: sampling, extraction by soxhlet technique, cleanup by Florisil column and analysis by Gas Chromatography-Flame Ionized Detector (GC-FID). The steps are described in the next section.

3.2 Study area

The study area is in Pretoria within Tshwane metropolis. Pretoria is a city located in the northern part of Gauteng Province, South Africa. It is one of the country's three capital cities, serving as the executive (administrative) and national capital; the others are Cape Town, the legislative capital, and Bloemfontein, the judicial capital. Pretoria is contained within the City of Tshwane Metropolitan Municipality. The geographical site of the study area is represented in the next page.

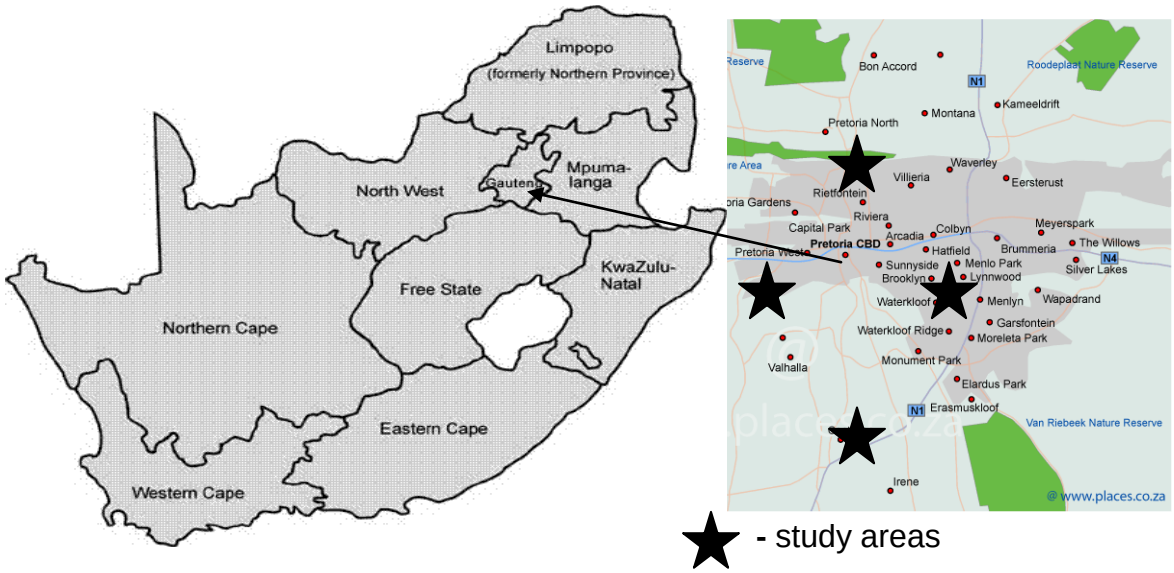

Figure 3.1: Map of South Africa

3.3 Samples and Reagents

3.3.1 Samples

The food samples that were used to investigate the presence of phthalate esters were the following:

1. Cheese
2. Polony and
3. Vienna
4. The thin plastic film wrappers of these foods were also investigated for the presence of phthalate esters.

A total number of 115 food samples (Vienna, Polony and Cheese) were purchased from food stores and analyzed during the study. The sampling method adopted was the non-probability judgmental method since the population of analyzed samples comes from the same city (Tshwane) even though the samples are available in other cities of the country.

Cheese **Polony** **Vienna**

Figure 2.2: Pictorial representation of analyzed food samples

3.3.2 Chemicals/reagents

The percentage purities and suppliers of the chemical used in this research work are as presented below:

- Hexane 97% purity was purchased from Merck chemicals, South Africa
- Methanol 98% purity from Merck chemicals, South Africa
- Dimethyl phthalate 99% purity from Merck chemicals, South Africa
- Dibutyl phthalate 97% purity from Merck chemicals, South Africa
- Benzylbutyl phthalate 96% purity from Merck chemicals, South Africa
- Di-n-butyl phthalate 97% purity from Supelco chemicals, South Africa
- Di-ethylhexyl adipate 98% purity from Merck chemicals, South Africa
- Chlorotetradecane (CHLORO) 95% purity from Merck chemicals, South Africa
- Dichloromethane 98% purity from Merck chemicals, South Africa
- Acetone 97% purity from Merck chemicals, South Africa

3.3.3 Stock and working solutions

Stock solutions of the phthalate ester standards were as stated under chemicals/regents above. They all had a concentration of 1000 µg/L. Working solutions were then prepared from the stock through serial dilution using hexane

solvent. 10 µg/L concentrations of working solutions of all the phthalates were then prepared by diluting 1ml of the stock solution by 100ml of hexane solvent.

3.4 Sampling protocol

Same brand of food samples (i.e. the same make of each of the food samples) [actual names of analysed food products were not stated/provided in the dissertation since this have litigation implication. The research work is an investigative academic work without recourse or collaboration with food producers] packed in thin plastic film (plastic wrapped) were purchased from commercial stores within the four areas (i.e. North, East, South and West) of Pretoria within Tshwane Metropolis. Samples were taken to the laboratory immediately after purchase and stored in the fridge at 4^{o}C until extracted. Extraction of food samples were carried out within 24-48 hours in order to reduce the storage time.

3.5 Sample pretreatment:

- Polony and vienna samples were separately cut into small pieces, placed onto petri-dishes and dried in the oven at 50^{o}C for 24 hours.
- The dried samples were then grinded using pestle and mortar to increase the surface area.
- Cheese samples were just cut into small pieces, weighed and transferred to extraction apparatus.

3.6 Extraction process:

- About 1 g of the powdered samples (Polony and vienna) were placed in extraction thimbles and transferred to soxhlet apparatus. For the cheese samples, 1g was simply weighed into the extraction thimble.

- 120 ml of extracting solvent (hexane) was placed into 250 ml round bottomed flask.
- Soxhlet extraction set up was connected, opened the water tap to run into the condenser.
- All samples were extracted at moderate heat (60^{o}C- 80^{o}C) for four hours.
- Extracts were allowed to cool to room temperature and evaporated to about 5 ml using rotor evaporator.
- The extracts were then passed through column (florisil column) cleanup and eluted with 10 ml of hexane.
- Extracts were evaporated (using rotor evaporator) again to about 2 ml and transferred to sample vials and stored in the fridge (4^{o}C) until they were analyzed.
- Avoidance of contact of the food samples as well as all processes during the experimental and analysis state with plastic was ensured. This is required in order to prevent cross contamination of the samples with phthalate esters. Below is an image of soxhlet apparatus.

Figure 3.3: Pictorial representation of the Soxhlet extraction process

3.7 Cleanup method of extracts by solid phase adsorbent:

- Florisil column was prepared by putting a small piece of glass wool into the tip of the glass cleanup column. This will ensure the containment of the adsorbent within the column. About 10 cm or 2g of Florisil adsorbent was transferred into the column putting another thin piece of glass wool on top of the Florisil.
- The column was conditioned by saturating it with the same solvent, i.e. hexane.

3.8 Experimental

3.8.1 Method for quality assurance

The extraction process as described above for the analysis of foods samples was used for the quality assurance experiment of the method. In the absence of Standards Reference Materials (SRM) of the analyzed food samples, standard addition method was adopted. This was carried out by spiking/adding known amount of the phthalate ester standards to pre-extracted food samples and the percentage recovery calculated.

3.8.2 Instrumentation

Clarus 600 Gas Chromatography that was fitted to the Flame-Ionization Detector (FID), linked to Auto Sampler with Elite-5 column and powered by the TotalChrome software and supplied by Perkin Elmer, South Africa was used in the detection and analyses of the phthalate esters in food samples. The image of the instrument is at the next page.

Figure 3.4: Pictorial representation of the Clarus 600 GC-FID instrument

3.8.3 0ptimization of Gas Chromatography (GC-FID)

- Gas Chromatography equipped with Flame Ionized Detector was used in the optimization of the phthalate esters in all extracts.
- The GC oven was programmed as follows: the temperature was initially held at 70^{o}C for 1 minute, heated from 70^{o}C to 150^{o}C at 30^{o}C/min, then held at 150^{o}C for 1 minute, heated from 150^{o}C to 290^{o}C at 25^{o}C/min. The helium flow was 1ml/min.
- The GC-FID was calibrated by the injection of mixture of four different levels of concentration (0.2, 0.4, 0.6 and 0.8 ppm) of all phthalate esters standards.
- Chromatogram of the mixture of analyzed phthalate esters were prepared and obtained by adding together 1ml of 1mg/l of DMP; 1ml of 1mg/l of DEHA; 1ml of 1mg/l of DBP; 1ml of 1mg/l of BBP; 1ml of 10mg/l of DnBP and 1ml of 1mg/l of internal standard was injected into the GC. Good resolution of the mixture of standards was obtained as shown in the next page, Fig 3.5.

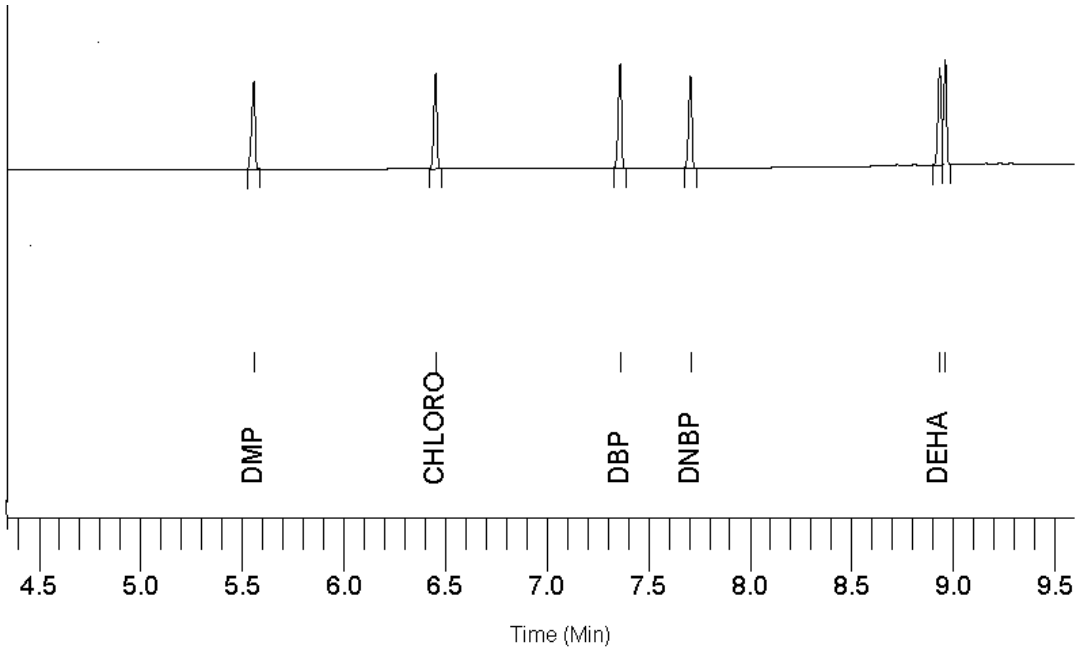

Figure 3.5: Gas chromatogram of phthalate ester standards (DMP, ISTD (CHLORO), DBP, DnBP, DEHA and BBP)

3.9 Analysis of phthalate esters in plastic wrappers

3.9.1 Sample treatment

Plastic wrappers were thoroughly cleaned and air-dried. They were then shredded into small pieces using acetone cleaned scissors in order to increase the surface area and facilitate quick dissolution in the organic solvent dichloromethane.

3.9.2 Experimental

50 mg of thin plastic wrapper films were shredded into smaller pieces hence ensuring faster dissolution. The plastic materials were dissolved by adding 5ml of dichloromethane solvent. This was evaporated to just dryness and 2ml of methanol was added to precipitate the polymer before being analyzed.

3.9.3 Instrumentation

The same method that was used to analyse food samples was also applied.

CHAPTER 4

RESULTS AND DISCUSSION

4.1 Introduction

This chapter presents the results of the analysis of Phthalate Esters in the food samples and food wrappers across all the sampling period. Results are presented in tabular form containing the phthalate standards, the foods samples and the various parameters that were evaluated.

Table 4.1.1: Retention times (RT n =3) and IDL (n=6) of PE standards

Phthalate Ester Standards	Retention times (minutes)			Average RT±SD	Instrumental Detection limit (IDL) (µg/kg)
DMP	5.54	5.56	5.55	5.55 ± 0.01	0.05
CHLORO	6.44	6.46	6.45	6.45 ± 0.01	0.05
DBP	7.35	7.36	7.36	7.36 ± 0.01	0.04
DnBP	7.69	7.71	7.70	7.70 ± 0.01	0.04
DEHA	8.92	8.92	8.93	8.92 ± 0.01	0.05
BBP	8.95	8.95	8.96	8.95 ± 0.01	0.03

Table 4.1.1 above revealed the retention times of the Phthalate Ester standards and the Instrumental detection limits (IDL) of the standards relative to the instrument. As could be seen above, the PE standards are well separated from each other (with reference to their RT's) which make detection, identification and ultimately, quantification of the unknown samples in the extract easy. The instrumental detection limits of analyzed phthalate ester standards as shown above revealed detectable levels of 0.05 µg/kg for DMP and DEHA; 0.04 µg/kg for DBP and DnBP, and 0.03 µg/kg for BBP. These levels of instrumental detections are comparable with levels that were reported by (Fatoki et al. 2009) in previous studies involving phthalate esters.

Table 4.1.2: Response Factors (RF) of all the standards within the same samples

Cheese			
Standards	**Replicates RF's**		**Average RF±SD**
DMP	0.934	0.940	0.94 ± 0.004
DBP	1.22	1.24	1.23 ± 0.156
BBP	1.58	1.61	1.56 ± 0.021
DEHA	1.03	1.04	1.04 ± 0.008
DnBP	$7x10^{-4}$	$7x10^{-4}$	$7x10^{-4} \pm 1.0x10^{-6}$
Vienna			
DMP	0.890	0.929	0.910 ± 0.028
DBP	1.120	1.040	1.08 ± 0.057
BBP	1.510	1.390	1.45 ± 0.085
DEHA	0.882	0.450	0.650 ± 0.305
DnBP	0.006	0.006	$0.003 \pm 1.0x10^{-4}$
Polony			
DMP	1.210	1.290	1.25 ± 0.057
DBP	1.440	1.690	1.57 ± 0.177
BBP	2.560	2.010	2.29 ± 0.389
DEHA	2.050	1.480	1.77 ± 0.403
DnBP	0.0006	0.022	0.0113 ± 0.015

The response factor is an indication of the response of the phthalate ester standards relative to that of the internal standard. Results from Table 4.1.2 above revealed that BBP is more sensitive to the instrument than DBP, DEHA, DMP and DnBP respectively.

4.2 Resolution of the mixture of phthalate esters standards

Based on the optimized GC conditions, good resolutions of the peaks of the standards were obtained as shown in Figure 4.1 below:

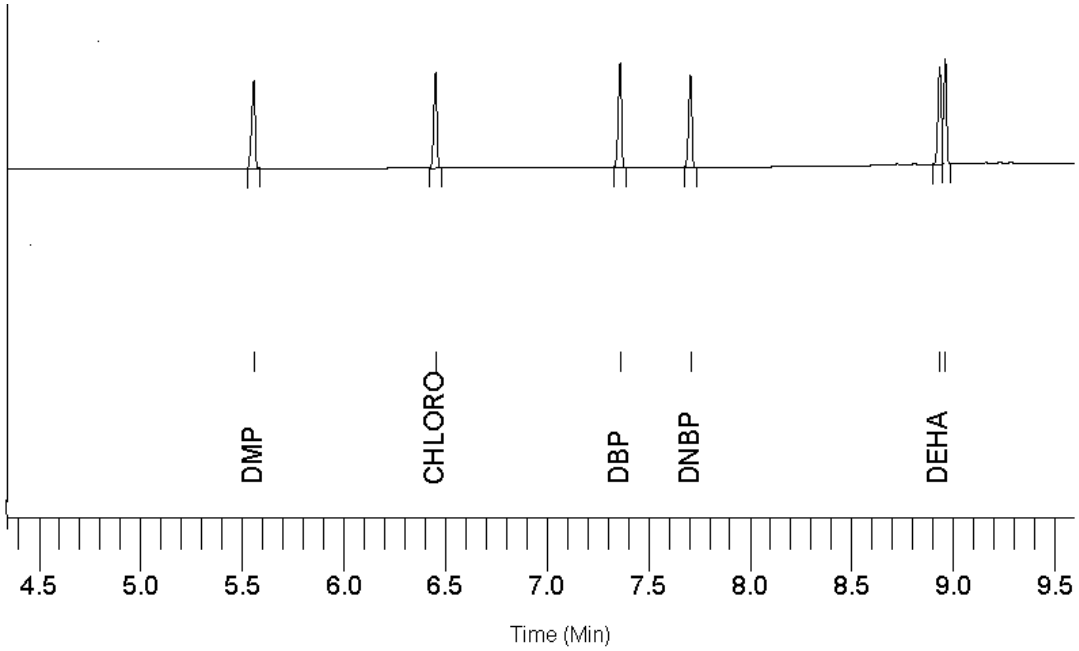

Figure 4.1: Gas chromatogram of mixture of standards

4.3 Quality assurance process

Table 4.2 below revealed the results of the quality assurance analysis of the food samples.

Table 4.2: Percentage (%) recoveries of Phthalates Ester standards from spiked samples (Cheese, Polony & Vienna)

Phthalate Esters	Cheese (%)		Polony (%)		Vienna (%)	
	Replicates	Mean	Replicates	Mean	Replicates	Mean
DMP	71		33		80	
	78	75	35	34	103	92
DBP	86		27		92	
	89	88	39	33	106	99

DnBP	86		30		90	
	89	88	101	66	104	97
DEHA	88		61		86	
	92	90	44	53	52	69
BBP	86		51		95	
	89	88	40	46	103	99

The percentage recovery of the phthalate esters ranged from 75 – 90 % in cheese; 33- 66 % in polony and 69 – 99 % in vienna sample. These recoveries are quite acceptable and applicable to the analysis and quantitation of the compounds in the samples with the exception of DBP (33%); DMP (34%) and BBP (46 %) in polony samples. These relatively low recoveries in spiked samples might be due to sample loss during extraction and cleanup processes. Similar quality assurance results have been reported by (Balafas et al., 1999).

4.4 Levels of phthalate esters in analyzed food samples

The levels of phthalate esters obtained from the analysed food samples are as presented in Table 4.3 - 4.20 below.

Table 4.3 in the next page reports on the level of phthalate esters in food samples purchased on the 3rd of March 2009. From the results, it could be seen that the vienna from the north contained more DMP than vienna from east and west respectively, DBP and BBP were more contained in vienna from the east than west and north respectively and DnBP was only detected in vienna from the east while DEHA proved to be below detection limit from all three sites.

Temperature variations and geographical difference might have been the reason for such results variations.

In polony, DMP and DBP were more detected from east, north and west respectively, BBP was highly detected form east, west and north respectively while DnBP and DEHA was below detection limits from all sites. The polonies contained more PEs than the viennas from the same sites.

Table 4.3: Levels of Phthalate Esters (µg/kg) in analyzed food samples collected on 3rd March 2009

Sample name	Sampling site (Pretoria)	DMP	DBP	DnBP	DEHA	BBP
Vienna	North	0.816	0.078	bdl	bdl	0.033
Vienna	West	bdl	0.081	bdl	bdl	0.048
Vienna	East	0.200	0.124	0.071	bdl	0.096
Polony	East	0.241	0.314	bdl	bdl	0.344
Polony	North	0.147	0.110	bdl	bdl	0.039
Polony	West	0.118	bdl	bdl	bdl	0.042

NB: bdl = below detection limit

Table 4.4 in the next page shows the results of the analysis of phthalate esters in food samples purchased on the 18th April 2009. The results showed that DMP, DnBP and DEHA were below detection limit in Polony from Pretoria north, south and west. DBP and BBP were detected from the Pretoria north only.

In cheese, DMP and BBP were detected more in the sample from Pretoria north than Pretoria south while they were below detection limit from Pretoria west site sample. DBP was more detected in sample from Pretoria south than Pretoria north and below detection limit in the sample from Pretoria west. DnBP and DEHA were below detection limit from samples from all three sites. The

concentrations of all the detected PEs were higher in the cheese samples than in polony samples.

Table 4.4: Levels of Phthalate Esters (µg/kg) in analyzed food samples collected on 18th April 2009

Sample name	Sampling site (Pretoria)	DMP	DBP	DnBP	DEHA	BBP
Polony	North	bdl	0.110	bdl	bdl	0.041
Polony	South	bdl	bdl	bdl	bdl	bdl
Polony	West	bdl	bdl	bdl	bdl	bdl
Cheese	North	0.086	0.054	bdl	bdl	0.050
Cheese	South	0.083	0.071	bdl	bdl	0.039
Cheese	West	bdl	bdl	bdl	bdl	bdl

Table 4.5 in the next page reports on the levels of phthalate esters in vienna and polony food samples purchased on the 20th April 2009. The results shows that DMP was more in vienna from the Pretoria west than Pretoria north while it was below detection limit in Pretoria east. DBP and BBP were more detected in vienna from Pretoria north than Pretoria east and west respectively. DMP and DnBP were below detection limit in vienna from Pretoria east.

All PEs were below detection limit in analyzed polony samples from Pretoria north. DMP and BBP were more in the polony from Pretoria east than Pretoria west, DBP was more in polony from Pretoria west than east. DnBP was only detected in polony from Pretoria west and in vienna from Pretoria north. DEHA was below detection limit from all samples from all three sites. Viennas contained high values of PE's compared to polonies.

Table 4.5: Levels of Phthalate Esters (µg/kg) in analyzed food samples collected on 20th April 2009

Sample name	Sampling site (Pretoria)	DMP	DBP	DnBP	DEHA	BBP
Vienna	East	bdl	0.092	bdl	bdl	0.673
Vienna	North	0.169	0.209	0.047	bdl	0.177
Vienna	West	0.185	0.081	bdl	bdl	0.086
Polony	East	0.149	0.052	bdl	bdl	0.047
Polony	West	bdl	0.094	0.057	bdl	0.035
Polony	North	bdl	bdl	bdl	bdl	bdl

Table 4.6 below reports on the levels of phthalate esters in polony purchased on the 11th August 2009. The results show that all the PEs were below detection limits from all different areas of Pretoria, i.e. Pretoria north, Pretoria south and Pretoria east.

Table 4.6: Levels of Phthalate Esters (µg/kg) in analyzed food samples collected on 11th August 2009

Sample name	Sampling site (Pretoria)	DMP	DBP	DnBP	DEHA	BBP
Polony	North	bdl	bdl	bdl	bdl	bdl
Polony	North	bdl	bdl	bdl	bdl	bdl
Polony	South	bdl	bdl	bdl	bdl	bdl
Polony	South	bdl	bdl	bdl	bdl	bdl
Polony	East	bdl	bdl	bdl	bdl	bdl
Polony	East	bdl	bdl	bdl	bdl	bdl

Table 4.7 below reports on levels of phthalate esters in foods purchased on the 12th August 2009. The results show that DMP, DBP and BBP were highly detected in cheese samples purchased from Pretoria south than in the north. DnBP and DEHA were found to be below detection limits from both sites. Only DEHA was below detection limit on vienna from Pretoria south while all PEs were below detection limit in vienna samples from Pretoria North. BBP was more detected on polony samples collected from the north of Pretoria than from the south while BBP was more detected in samples from the south than the north. High detections were obtained from cheese, polony and vienna respectively.

Table 4.7: Levels of Phthalate Esters (µg/kg) in analyzed food samples collected on 12th August 2009

Sample name	Sampling site (Pretoria)	DMP	DBP	DnBP	DEHA	BBP
Cheese	South	0.215	0.153	bdl	bdl	0.101
Cheese	North	0.072	0.049	bdl	bdl	0.042
Vienna	South	0.084	0.133	0.120	bdl	0.035
Vienna	North	bdl	bdl	bdl	bdl	bdl
Polony	North	bdl	0.076	bdl	bdl	0.100
Polony	South	0.177	0.087	bdl	bdl	0.041

Table 4.8 in the next page reports on levels of phthalate esters in polony purchased on the 13th August 2009. The results showed that DMP and DBP were highly detected in the analyzed polony samples from the east than from the south while BBP was detected highly from Pretoria south samples than from the east. DnBP and DEHA were below detection limits in samples from both sites. DEHA was only detected in sample from the west of Pretoria while all the other PEs were below detection limits.

Table 4.8: Levels of Phthalate Esters (µg/kg) in analysed food samples collected on 13th August 2009

Sample name	Sampling site (Pretoria)	DMP	DBP	DnBP	DEHA	BBP
Polony	East	0.126	0.095	bdl	bdl	0.036
Polony	South	0.080	0.064	bdl	bdl	0.040
Polony	South	0.077	0.611	bdl	bdl	0.049
Polony	West	bdl	bdl	bdl	0.075	bdl
Polony	West	bdl	bdl	bdl	bdl	bdl
Polony	East	bdl	bdl	bdl	bdl	bdl

Table 4.9 below reports on levels of phthalate esters in cheese and vienna food samples purchased on 31st August 2009. The results show that DMP, DBP and BBP were detected in cheese samples that were purchased from this sampling site. Values of DMP obtained ranged from 0.057 - 0.161 µg/kg; DBP ranged from 0.065 – 0.129 µg/kg and BBP from 0.037 – 0.130 µg/kg. DnBP and DEHA were not detected in all three cheese samples.

Table 4.9: Levels of Phthalate Esters (µg/kg) in analyzed food samples collected on 31st August 2009

Sample name	Sampling site (Pretoria)	DMP	DBP	DnBP	DEHA	BBP
Cheese	East	0.161	0.081	bdl	bdl	0.130
Cheese	East	bdl	0.065	bdl	bdl	0.037
Cheese	East	0.057	0.129	bdl	bdl	0.070
Vienna	East	bdl	bdl	bdl	bdl	bdl
Vienna	East	bdl	bdl	bdl	bdl	bdl
Vienna	East	bdl	bdl	bdl	bdl	bdl

Table 4.10 below reports on levels of phthalate esters in vienna and polony food samples purchased on the 04th September 2009. The results show that BBP and DBP were detected in viennas (duplicate) from Pretoria west while DMP, DnBP and DEHA were below detection limits. DBP and BBP were detected in the polony from Pretoria east while DMP, DBP and DEHA were found to be below detection limits. All PE's were below detection limits in the polony from Pretoria west. Vienna from Pretoria west and polony from Pretoria east have almost the same amount of DBP and BBP.

Table 4.10: Levels of Phthalate Esters (µg/kg) in analyzed food samples collected on 04th September 2009

Sample name	Sampling site (Pretoria)	DMP	DBP	DnBP	DEHA	BBP
Vienna	West	bdl	0.074	bdl	bdl	0.070
Vienna	West	bdl	bdl	bdl	bdl	0.036
Polony	East	bdl	0.063	bdl	bdl	0.064
Polony	East	bdl	0.076	bdl	bdl	0.079
Polony	West	bdl	bdl	bdl	bdl	bdl
Polony	West	bdl	bdl	bdl	bdl	bdl

Table 4.11 in the next page reports on the levels of phthalate esters in cheese and vienna food samples purchased on the 07th September 2009. The results show that DMP, DBP and BBP were highly detected in cheese samples from Pretoria north than the ones from Pretoria south. DnBP and DEHA were found to be below detection limits in both the samples from Pretoria south and Pretoria north. All PE's in the vienna samples from Pretoria east were below detection limits.

Table 4.11: Levels of Phthalate Esters (µg/kg) in analyzed food samples collected on 07th September 2009

Sample name	Sampling site (Pretoria)	DMP	DBP	DnBP	DEHA	BBP
Cheese	South	bdl	0.071	bdl	bdl	0.040
Cheese	North	0.700	0.087	bdl	bdl	0.100
Cheese	North	0.131	0.256	bdl	bdl	0.643
Cheese	South	bdl	bdl	bdl	bdl	0.420
Vienna	East	bdl	bdl	bdl	bdl	0.031
Vienna	East	bdl	bdl	bdl	bdl	0.075

Table 4.12 below reports on the levels of phthalate esters in food samples purchased on the 19th January 2010. The results show that DMP and BBP were detected only in the cheese samples from Pretoria north, DBP, DnBP and DEHA were below detection limits from the very same cheese samples. DMP was highly detected than BBP. All PEs were found to be below detection limits from vienna and polony from the same site, Pretoria north.

Table 4.12: Levels of Phthalate Esters (µg/kg) in analyzed food samples collected on 19th January 2010

Sample name	Sampling site (Pretoria)	DMP	DBP	DnBP	DEHA	BBP
Cheese	North	0.071	bdl	bdl	bdl	0.048
Polony	North	bdl	bdl	bdl	bdl	bdl
Vienna	North	bdl	bdl	bdl	bdl	bdl
Cheese	North	bdl	bdl	bdl	bdl	0.031
Polony	North	bdl	bdl	bdl	bdl	bdl
Vienna	North	bdl	bdl	bdl	bdl	bdl

Table 4.13 below reports on the levels of phthalate esters in food samples purchased on the 20th January 2010. The results show that DMP, DBP and BBP were detected in cheese sample from Pretoria south with DnBP and DEHA being below detection limits. BBP was highly detected than BBP and DMP respectively. All PEs in polony and vienna from Pretoria south were below detection limits.

Table 4.13: Levels of Phthalate Esters (µg/kg) in analyzed food samples collected on 20th January 2010

Sample name	Sampling site (Pretoria)	DMP	DBP	DnBP	DEHA	BBP
Cheese	South	0.114	0.384	bdl	bdl	1.81
Vienna	South	bdl	bdl	bdl	bdl	bdl
Cheese	South	bdl	bdl	bdl	bdl	bdl
Polony	South	bdl	bdl	bdl	bdl	bdl
Vienna	South	bdl	bdl	bdl	bdl	bdl
Polony	South	bdl	bdl	bdl	bdl	bdl

Table 4.14 below reports on the levels of phthalate esters in food samples purchased on the 21st January 2010. The results show that all the PE's were below detection from all the food samples (cheese, polony and vienna) purchased from Pretoria south.

Table 4.14: Levels of Phthalate Esters (µg/kg) in analyzed food samples collected on 21st January 2010

Sample name	Sampling site (Pretoria)	DMP	DBP	DnBP	DEHA	BBP
Vienna	South	bdl	bdl	bdl	bdl	bdl
Polony	South	bdl	bdl	bdl	bdl	bdl
Vienna	South	bdl	bdl	bdl	bdl	bdl
Cheese	South	bdl	bdl	bdl	bdl	bdl

Polony	South	bdl	bdl	bdl	bdl	bdl
Cheese	South	bdl	bdl	bdl	bdl	bdl

Table 4.15 below reports on the levels of phthalates esters in food purchased on the 27th January 2010. The results show that DBP and BBP were detected in cheese samples from Pretoria east and Pretoria north while DMP, DnBP and DEHA were below detection limits. All PE's were below detection limit in cheese sample from Pretoria west. All PEs were below detection limits in all polony samples from all three sites, i.e. Pretoria north, east and the south.

Table 4.15: Levels of Phthalate Esters (µg/kg) in analyzed food samples collected on 27th January 2010

Sample name	**Sampling site (Pretoria)**	**DMP**	**DBP**	**DnBP**	**DEHA**	**BBP**
Polony	North	bdl	bdl	bdl	bdl	bdl
Cheese	East	bdl	0.081	bdl	bdl	0.031
Polony	East	bdl	bdl	bdl	bdl	bdl
Polony	West	bdl	bdl	bdl	bdl	bdl
Cheese	North	bdl	0.074	bdl	bdl	0.043
Cheese	West	bdl	bdl	bdl	bdl	bdl

Table 4.16 in the next page reports on the levels of phthalate esters in cheese food samples purchased on the 21st February 2010. The results show that DMP and BBP were detected in cheese samples from Pretoria north while DBP, DnBP and DEHA were below detection limits. All PE's were found to be below detection limits in cheese samples from both Pretoria south and west.

Table 4.16: Levels of Phthalate Esters (µg/kg) in analyzed food samples collected on 21st February 2010

Sample name	Sampling site (Pretoria)	DMP	DBP	DnBP	DEHA	BBP
Cheese	North	0.091	bdl	bdl	bdl	0.078
Cheese	North	0.107	bdl	bdl	bdl	0.163
Cheese	South	bdl	bdl	bdl	bdl	bdl
Cheese	South	bdl	bdl	bdl	bdl	bdl
Cheese	West	bdl	bdl	bdl	bdl	bdl
Cheese	West	bdl	bdl	bdl	bdl	bdl

Table 4.17 below reports on the levels of phthalate esters in polony food samples purchased on the 02nd February 2010. The results show that only DnBP was detected in polony sample from Pretoria north while DMP, DBP, DEHA and BBP were below detection limits. All PE's were below detection limits in polony samples from both Pretoria west and south.

Table 4.17: Levels of Phthalate Esters (µg/kg) in analyzed food samples collected on 02nd February 2010

Sample name	Sampling site (Pretoria)	DMP	DBP	DnBP	DEHA	BBP
Polony	North	bdl	bdl	0.074	bdl	bdl
Polony	West	bdl	bdl	bdl	bdl	bdl
Polony	West	bdl	bdl	bdl	bdl	bdl
Polony	South	bdl	bdl	bdl	bdl	bdl
Polony	South	bdl	bdl	bdl	bdl	bdl
Polony	North	bdl	bdl	bdl	bdl	bdl

Table 4.18 below reports on the levels of phthalates esters in vienna food samples purchased on the 03rd February 2010. The results show that only DMP was detected in vienna sample bought at Pretoria east while BBP, DnBP, DEHA and BBP were below detection limits from the same sample. All PE's were found to be below detection limits in both vienna samples from the north and west of Pretoria.

Table 4.18: Levels of Phthalate Esters (µg/kg) in analyzed food samples collected on 03rd February 2010

Sample name	Sampling site (Pretoria)	DMP	DBP	DnBP	DEHA	BBP
Vienna	East	0.083	bdl	bdl	bdl	bdl
Vienna	West	bdl	bdl	bdl	bdl	bdl
Vienna	North	bdl	bdl	bdl	bdl	bdl
Vienna	East	bdl	bdl	bdl	bdl	bdl
Vienna	North	bdl	bdl	bdl	bdl	bdl
Vienna	West	bdl	bdl	bdl	bdl	bdl

Table 4.19 in the next page report on the levels of phthalate esters on cheese samples purchased on the 09th February 2010. All PE's were below detection limits from the replicates cheese samples from Pretoria north and Pretoria south. Only DBP and BBP were detected from cheese sample from Pretoria east and the others (DMP, DnBP and DEHA) were below detection limits.

Table 4.19: Levels of Phthalate Esters (µg/kg) in analyzed food samples collected on 09th February 2010

Sample name	Sampling site (Pretoria)	DMP	DBP	DnBP	DEHA	BBP
Cheese	East	bdl	0.094	0.094	bdl	0.082
Cheese	South	bdl	bdl	bdl	bdl	bdl
Cheese	North	bdl	bdl	bdl	bdl	bdl
Cheese	South	bdl	bdl	bdl	bdl	bdl
Cheese	East	bdl	bdl	bdl	bdl	0.035
Cheese	North	bdl	bdl	bdl	bdl	bdl

Table 4.20 below report on the levels of phthalate esters in food samples purchased on the 10th February 2010. The results show that DnBP, DEHA and BBP were detected in one vienna sample, the other two all PE's were below detection limit and so were the polony samples (triplicate) from the same site, i.e. Pretoria east.

Table 4.20: Levels of Phthalate Esters (µg/kg) in analyzed food samples collected on 10th February 2010

Sample name	Sampling site (Pretoria)	DMP	DBP	DnBP	DEHA	BBP
Vienna	East	bdl	bdl	0.700	0.281	0.159
Vienna	East	bdl	bdl	bdl	bdl	bdl
Polony	East	bdl	bdl	bdl	bdl	bdl
Polony	East	bdl	bdl	bdl	bdl	bdl
Vienna	East	bdl	bdl	bdl	bdl	bdl
Polony	East	bdl	bdl	bdl	bdl	bdl

4.5 Results of PEs from analyzed polymer samples (i.e. food wrappers)

Phthalate Esters has been reported to migrate from the wrapper (plastic thin film) into the food content in which the food is presented. Chemically, PEs are tightly bound to the polymeric unit of the plastic wrapper/thin film, hence the “leaching” from the wrapper into the food content.

Since the possible prevalence of PEs in foods are investigated, it becomes logical to also investigate the primary source of the presence and level of the PEs in the wrappers. Results of the level of PEs in selected wrappers of food samples are as presented in Tables 4.21- 4.23 below.

Table 4.21: Levels of Phthalate Esters (µg/kg) in analyzed food wrappers samples collected on 11th July 2011 from Pretoria North

Sample name	Sampling site (Pretoria)	DMP	DBP	DnBP	DEHA	BBP
Cheese	North	bdl	0.117	Bdl	bdl	14.1
Polony	North	bdl	0.152	3.370	0.088	0.040
Vienna	North	bdl	0.116	bdl	bdl	0.047

Table 4.21 above reports on the levels of phthalate esters in food wrappers samples purchased on the 11th July 2011. The results show that DBP and BBP were detected in all three different kinds of wrappers (cheese, polony and vienna) while DnBP and DEHA were only detected from polony wrapper sample. DnBP and DEHA were found to be below detection limits in cheese and vienna plastic wrappers. DMP was below detection limit in all samples. Polony wrapper contained a slightly more DBP than both cheese and vienna wrappers that had almost the same values while the cheese wrapper contained a lot of BBP than polony and vienna plastic wrappers.

Table 4.22 Levels of Phthalate Esters (µg/kg) in analyzed food wrappers samples collected on 11th July 2011 from Pretoria West

Sample name	Sampling site (Pretoria)	DMP	DBP	DnBP	DEHA	BBP
Cheese	West	bdl	bdl	0.150	bdl	4.840
Polony	West	bdl	0.071	2.200	bdl	0.052
Vienna	West	bdl	bdl	0.164	bdl	bdl

The level of PEs in analysed food wrappers purchased from the west of Pretoria sapling site is as presented in Table 4.22 above. The results show that only DnBP was detected in all three different kinds of wrappers (cheese, polony and vienna). DBP was only detected from polony plastic wrapper and below detection limit in cheese and vienna plastic samples. DMP and DEHA were below detection limits in all samples. Cheese contained a lot more of BBP while vienna sample contained none, it was below detection limit.

Table 4.23 in the next page also reports on the levels of phthalate esters in food wrappers samples purchased from the eastern part of Pretoria. Results obtained revealed that DBP, DnBP and BBP were detected in all plastic wraps from the food samples while DMP and DEHA were below detection limits in all samples. The levels of DBP and DnBP obtained from plastic wrapper from polony samples were lesser than that obtained from both the cheese and vienna wrappers. The highest level of BBP was detected in the wrapper of cheese sample. This high level gave credence on the tendency of the PE to migrate with increasing level over time into the food sample in which it was packaged

Table 4.23 Levels of Phthalate Esters (µg/kg) in analyzed food wrappers samples collected on 11th July 2011 from Pretoria east

Sample name	Sampling site (Pretoria)	DMP	DBP	DnBP	DEHA	BBP
Cheese	East	bdl	0.137	0.103	bdl	14.20
Polony	East	bdl	0.083	0.069	bdl	1.880
Vienna	East	bdl	0.121	0.081	bdl	0.187

4.6 Representative chromatograms from analyzed plastic wrappers

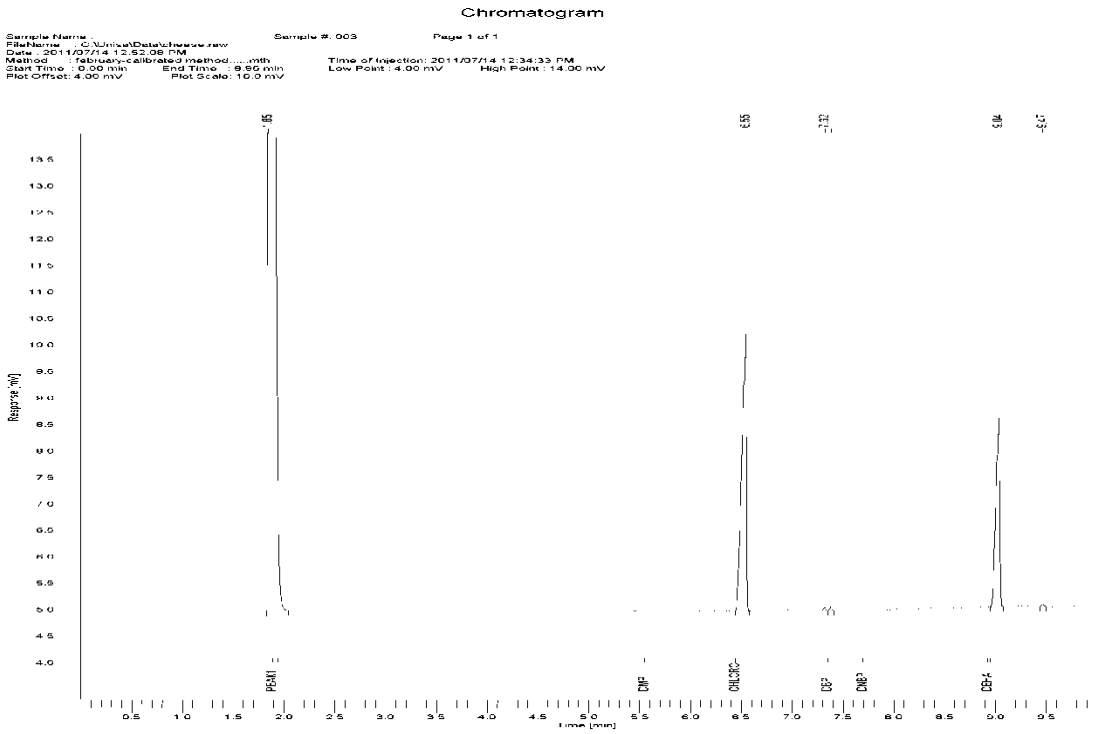

Figure 4.2: Representative chromatogram of PEs from analysed plastic wrapper of cheese sample

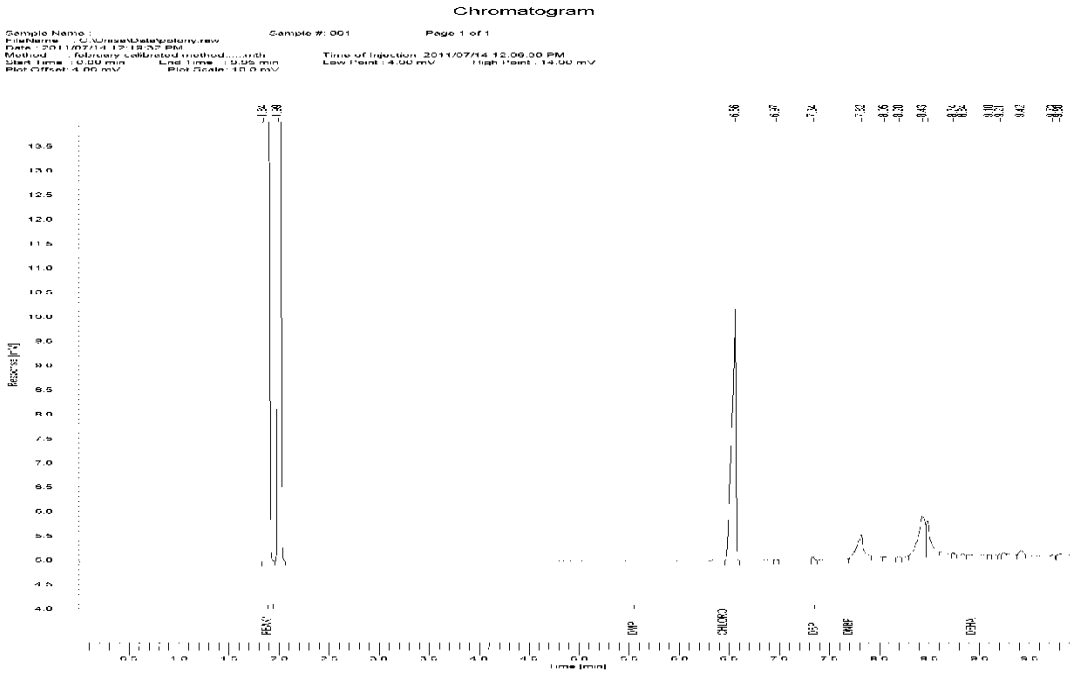

Figure 4.3: Representative chromatogram of PEs from analysed plastic wrapper of polony sample

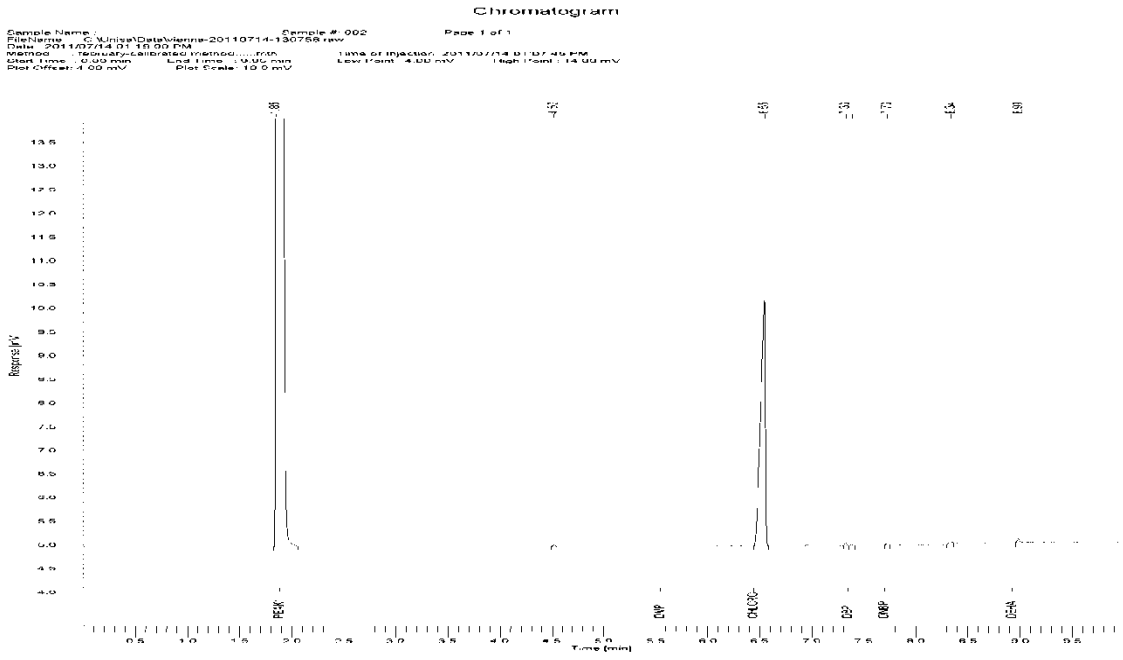

Figure 4.4: Representative chromatogram of PEs from analysed plastic wrapper of vienna sample.

CHAPTER 5

CONCLUSION AND RECOMMENDATIONS

5.1 Introduction

The main objective of the study was to carry out a survey of the prevalence of endocrine disrupting phthalate esters in selected consumed foods and food wrappers around Tshwane metropolis. In order to achieve this, the following investigations were also carried out: evaluation and optimization of soxhlet technique for the extraction of phthalates esters in samples, determination and quantifying, using the optimized method, the level of phthalate esters in samples, comparing results obtained with related data in other parts of the world and with international acceptable safety limits. In this concluding part of the project, the status of the research problem, research hypothesis (in terms of acceptance or rejection) and the findings on the extent of prevalence of Endocrine Disrupting Phthalate Esters in selected foods and food wrappers around Tshwane Metropolis are unraveled. It also presents applicable recommendations with regards to results obtained.

5.2 Ways to reduce phthalate esters intake via food chain

Phthalates can also enter food items during processing, quite often due to the use of PVC in food production and processing systems. Quite a number of studies of fat-containing foods have suggested that the presence of phthalate esters in food is largely due to general contamination of the environment and food chain, so ingestion of phthalates in food can occur irrespective of the packaging and/or processing involved.

People may particularly want to reduce their ingestion of phthalates. One way to reduce ingestion is to avoid consuming foods packaged with flexible PVC contained materials, although this is difficult since almost all packaging materials consists of few phthalates esters. Rather make sure that packaged foods are

consumed as soon as possible so as to reduce the storage period because the longer the foods stay packaged the more vulnerable to phthalates contamination they becomes.

Since phthalates are soluble in fats, it becomes easy for them to migrate from PVC packaging materials to fatty foods. Therefore one should reduce the intake of fats containing food such as cheese, vienna and polony since it has been confirmed that these foods contain endocrine disruptors in the form of phthalates due to the packaging materials used.

5.3 Summary

The phthalates were eluted from the gas chromatographic column in the order DMP, DBP, DnBP, DEHA and BBP. The retention time (in minutes) for phthalates esters using the optimized GC conditions were DMP-5.55, DBP-7.36, DnBP-7.70, DEHA-8.92 and BBP-8.95 (Fig 4.1). The instrument detection limits of the GC system for the phthalates were DMP-0.05 µg/kg, DBP-0.04 µg/kg, DnBP-0.04 µg/kg, DEHA-0.05 µg/kg, and BBP-0.03 µg/kg.

The levels of phthalates found in the food samples from selected commercial stores (all sites) within the Tshwane Metropolis are as shown in Table 4.3 - 4.20. Phthalates concentration levels ranged as follows: DMP; from below detection limit to 0.70 µg/kg in cheese sample, from below detection limit to 0.24 µg/kg in polony sample and from below detection limit to 0.86 µg/kg in vienna sample, DBP; from below detection limit to 0.38 µg/kg in cheese sample, from below detection limit to 0.61 µg/kg in polony sample and from below detection limit to 0.209 µg/kg in vienna sample, DnBP; from below detection limit to 0.11 µg/kg in cheese sample, from below detection limit to 0.07 µg/kg in polony sample and from below detection limit to 0.70 µg/kg in vienna sample, DEHA; below detection limit in cheese sample, from below detection limit to 0.075 µg/kg in polony sample and from below detection limit to 0.28 µg/kg in vienna sample, BBP; from below detection limit to 1.81 µg/kg in cheese sample, from below detection limit

to 0.344 µg/kg in polony sample and from below detection limit to 0.68 µg/kg in vienna sample. These data suggest that BBP and DnBP have a more significant role in food samples than DMP, DBP and DEHA.

The levels of phthalates found in the food wrappers samples from the three representative sites (north, west and east) of Tshwane Metropolis are shown in Table 4.21 – 4.23. Phthalates concentration levels ranged as follows: DMP; below detection limit in all samples from all sites, DBP; from below detection limit to 0.14 µg/kg in cheese wrapper, from 0.01 - 0.15 µg/kg in polony wrapper and from below detection limit to 0.1 µg/kg in vienna wrapper, DnBP; from below detection limit to 0.15 µg/kg in cheese wrapper, from 0.01 – 3.4 µg/kg in polony wrapper and from below detection limit to 0.2 µg/kg in vienna wrapper, DEHA; from below detection limit to 0.088 µg/kg in polony wrapper while it was below detection limit in both cheese and vienna wrappers, BBP; from below 4.84 - 14.2 µg/kg in cheese wrapper, from 0.04 – 1.9 µg/kg in polony wrapper and from below detection limit to 0.2 µg/kg in vienna wrapper. These data also suggest that BBP and DnBP have a more significant role in wrappers samples than DMP, DBP and DEHA. It is therefore in agreement with the results obtained from the food samples.

Overall, the highest concentrations of plasticisers were detected in the most flexible printed polyethylene materials; hence the ultimate source of plasticisers the may be due to the printing ink and flexibility. Since the concentrations of phthalates on the plastic wrappers are a bit higher than the ones found on foods samples, we can safely conclude that the ones on food migrated from the food wrappers. The rate of migration seems to be a bit lower for BBP since there is large difference in concentrations from foods and food wrappers samples. The below detection limit of DMP in most foods samples is corroborated by its non-detection from the food wrappers in all samples. Similar study confirmed the absence/ below detection limit of DMP in most packaging materials (Bafalas et al 2008).

The level of DnBP exposure in food samples is expected to be in the low parts per billion ranges. In Canada, the estimated daily intake is 1.9–5.0 µg/kg body weight per day (ATSDR, 2001). DBP's potential risk to human health has been and continues to be investigated. Researchers have evaluated its developmental and reproductive effects, systemic effects and genetic toxicity. Based on studies by the National Toxicology Program, the IPCS determined a tolerable daily intake of 66 µg/kg body weight per day for DBP (IPCS, 1999a; CERHR, 2003).

Based on the National Toxicology Program (NTP) bioassay reports of increased pancreatic lesions in male rats, a tolerable daily intake of 1300 µg/kg body weight per day has been calculated for BBP by the IPCS, 1999a. Studies were done on rats not humans (Bets, 2008).

According to some health organizations (IPCS, 1999b; ATSDR, 2001) the phthalates levels found in this study all fall within the international acceptable safety limits; i.e. 1.9–5.0 µg/kg DnBP, 66 µg/kg DBP and 1300 µg/kg BBP. Though studies only reported the health effects of some phthalates e.g. BBP on rodents, it is still believed those effects might also apply to humans.

According to the European Union Commission Regulation (EU, 2011), the allowed migration limit of these phthalates from the plastic to foods are indicated below; i.e. DBP, only to be used as plasticizer in repeated use materials and articles contacting non-fatty foods and technical support agent in polyolefin in concentrations limit of up to 0,05 % in the final product. BBP, only to be used as plasticizer in repeated use materials and articles; plasticizer in single-use materials and articles contacting non-fatty foods and technical support agent in concentrations limit of up to 0,1 % in the final product. DEHA, Only to be used as plasticizer in repeated use materials and articles contacting non-fatty foods and technical support agent in concentrations limit of up to 0,1 % in the final product. DMP, a detection limit of 0,01 mg substance per kg food is applicable unless specified differently for an individual substance.

Some of the health concerns associated with the exposure to phthalates are indicated in the table 5.1 below:

Table 5.1: Health concerns from various world organizations

Phthalates	**All the listed phthalates are subject to bans or restrictions in the European organizations (Risk Assessments)**				
	CPSC (Consumer Product Safety Commission)	**EU** (European Union)	**CERHR** (U.S. Center for the Evaluation of Risks to Human Reproduction (All CERHR reports are expressed in levels of concern)	**CIR** (Cosmetic Ingredient Review)	**CDC** (Centers for Disease Control and Prevention biomonitoring studies)
DEHA	No demonstrated health risk	Preliminary conclusion: safe for general public (excluding neonates)	serious concern for critically ill neonates; concern for infants under one; some concern for toddlers over one and male offspring of exposed pregnant women; minimal concern for general public		average exposure 10-33 times below EPA reference dose 2 (safety level)
DBP/DnBP	No demonstrated health risk	no concern for consumers	Minimal concern for fetal developmental effects for pregnant women with typical exposure; some concern for male fetal development in women with high exposure (conclusion based on exposure estimates that turned out to be higher than actual)	safe as used in current applications and concentrations	average exposure more than 100 times below EPA reference dose
BBP	No demonstrated health risk	no concern for children or adults	Minimal concern for developmental effects in children and fetuses; negligible concern for reproductive effects in adult males.		average exposure 400 times below EPA reference dose

Available:Http://www.harmancorp.com/images/PhthalateRiskAssessment.pdf; (10 February 2010)

Since phthalates are used worldwide as plasticizers in PVC materials. Many consumer products contain some phthalates resulting in ubiquitous exposure of the population. Though foodstuffs are regarded as the main source of exposure to the general population, other sources such as medical devices may be predominant, i.e. via use of medical devices or pharmaceutical containing phthalates. Because of their chemical properties, exposure to phthalate esters does not result in bioaccumulation. Since it was proved that they are endocrine disrupters in the previous studies (Swan et al., 2005; Sathyanarayana et al., 2008), further studies are necessary to shed more light on how to minimize the intake of phthalates and possible to find an alternative packaging materials.

5.4 Recommendations

In order to prevent and/or minimize the occurrence of phthalate esters, and on the basis of the comprehensive knowledge of the effects these esters exert on living organisms as well as of the results of the studies of the occurrence of phthalates in the environment and food chain, it is necessary to monitor DnBP, DEHA and DBP levels in food and feed ingredients to identify hazardous substances and sources of phthalate contamination. A suitable indicator of DnBP, DEHA and DBP contamination is cheese, polony and vienna.

To reduce the phthalates contamination risk, it is necessary to monitor packaging materials used for storing foodstuffs and to watch closely colored prints, adhesives and other elements that are in contact with foods. The best way to achieve this would be for South African Government Departments such as the Department of Agriculture and Department of Science and Technology to employ competitive regulation agency that would oversee all the packaging industries and make sure that all the precautions are adhered to since there is currently no specific tolerable daily intake/limits of phthalate esters in the country. In addition, employing the health inspectors that would see to it that departmental stores and local supermarkets do not sell foodstuff that have passed their shelf lives.

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Appendices

Appendix 1: Phthalates report and chromatogram of polony sample from Pretoria east

Software Version	: 6.3.2.0646	Date	: 2011/02/09 10:59:38 AM
Sample Name	:	Data Acquisition Time	: 2011/02/08 01:40:30 PM
Instrument Name	: Clarus GC	Channel	: A
Rack/Vial	: 0/13	Operator	: Unisa
Sample Amount	: 1.000000	Dilution Factor	: 1.000000
Cycle	: 3		

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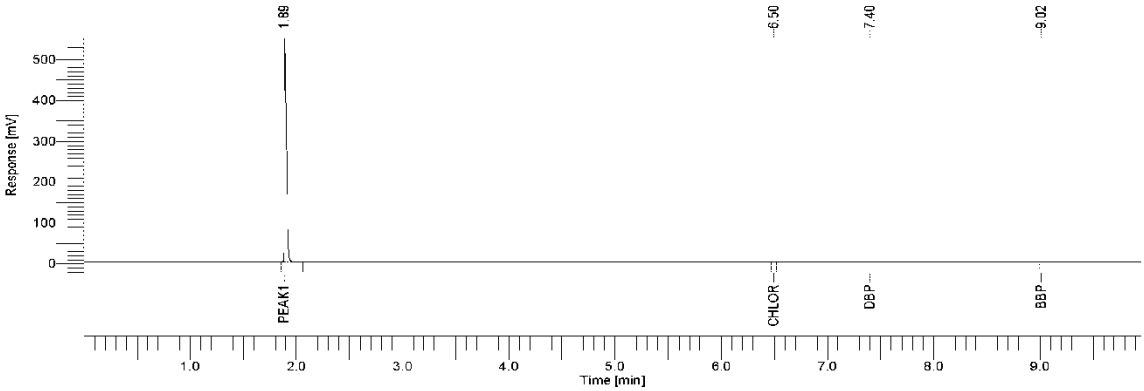

Phthalates Report

Peak #	Component Name	Time [min]	Area [uV*sec]	Area [%]	Raw Amount	Adjusted Amount	Amount [Norm. %]	Peak Search
1	peak1	1.890	764745.05	99.97	0.0386	0.0386	23.26	----------
-	peak2	1.943	0.00	0.00	0.0000	0.0000	0.00	----------
-	dmp	5.549	0.00	0.00	0.0000	0.0000	0.00	----------
2	chloro	6.496	118.65	0.02	----------	----------	0.00	----------
3	dbp	7.402	35.14	0.00	0.0631	0.0631	37.99	----------
-	dnbp	7.699	0.00	0.00	0.0000	0.0000	0.00	----------
-	deha	8.926	0.00	0.00	0.0000	0.0000	0.00	----------
4	bbp	9.016	40.70	0.01	0.0643	0.0643	38.75	----------
			764939.54	100.00	0.1660	0.1660	100.00	0.0000

Appendix 2: Phthalates report and chromatogram of polony sample from Pretoria west

Software Version	: 6.3.2.0646	Date	: 2011/02/09 10:24:04 AM
Sample Name	:	Data Acquisition Time	: 2011/02/08 02:50:56 PM
Instrument Name	: Clarus GC	Channel	: A
Rack/Vial	: 0/18	Operator	: Unisa
Sample Amount	: 1.000000	Dilution Factor	: 1.000000
Cycle	: 1		

Result File :
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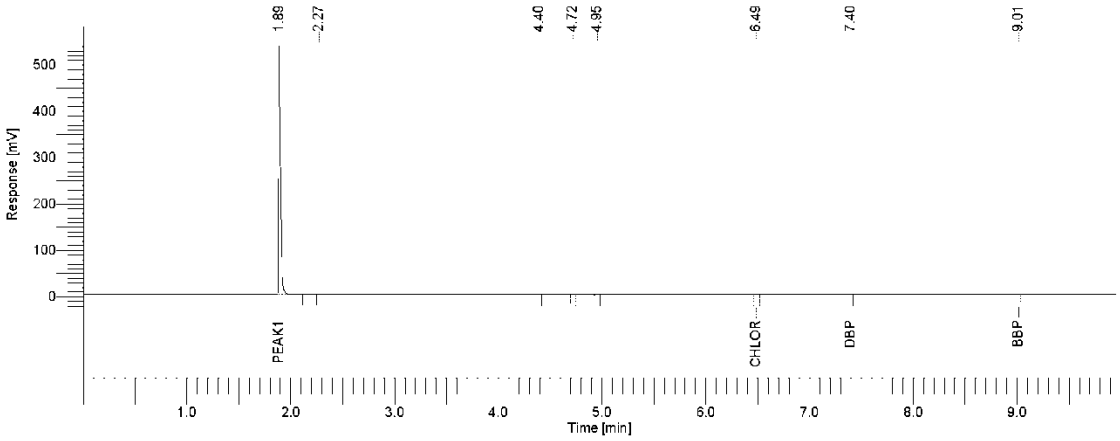


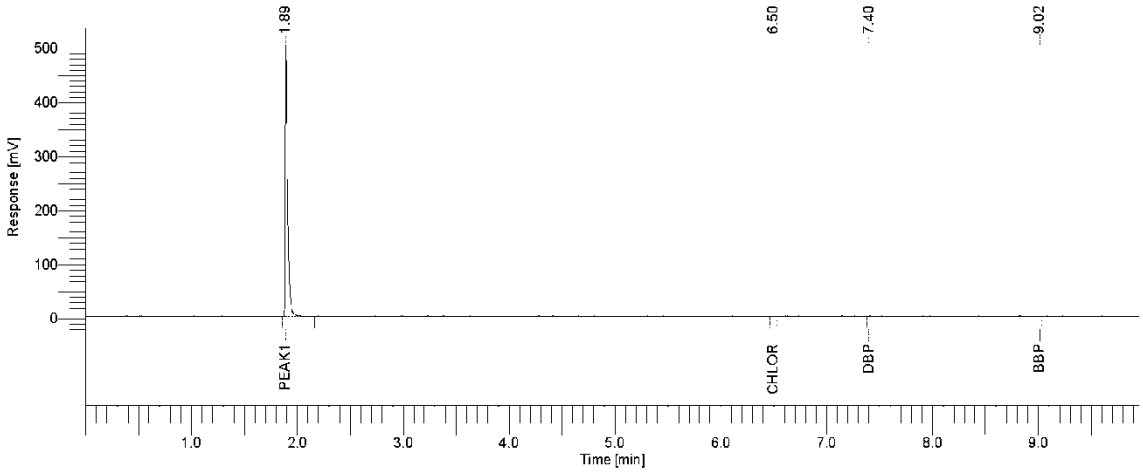
Phthalates Report

Peak #	Component Name	Time [min]	Area [uV*sec]	Area [%]	Raw Amount	Adjusted Amount	Amount [Norm. %]	Peak Search
1	peak1	1.885	721980.80	99.94	0.0340	0.0340	18.91	----------
-	peak2	1.943	0.00	0.00	0.0000	0.0000	0.00	----------
2		2.273	94.70	0.01	0.0001	0.0001	0.05	----------
3		4.396	26.69	0.00	0.0000	0.0000	0.01	----------
4		4.719	55.85	0.01	0.0001	0.0001	0.03	----------
5		4.951	39.65	0.01	0.0000	0.0000	0.02	----------
-	dmp	5.549	0.00	0.00	0.0000	0.0000	0.00	----------
6	chloro	6.489	142.93	0.02	----------	----------	0.00	----------
7	dbp	7.395	43.92	0.01	0.0758	0.0758	42.18	----------
-	dnbp	7.699	0.00	0.00	0.0000	0.0000	0.00	----------
-	deha	8.926	0.00	0.00	0.0000	0.0000	0.00	----------
8	bbp	9.010	44.31	0.01	0.0697	0.0697	38.80	----------
			722428.85	100.00	0.1797	0.1797	100.00	0.0000

Appendix 3: Phthalates report and chromatogram of Vienna sample from Pretoria east

Software Version	: 6.3.2.0646	Date	: 2011/02/09 10:25:39 AM
Sample Name	:	Data Acquisition Time	: 2011/02/08 03:05:03 PM
Instrument Name	: Clarus GC	Channel	: A
Rack/Vial	: 0/19	Operator	: Unisa
Sample Amount	: 1.000000	Dilution Factor	: 1.000000
Cycle	: 1		

Result File :
Sequence File : C:\Unisa\Sequences\ANALYSIS RUNTIME.seq



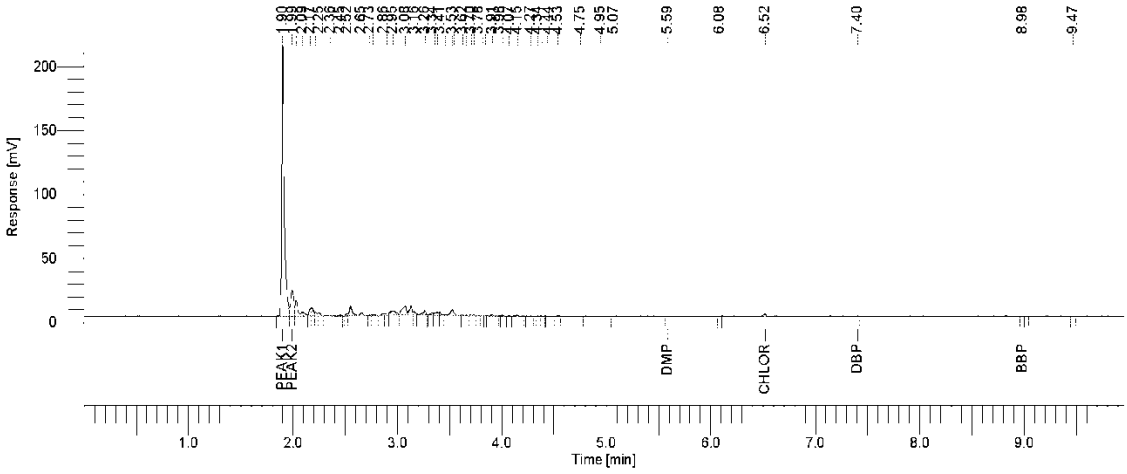
Phthalates Report

Peak #	Component Name	Time [min]	Area [uV*sec]	Area [%]	Raw Amount	Adjusted Amount	Amount [Norm. %]	Peak Search
1	peak1	1.891	755513.36	99.95	0.0376	0.0376	20.70	----------
-	peak2	1.943	0.00	0.00	0.0000	0.0000	0.00	----------
-	dmp	5.549	0.00	0.00	0.0000	0.0000	0.00	----------
2	chloro	6.497	262.86	0.03	----------	----------	0.00	----------
3	dbp	7.402	42.86	0.01	0.0743	0.0743	40.87	----------
-	dnbp	7.699	0.00	0.00	0.0000	0.0000	0.00	----------
-	deha	8.926	0.00	0.00	0.0000	0.0000	0.00	----------
4	bbp	9.015	44.39	0.01	0.0698	0.0698	38.43	----------
			755863.48	100.00	0.1817	0.1817	100.00	0.0000

Appendix 4: Phthalates report and chromatogram polony sample from Pretoria north

Software Version	: 6.3.2.0646	Date	: 2011/02/09 10:26:24 AM
Sample Name	:	Data Acquisition Time	: 2011/02/08 03:19:11 PM
Instrument Name	: Clarus GC	Channel	: A
Rack/Vial	: 0/20	Operator	: Unisa
Sample Amount	: 1.000000	Dilution Factor	: 1.000000
Cycle	: 1		

Result File :
Sequence File : C:\Unisa\Sequences\ANALYSIS RUNTIME.seq



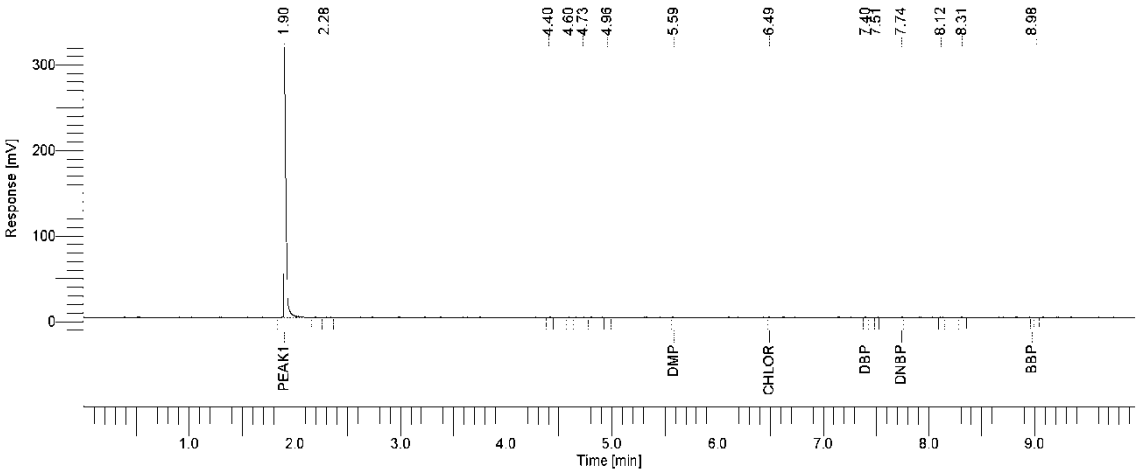
Phthalates Report

Peak #	Component Name	Time [min]	Area [uV*sec]	Area [%]	Raw Amount	Adjusted Amount	Amount [Norm. %]	Peak Search
1	peak1	1.899	342439.81	62.05	-0.0073	-0.0073	-0.91	----------
2	peak2	1.993	41133.36	7.45	0.3519	0.3519	43.67	----------
3		2.030	19963.98	3.62	0.0200	0.0200	2.48	----------
4		2.093	7193.80	1.30	0.0072	0.0072	0.89	----------
5		2.166	5778.66	1.05	0.0058	0.0058	0.72	----------
6		2.184	10729.27	1.94	0.0107	0.0107	1.33	----------
7		2.215	4498.24	0.82	0.0045	0.0045	0.56	----------
8		2.250	3924.73	0.71	0.0039	0.0039	0.49	----------
9		2.360	372.91	0.07	0.0004	0.0004	0.05	----------
10		2.453	811.56	0.15	0.0008	0.0008	0.10	----------
11		2.480	468.46	0.08	0.0005	0.0005	0.06	----------
12		2.519	1825.61	0.33	0.0018	0.0018	0.23	----------
13		2.554	16448.35	2.98	0.0164	0.0164	2.04	----------
14		2.653	4325.70	0.78	0.0043	0.0043	0.54	----------
15		2.733	476.06	0.09	0.0005	0.0005	0.06	----------
16		2.770	1412.42	0.26	0.0014	0.0014	0.18	----------
17		2.864	1953.58	0.35	0.0020	0.0020	0.24	----------
18		2.900	2895.14	0.52	0.0029	0.0029	0.36	----------

Appendix 5: Phthalates report and chromatogram of Vienna sample from Pretoria south

Software Version	: 6.3.2.0646	Date	: 2011/02/09 10:27:43 AM
Sample Name	:	Data Acquisition Time	: 2011/02/08 03:47:29 PM
Instrument Name	: Clarus GC	Channel	: A
Rack/Vial	: 0/22	Operator	: Unisa
Sample Amount	: 1.000000	Dilution Factor	: 1.000000
Cycle	: 1		

Result File :
Sequence File : C:\Unisa\Sequences\ANALYSIS RUNTIME.seq



Phthalates Report

Peak #	Component Name	Time [min]	Area [uV*sec]	Area [%]	Raw Amount	Adjusted Amount	Amount [Norm. %]	Peak Search
1	peak1	1.901	395364.26	99.52	-0.0016	-0.0016	-0.42	----------
-	peak2	1.943	0.00	0.00	0.0000	0.0000	0.00	----------
2		2.282	191.24	0.05	0.0002	0.0002	0.05	----------
3		4.404	98.65	0.02	0.0001	0.0001	0.03	----------
4		4.598	115.82	0.03	0.0001	0.0001	0.03	----------
5		4.727	351.67	0.09	0.0004	0.0004	0.09	----------
6		4.957	139.13	0.04	0.0001	0.0001	0.04	----------
7	dmp	5.587	42.75	0.01	0.0843	0.0843	22.66	----------
8	chloro	6.493	121.67	0.03	----------	----------	0.00	----------
9	dbp	7.401	83.37	0.02	0.1329	0.1329	35.73	----------
10		7.506	35.34	0.01	0.0000	0.0000	0.01	----------
11	dnbp	7.739	66.56	0.02	0.1196	0.1196	32.14	----------
12		8.116	224.20	0.06	0.0002	0.0002	0.06	----------
13		8.315	355.54	0.09	0.0004	0.0004	0.10	----------
-	deha	8.926	0.00	0.00	0.0000	0.0000	0.00	----------
14	bbp	8.981	21.13	0.01	0.0353	0.0353	9.48	----------
15		9.018	51.91	0.01	0.0001	0.0001	0.01	----------
			397263.26	100.00	0.3721	0.3721	100.00	0.0000

Appendix 6: Phthalates report and chromatogram of polony sample from Pretoria south

Software Version : 6.3.2.0646		Date	: 2011/02/09 10:28:49 AM
Sample Name :		Data Acquisition Time	: 2011/02/08 04:01:39 PM
Instrument Name : Clarus GC		Channel	: A
Rack/Vial : 0/23		Operator	: Unisa
Sample Amount : 1.000000		Dilution Factor	: 1.000000
Cycle : 1			

Result File :
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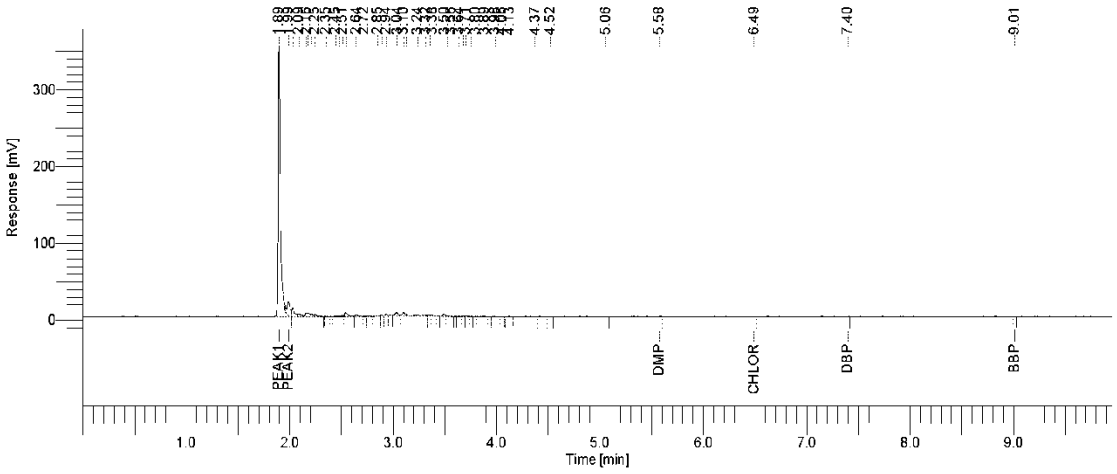


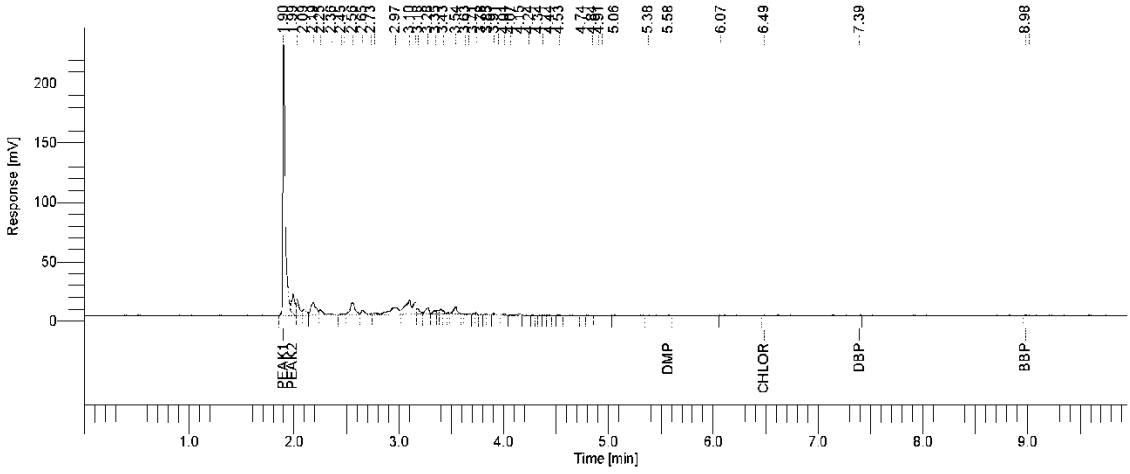
Phthalates Report

Peak #	Component Name	Time [min]	Area [uV*sec]	Area [%]	Raw Amount	Adjusted Amount	Amount [Norm. %]	Peak Search
1	peak1	1.894	503004.88	78.87	0.0101	0.0101	1.56	----------
2	peak2	1.991	42200.11	6.62	0.3616	0.3616	55.76	----------
3		2.028	19493.90	3.06	0.0195	0.0195	3.01	----------
4		2.090	7307.10	1.15	0.0073	0.0073	1.13	----------
5		2.161	4929.11	0.77	0.0049	0.0049	0.76	----------
6		2.181	6356.76	1.00	0.0064	0.0064	0.98	----------
7		2.213	3566.91	0.56	0.0036	0.0036	0.55	----------
8		2.245	3158.66	0.50	0.0032	0.0032	0.49	----------
9		2.355	150.38	0.02	0.0002	0.0002	0.02	----------
10		2.447	420.17	0.07	0.0004	0.0004	0.06	----------
11		2.476	215.80	0.03	0.0002	0.0002	0.03	----------
12		2.512	1158.07	0.18	0.0012	0.0012	0.18	----------
13		2.543	9686.73	1.52	0.0097	0.0097	1.49	----------
14		2.644	2572.58	0.40	0.0026	0.0026	0.40	----------
15		2.723	293.13	0.05	0.0003	0.0003	0.05	----------
16		2.761	623.28	0.10	0.0006	0.0006	0.10	----------
17		2.850	694.06	0.11	0.0007	0.0007	0.11	----------
18		2.893	526.88	0.08	0.0005	0.0005	0.08	----------

Appendix 7: Phthalates report and chromatogram from cheese sample from Pretoria east

Software Version	: 6.3.2.0646	Date	: 2011/02/09 10:29:32 AM
Sample Name	:	Data Acquisition Time	: 2011/02/08 04:15:50 PM
Instrument Name	: Clarus GC	Channel	: A
Rack/Vial	: 0/24	Operator	: Unisa
Sample Amount	: 1.000000	Dilution Factor	: 1.000000
Cycle	: 1		

Result File :
Sequence File : C:\Unisa\Sequences\ANALYSIS RUNTIME.seq



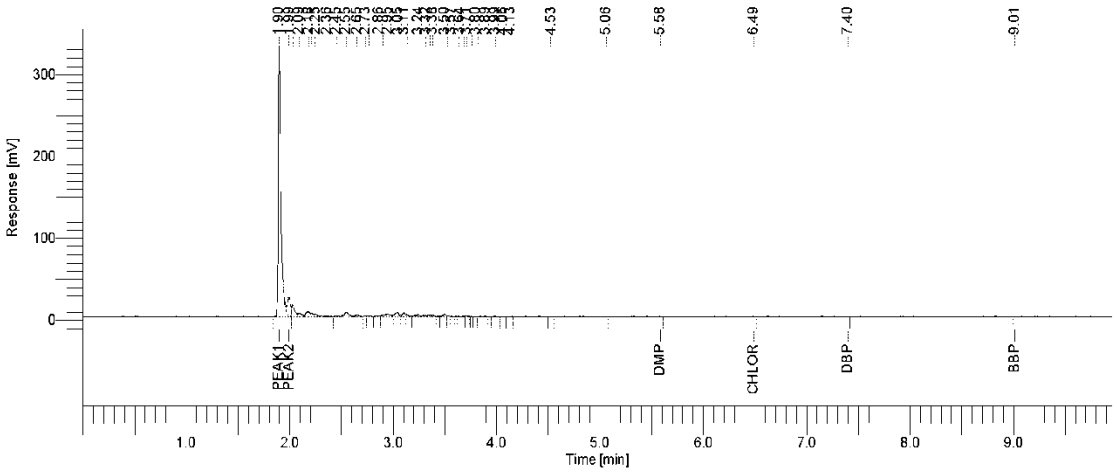
Phthalates Report

Peak #	Component Name	Time [min]	Area [uV*sec]	Area [%]	Raw Amount	Adjusted Amount	Amount [Norm. %]	Peak Search
1	peak1	1.899	345596.62	49.89	-0.0070	-0.0070	-0.71	----------
2	peak2	1.993	36320.10	5.24	0.3080	0.3080	31.36	----------
3		2.030	27368.25	3.95	0.0274	0.0274	2.79	----------
4		2.092	12347.56	1.78	0.0123	0.0123	1.26	----------
5		2.185	36795.27	5.31	0.0368	0.0368	3.75	----------
6		2.250	11541.87	1.67	0.0115	0.0115	1.18	----------
7		2.360	1214.62	0.18	0.0012	0.0012	0.12	----------
8		2.453	982.08	0.14	0.0010	0.0010	0.10	----------
9		2.478	880.36	0.13	0.0009	0.0009	0.09	----------
10		2.558	33838.62	4.88	0.0338	0.0338	3.45	----------
11		2.655	8724.93	1.26	0.0087	0.0087	0.89	----------
12		2.733	895.63	0.13	0.0009	0.0009	0.09	----------
13		2.769	3425.65	0.49	0.0034	0.0034	0.35	----------
14		2.970	36521.64	5.27	0.0365	0.0365	3.72	----------
15		3.105	44376.65	6.41	0.0444	0.0444	4.52	----------
16		3.154	22295.94	3.22	0.0223	0.0223	2.27	----------
17		3.180	8640.07	1.25	0.0086	0.0086	0.88	----------
18		3.278	13728.53	1.98	0.0137	0.0137	1.40	----------

Appendix 8: Phthalates report and chromatogram of cheese sample from Pretoria south

Software Version	: 6.3.2.0646	Date	: 2011/02/09 10:30:54 AM
Sample Name	:	Data Acquisition Time	: 2011/02/08 04:44:30 PM
Instrument Name	: Clarus GC	Channel	: A
Rack/Vial	: 0/26	Operator	: Unisa
Sample Amount	: 1.000000	Dilution Factor	: 1.000000
Cycle	: 1		

Result File :
Sequence File : C:\Unisa\Sequences\ANALYSIS RUNTIME.seq



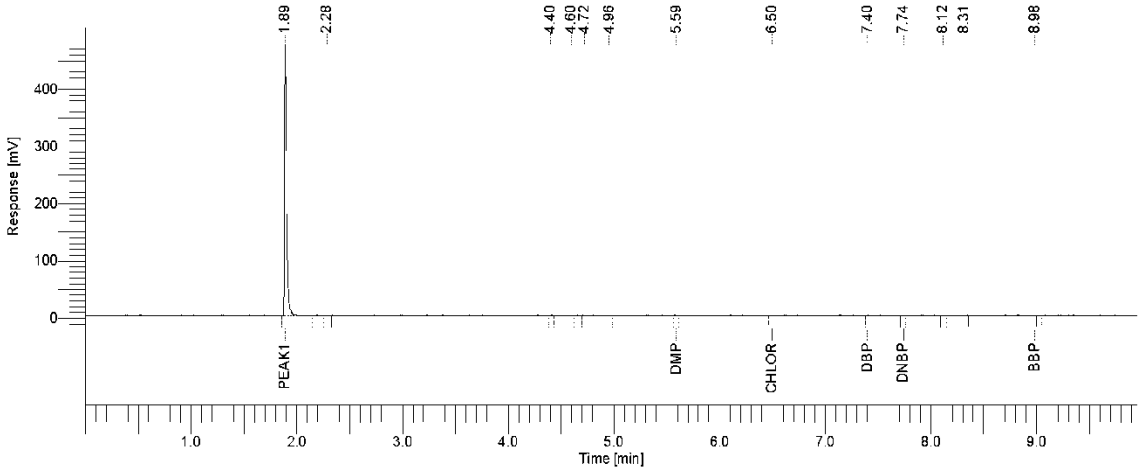
Phthalates Report

Peak #	Component Name	Time [min]	Area [uV*sec]	Area [%]	Raw Amount	Adjusted Amount	Amount [Norm. %]	Peak Search
1	peak1	1.896	547688.84	73.61	0.0150	0.0150	1.89	----------
2	peak2	1.993	50914.39	6.84	0.4412	0.4412	55.54	----------
3		2.030	27625.92	3.71	0.0276	0.0276	3.48	----------
4		2.093	9915.35	1.33	0.0099	0.0099	1.25	----------
5		2.183	16169.43	2.17	0.0162	0.0162	2.04	----------
6		2.213	5261.47	0.71	0.0053	0.0053	0.66	----------
7		2.248	6128.39	0.82	0.0061	0.0061	0.77	----------
8		2.359	897.17	0.12	0.0009	0.0009	0.11	----------
9		2.452	828.06	0.11	0.0008	0.0008	0.10	----------
10		2.549	13818.15	1.86	0.0138	0.0138	1.74	----------
11		2.650	3680.51	0.49	0.0037	0.0037	0.46	----------
12		2.734	732.49	0.10	0.0007	0.0007	0.09	----------
13		2.767	1546.87	0.21	0.0015	0.0015	0.19	----------
14		2.857	2632.65	0.35	0.0026	0.0026	0.33	----------
15		2.905	2553.84	0.34	0.0026	0.0026	0.32	----------
16		2.947	8559.26	1.15	0.0086	0.0086	1.08	----------
17		3.045	11934.42	1.60	0.0119	0.0119	1.50	----------
18		3.107	7813.31	1.05	0.0078	0.0078	0.98	----------

Appendix 9: Phthalates report and chromatogram of Vienna sample from Pretoria east

Software Version	: 6.3.2.0646	Date	: 2011/02/09 10:32:08 AM
Sample Name	:	Data Acquisition Time	: 2011/02/08 04:58:48 PM
Instrument Name	: Clarus GC	Channel	: A
Rack/Vial	: 0/27	Operator	: Unisa
Sample Amount	: 1.000000	Dilution Factor	: 1.000000
Cycle	: 1		

Result File :
Sequence File : C:\Unisa\Sequences\ANALYSIS RUNTIME.seq



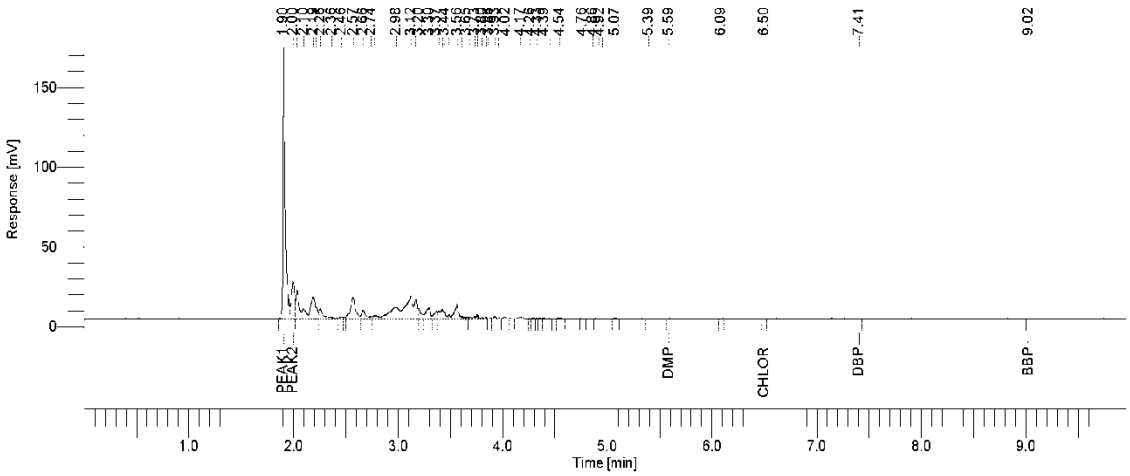
Phthalates Report

Peak #	Component Name	Time [min]	Area [uV*sec]	Area [%]	Raw Amount	Adjusted Amount	Amount [Norm. %]	Peak Search
1	peak1	1.889	610835.75	99.77	0.0219	0.0219	4.26	----------
-	peak2	1.943	0.00	0.00	0.0000	0.0000	0.00	----------
2		2.282	70.49	0.01	0.0001	0.0001	0.01	----------
3		4.404	29.99	0.00	0.0000	0.0000	0.01	----------
4		4.598	48.01	0.01	0.0000	0.0000	0.01	----------
5		4.725	138.45	0.02	0.0001	0.0001	0.03	----------
6		4.956	44.42	0.01	0.0000	0.0000	0.01	----------
7	dmp	5.592	113.30	0.02	0.2003	0.2003	39.00	----------
8	chloro	6.496	320.54	0.05	----------	----------	0.00	----------
9	dbp	7.401	77.02	0.01	0.1237	0.1237	24.10	----------
10	dnbp	7.739	37.17	0.01	0.0708	0.0708	13.80	----------
11		8.116	139.26	0.02	0.0001	0.0001	0.03	----------
12		8.314	232.24	0.04	0.0002	0.0002	0.05	----------
-	deha	8.926	0.00	0.00	0.0000	0.0000	0.00	----------
13	bbp	8.982	61.98	0.01	0.0959	0.0959	18.69	----------
14		9.020	89.82	0.01	0.0001	0.0001	0.02	----------
			612238.46	100.00	0.5134	0.5134	100.00	0.0000

Appendix 10: Phthalates report and chromatogram of cheese sample from Pretoria east

Software Version	: 6.3.2.0646	Date	: 2011/02/09 10:32:46 AM
Sample Name	:	Data Acquisition Time	: 2011/02/08 05:13:06 PM
Instrument Name	: Clarus GC	Channel	: A
Rack/Vial	: 0/28	Operator	: Unisa
Sample Amount	: 1.000000	Dilution Factor	: 1.000000
Cycle	: 1		

Result File :
Sequence File : C:\Unisa\Sequences\ANALYSIS RUNTIME.seq



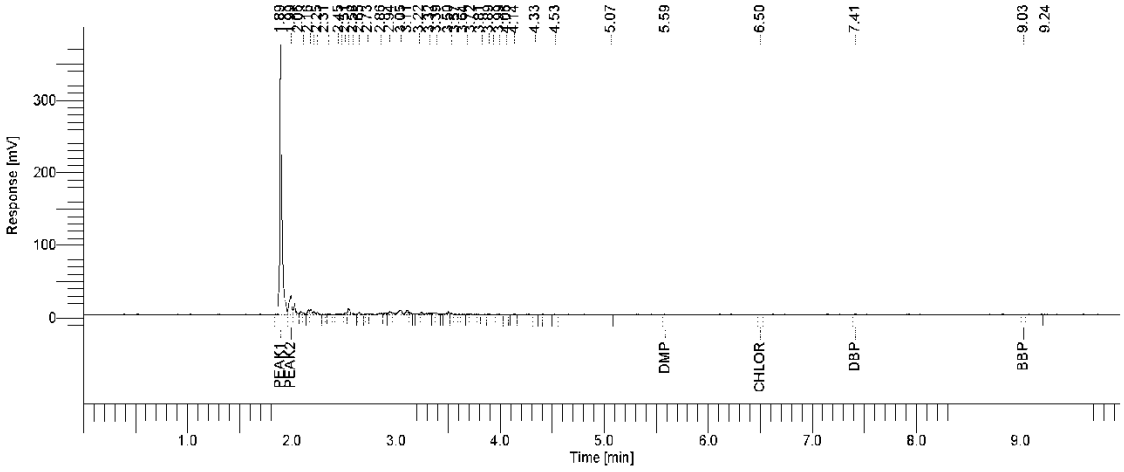
Phthalates Report

Peak #	Component Name	Time [min]	Area [uV*sec]	Area [%]	Raw Amount	Adjusted Amount	Amount [Norm. %]	Peak Search
1	peak1	1.904	251438.36	33.34	-0.0172	-0.0172	-1.46	----------
2	peak2	1.996	51568.83	6.84	0.4471	0.4471	37.74	----------
3		2.034	35779.43	4.74	0.0358	0.0358	3.02	----------
4		2.096	17227.86	2.28	0.0172	0.0172	1.45	----------
5		2.190	38141.36	5.06	0.0381	0.0381	3.22	----------
6		2.218	9877.49	1.31	0.0099	0.0099	0.83	----------
7		2.256	17404.50	2.31	0.0174	0.0174	1.47	----------
8		2.363	3170.90	0.42	0.0032	0.0032	0.27	----------
9		2.459	2466.44	0.33	0.0025	0.0025	0.21	----------
10		2.484	1726.69	0.23	0.0017	0.0017	0.15	----------
11		2.570	46224.66	6.13	0.0462	0.0462	3.90	----------
12		2.665	14002.51	1.86	0.0140	0.0140	1.18	----------
13		2.740	2353.50	0.31	0.0024	0.0024	0.20	----------
14		2.778	7225.27	0.96	0.0072	0.0072	0.61	----------
15		2.983	51353.84	6.81	0.0514	0.0514	4.33	----------
16		3.124	59681.22	7.91	0.0597	0.0597	5.04	----------
17		3.172	27264.98	3.62	0.0273	0.0273	2.30	----------
18		3.197	12990.28	1.72	0.0130	0.0130	1.10	----------

Appendix 11: Phthalates report and chromatogram of polony sample from Pretoria south

Software Version	: 6.3.2.0646	Date	: 2011/02/09 10:34:08 AM
Sample Name	:	Data Acquisition Time	: 2011/02/08 05:41:41 PM
Instrument Name	: Clarus GC	Channel	: A
Rack/Vial	: 0/30	Operator	: Unisa
Sample Amount	: 1.000000	Dilution Factor	: 1.000000
Cycle	: 1		

Result File :
Sequence File : C:\Unisa\Sequences\ANALYSIS RUNTIME.seq



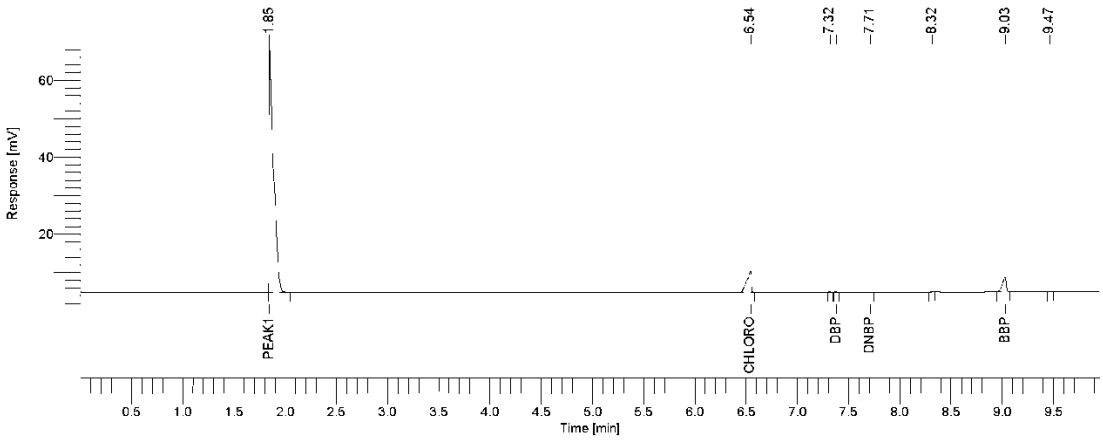
Phthalates Report

Peak #	Component Name	Time [min]	Area [uV*sec]	Area [%]	Raw Amount	Adjusted Amount	Amount [Norm. %]	Peak Search
1	peak1	1.889	519427.07	74.59	0.0119	0.0119	0.94	----------
2	peak2	1.989	45103.79	6.48	0.3881	0.3881	30.61	----------
3		2.026	18133.25	2.60	0.0181	0.0181	1.43	----------
4		2.062	854.13	0.12	0.0009	0.0009	0.07	----------
5		2.088	4754.62	0.68	0.0048	0.0048	0.37	----------
6		2.111	1644.82	0.24	0.0016	0.0016	0.13	----------
7		2.160	7193.49	1.03	0.0072	0.0072	0.57	----------
8		2.181	7371.31	1.06	0.0074	0.0074	0.58	----------
9		2.212	4199.22	0.60	0.0042	0.0042	0.33	----------
10		2.245	3011.23	0.43	0.0030	0.0030	0.24	----------
11		2.308	84.06	0.01	0.0001	0.0001	0.01	----------
12		2.355	267.98	0.04	0.0003	0.0003	0.02	----------
13		2.448	745.14	0.11	0.0007	0.0007	0.06	----------
14		2.479	355.35	0.05	0.0004	0.0004	0.03	----------
15		2.514	2123.42	0.30	0.0021	0.0021	0.17	----------
16		2.548	10574.90	1.52	0.0106	0.0106	0.83	----------
17		2.583	1123.57	0.16	0.0011	0.0011	0.09	----------
18		2.646	3035.10	0.44	0.0030	0.0030	0.24	----------

Appendix 12: Phthalates report and chromatogram of cheese plastic wrapper

Software Version	: 6.3.2.0646	Date	: 2011/07/14 01:32:51 PM
Sample Name	:	Data Acquisition Time	: 2011/07/14 01:21:49 PM
Instrument Name	: Clarus GC	Channel	: A
Rack/Vial	: 0/38	Operator	: Unisa
Sample Amount	: 1.000000	Dilution Factor	: 1.000000
Cycle	: 1		

Result File :
Sequence File : C:\Unisa\Sequences\plastics analysis.seq



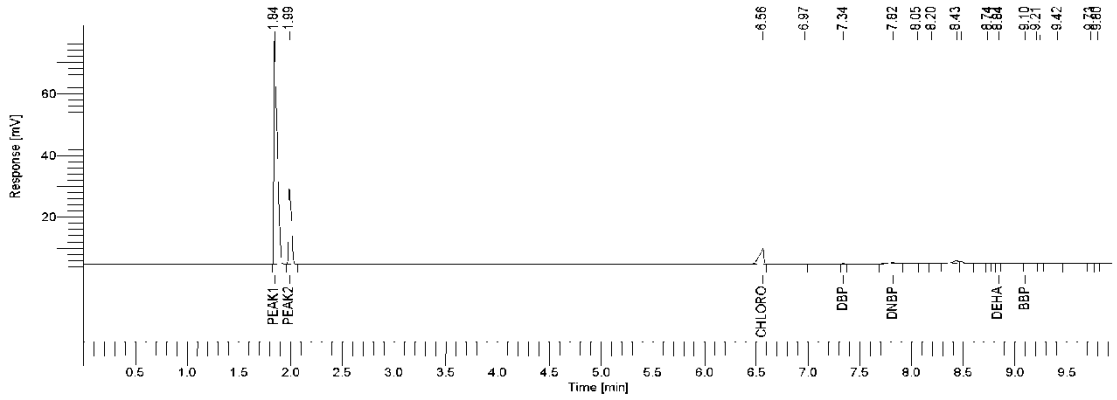
Phthalates Report

Peak #	Component Name	Time [min]	Area [uV*sec]	Area [%]	Raw Amount	Adjusted Amount	Amount [Norm. %]	Peak Search
1	peak1	1.848	189873.95	87.66	-0.0239	-0.0239	-0.17	----------
-	peak2	1.943	0.00	0.00	0.0000	0.0000	0.00	----------
-	dmp	5.549	0.00	0.00	0.0000	0.0000	0.00	----------
2	chloro	6.541	16812.66	7.76	----------	----------	0.00	----------
3		7.319	73.59	0.03	0.0001	0.0001	0.00	----------
4	dbp	7.380	85.84	0.04	0.1365	0.1365	0.94	----------
5	dnbp	7.713	56.52	0.03	0.1029	0.1029	0.71	----------
6		8.319	38.55	0.02	0.0000	0.0000	0.00	----------
-	deha	8.926	0.00	0.00	0.0000	0.0000	0.00	----------
7	bbp	9.032	9585.61	4.43	14.2404	14.2404	98.51	----------
8		9.465	71.06	0.03	0.0001	0.0001	0.00	----------
			216597.77	100.00	14.4561	14.4561	100.00	0.0000

Appendix 13: Phthalates report and chromatogram of polony plastic wrapper

Software Version : 6.3.2.0646		Date	: 2011/07/14 12:20:24 PM
Sample Name :		Data Acquisition Time	: 2011/07/14 12:06:30 PM
Instrument Name : Clarus GC		Channel	: A
Rack/Vial : 0/36		Operator	: Unisa
Sample Amount : 1.000000		Dilution Factor	: 1.000000
Cycle : 1			

Result File :
Sequence File : C:\Unisa\Sequences\plastics analysis.seq



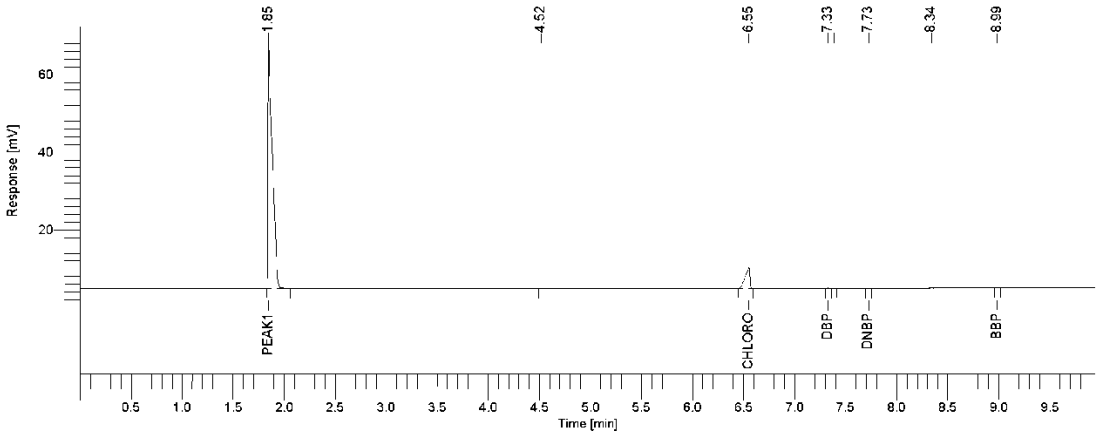
Phthalates Report

Peak #	Component Name	Time [min]	Area [uV*sec]	Area [%]	Raw Amount	Adjusted Amount	Amount [Norm. %]	Peak Search
1	peak1	1.844	169081.76	70.59	-0.0262	-0.0262	-0.65	----------
2	peak2	1.990	47338.80	19.76	0.4085	0.4085	10.12	----------
-	dmp	5.549	0.00	0.00	0.0000	0.0000	0.00	----------
3	chloro	6.562	14869.79	6.21	----------	----------	0.00	----------
4		6.968	30.62	0.01	0.0000	0.0000	0.00	----------
5	dbp	7.340	96.70	0.04	0.1523	0.1523	3.77	----------
6	dnbp	7.822	2025.26	0.85	3.3696	3.3696	83.44	----------
7		8.053	19.51	0.01	0.0000	0.0000	0.00	----------
8		8.197	41.58	0.02	0.0000	0.0000	0.00	----------
9		8.433	3589.36	1.50	0.0036	0.0036	0.09	----------
10		8.484	1913.42	0.80	0.0019	0.0019	0.05	----------
11		8.738	46.61	0.02	0.0000	0.0000	0.00	----------
12	deha	8.841	45.13	0.02	0.0879	0.0879	2.18	----------
13	bbp	9.100	24.51	0.01	0.0403	0.0403	1.00	----------
14		9.206	25.18	0.01	0.0000	0.0000	0.00	----------

Appendix 14: Phthalates report and chromatogram of Vienna plastic wrapper

Software Version : 6.3.2.0646		Date	: 2011/07/14 01:18:22 PM
Sample Name :		Data Acquisition Time	: 2011/07/14 01:07:46 PM
Instrument Name : Clarus GC		Channel	: A
Rack/Vial : 0/37		Operator	: Unisa
Sample Amount : 1.000000		Dilution Factor	: 1.000000
Cycle : 1			

Result File :
Sequence File : C:\Unisa\Sequences\plastics analysis.seq



Phthalates Report

Peak #	Component Name	Time [min]	Area [uV*sec]	Area [%]	Raw Amount	Adjusted Amount	Amount [Norm. %]	Peak Search
1	peak1	1.848	187504.88	91.58	-0.0242	-0.0242	-6.63	----------
-	peak2	1.943	0.00	0.00	0.0000	0.0000	0.00	----------
2		4.517	44.65	0.02	0.0000	0.0000	0.01	----------
-	dmp	5.549	0.00	0.00	0.0000	0.0000	0.00	----------
3	chloro	6.553	16863.43	8.24	----------	----------	0.00	----------
4	dbp	7.328	75.41	0.04	0.1214	0.1214	33.25	----------
5		7.387	36.28	0.02	0.0000	0.0000	0.01	----------
6	dnbp	7.725	43.07	0.02	0.0806	0.0806	22.07	----------
7		8.340	55.94	0.03	0.0001	0.0001	0.02	----------
-	deha	8.926	0.00	0.00	0.0000	0.0000	0.00	----------
8	bbp	8.987	123.45	0.06	0.1872	0.1872	51.27	----------
			204747.11	100.00	0.3652	0.3652	100.00	0.0000