

Evaluation of maleate, fumarate, and succinate diesters as potential green plasticizers

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Être homme, c'est précisément être responsable. C'est connaître la honte en face d'une misère qui ne semblait pas dépendre de soi. C'est être fier d'une victoire que les camarades ont remportée. C'est sentir, en posant sa pierre, que l'on contribue à bâtir le monde.

-Antoine de Saint-Exupéry

Gewidmet meinen lieben Eltern Eda & Karl-Heinz, sowie Thilo mit Marla.

Abstract

The industrial success of poly(vinyl chloride) (PVC) would not have been possible without compounds known as plasticizers, the most important class of additives to brittle polymers such as PVC. Plasticizers facilitate the processing of PVC and render the final product soft and flexible, and plasticizer content can reach up to 50 weight-percent. Due to the ever-increasing use of flexible PVC, plasticizers are produced in the range of several million metric tonnes per year. The most important class of plasticizers are phthalate diesters and di (2-ethylhexyl) phthalate (DEHP) is the most frequently used compound. The vast majority of plasticizers is simply blended with a polymer at elevated temperatures and thus is not bound to the polymer matrix, which results in their tendency to leach from the material over time, ultimately ending up in the environment. Once in the environment, the microbial biodegradation rates of phthalates are low, leading to their omnipresence in environmental samples. Moreover, the metabolites produced during the microbial breakdown are also stable compounds, as for example the corresponding monoester to DEHP, mono (2-ethylhexyl) phthalate (MEHP). Many studies have been carried out to investigate the toxicological effects of phthalates and their metabolites, and a variety of negative effects have been reported including endocrine disrupting and anti-androgenic effects. As a result, six phthalates have been banned in toys and childcare articles in Canada and other major markets. This ban has led to increased efforts to develop efficient plasticizers that are less environmentally persistent and show a lower toxicity profile.

The research reported in this thesis aimed at evaluating plasticizer effectiveness and biodegradation rates of diesters synthesised from four-carbon diacids esterified with a linear alcohols ranging from ethanol (C2) to octanol (C8), and one branched alcohol, 2-ethylhexanol. The diacids were chosen due to their structural resemblance to the phthalate molecule, of which two were unsaturated (i.e., maleate: *cis*; fumarate: *trans*) and one saturated (i.e., succinate). The goal of this thesis was to gain insight on the influence of the geometry of the central structure and the length and branching of the side chains on plasticizer efficiency and biodegradability. The plasticizer candidates were blended with PVC at varying concentrations and the plasticizer effectiveness was evaluated by the measurement of the glass transition temperature T_g , tensile testing, dynamic mechanical thermal analysis (DMTA), and surface

hardness. The obtained results were then compared to similar blends containing the commercial plasticizers DEHP and DINCH[®]. Biodegradation properties were assessed by exposing the common soil bacterium *Rhodococcus rhodocrous* (ATCC 13808) to candidate plasticizers with hexadecane as carbon source.

The results revealed important differences arising from central structures and indicated that the saturated (succinate) and the *cis*-structure (maleate) made for the most efficient plasticizers. Further studies of the succinate revealed that any groups hindering free rotation around the central chain of the molecule reduced its plasticizing efficiency. Side chain length was important and maximum efficiency occurred for a side chain length around six carbons. The added ethyl branch in those diesters with 2-ethylhexyl side chains was not found to have an enhancing effect on plasticizer effectiveness.

The biodegradation experiments revealed a crucial influence of the central structure, as the saturated compound (succinate) was rapidly degraded and metabolite buildup was small and only transient. Between the two unsaturated compounds, those with the ester groups in *trans* position (fumarate) were more quickly degraded than those in *cis*-position (maleate); the latter showing almost no degradation over the course of the experiment. This could also explain the observed slow biodegradation of phthalate diesters, in which the ester groups are in a similar arrangement as in the maleate. Slower degradation rates were found with increasing side chain length as well as branching, but effects were not as important as that of the central structure. Nonetheless, when linear side chains were used, no or only small, transient buildup of the released alcohol was found, contrarily to 2-ethylhexanol which built up significantly during the according experiments.

Taken together, dibutyl and dihexyl diesters of succinic- and maleic acid showed the most promising results in terms of plasticizer efficiency and biodegradability, yet further toxicological experiments are required to determine if these are truly "green" plasticizers.

Résumé

Le succès industriel du polymère poly(chlorure de vinyle) (PVC) n'aurait pas été possible sans des molécules appelées plastifiants, qui représentent l'additif le plus important du PVC. Les plastifiants facilitent les étapes de production du PVC et rendent les produits finaux souples et flexibles. Plusieurs millions de tonnes de plastifiants sont produits chaque année. Les diesters de phthalate comme le di (2-éthylhexyle) phthalate (DEHP) représentent le type le plus important de plastifiant. Les plastifiants sont mélangés avec des polymères à des températures élevées, mais généralement, aucun lien chimique n'est établi entre le plastifiant et le polymère. C'est pour cette raison que les plastifiants ont tendance à se retirer du mélange au fil du temps, pour ensuite finir comme polluant dans l'environnement. Le taux de biodégradation des phthalates dans l'environnement est faible, ils sont donc omniprésents. Les métabolites qui sont produits pendant ce processus sont stables. Un exemple d'un tel métabolite est le monoester mono (2-éthylhexyle) phthalate (MEHP) pour DEHP. Plusieurs études ont démontré des mécanismes toxiques des phthalates et leurs métabolites. Les effets les plus lancinants affectent le système endocrinien, résultant dans des effets anti-androgènes. Basé sur ces résultats, six phthalates ont été bannis de jouets et d'articles de puériculture dans le Canada et d'autres marchés majeurs. Par conséquent, le développement de nouveaux plastifiants efficaces, moins persistants et toxiques dans l'environnement est nécessaire.

Cette thèse vise à évaluer l'efficacité du plastifiant et les taux de biodégradations de plusieurs diesters synthétisés avec des alcools linéaires, qui variaient d'une longueur de deux (éthanol) à huit (octanol) charbons, et un alcool ramifié, soit 2-éthylhexanol. Les acides dicarboxyliques utilisés ont été choisis à cause de leur similitude à la structure du phthalate, et un total de trois structures centrales différentes ont été évaluées. Celles-ci comprenaient deux structures insaturées, le maléate (*cis*) et le fumarate (*trans*), et une structure saturée, le succinate. L'objectif de cette thèse était d'ailleurs de mieux comprendre l'influence de la géométrie de la partie centrale de la molécule et de la longueur de la chaîne latérale sur l'efficacité du plastifiant et le taux de biodégradation. Les molécules étudiées étaient mélangées à différentes concentration avec du PVC non-plastifié, et leur efficacité du plastifiant a été évaluée à l'aide de plusieurs essais, dont la détermination de la température de transition vitreuse (T_g), des essais de traction, l'analyse thermomécanique dynamique

(ATMD), et la dureté superficielle. Les résultats obtenus étaient ensuite comparés avec des résultats d'essais semblables avec des plastifiants commerciaux comme DEHP et DINCH®. Les taux de biodégradation ont été évalués à l'aide d'une bactérie courante dans le sol, *Rhodococcus rhodocrous* (ATCC 13808) et hexadécane comme source de carbone.

Les résultats obtenus montraient que les plastifiants les plus efficaces contenaient des structures centrales insaturées en configuration *cis* (maléate) ou saturées (succinate). La capacité de rotation autour de la chaîne centrale du succinate était un facteur important dans l'efficacité du plastifiant, et l'attachement de groupes chimiques qui empêchaient cette capacité de rotation menait à des plastifiants moins efficaces. La longueur de la chaîne latérale jouait aussi un rôle important : l'efficacité maximale des plastifiants était atteinte quand cette chaîne latérale était formée d'environ six charbons. Quant à la chaîne ramifiée, l'addition d'une branche éthylique au diester n'améliorait pas l'efficacité du plastifiant, mais l'efficacité trouvée était semblable à celle de la molécule avec la même longueur.

Les expériences de biodégradation révélaient l'importance de la géométrie de la structure centrale : les molécules saturées (succinates) étaient biodégradées rapidement, et l'accumulation de métabolites était faible et seulement transitoire. Entre les structures centrales contentant des liens non-saturés, les diesters en conformation *trans* étaient biodégradés plus rapidement que ceux en conformation *cis*, et ces derniers ne montraient pas des taux de biodégradation très faible. Cela aide à expliquer la relative résistance des diesters de phthalate à la biodégradation, vu que la position des deux groupes esters est très similaire entre les maléates (*cis*) et les phthalates. Au niveau de la chaîne latérale, des taux de biodégradation inférieurs étaient observés avec la croissance de la longueur de la chaîne latérale, mais la structure de la partie centrale de la molécule était plus importante ultimement. Néanmoins, quand les chaînes latérales étaient linéaires, aucun ou seulement très peu d'accumulation d'alcool libéré a été observé, tandis que 2-éthylhexanol s'accumulait pendant les expériences avec les molécules contenant des ramifications.

En conclusion, les maléates et succinates de dibutyl et dihexyl semblent être les candidates les plus prometteuses en termes d'efficacité du plastifiant et de biodégradabilité, néanmoins plus d'expériences sur la possible toxicité de ces substances sont requises pour assurer que ceux-ci sont vraiment des plastifiants « verts ».

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Contribution of Authors

This thesis is manuscript-based and contains five manuscripts, one of which is a review article. In all cases, I have researched and prepared the entire article in consultation with my supervisors. Furthermore, all necessary synthesis, experimental design and execution, and analysis of the data was carried out by me. NMR spectroscopy was carried out by Dr. Frederick Morin in the Department of Chemistry, but interpretation of the spectra was my own work. For the manuscripts #3-5 (see below), co-authors include summer students (Patrick Dodd, Tobin Brown, Sarah Shipley), as well as a German Master's student on an internship comparable to a summer project (Aurélie Börmann). In all cases, these students were trained by me and carried out independent projects under my supervision, in conjunction with my supervisors. More specifically, Patrick Dodd was responsible for extrusion and material testing (Tg and tensile testing) of the succinates with modified central alkyl chain between the ester bonds. Tobin Brown was responsible for the biodegradation experiments of dihexyl-, monohexyl-, dioctyl-, and monooctyl- maleates. Sarah Shipley was responsible for the extrusion and collection of one set of data of tensile testing of the series of succinates and maleates, DEHP, and DINCH[®]. Aurélie Börmann helped in the DMTA study design and was responsible for extrusion and DMTA experiments of the series of succinates and maleates, DEHP, and DINCH®. My Ph.D. supervisors, Prof. Richard L. Leask and Jim A. Nicell, as well as my former supervisors Prof. David G. Cooper and Prof. Milan Maric supervised and helped in guiding the research, proofreading and editing the manuscripts. Prof. Viviane Yargeau proofread and edited the review article (#1 in this list), which is presented in Chapter 4. The following manuscripts are part of this thesis, including information on their publication:

- Erythropel, H.C., Maric, M., Nicell, J.A., Leask, R.L., and Yargeau, V. (2014). "Leaching of the plasticizer di (2-ethylhexyl) phthalate (DEHP) from plastic containers and the question of human exposure." <u>Applied Microbiology and Biotechnology</u> 98(24): 9967-9981. (Chapter 4)
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- Erythropel H.C., Shipley, S., Börmann, A., Maric, M., Nicell, J.A., and Leask, R.L. "Designing Green Plasticizers: Influence of Molecule Geometry and Alkyl Chain Length on the Plasticizing Effectiveness of Diester Plasticizers in PVC Blends". <u>Polymer</u>, accepted (DOI: 10.1016/j.polymer.2016.02.031) (Chapter 8)

1 Introduction

Plasticizers are compounds that are added to polymeric resins such as PVC in order to render them malleable and flexible. A plasticizer is literally translated to "soft-maker" in several languages, such as German, the country where much of the early research on plasticizers was carried out starting in the 1930s. The American Society for Testing and Materials (ASTM) defines a plasticizer as "a substance incorporated in a [polymeric] material to increase its workability, flexibility, or distensibility" (ASTM D-883 2012).

1.1 PVC

The polymer poly(vinyl chloride) (PVC) was discovered in the 19th century, but it was not until the early 20th century that the rise of PVC usage began, leading to PVC being the third-largest selling commodity plastic after polyethylene (PE) and polypropylene (PP) today (Matthews 1996, Modern Plastics 2001, American Chemistry Council 2015, PlasticsEurope 2015). Although PVC is a hard and brittle material that is very difficult to process, it is unique in that it can be readily blended with many other materials, which means that many functions can be added to PVC by blending in external compounds (Wilkes et al. 2005). Plasticizers played a key role in the development and commercial success of PVC products, because they enabled an easy processability of PVC (Wilkes et al. 2005). Some of the advantages of PVC include its low production cost and its versatility for use in a wide variety of products (Matthews 1996). PVC contains one chlorine-atom on every second carbon of the backbone as shown in Figure 1.1.



Figure 1.1. Chemical structure of poly(vinyl chloride) (PVC), and indication of the weak dipole present (i.e., δ - and δ +).

Since chlorine is more electronegative than carbon, a weak dipole moment results between these two atoms, as indicated with δ symbols in Figure 1.1. Due to this dipole moment inherent in the PVC chain, individual chains interact with each other and their movement relative to each other is constricted because of the weak interactions between chains (Leuchs 1956, Sears and Darby 1982). As a result of this microscopic effect, PVC is macroscopically rigid and brittle at room temperature. Once the glass transition temperature T_g is reached, enough energy is in the system to overcome these restrictive forces and the polymer becomes macroscopically flexible as chains are able to move past one another more easily on a microscopic level (Sears and Darby 1982). The T_g for unplasticized PVC is 82 °C (Wilkes et al. 2005). It is assumed that PVC is mainly amorphous but that some regions of crystallinity exist. The concept of anti-plasticization is used to prove this (Sears and Darby 1982).

1.2 External and Internal Plasticization

A distinction is made between external and internal plasticizers, however the following work will focus on external plasticization and any use of the word "plasticizer" refers to external plasticizers from here on, unless otherwise specified.

Briefly, internal plasticization usually refers to compounds that are part of the polymer backbone and thus chemically attached to the polymer itself. Their mechanism of action is similar to external plasticizers; i.e., the disruption of the interaction of the neighbouring polymer chains. As internal plasticizers often need to be custom-tailored for each resin, the development of such an internal plasticizer usually results in higher production costs of the internally plasticized resin (Sears and Darby 1982). There are also efforts to make plasticizers that can be chemically bonded to the polymer chain once these are blended (Navarro et al. 2010). Internally plasticized resins often exhibit a decreased range of workable temperatures compared to externally plasticized resins, resulting in the need of the addition small amounts of external plasticizer to internally plasticized resins (Sears and Darby 1982).

External plasticization is the addition of a usually relatively low molecular weight compound to a polymer, yet no chemical bond is created between resin and plasticizer. This type of plasticization is by far the more important one due to practical and economic reasons (Sears and Darby 1982). Because external plasticizers are not bound to the polymer matrix, they have a tendency to leach from the blend, leading to human exposure and ultimately the entry of these compounds into the environment (Wams 1987). Please see Chapter 4 where this is discussed in greater detail.

1.3 Phthalates

Phthalates are the most important class of plasticizers worldwide, accounting for 88 % of all plasticizers used in PVC, which translates to approximately 5 million metric tonnes annually (Bisig, 2009). These compounds are produced through the esterification of the diacid phthalic acid with two alcohols, yielding phthalate diesters. In these phthalates, the two ester groups are in *ortho*-position to one another with respect to the benzene ring, which is shown in Figure 1.2.



Figure 1.2. Structure of six phthalates recently banned in quantities above 0.1 wt.-% in children's toys in Canada (HPA 2010), the United States (CPSIA 2008), Europe (EU 2005), and Japan (Bureau Veritas 2010). DEHP – di (2-ethylhexyl) phthalate, DBP – dibutyl phthalate, DNOP – di-n-octyl phthalate, DINP – diisononyl phthalate, DIDP – diisodecyl phthalate, BBP – benzyl butyl phthalate.

The compound di (2-ethylhexyl) phthalate (DEHP) is the most important phthalate, with a production rate of approximately 2.6 million metric tonnes annually, or 54 % of overall phthalate production (Bisig 2009). External plasticizers tend to leach from blends with PVC and studies have shown that DEHP and other phthalates are ubiquitous contaminants on a global scale (Wams 1987) due to the high yearly production volumes, their rather slow biodegradation kinetics, and their tendency to migrate to lipophilic environments (Wams 1987, Staples et al. 1997, ATSDR 2002, Heinemeyer et al. 2013). Furthermore, their biodegradation rates in lipophilic environments are further reduced due to the often present anaerobic conditions (Wams 1987, Staples et al. 1997). Studies have found phthalates in various environmental matrices including soils (Bauer and Herrmann 1997, Cartwright et al. 2000), surface water (Horn et al. 2004), indoor air (Edwards et al. 2001), and the atmosphere (Thuren and Larsson 1990). Several recent studies reported findings of DEHP and other phthalates in samples of bottled water (Bošnir et al. 2007, Leivadara et al. 2008, Diana and Dimitra 2011, Keresztes et al. 2013, Guart et al. 2014), yet the source of this contamination remained speculative. Unfortunately, there commonly exist misconceptions about the kinds of materials that contain plasticizers, resulting in turn in misconceptions about the source of plasticizer exposure. Therefore, it is important to identify the predominant routes of human exposure to DEHP and other phthalates, and these routes of exposure need to be taken into consideration when designing alternative, green plasticizers.

The microbial biodegradation mechanism of phthalates is discussed in more detail in Sections 3.4 and 4.8, but in brief, a first hydrolysis step yields a stable metabolite, the corresponding monoester mono (2-ethylhexyl) phthalate (MEHP) and 2-ethylhexanol, in the case of DEHP, which is followed by a second hydrolysis step to yield another alcohol and phthalic acid. The liberated 2-ethylhexanol is further oxidized to 2-ethylhexanoic acid, yet further degradation of it is slow due to the ethyl-branch on the β -carbon, which hinders further breakdown by β -oxidation (Schaeffer et al. 1979, Nalli et al. 2002). The mineralization pathway of the liberated phthalic acid is shown in Figure 3.6, as reproduced from Staples et al. (1997).

DEHP and the stable metabolites MEHP and 2-ethylhexanoic acid have been thoroughly studied and a broad range of toxic effects on humans and animals have been found (Akingbemi et al. 2001, Foster et al. 2001, Gazouli et al. 2002, Akingbemi et al. 2004, Horn et

al. 2004). The most problematic compound is the monoester MEHP, which is believed to be an endocrine disruptor, leading to anti-androgenic effects (Gray and Gangolli 1986, Richburg and Boekelheide 1996, Lovekamp-Swan and Davis 2003, Swan et al. 2005a, Pant et al. 2008, Fan et al. 2010, Piche et al. 2012). Also, 2-ethylhexanoic acid has also been shown to be more toxic than DEHP (Nalli et al. 2002). The literature pertaining to toxicity is reviewed in Section 3.3. The findings of these studies have recently caused several countries including Canada (HPA 2010), the United States (CPSIA 2008), the European Union (EU 2005), and Japan (Bureau Veritas 2010), which constitute key markets for soft PVC articles, to ban six phthalate plasticizers in concentrations above 0.1 wt.-% from children's toys and -articles, as these are one of the most susceptible population group to the effects of phthalates. The six banned phthalates are: di (2-ethylhexyl)- (DEHP), dibutyl- (DBP), di-n-octyl- (DNOP), diisononyl- (DINP), diisodecyl- (DIDP), and benzyl butyl phthalate (BBP). Their structures are shown in Figure 1.2.

1.4 "Green" Plasticizers

Owing to the adverse effects of phthalates and the increased regulatory pressure described above, there is a growing incentive to develop alternative, "green" plasticizers. The three main criteria for such a compound are:

- 1. Good plasticizing properties of PVC, as compared to currently used, commercial plasticizers;
- 2. Quick biodegradation rates once in the environment, which should equally be true for any metabolites formed during the process;
- 3. Low or no toxicity of the parent compound and the metabolites.

Many alternatives plasticizers have been proposed, or are under development, and some of these are further discussed in Section 3.5. However, the mechanism of plasticization is not fully understood (see also Section 3.1) and few systematic studies have been carried out that examine favourable structural features for the plasticization of PVC. However, it has been established that ester-containing compounds often make good plasticizers as the attractive forces between the carbonyl group in the ester and the dipole stemming from the carbon-chlorine bond in the PVC chains are of the same order of magnitude (Smyth 1955, Leuchs

1956, Daniels 2009). Studies examining the effect of the chemical structure of plasticizers have focussed mainly on the compatibility of the plasticizer with PVC rather than the effectiveness of the plasticizer to improve material properties, and these studies often focussed on one part of the molecule, such as the alkyl-chain length in mono- and diesters. Examples for such studies are those by Doty and Zable (1946), Würstlin and Klein (1956), and van Veersen and Meulenberg (1972).

Replacement green plasticizers need to be extensively screened for risk of human and environmental toxicity. Risk is generally defined as a combination of toxicity and exposure (Casarett et al. 2013). In order to conduct such risk assessments in a complete and meaningful way, knowledge of the breakdown pattern of the plasticizer is essential. For example, as has been reported for the plasticizer DEHP, stable and problematic products are formed upon biodegradation, including MEHP and 2-ethylhexanoic acid (Wams 1987, Staples et al. 1997, Nalli et al. 2002, Frederiksen et al. 2007). A green alternative to DEHP would thus ideally be not only benign in itself but also avoid the buildup of stable metabolites that could lead to ecological and human health toxicity. If, however, such stable metabolites were observed, their testing along with the parent compound is of crucial importance. Biodegradation protocols that can be used to quantify the removal of the parent compound but also monitor and quantify appearing metabolites have been established. For example, one biodegradation protocol is based on the use of the common soil bacterium *Rhodococcus rhodocrous* (Nalli et al. 2002). This bacterial strain is known to be able to degrade hydrophobic compounds such as most plasticizers (Jones and Goodfellow 2012).

When evaluating "green" alternatives, the human health risk including cancerogenicity, skin sensitization, genotoxicity, as well as reproductive-, developmental-, neurological-, and systemic toxicity, and both acute and chronic ecotoxicity to a variety of animals and organisms should be taken into account (US EPA 2005). Since the testing for these many endpoints for newly proposed compounds is labour-intensive and expensive, efforts are on-going to determine structure-activity relationships that could aid in the process of pre-selecting appropriate compounds before they undergo extensive testing (Voutchkova et al. 2010). Similar efforts also exist for biodegradation properties of a broad spectrum of compounds (Mani et al. 1991), but the latter focus primarily on biodegradation end-points that report

mineralization of the compound, as per the OECD method (OECD 2006, OECD 2014). The OECD methods are described in more detail in Section 3.4.

Note that the level to which a compound is considered green must also take into account how it is produced. Ideally, the compound would be sourced from renewable materials, as outlined by the Twelve Principles of Green Chemistry (Anastas and Warner 2000). Recently, several companies have started to produce succinic acid by fermentation with various microorganisms (Debabov 2015), and if the alcohol used to create the ester-bond was also renewably-sourced, the succinate compounds would be of particular interest.

In this thesis, several diester compounds with varying side chain length and degree of branching were evaluated as potential green plasticizers, which structurally resemble phthalate diesters (i.e., maleate, fumarate, and succinate). The work is part of a larger research effort which also includes the evaluation of the mammalian toxicity of these compounds and their metabolites.

2 Objectives

To produce green alternatives to phthalates plasticizers, research is required to develop alternative compounds that not only function well as plasticizers, but are also environmentally and toxicologically benign. Additionally, a good understanding of the routes of human exposure to plasticizers and their metabolites is important to set priorities in the design of alternative, green plasticizers. The goal of this work was to design compounds that resemble the phthalate molecule to retain plasticizer functionality, but which would biodegrade more quickly, and have a lower toxicity profile. In light of this, studies were conducted to determine how the central group structure and the side chain length and branching of diester molecules affect plasticizer performance in blends with PVC, the biodegradation rate, and the buildup of metabolites. More specifically, in order to advance toward the long-term goal of creating green plasticizers, the following objectives were pursued:

- Quantify levels of plasticizer in various products that lead to human exposure to DEHP using literature sources;
- Evaluate and compare the biodegradation rates of pure candidate compounds, monitor the appearance of metabolites, and establish the biodegradation pathway.
- Identify detected metabolites during the biodegradation experiments, quantify their build-up, and assess their impact on the microbial growth.
- Evaluate and compare the T_g reduction efficiencies of the candidate compounds in blends with PVC to commercial plasticizers, as well as to identify the influence of central group and side chains of the candidate compounds on the T_g reduction efficiency;
- 5) Evaluate and compare several mechanical properties of PVC plasticized with the candidate compounds and compare the results to blends prepared with commercial plasticizers, as well as to identify the influence of central group and side chains of the candidate compounds on the mechanical properties of blends of these with PVC.

3 Literature Review

3.1 Plasticization Theories

Due to the vast variety of polymers containing different chemical moieties along its backbone, many different plasticizers exist for many different polymers and applications. However, the mechanism of plasticization is not fully understood to date. Several plasticization theories exist, which partially build on each other. The most important ones (pertaining mainly to PVC) will be summarized briefly in the following paragraphs, while more detailed descriptions of these theories can be found elsewhere, for example in Sears and Darby (1982), Matthews (1996), or Marcilla and Beltrán (2012).

3.1.1 Lubricity and Gel Theory

The first theories developed were the lubricity and the gel theory, rapidly following the increasing commercial use of PVC in the 1940s. Both theories assume that the plasticizer molecules break up either PVC chain-chain interactions along gliding planes (lubricity theory) or tridimensional networks of PVC (gel theory), thereby allowing more PVC chain movement, resulting in a macroscopically more flexible blends once enough plasticizer was added. Experimentally, this would manifest itself by a lower T_g of the polymer blend with increasing amount of plasticizer (Kirkpatrick 1940, Doolittle 1944, Doolittle 1946, Stickney and Cheyney 1948, Sears and Darby 1982, Marcilla and Beltrán 2012). Subsequently, Doolittle (1947) suggested a solvation-desolvation equilibrium between plasticizer molecules and polymer chains similarly to a solvent-solute system, suggesting the solvent power of a plasticizer is its most important characteristic.

Based on the lubricity theory, Leuchs (1956) visualized two different types of plasticizer action based on the chemical moieties present in the plasticizer molecule (parts of the paper by Leuchs (1956) are available in English in the publications of Buszard (1984) and Titow (1990)). Table 3.1 summarizes the kinds of chemical moieties used in plasticizer structures. A plasticizer forcibly needs a polar part to be able to interact with PVC chains and assure compatibility, however compounds that contain only polar parts (combination no.1) are unsuitable as plasticizers, as they cannot break up any PVC-chain interactions, but instead

reinforce the attractive forces that render PVC brittle (Sears and Darby 1982). For molecules containing polarized moieties (combination no. 2), such as diphenyl phthalate, Leuchs (1956) coined the term "hinge type plasticizers" (Note: Boyer and Spencer (1947) called these "solvent type plasticizers"). Reference to a hinge is made because these plasticizers are able to transmit the polar attraction between PVC chains through their polarizable group, but attractive forces are generally lowered, and by being placed between these two chains, some motion of these chains towards one another is made possible. Hinge or solvent type plasticizers are easily blended with PVC, and often no heat is needed to do so, yet the cold properties are not very good, meaning blends with PVC become brittle at higher temperatures compared to the screen or oil type plasticizers discussed below (Leuchs 1956).

Combinations no. 3 and 4 shown in Table 3.1 were termed "screen type plasticizers" by Leuchs (1956) (Note: Boyer and Spencer (1947) called these "oil type plasticizers"), as they also contain non-polar parts. While the polar and polarizable parts assure the compatibility of the plasticizer with the resin, the non-polar parts physically separate PVC chains from each other, reducing their interaction thus acting as a screen and allowing for more motion of the chains against each other. On the other hand, the non-polar parts reduce the compatibility of the screen or oil type plasticizers with PVC, meaning that heat is usually required in the blending process. The plasticizers of combination 3 have particularly good cold properties and are often used as secondary plasticizers (Leuchs 1956).

Sometimes compounds that could be good plasticizers due to their non-polar parts, but with low compatibility to PVC due to little polarity (combination no. 5 and 6), are mixed with another plasticizer, which is more compatible with PVC. These latter compounds are called primary plasticizers, while the former are called secondary plasticizers or sometimes fillers, as they only function in combination with other compounds, primary plasticizers. Finally, a compound that only contains non-polar parts, such as a simple alkane (combination no. 7), is not miscible with PVC and therefore unsuitable as plasticizer.

Combination	part of the molecule			Remarks
No.	Polar	Polarized	Non-polar	
1	+	-	-	unsuitable as plasticizer
2	+	+	-	
3	+	-	+	primary plasticizer
4	+	+	+	
5	-	+	-	secondary plasticizer or
6	-	+	+	filler
7	-	-	+	unsuitable as plasticizer

Table 3.1. Possible combinations of plasticizer-structures, + group(s) present, - group(s) not present. Adapted and translated from Leuchs (1956) with permission.

3.1.2 Free Volume Theory

The free volume theory was developed in the 1950s and 1960s. It is based on the observation that when cooling amorphous materials such as PVC past its glass transition temperature T_g , the linear change in volume with regards to temperature changes slope to a lower value and the polymer is effectively "vitrified". Figuratively, this means that once below the T_g , the rearrangement of molecules almost comes to a standstill, or is significantly slowed down, resulting in the retention of approximately the same amount of free volume between chains for temperatures below the T_g (Fox and Flory 1950, Sears and Darby 1982). As a result, a state of perfect molecule arrangement, which should be expected at T = 0K, cannot be reached (see Figure 3.1). The onset of this change in slope is however dependant on the cooling rate of the experiment and if an infinitively small cooling rate could be applied, no vitrification would occur and hence a minimum volume V_0 (at T = 0K) would be reached due to the perfect arrangement of molecules in the lattice. This was a theoretical approach of course, and V_0 can only be obtained mathematically. The difference in volume between this ideal case and the

measured volume extrapolated to T = 0K, is called the free volume (Jenckel 1937, Ueberreiter and Kanig 1952, Jenckel et al. 1962, Sears and Darby 1982).



Figure 3.1. Free volume in an ideal and a real crystal, and a liquid. Reproduced from Sears and Darby (1982) with permission. Copyright© 1982 by John Wiley & Sons, Inc.

An increase in the free volume of a polymer results in a plasticizing effect (Sears and Darby 1982). If plasticization is desired, the necessary increase in free volume can be achieved by allowing for more molecular movement of the polymer chains by several means, and these are shown in Figure 3.2A-D (Sears and Darby 1982). The depicted sources of increased free volume are (A) an increase in the number of end groups present in the polymer backbone, which essentially means reducing the number of repeat units in the polymer chain; (B) increasing the amount of side chains attached to the polymer backbone (which is the concept of internal plasticization); (C) enhancing backbone mobility by creating void spaces through molecular geometry (i.e., such as present in natural rubber); and (D) the addition of external plasticizers. Another obvious way of increasing chain motion would be by raising the temperature (Sears and Darby 1982). Since plasticizers are usually small molecules compared to the polymer with which they are blended, they contain a higher proportion of end groups, thus increasing the above-described motion and effectively acting as plasticizer (Fox and Flory 1950, Boyer 1963, Kanig 1963, Sears and Darby 1982, Marcilla and Beltrán 2012).

The free volume theory allowed to develop, for example, the prediction of T_g values for polymer-plasticizer blends. However, the theory does not take into account the compatibility (i.e., miscibility of polymer and plasticizer), nor the permanence (i.e., migratory

loss of the plasticizer) of these compounds. For PVC, this model would predict that short alkanes would be good plasticizers because of their large amount of end groups per volume, yet these short alkanes would not be miscible with PVC and they would be volatile and would quickly leach from the material if they could be successfully blended with PVC (Hansen 1965, Matthews 1996). The free volume theory was the basis for several mathematical models that could predict the T_g of polymer-plasticizer blends (Marcilla and Beltrán 2012) and an overview of several such models is provided by Mauritz et al. (1990).



Figure 3.2. Sources of free volume for plasticization: A, chain end motion; B, side chain motion; C, main chain "crankshaft" motion; D, external plasticizer motion. Reproduced from Sears and Darby (1982) with permission. Copyright© 1982 by John Wiley & Sons, Inc.

3.1.3 Generalized Structure Theories

A generalized structure theory was proposed by Sears and Darby (1982) to visualize plasticizer action, with specific attention to the effect of anti-plasticization, as shown in Figure 3.3. This effect is observed when small amounts of plasticizer are added to a resin such as PVC and a stiffening of the material is observed. Because more chain-movement is possible due to

the addition of small amounts of plasticizer, ordered crystalline zones grow slightly, resulting in higher macroscopic stiffness, explaining this effect. Only once a certain threshold of plasticizer-content is reached, a decrease in stiffness is observed (Sears and Darby 1982). This effect was described as early as 1935 by Brous and Semon (1935).



Figure 3.3. Stylized concept of plasticization of a resin, explaining antiplasticization and plasticization. Ordered regions represent crystallinity. Reproduced from Sears and Darby (1982) with permission. Copyright© 1982 by John Wiley & Sons, Inc.

3.2 Aspects of Plasticization

The three major aspects of plasticization were defined by Boyer (1951) as compatibility, efficiency, and permanence, as reproduced in Figure 3.4. These three properties are intricately connected to one another and a careful balance between the three is necessary for any good plasticizer. For example, regarding compatibility, a plasticizer that is not compatible (i.e., not miscible) with the polymer would be useless, as would a plasticizer that would quickly be removed from the blend (pertaining to permanence), although this could be desirable in certain applications. Finally, the higher the plasticizer efficiency, the smaller the quantity of plasticizer necessary to achieve a certain degree of flexibility, which in turn would lead to decreased material need and could translate into lower costs and/or reduced resource requirements.

For each of the three key properties, the most important factors are listed on the outside of the triangle in Figure 3.4. For example, the compatibility of a plasticizer with a polymer has an influence on both efficiency (i.e., if the plasticizer does not mix well with the polymer and is not dispersed very well, it would likely be a less efficient plasticizer as measured for example by T_g) and permanence (i.e., the plasticizer would likely phase-separate quickly and possibly leach out of the blend). Similarly, while a large diffusion rate would indicate good compatibility between polymer and plasticizer, low permanence could result, as plasticizer molecules could easily diffuse to the surface of the blend and be washed out. From this, Boyer (1951) drew the conclusion that all three properties are related to one another and that all three major aspects need to be dealt with sufficiently in the design of plasticizer molecules to optimize all three major aspects.



Figure 3.4. Schematic representations of relationships between three important properties of the plasticizers, namely, compatibility, efficiency, and permanence. Reproduced from (Boyer 1951) with permission.

The following sections describe some literature that pertains to the three major aspects of plasticizer behaviour as shown in Figure 3.4. This literature review however is not intended to be exhaustive, but rather to provide some examples of the measurements performed to assess these three aspects.

3.2.1 Compatibility

The compatibility or miscibility of plasticizers with polymers is closely linked to the solvent power of the plasticizer towards the polymer in question (Immergut and Mark 1965, Wilkes et al. 2005). It is very important that the attractive forces between plasticizer and polymer are in the same order of magnitude, otherwise the plasticizer molecules and PVC chains would prefer to interact with each other respectively, and phase separation would rapidly occur (Sears and Darby 1982). Compatibility of plasticizers and PVC has been studied both experimentally and through modelling approaches. Experimental methods include

dissolving small amounts of PVC in plasticizer to determine dissolution or fusion temperature, cloud point, and viscosities (Jenckel and Rehage 1951, Würstlin and Klein 1952b, Würstlin and Klein 1952a, Leuchs 1956, Würstlin and Klein 1956, Luther et al. 1962, Würstlin 1962), determination of dielectric constants of the pure plasticizer (Darby et al. 1967), FT-IR spectroscopy to establish interaction between PVC and ester groups present in the plasticizer (Iskandar et al. 1983, Varnell et al. 1983, Braun et al. 1989, Beltrán et al. 1997, Audic et al. 2003, Lai et al. 2012), and NMR spectroscopy (Clayden and Howick 1993, Cadogan and Howick 1996). Another way of establishing the mentioned fusion temperature is by Brabender torque rheometry (Schmidt 1951, Hartmann 1955, Hartmann 1956, Blake 1962, Touchette et al. 1964, McKinney 1965, Ramos-de Valle and Gilbert 1990).

Models to assess compatibility of plasticizers and polymers include the Flory-Huggins interaction parameter μ (Flory 1941, Huggins 1942, Flory and Rehner 1943), which indicates compatibility up to a certain threshold value (usually up to 0.55). This parameter can also be determined experimentally through swelling experiments (Doty and Zable 1946, Gee 1946a, Gee 1946b). Anagnostopoulos et al. (1960) then related solid-gel transition temperatures to the Flory-Huggins interaction parameter introducing the parameter χ , and Bigg (1975) further developed the model, introducing an activity parameter α . Another model is the cohesive energy density (CED), which assigns a solubility parameter δ to any chemical compound based on the heat of mixing (Hildebrand 1929, Scatchard 1931). In CED, the closer the values of δ between solvent and polymer, the better the compatibility. Small (1953) used this as a basis to develop an additive method for estimating solubility parameters based on group contributions. Tables with δ values for many compounds are available (van Krevelen 1965, Hoy 1970, Koenhen and Smolders 1975, Mark 1996, van Krevelen and te Nijenhuis 2009) and Chapter 7 in van Krevelen and te Nijenhuis (2009) contains a good review on the topic.

A somewhat simpler model was devised in a series of publications by van Veersen and Meulenberg (1964, 1966, 1967, 1972), who introduced the ratio Ap/Po of non-polar (Ap stands for "apolair", from Dutch) to polar parts in plasticizers and the authors were able to relate the ratio Ap/Po, and variations of these, to the compatibility of polymer-plasticizer blends.

3.2.2 Efficiency

The efficiency of a plasticizer is another important aspect as it determines how much plasticizer is needed to achieve a desired property, which in turn can have a direct impact on the overall cost of a product. Plasticizer efficiency is a broad term as many material properties of blends are important and most studies look at several parameters (Wilkes et al. 2005). These include, but are not limited to, the Tg reduction potential (Würstlin and Klein 1955, Mauritz et al. 1990, Gil et al. 2006, Rahman and Brazel 2006, Stuart et al. 2010), tensile and compression testing to determine modulus and tensile strength (Taylor and Tobolsky 1964, Lindström and Hakkarainen 2006, Firlotte et al. 2009, Calò et al. 2011, Kendall and Siviour 2014), dynamic mechanical thermal analysis (DMTA) (Ballard et al. 1987, Oksman et al. 2003, De Zarraga et al. 2004), hardness (Titow 1990, Calò et al. 2011, Clarizio and Tatara 2012), and specific gravity (Brookman et al. 1993, Bee et al. 2014). Additionally, rheological studies can determine the flow behaviour of melted blends, an important processing parameter (Leblanc et al. 2007).

3.2.3 Permanence

The third important aspect is the permanence of the plasticizer in the blend as it impacts the life span of a plasticized product directly. If the plasticizer leaches quickly from the blend, the life span of the product for its intended use could be very short (Matthews 1996). On the other hand, the ecological and toxicological profile of a plasticizer is also affected by the leaching rate, as higher leaching rates would result in higher entry of the plasticizer into the human body and the environment (Wams 1987, Wypych 2012). This will be further discussed in the manuscript in Chapter 4.

A plasticizer molecule on the surface of a blend can be removed from it by volatilisation (transfer into the gas phase), extraction (transfer into a liquid), or migration (transfer into a solid or semi-solid material with intimate surface contact) (Titow 1990). Once the plasticizer molecule is removed from the surface, further leaching of the plasticizer from the blend can only occur once more plasticizer molecules have diffused to the surface of the blend. Based on work by Liebhafsky et al. (1942) and Small (1947), Quackenbos (1954) expanded the idea that the determining step in leaching of plasticizer from a blend with PVC is the migration of the
plasticizer inside the blend (resulting in plasticizer loss correlated to the square root of time according to Fick's law of diffusion), or its removal from the surface of the blend (resulting in a linear dependence of plasticizer loss over time), depending on the conditions. Leaching rates were limited by diffusion only in experiments in vacuum or in convection ovens at elevated temperatures, in liquid solvents with sufficiently high flow rates, and in contact with solids in which the plasticizer had a high solubility (Quackenbos 1954, Sears and Darby 1982). When considering volatilisation, most plasticized PVC applications are not used at such extremes as vacuum and high temperatures, meaning that loss of plasticizer to air is usually dependant on the removal from the surface rather than the diffusion within the blend. Furthermore, Quackenbos (1954) showed experimentally that loss to air is positively correlated to the vapor pressure of a plasticizer, which could be used to predict the service life of a plasticized product for atmospheric use. Similarly, a series of papers by Reed and coworkers showed that loss of plasticizer is positively correlated to the time and to temperature and negatively correlated to the thickness of the material (Reed 1943, Reed and Connor 1948, Reed and Harding 1949, Reed et al. 1954).

While a diffusion-limited leaching rate is rarely encountered in atmospheric environments, it is more common when leaching experiments are carried out in solvents and solids, for example through "rub-off" (Quackenbos 1954). It is important to realize that in such experiments, the matrix into which a plasticizer leaches is irrelevant as long as the plasticizer is removed from the surface of the blend more quickly than the diffusion of more plasticizer molecules occurs (Quackenbos 1954). Several studies in diffusion-limited conditions have found the diffusion coefficient to increase with temperature and with plasticizer content using various techniques (Liebhafsky et al. 1942, Small 1947, Knappe 1954, Quackenbos 1954, Luther and Meyer 1960, Hellwege et al. 1961, Knappe 1962, Sears and Darby 1982). This higher mobility of the plasticizer molecules within the blend is inherent to the concept of plasticization: plasticization aims at creating more movement of the polymer chains to one another, which in turn increases the plasticizer mobility within the blend (Liebhafsky et al. 1942, Quackenbos 1954).

Besides temperature and plasticizer concentration, the chemical structure of the plasticizer molecule plays a crucial role in their diffusivity. For example, Luther and Meyer (1960) could show lower diffusion coefficients of dialkyl phthalates with increased alkyl side

chain length, as well as with increased branching (for DEHP compared to n-alkyl phthalates). Other studies reported reduced leaching rates with increasing alkyl side chain length: Patuska (1984) of dialkyl phthalates into polystyrene, and Kastner et al. (2012) of maleates, fumarates, and succinates into RO-water.

The diffusivity of plasticizers closely relates to the compatibility of the plasticizer with the polymer (also see Section 3.2.1). In brief, the better the solvent power of the plasticizer towards the polymer, the higher its diffusivity will be inside the blend (Sears and Darby 1982). Hence, permanence and compatibility often stand diametrically opposed to each other and a balance between the two has to be found, as expressed in Figure 3.4.

While leaching into liquids generally obeys the principles laid out above, matters are often complicated by the liquid penetrating into the blend, thereby swelling it, as shown for example by Kastner et al. (2012) where water swelled PVC blended with a range of different plasticizers. Depending on the nature of the plasticizer and the extractant and the solubilities of these in each other, washing out of the plasticizer can also occur, further complicating the matter (Sears and Darby 1982). For this reason, leaching tests into liquids usually require the use of several solvents, such as outlined for example in ASTM D-1239 (2014).

More detailed information on permanence, leaching, and diffusion of plasticizers in polymers can be found in Chapter 6 of Sears and Darby (1982), and Chapter 7 of Wypych (2012).

3.2.3.1 Leaching Studies

Since plasticized PVC applications are used in various environments, applied leaching studies are performed to include all three above-mentioned states of aggregation. Most commonly, leaching studies into liquids are performed in which a specimen is suspended in a solvent, for which ASTM standard methods exist (ASTM D-543 2014, ASTM D-1239 2014). ASTM D-1239 (2014) requires immersion at room temperature for 24h in the following solvents: distilled water, soapy water (1 %), cottonseed oil, mineral oil, kerosene, and ethyl alcohol (50 % in water) to simulate many different liquid matrices. Since plasticized PVC is used extensively in hospital environments and in food packaging and since direct exposure to humans can result from plasticizer leaching, many studies have been conducted on these topics

(Jaeger and Rubin 1972, Rock et al. 1978, Peck et al. 1979, Demore et al. 2002, Buchta et al. 2005, Inoue et al. 2005, Sørensen 2006, Carrillo et al. 2007, Del Carlo et al. 2008, Bagel et al. 2011, Kastner et al. 2012, Rose et al. 2012, Russo et al. 2012, Veiga et al. 2012); see also Section 4.6.2. However, leaching studies into air are also regularly performed (Nobis 1957, Rössig 1958, Aloul and Shen 2002, Hill et al. 2003, Clausen et al. 2010), as well as diffusion studies into solids, which treat most commonly the diffusion of plasticizer from food wrapping into foodstuff (Zöhrer and Merz 1955, Castle et al. 1988, Page and Lacroix 1992, Tsumura et al. 2001, Cao 2010); see also Section 4.6.1.

3.2.3.2 Strategies to Reduce Leaching of Plasticizers

Owing to the possible direct exposure to humans in sensitive applications such as food wrapping and hospital equipment, efforts have been made to reduce leaching of plasticizer in those kinds of applications by surface modification (Moulay 2010). However, any modification to the surface will ultimately have an impact on the price of the final product.

Surface modification methods include grafting (Krishnan et al. 1991, Lakshmi and Jayakrishnan 1998a, Babukutty et al. 1999, Balakrishnan et al. 2005), crosslinking (Petit and Ladang 1995, Lambert et al. 1997, Jayakrishnan and Lakshmi 1998, Lakshmi and Jayakrishnan 1998b, Audic et al. 2001, Gourlay et al. 2010, Ambrogi et al. 2012), and radiation treatment to change the surface chemistry (Ito et al. 2005). Chiellini et al. (2013) published a comprehensive overview of surface modification techniques.

Another strategy to avoid leaching of the plasticizer was proposed by Navarro et al. (2010), who synthesized phthalate-based plasticizers that could chemically react with the polymer once blended with it, to essentially achieve internal plasticization. Classically, internal plasticization is achieved in the polymerization step of the polymer, by co-polymerizing with an appropriate monomer that already contains the plasticizing group (Sears and Darby 1982).

3.3 Toxicity of Phthalates

Phthalates are ubiquitous and there are multiple pathways of exposure for humans, animals, and the environment (Wams 1987). Many studies have investigated the impact of phthalates and their breakdown products, particularly on human health. This section aims at

providing a brief overview, but more detailed information regarding routes of exposure and health impacts can be found elsewhere (Wormuth et al. 2006, Frederiksen et al. 2007, UBA 2012b).

Once phthalates enter the human body, they are chemically transformed and generally quickly excreted (Anderson et al. 2001, Koch et al. 2005). While the general metabolism pathways of various phthalates in the body are similar, the size and shape of the side chain of the particular phthalate molecule have an influence on the quantity of metabolites that are produced and their residence time in the body (Koch et al. 2005). The metabolites can be detected in urine and feces of humans using analytical techniques (Anderson et al. 2001). Figure 3.5 provides a detailed outline of the most important metabolites of DEHP formed in the human body, as shown by Koch et al. (2005).



Figure 3.5. Metabolism of DEHP in the human body according to Albro et al. (1982), Peck and Albro (1982), Schmid and Schlatter (1985), and ATSDR (2002). Major metabolites as found by Koch et al. (2005) are highlighted in gray. Reproduced from Koch et al. (2005) with permission.

The most significant concern with phthalates and their metabolites is their potential to act as endocrine disruptors. In males, phthalates can produce anti-androgenic effects (the male sex hormone testosterone is the most important androgen), resulting in feminization, as detected for example by a reduced anogenital distance (AGD) (Frederiksen et al. 2007, Habert et al. 2010). Animal studies have shown that the biggest effects of phthalates result from exposure during pregnancy (prenatal) and right after birth (perinatal), for example through lactation (Frederiksen et al. 2007, Habert et al. 2014). These effects include reduced testosterone levels and sperm counts, testicular malformations (i.e., testicular atrophy, cryptorchidism), and changes in cell behaviour related to sperm production and storage (i.e., in Sertoli cells) (Richburg and Boekelheide 1996, Mylchreest et al. 1999, Foster et al. 2000, Gray Jr et al. 2000, Parks et al. 2000, Lee et al. 2004, Dalsenter et al. 2006, Foster et al. 2006). Effects of pre- and perinatal exposure to females is somewhat less understood, but some studies showed masculinization, as observed by increased AGDs, and reduced levels of the female sex hormone estradiol (Piepenbrink et al. 2005, Dickerson et al. 2012, Martinez-Arguelles et al. 2013, Hannon and Flaws 2015). A particular focus lies on the primary metabolite of DEHP, mono (2-ethylhexyl) phthalate (MEHP) (see Figure 3.5), which is produced within the body during the DEHP removal process, through lipases that hydrolyse DEHP. MEHP is believed to play an important role in the endocrine-disrupting effects observed (Richburg and Boekelheide 1996, Hauser et al. 2007, Fan et al. 2010, Campioli et al. 2011, Muczynski et al. 2012, Piche et al. 2012). However, a recent review article by Habert et al. (2014) suggests that phthalate effects could be species-dependant.

Similar experiments on humans are not ethical for obvious reasons, but studies have looked at pre- and perinatal environmental phthalate exposure to male infants and also found anti-androgenic effects (Swan et al. 2005b, Lottrup et al. 2006, Main et al. 2006, Frederiksen et al. 2007).

3.4 Biodegradation Studies

The Organisation for Economic Co-operation and Development (OECD) published a wide range of guidelines for the testing of chemicals and Section 3 particularly treats the topic of degradation and accumulation (OECD 1981-2014). Within these, tests 301A-F (OECD 1992) and 310 (OECD 2014) are concerned with specific aerobic biodegradation protocols,

determining whether or not a compound is readily biodegradable. The types of microorganisms used are broad and usually a mixture obtained from activated sludge, sewage effluent, surface water, or soil is used. The monitoring of the biodegradation process is achieved by measurements related to the breakdown of the contaminant such as dissolved organic carbon (DOC) or O₂ consumption, as well as measurements to monitor microbe metabolism such as CO₂ evolution, O₂ consumption, or dissolved oxygen (OECD 1992, OECD 2014). Test 311 (OECD 2006) deals with anaerobic degradation, using pressure and measurements of inorganic carbon as monitoring tools.

The main purpose of the OECD tests 301 and 310 is to test for rapid biodegradability, which usually involves a certain pass level that needs to be reached within 28 days (OECD 1992, OECD 2014). However, if a pass level is not reached, the tests cannot necessarily establish whether it is the parent compound or a metabolite that is recalcitrant. In such a case, or even if the parent compound is readily biodegradable, analytical methods can be used to determine what kinds of intermediates are formed.

Other studies used pure microorganisms, or defined mixtures, to evaluate breakdown patterns and biodegradation rates. The microorganisms used include bacteria, fungi, yeasts, and algae (Engelhardt et al. 1977, Eaton and Ribbons 1982, Nalli et al. 2002, Begum et al. 2003, Gartshore et al. 2003, Yan and Pan 2004, Nalli et al. 2006b, Qiu et al. 2006, Baek et al. 2009, Luo et al. 2009, Sauvageau et al. 2009). Biodegradation rates are strongly influenced by the availability of oxygen, and rates are significantly higher in aerobic environments (Engelhardt et al. 1977, Staples et al. 1997, Nalli et al. 2006b, Liang et al. 2008, Baek et al. 2009). However, as phthalates are highly lipophilic (ATSDR 2002), they tend to accumulate in lipid-rich environments, in which anaerobic conditions often prevail, slowing down biodegradation of phthalates and other environmental contaminants (Staples et al. 1997). Many more studies exist on this topic, reviewed for example by Wams (1987), the OECD (1995), Staples et al. (1997), and Benjamin et al. (2015).

The general breakdown pathway for diesters such as phthalates is the subsequent hydrolysis of the two ester bonds present, which is carried out by esterases that are commonly produced by many microorganisms (Staples et al. 1997). These two hydrolysis steps are rather slow for phthalates diesters of higher molecular weight and thus size (such as DEHP), meaning that not only is the degradation of the parent compound DEHP slow, but also that of the primary

metabolite mono (2-ethylhexyl) phthalate (MEHP). Some studies suggest that MEHP is more stable than DEHP (Ejlertsson et al. 1996, Jonsson et al. 2003). During the hydrolysis process, two units alcohol are liberated per diester molecule, and the further microbial degradation is dependant on the degree of branching present: while linear alcohols are easily broken down by β -oxidation, branched compounds can be more recalcitrant, especially when β -oxidation is hindered by alkyl substituents on the β carbon (Nalli et al. 2006a). Once the two alcohol units are liberated, phthalic acid results, and it is further mineralized as shown in Figure 3.6 as adapted from Staples et al. (1997).



Figure 3.6. General biodegradation pathway for phthalate esters in the environment. Reproduced from Staples et al. (1997) with permission.

3.4.1 Biodegradation Kinetics

In several plasticizer biodegradation studies, first-order kinetics with regards to the plasticizer concentration were found for the first hydrolysis step of dialkyl phthalates (Staples et al. 1997, Wang et al. 2000, Gavala et al. 2003). This model was sufficient for phthalates with side chains of up to six carbons, yet for larger phthalates such as DEHP, such first-order kinetics were only able to accurately describe the first four weeks of the experiment. A fractional power law was proposed to adequately fit the experimental data subsequently (Roslev et al. 1998, Madsen et al. 1999). This change in observed biodegradation rate may be due to the residence time of the organic compound, especially in experiments inside soil matrices (Roslev et al. 1998). Inside a soil matrix, the organic compound can adsorb to particles and hence become less bioavailable over time, an effect referred to as "aging" (Bollag 1992, Alexander 1995, Hatzinger and Alexander 1995, Pignatello and Xing 1996). More detailed information on DEHP biodegradation in by bacteria in soils is available in a book chapter by Kumar (2013).

3.5 Existing Alternative Plasticizers

Due to the aforementioned regulatory pressure on several phthalates (EU 2005, CPSIA 2008, Bureau Veritas 2010, HPA 2010), many alternative plasticizers have been proposed and some are already heavily used. The study by Biedermann-Brem et al. (2008) identified the most important replacements for phthalates in various children's articles in Europe. These compounds (shown in Figures 3.7, 3.8, and 3.9 below) were diisononyl cyclohexane-1,2-dicarboxylate (called Hexamoll® DINCH®), di (2-ethylhexyl) terephthalate (DEHTP), citrates, dibenzoates, and poly(adipate), and they will be discussed in the appropriate sections below. Other compounds included were epoxidized soy bean oil and 2,2,4-trimethyl-1,3-pentanediol-diisobutyrate (TXIB), but the latter two were used only in smaller quantities and mixed with another primary plasticizer. Another commonly used plasticizer is di (2-ethylhexyl) adipate. The company Dow Chemical introduced a plasticizer named "EcolibriumTM" in 2010 and the company claims that it is produced from renewable resources, yet this cannot be confirmed as no chemical structure was communicated (Dow Chemical 2010). Ionic liquids have also been proposed as alternative plasticizers, particularly due to their

very low vapor pressure (Rahman and Brazel 2006, Choi et al. 2011), which is important for the permanence of a plasticizer within a PVC blend (see Section 3.2.3).

Good overviews of alternative plasticizers have been written such as those by Sears and Darby (1982), Rahman and Brazel (2004), Wypych (2012), Beach et al. (2013), Chiellini et al. (2013), and Bocqué et al. (2016). Some of these alternative compounds will be discussed here and the chemical structures of those compounds can be found in Figures 3.7, 3.8, and 3.9.

3.5.1 Oligomeric and Polymeric Plasticizers

Oligomeric and polymeric plasticizers are advantageous because of their reduced leaching rates owing to higher molecular weight, yet compatibility with PVC can be problematic and plasticizing effectiveness is often worse compared to smaller molecular weight plasticizers (Moorshead 1962, Prud'Homme 1989, Titow 1990, Wypych 2012, Chiellini et al. 2013). Among others, compounds considered include poly(caprolactone) (PCL) due to ready biodegradability of the monomer (Shi et al. 2011) and poly(adipate) (PA), a polymer comprised of units of the diacid adipic acid and a diol of varying chain length, as denoted by R' in Figure 3.7 (Sears and Darby 1982, Ežerskis et al. 2007, Lindström and Hakkarainen 2007, Biedermann-Brem et al. 2008). Other diacids such as succinic-, glutaric-, and sebacic acid can also be used (Titow 1990).



Figure 3.7. Structures of oligomeric and polymeric plasticizers discussed.

3.5.2 Aromatic Plasticizers

Several plasticizers exist that have a certain resemblance to DEHP, for example di (2-ethylhexyl) terephthalate (DEHTP, sometimes misleadingly called DOTP), in which the ester groups are in *para*-position on the benzene ring, and tris (2-ethylhexyl) trimellitate (TEHTM, sometimes misleadingly called TOTM), which is a tri-substituted benzene ring. Both compounds contain 2-ethylhexanol, which has been shown to be difficult to biodegrade (Nalli et al. 2002, Nalli et al. 2006b). From toxicity studies, it seems that DEHTP is a safe alternative (Chiellini et al. 2013), while TOTM showed signs of estrogenic behaviour (ter Veld et al. 2006, Chiellini et al. 2013). Another class of compounds are dibenzoates, which are comprised of one or more repeat units of a diol, capped with benzoic acid on each side (Sears and Darby 1982). Recent work has shown that the ether bonds in compounds such as diethylene glycol dibenzoate (DEGDB) and dipropylene glycol dibenzoate (DPGDB) reduce the biodegradability of these compounds (Kermanshahi pour et al. 2009a, Kermanshahi pour et al. 2009b). A compound in which the oxygen atom was replaced by a carbon atom (1,5-pentanediol dibenzoate; 1,5PDB) showed comparable plasticizer properties and increased biodegradation rates (Firlotte et al. 2009).



Figure 3.8. Structures of aromatic plasticizers discussed.

3.5.3 Non-aromatic Plasticizers

BASF hydrogenated of The German company the aromatic portion di (isononyl phthalate (DINP), to make diisononyl cyclohexane-1,2-dicarboxylate, a mixture of isomers which is sold as Hexamoll® DINCH® (Wadey 2003) (called DINCH® for simplicity from here on). The production capacity was recently increased to 200,000 metric tonnes per year (BASF 2014) and DINCH® seems to be the most important phthalate substitute (Biedermann-Brem et al. 2008). DINCH® is marketed as a plasticizer for "sensitive applications" based on various toxicological studies (Wadey 2003). However, several recent studies in the US and Germany now show that DINCH® and its metabolites can be found increasingly in urine samples (CDC 2012, Schütze et al. 2012, Silva et al. 2013, Schütze et al. 2014, Calafat et al. 2015) and house dust (Fromme et al. 2016). One recent study showed altered gene expression pathways in Sertoli cells exposed to DINCH® (Nardelli et al. 2015).

Citrates are another group of saturated compounds that are based on citric acid, in which all three carbonyl groups are esterified, sometimes also including the alcohol group present in the molecule (Sears and Darby 1982). According to the study on phthalate replacements in children's articles by Biedermann-Brem et al. (2008), acetyl-tributyl-citrate (ATBC; also called Citroflex A) was used frequently, however often in combination with another plasticizer. Several other citrates exist, differing in the chain length of the alcohol used to create the ester bond (Sears and Darby 1982). Citrates are generally regarded as non-toxic and are often used in "sensitive applications with toxicological and environmental constraints" (Wilkes et al. 2005, Chiellini et al. 2013).

Aliphatic saturated diesters are another group of compounds that are used as, or proposed as plasticizers (Titow 1990). These compounds vary mainly in the length of the central carbon chain and the alkyl side chains. Many combinations are possible and these compounds have been explored early after the rise of PVC. Among others, Würstlin and Klein (1956) investigated the compatibility of a range of aliphatic saturated diesters with PVC, ranging from central chain lengths of C2 (oxalic acid) to C8 (sebacic acid), and side chain lengths from C1 (methyl) to C8 (octyl) (Würstlin and Klein 1956). These types of compounds generally show good low temperature flexibility in PVC blends (Sears and Darby 1982). Several commercial plasticizers based on adipic acid (C6), azelaic acid (C9), and sebacic acid (C10) exist, most often as the corresponding di (2-ethylhexyl) diester (Sears and Darby 1982).

Recent work has focussed on succinic acid (C4) esterified with linear side chains as possible phthalate replacement (Stuart et al. 2010), partly owing to the increased fermentative production of succinic acid through renewable sources (Debabov 2015).



Figure 3.9. Structures of non-aromatic plasticizers discussed.

4 Leaching of the Plasticizer Di (2-Ethylhexyl) Phthalate (DEHP) from Plastic Containers and the Question of Human Exposure.

4.1 Preface

The following manuscript was published in 2014 in the Journal Applied Microbiology and Biotechnology, 98(24): pp. 9967-9981. As described in Section 3.2.3, leaching of plasticizers is of significant concern, especially when the leached compounds enter the body directly, such as from foodstuff or hospital equipment. The manuscript deals particularly with leaching of DEHP due to its relevance as an industrial compound in widespread use. DEHP has been reported to have been found repeatedly in water bottles, however water bottles are not usually made from PVC, let alone plasticized PVC. The manuscript thus focussed on quantities of DEHP found in different samples, such as in water bottles, beverages, foodstuff, and DEHP leached from hospital equipment such as blood bags and flexible tubing. Also, levels of DEHP found in various aqueous samples from the environment, such as rain and surface water, were presented.

The manuscript relates the levels of DEHP found in the environment and food to each other, to show which of the reported foods and beverages lead to the highest chronic exposure. It was found that fatty foods contain levels of DEHP of orders of magnitude higher than drinking water samples, which is likely due to its lipophilic nature (ATSDR 2002), concluding that the consumption of water is probably a subordinate source of DEHP exposure.

Exposure to DEHP in a hospital environment is acute rather than chronic due to the limited time spent by the average person in a hospital environment, and therefore comparisons to the levels reported in foodstuff were deemed difficult. Problematic however are the cases of patients that need regular or long-term hospital treatment, such as dialysis patients or neonates.

Additionally, DEHP-levels deemed safe by several authorities are presented in the manuscript, as well as an overview of the environmental breakdown pattern. Finally, the manuscript advocates for the replacement of DEHP and other phthalates based on the precautionary principle, due to their ubiquitous presence.

Leaching of the Plasticizer Di (2-Ethylhexyl) Phthalate (DEHP) from Plastic Containers and the Question of Human Exposure.

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4.2 Abstract

Di (2-ethylhexyl) phthalate (DEHP) is a widely used plasticizer to render poly(vinyl chloride) (PVC) soft and malleable. Plasticized PVC is used in hospital equipment, food wrapping, and numerous other commercial and industrial products. Unfortunately, plasticizers can migrate within the material and leach out of it over time, ending up in the environment and, frequently, the human body. DEHP has come under increased scrutiny as its breakdown products are believed to be endocrine disruptors and more toxic than DEHP itself. DEHP and its breakdown products have been identified as ubiquitous environmental contaminants, and daily human exposure is estimated to be in the microgram per kilogram level. The objective of this review is to summarize and comment on published sources of DEHP exposure and to give an overview of its environmental fate. Exposure through bottled water was examined specifically, as this concern is raised frequently, yet only little exposure to DEHP occurs through bottled water, and DEHP exposure is unlikely to stem from the packaging material itself. Packaged food was also examined and showed higher levels of DEHP contamination compared to bottled water. Exposure to DEHP also occurs in hospital environments, where DEHP leaches directly into liquids that passed through PVC/DEHP tubing and equipment. The latter exposure is at considerably higher levels compared to food and bottled water, specifically putting patients with chronic illnesses at risk. Overall, levels of DEHP in food and bottled water were below current tolerable daily intake (TDI) values. However, our understanding of the risks of DEHP exposure is still evolving. Given the prevalence of DEHP in our atmosphere and environment, and the uncertainty revolving around it, the precautionary principle would

suggest its phase-out and replacement. Increased efforts to develop viable replacement compounds, which necessarily includes rigorous leaching, toxicity, and impact assessment studies, are needed before alternative plasticizers can be adopted as viable replacements. <u>Keywords:</u> DEHP, Phthalate, Plasticizer, Leaching, Human exposure, Environmental contamination

4.3 Introduction

The use of polymers is increasing on a worldwide scale (PEMRG 2013) and along with it is the use of the many compounds added to these polymers to serve a broad variety of functions (Sears and Darby 1982). Such additives include plasticizers, coloring agents, flow aids, heat stabilizers and solvents. Due to their widespread and growing use, there is an increasing interest and concern as to the impacts that these additives have on humans, animals and the environment. This is especially important given that most of these additives are not chemically bound to the polymer chains, which means that they can migrate within the material and leach out over time. Furthermore, since these additives are usually small molecules compared to the large polymer chains, they are more prone to dissolve in the aqueous environment, thereby contributing to the risk of exposure of humans, animals and microbes.

Plasticizers account for a large fraction of additives to polymers (Murphy 2001) and can represent as much as 40 % by weight of such materials (ATSDR 2002). These molecules interact with the polymer chains to render the material more flexible and malleable. The most important class of plasticizers in widespread use are phthalate diesters and, within these phthalates, DEHP (Figure 4.1) is the most important (Murphy 2001). DEHP has also been the source of the greatest concern. For example, several studies have suggested that it is toxic and can disrupt normal endocrine function in humans (see Section 4.4). Furthermore, the US Environmental Protection Agency (US EPA) has expressed its concern "about phthalates because of their toxicity and the evidence of pervasive human and environmental exposure to them" (US EPA 2012). Due to these concerns and growing evidence of health impacts, certain phthalates have been banned for use in children's toys in the European Union (EU 2005), the United Stated (CPSIA 2008) and Canada (HPA 2010).

There have been recent reports of phthalates, specifically DEHP, in water bottles made from polyethylene terephthalate (PET) (see Table 4.1 and Section 4.5). PET is a hard material that is used to make containers, especially for use by the beverage industry. However, it does not contain, and is not blended, with DEHP. A plasticizers' role is to soften hard materials. PET also cannot release DEHP, as the terephthalate used to make the backbone of the polymer is a different chemical compound, while DEHP is a low molecular weight additive to the polymer resin. Contamination from DEHP in water bottles must stem from a processing step before bottling, or from the cap liner used.

There is growing public awareness regarding the human and environmental impacts associated with the use of DEHP owing to numerous recent scientific reports and increases in the regulation of this compound in various countries around the world. This awareness is also coupled with misconceptions about their use. Therefore, this article aims to clarify the risk of exposure to DEHP via liquids stored in plastic but also glass containers, including water, beverages and food, and in various types of hospital equipment. Measured and estimated levels of DEHP in a variety of samples stored in commercial and clinical containers reported in the literature are presented. The article also summarizes levels of phthalates present in the environment, resulting in a human "background exposure".



Figure 4.1. Chemical structures of chemical compounds discussed.

4.4 Varieties of Phthalates: Phthalates and Terephthalates

Because of a worldwide increase in the consumption of bottled water (Beverage Marketing 2011), the materials used to make these water bottles have come under increased scrutiny. Polymers such as polyethylene (PE; Recycling code #2) and polyethylene terephthalate (PET, also known as PETE; Recycling code #1; see Figure 4.1) are used in lieu of glass, mainly due to their low density and durability. The 2011 Beverage Market Report reported that "plastic packaging is preferred over glass in almost every country" and that "PET is the most dynamic and rapidly growing segment" (Beverage Marketing 2011). PET is a polyester that is produced either from the esterification reaction of terephthalic acid with ethylene glycol (see Figure 4.1) and subsequent polymerization or, alternatively, by the

transesterification of dimethyl terephthalate with ethylene glycol and subsequent polymerization. Once polymerized, the resulting polyester is a hard and rather brittle material. The only means by which some terephthalic acid, or an ester of it, could leach from it is by gradual depolymerization of the polymer chains forming the material. Figure 4.1 shows that the two carbonyl functions of terephthalic acid are in *para*-position to one another, on opposite ends of the aromatic ring. Terephthalic acid was originally reported to be non-toxic (Hoshi et al. 1968), but later studies involving higher levels of exposure to this compound suggested that it may impair testicular functions (Cui et al. 2004). An extensive review on terephthalic acid has been recently published by Ball et al. (2011). Although terephthalic acid has a certain resemblance in terms of molecular structure to DEHP, it cannot leach out of the final product and is chemically distinct from DEHP. Hence, it will not be dealt with further in this review article.

Phthalate plasticizers such as DEHP are synthesised from phthalic acid, in which the two carbonyl groups are in *ortho*-position to one another; i.e., on neighbouring carbon atoms in the aromatic ring (Figure 4.1). Phthalic acid is esterified with various alcohols, but most commonly with 2-ethylhexanol, to form liquid DEHP (Figure 4.1). This is the final form and it is not further polymerized as PET, but it remains as a small molecule additive. Of all DEHP produced globally, 95 % is used as a plasticizer to render hard and brittle polymers more flexible and malleable (ATSDR 2002). Poly(vinyl chloride) (PVC; Recycling code #3) is the most important of these brittle polymers, where an estimated 80 % of all plasticizers produced are used to plasticize PVC (Stevens 1999, Murphy 2001). Of these plasticizers, the most common is DEHP, which accounts for approximately 50 % of all plasticizers used in PVC (Murphy 2001). Its production was estimated to be about 2 million metric tons per year in 2004 (AgPU 2006). Some typical applications of plasticized PVC include medical equipment such as hospital tubing and blood bags, food wrapping, wire and cable insulation, and automobile parts (Rahman and Brazel 2004, AgPU 2006).

Plasticization (i.e., the functional effect of plasticizers) is caused by various interactions between the small plasticizer and the polymer (Wypych 2012). The interaction of the polar carbonyl functionalities in DEHP with the polar carbon-chloride bonds in the polymer chains of PVC makes the two compounds compatible, but it is countered by the lack of interaction between the non-polar parts of the DEHP. As a result, in the presence of plasticizers, the long PVC chains interact less with each other and the material is rendered more flexible and malleable (Wypych 2012). To achieve this, a large quantity of DEHP is usually needed. For instance, plasticized PVC can contain up to 40 % of plasticizer by weight (ATSDR 2002). It is important to recognize that the plasticizer is not chemically bound to the PVC, resulting in the possibility of DEHP migrating within the material and reaching the surface of the blend, where it can ultimately leach out of the material (Kastner et al. 2012). Ultimately, due to this behaviour, DEHP has been labelled a ubiquitous environmental contaminant as far back as 28 years ago (Wams 1987).

DEHP is particularly well studied because of concerns about its wide range of toxic effects (Akingbemi et al. 2001, Foster et al. 2001, Gazouli et al. 2002, Akingbemi et al. 2004, Horn et al. 2004). The US EPA has expressed its concern about phthalates "because of their toxicity and the evidence of pervasive human and environmental exposure to them" (US EPA 2012). The breakdown pattern of DEHP is very important, because stable metabolites are produced. such as 2-ethylhexanol, 2-ethylhexanoic acid, and its monoester. mono (2-ethylhexyl) phthalate (MEHP), each of which have been shown to be more toxic than DEHP itself (Horn et al. 2004, Nalli et al. 2006a, Nalli et al. 2006b, Nalli et al. 2006c). Special attention has been given to MEHP, as it is believed to be an endocrine disruptor and has been linked to antiandrogenic activities in humans (Richburg and Boekelheide 1996, Swan et al. 2005a, Pant et al. 2008, Fan et al. 2010, Piche et al. 2012). Such findings eventually led to its ban in applications such as children's toys in much of the Western world (EU 2005, CPSIA 2008, HPA 2010). An extensive overview of DEHP has been provided in reports from the U.S. Department of Health and Human Services (ATSDR 2002) and the European Union (EU 2008).

4.5 Bottled Water

Although DEHP is not used to make water bottles, DEHP has been detected in many samples from water bottles regardless of its material, including PET, PE and glass (Table 4.1). This is unexpected and suggests that contamination does not stem from material of which the bottle is made, but rather through a different route of contamination. The quantities of DEHP found in bottled water have been reported in the range of 0 to 1 μ g/L, with the exception of three studies: Bošnir et al. (2007) found an average of 8.78 μ g/L, Keresztes et al. (2013)

reported values of up to 1.7 μ g/L, and Guart et al. (2014) reported values of up to 13.0 μ g/L. The variability in the concentrations of DEHP found in the different bottled waters shown in Table 4.1 could possibly be due to regional differences in the prevalence of contaminants such as DEHP.

Some of the studies shown in Table 4.1 looked directly at the content of DEHP of the water stored in PET, PE and glass containers. Given that DEHP is not used in the manufacturing of these bottles, the type of material of the bottle is obviously not as important as the source of the water. The water bottles were bought in local stores, so that a certain shelf life already had passed, and the DEHP content of the water was analyzed immediately thereafter (Bošnir et al. 2007, Cao 2008, Martine et al. 2013). Other studies involved the purchase of the bottles in local stores, but then monitored the concentration of DEHP in the water over time. Overall, the results have been very variable from study to study and no clear trend can be seen.

When examining the levels of DEHP in the bottled water over time, some studies report increasing concentrations (Casajuana and Lacorte 2003, Leivadara et al. 2008, Keresztes et al. 2013, Guart et al. 2014) and others report constant or even decreasing levels of DEHP (Al-Saleh et al. 2011, Diana and Dimitra 2011, Keresztes et al. 2013, Guart et al. 2014). A decrease of DEHP concentration would mean that some kind of breakdown had occurred, which seems unlikely. Even within individual studies, both increases and decreases of DEHP concentration have been reported over time (Keresztes et al. 2013, Guart et al. 2014).

Some of the studies on bottled water also dealt with the effect of storage location and condition on DEHP concentrations in bottled water. These parameters compared storage inside or outside a building, with the main difference being storage temperature and exposure to direct sun light. No clear or consistent trends regarding the effects of storage condition on DEHP concentration have been produced, with contradictory observations of increasing concentrations (Al-Saleh et al. 2011, Diana and Dimitra 2011, Keresztes et al. 2013) and decreasing concentrations of DEHP (Leivadara et al. 2008) having been reported.

Finally, some studies reported higher concentrations of DEHP in still water compared to carbonated water (Keresztes et al. 2013), while others reported an opposite trend (Leivadara et al. 2008, Martine et al. 2013).

From these highly variable results, it can be seen that there was no overall trend of leaching of DEHP from the water bottles to the water contained therein. Furthermore, no standard protocols have been established for these kinds of measurements and the experimental setups were quite different throughout the presented studies, making it difficult to compare studies and draw substantiated conclusions. Some studies also lack supporting statistical analyses.

It is noteworthy to mention that three of the studies presented in Table 4.1 also included glass bottles. The study by Leivadara et al. (2008) reported that the contents of two of ten glass bottles contained DEHP above the limit of quantification (LOQ), with values between 0.1 μ g/L and 1.5 μ g/L, depending on storage conditions of the carbonated water. Similarly, Cao (2008) reported DEHP in the carbonated water contained in three tested glass bottles, at levels between 0.15 μ g/L and 0.24 μ g/L. The third study involving glass bottles is a recent and vast study in Spain by Guart et al. (2014), which looked at a large number of different bottle materials. It found that water bottled in glass with a metal cap (including a liner inside the cap) was most likely to contain detectable levels of DEHP, with 39 % of all samples after one year of storage (Guart et al. 2014) yielding positive results. The liner within the metal cap is a soft material and would thus likely contain a plasticizer which could be the source of contamination. This is discussed in greater detail in the following section. Overall, these results demonstrate that the bottle material is not the important factor, but that the DEHP was more likely in the water prior to bottling, or in the liner of the cap.

Table 4.1. Quantities of DEHP found in various liquids stored in plastic and glass containers (# of n - number of replicates; LOQ - limit of quantification).

Type of sample	Concentration of DEHP (µg/L)	# of n	Country of origin	Source	
Bottled water					
Water in PET bottles	< 0.7 - 1.07	150	Riyadh, Saudi-Arabia	a (Al-Saleh et al. 2011)	
Water in PET bottles	8.78	9	Croatia	(Bošnir et al. 2007)	
Water in PET bottles	0.12 ± 0.1	7	Canada	(Cao 2008)	
Water in polycarbonate bottle	0.22	1	Canada	(Cao 2008)	
Water in glass bottle	0.17 ± 0.05	3	Canada	(Cao 2008)	
Water in PET bottles	0-0.19	4	Catalonia, Spain	(Casajuana and Lacorte 2003)	
Water in PE bottles	0.15 - 0.33	3	Catalonia, Spain	(Casajuana and Lacorte 2003)	
Water in PET bottles	0.35 - 0.48	30	Thessaloniki, Greece	(Diana and Dimitra 2011)	
Still and carbonated water in glass bottles with metallic crown	< LOQ - 11.9	170	Spain	(Guart et al. 2014)	
Still and carbonated water in PET bottles	< LOQ - 13	448	Spain	(Guart et al. 2014)	
Water in PET bottle, non-carbonated	0.02 - 1.7	3	Hungary	(Keresztes et al. 2013)	
Water in PET bottles, carbonated	< 0.02	3	Hungary	(Keresztes et al. 2013)	
Water PET and glass bottles, carbonated	< 0.02 - 6.8	12	Mytilene, Greece	(Leivadara et al. 2008)	
Water PET and glass bottles, non-carbonated	< 0.02 - 0.2	14	Mytilene, Greece	(Leivadara et al. 2008)	
RO water stored in PET and glass bottles	< 0.02 - 0.06	5	Mytilene, Greece	(Leivadara et al. 2008)	

Type of sample	Concentration of DEHP (µg/L)	# of n	Country of origin	Source
Water in PET bottles, non-carbonated	0.125 ± 0.09	11	Paris, France	(Martine et al. 2013)
Water in PET bottles, carbonated	0.15 ± 0.07	4	Paris, France	(Martine et al. 2013)
Water in recycled PET bottles	0.1 - 0.7	15	Honduras, Nepal, Switzerland	(Schmid et al. 2008)
Food-containers				
Wine in glass bottle	< 2	3	La Rioja, Spain	(Carrillo et al. 2007)
Wine in glass bottle	< 24 - 242	52	Italy	(Del Carlo et al. 2008)
Wine in PE box	25 - 276	10	Italy	(Del Carlo et al. 2008)
Wine in glass bottle	2.4 - 16	6	Italy	(Russo et al. 2012)
Beer in glass bottle	4.7	3	China	(Ye et al. 2009)
Soft drinks	18.9	36	Croatia	(Bošnir et al. 2007)
RO water in vegetable cans (121 °C)	0-0.66	24	Mexico	(González-Castro et al. 2011)
RO water in plastic containers (121 °C)	0-0.23	22	Mexico	(González-Castro et al. 2011)
Raw milk	Ca. 7-30	18	Denmark	(Sørensen 2006)
Pasteurized and homogenised milk	Ca. 13-27	4	Denmark	(Sørensen 2006)
Leaching from PVC tubing (Terufusion® IV set, 1m)				
Into a Tween80 solution (2mg/ml) – dynamic testing 66-1125 ml/h	100 - 1,000	3	Japan	(Takehisa et al. 2005)

Type of sample	Concentration of DEHP (µg/L)	# of n	Country of origin	Source
Leaching from infusion sets (Mediplus Dual				
TIVA infusion sets, PVC/DEHP 2m)				
Into Diprivan© 1 % solution – dynamic testing	4,200	3	UK	(Rose et al. 2012)
12 ml/h				
Into Propoven© 1 % solution – dynamic	4,900	3	UK	(Rose et al. 2012)
testing 12 ml/h				
Into Intralipid© 10 % solution – dynamic	7,600	3	UK	(Rose et al. 2012)
testing 12 ml/h				
Leaching from infusion sets (Extension Set,				
Laboratoire Cair, PVC/DEHP/PE 1.5m)				
Into six lipid emulsions, all 20 % Intralipid©,	19,400 - 65,800	6	France	(Bagel et al. 2011)
Medialipid©, Structolipid©, Lipidem©,				
Clinoleic©, Omegaven© - static				
Into pure oils (olive, soybean, coconut, cod	1,700,000 -	6	France	(Bagel et al. 2011)
liver) - static	3,100,000			
Leaching from 5 different brands of PVC				
bags for injections (France)				
Into 0.9 % NaCl + 0.4mg/ml etoposide sol'n	17,000 - 25,000	8	France	(Demore et al. 2002)
Into 5 % dextrose + 0.4mg/ml etoposide sol'n	17,000 - 25,000	8	France	(Demore et al. 2002)

Type of sample	Concentration of DEHP (µg/L)	# of n	Country of origin	Source
Leaching from PVC bag (500ml) with aq. Solutions (Brazil, Baxter bags)				
Into 10 % Glucose sol'n	1900	2	Brazil	(Veiga et al. 2012)
Into 0.5 % (m/v) amino acid sol'n (Leu, His, Thr)	800 - 900	2	Brazil	(Veiga et al. 2012)
Into 0.9 % NaCl sol'n	300	2	Brazil	(Veiga et al. 2012)
Into purified water	100	2	Brazil	(Veiga et al. 2012)
Leaching from PVC/DEHP blood bags,				
containing CPD (citrate-phosphate-dextrose)				
Into human blood 4 °C (Fenwal)	50,000 - 65,000	3	Baltimore, US	(Jaeger and Rubin 1972)
Into whole blood 4 °C (Fenwal)	80,000 ± 10,000	3	Ottawa, Canada	(Rock et al. 1978)
Into whole blood 4 °C (Fenwal, adenine- enriched CPD)	152,000 ± 4,500	4	US	(Peck et al. 1979)
Into whole blood 4 °C (McGaw adenine- enriched CPD)	123,000 ± 22,400	4	US	(Peck et al. 1979)
Into whole blood	50,000 - 70,000	18	Japan	(Inoue et al. 2005)
Into pure plateletpheresis concentrate	2,090-10,670	5	Austria	(Buchta et al. 2005)
Into pure 35 % plateletpheresis concentrate + 65 % T-Sol (Baxter Healthcare)	500 - 3,250	4	Austria	(Buchta et al. 2005)
PVC/29 wt% DEHP				
Into RO water	8-20	3	Canada	(Kastner et al. 2012)

4.6 Potential Sources of Contamination with DEHP

DEHP has been a contaminant of concern for almost three decades (Wams 1987) and contamination, if not from the liner of the cap, could stem from any step in the production and bottling process (Leivadara et al. 2008, Diana and Dimitra 2011, Keresztes et al. 2013). To start with, the source of water could be contaminated as suggested by studies that revealed the presence of DEHP in rain water, surface waters, and tap water. For instance, several studies summarized in Table 4.2 showed that rain water samples contained DEHP in levels up to 39 µg/L (Cole et al. 1984, Ligocki et al. 1985, Thuren and Larsson 1990, Vethaak et al. 2005, Teil et al. 2006, Björklund et al. 2009, Berge et al. 2013). Such rain water will end up in the watershed and carry with it the DEHP contamination. Treated wastewaters from municipal or industrial sources that is released into rivers and streams would also add to this contamination burden (Horn et al. 2004, Vethaak et al. 2005, Barnabe et al. 2008, Beauchesne et al. 2008, Liang et al. 2008, Luo et al. 2014, Zolfaghari et al. 2014). Such contamination by DEHP has been found in surface water (Vitali et al. 1997, Yuan et al. 2002, Vethaak et al. 2005, Yuwatini et al. 2006), water from public fountains (Casajuana and Lacorte 2003), and river or creek water and melted snow (Horn et al. 2004). A portion of this contamination can end up in treated drinking water such as tap water (Horn et al. 2004, Martine et al. 2013) used as the source of bottled water. Such water may also be a source of contamination during the washing of water bottles prior to their being filled. Also, the use of PVC piping to bring water to the bottling plant and subsequently to fill the bottle could also be a source of contamination, thus exposing the water to DEHP – although PVC pipes are a hard material and would consequently only contain low concentrations of plasticizer. Further studies would be needed to examine this, where it would need to be established whether the mass transport of DEHP from the pipes into the water carried in them would be significant enough to account for such contamination. Later in the process, bottles are capped with a lid with a liner. The liner must be a softer material and, therefore, DEHP contamination could stem from it, as was found by Guart et al. (2014) and in a study on bottled beer in China by Ye et al. (2009). The German Federal Institute for Risk Assessment (BfR) released two advisory opinions stating that a substantial amount of DEHP can migrate from such liners of containers to the food contained in them (BfR 10/2005, BfR 25/2007).

Another important factor to consider is the possibility of DEHP contamination arising from the materials and solvents used in the laboratory conducting the analyses, especially during the preparation and analysis of the samples. Contamination from the use of plastic sampling bottles, caps with liners, filters, tubing *et cetera* must be accounted for during the sampling, preparation and analytical processes and eliminated, otherwise this would result in background levels of DEHP (Fankhauser-Noti and Grob 2007). The role that these factors play in sample contamination, especially for sensitive bio-assays, was demonstrated by Olivieri et al. (2012).

4.6.1 Food Containers

Studies on DEHP found in food containers (Table 4.1) report a broad range of contamination, showing again the ubiquity of DEHP. DEHP concentrations measured in food are generally higher than in water (Wormuth et al. 2006, Cao 2010, UBA 2012a, UBA 2012b, Martine et al. 2013). Again, glass bottles are not immune to DEHP contamination as revealed in studies where it has been reported in significant amounts in wine (Carrillo et al. 2007, Del Carlo et al. 2008, Russo et al. 2012). Overall, the results suggest contamination occurs during the preparation and filling of water, food-stuff or food at the source.

The concentrations of DEHP found in milk products that were stored in various containers (Sørensen 2006) were higher than those found in other liquids such as water, wine or beer placed in similar containers. This can be explained by the higher lipid content of the milk in which DEHP would have a higher solubility (ATSDR 2002). Part I of the study by the German Federal Environmental Agency (UBA 2012a) used literature data to show a correlation between increased DEHP content of milk and milk products with increasing fat content ($R^2 = 0.79$). Extensive reviews on phthalate esters in foods in Canada (Cao 2010) and Europe (Wormuth et al. 2006, UBA 2012a, UBA 2012b, Martine et al. 2013) are available and consistently report that the highest DEHP loads are found in the fattiest foods, such as citrus essential oils, fresh meat, fish terrine, chicken and mayonnaise (Wormuth et al. 2006, Cao 2010, UBA 2012b, Martine et al. 2013).

For the general population, the most important route of exposure to DEHP and phthalates is via foods, which has been reported to account for 80 % (UBA 2012a) or more than 90 % (Wormuth et al. 2006) of the daily intake of DEHP in adults. The study by

UBA (2012a) includes bottled water in the category of food uptake, but, as shown above, concentrations of DEHP are significantly higher in lipid-containing foodstuff compared to water. The estimated levels reported, however, are below the tolerable daily intake (TDI) or reference dose (RfD), both based on non-cancer effects, used in various jurisdictions: e.g., $50 \ \mu g/kg$ of bodyweight (kg bw) in the EU (EFSA 2005), $44 \ \mu g/kg$ bw in Canada (Health Canada 1996), and 22 $\mu g/kg$ bw in the USA (US EPA 1997). An uncertainty factor, which is used to translate the TDI to a no-observed-adverse-effect level (ANOEL; i.e., the dose at which no adverse effect of DEHP is expected), is also provided by each governing body. The uncertainty factors are 100 in the case of the EU and 1000 for both Canada and the USA, and translates to ANOELs of 5 mg/kg bw, 44 mg/kg bw and 22 mg/kg bw for the EU, Canada and the USA, respectively. An overview of how these levels are established is provided by the US Food and Drug Administration (US FDA 2001). The USA also defines a specific maximum limit for DEHP for bottled water, which is set at 6 $\mu g/L$ (US EPA 2013). When comparing exposure to DEHP through food and through bottled water specifically, levels found in food can be as much three orders of magnitude higher (Cao 2010, Martine et al. 2013).

4.6.2 Hospital Equipment

Over the past 10 years, DEHP has been banned for use in children's toys throughout the Western world (EU 2005, CPSIA 2008, HPA 2010), yet it is still heavily used in hospital equipment due to its very low cost (Blass 2001). DEHP also seems to have a certain stabilizing effect on blood platelets stored in PVC/DEHP blood storage bags (Horowitz et al. 1985). This heavy use is an important consideration given the extensive amounts of one-time use materials that are consumed in hospitals. As such, Table 4.1 provides data on the leaching of DEHP from some commonly used hospital equipment, such as infusion sets (Bagel et al. 2011, Rose et al. 2012), tubing (Takehisa et al. 2005), PVC storage bags (Demore et al. 2002, Veiga et al. 2012), and PVC blood bags (Jaeger and Rubin 1972, Rock et al. 1978, Peck et al. 1979, Buchta et al. 2005, Inoue et al. 2005). Concentrations of DEHP in leachates from such equipment are significantly greater than what has been reported in water and foods, as was discussed above. These differences range from three to six orders of magnitude higher than DEHP levels found in water and food, as shown in Table 4.1; for example, 50,000 – 70,000 µg/L in whole blood from blood bags (Inoue et al. 2005), 17,000 to 25,000 µg/L in aqueous solutions stored in PVC

bags (Demore et al. 2002), and 1,700,000 to 3,100,000 μ g/L in pure oils and 19,400 to 65,800 μ g/L in lipid emulsions in infusion sets (Bagel et al. 2011). Consistent with what has been observed for food containers, the more lipophilic the solution stored or passed through the container, the higher the amount of DEHP observed in the content, mostly due to higher solubility of DEHP under such conditions (ATSDR 2002). Another study supporting this trend shows that in patients undergoing dialysis a higher total amount of leached DEHP was retained in the patients' blood with increasing plasma lipid (cholesterol and triglycerides) concentration of these patients (Faouzi et al. 1999).

Table 4.2. Environmental concentrations of DEHP reported in various countries in different environmental compartments (# of n - number of replicates; LOQ - limit of quantification; dw – dry weight).

Type of sample	Concentration of DEHP	# of n	Country of origin	Source
	(µg/L) or (µg/kg)			
Creek water	47	1	Montréal, Canada	(Horn et al. 2004)
Melted snow	130	1	Montréal, Canada	(Horn et al. 2004)
Public fountains	0-0.33	7	Catalonia, Spain	(Casajuana and Lacorte 2003)
Rain water	0.77	3	Netherlands	(Vethaak et al. 2005)
Rain water	0.02 - 0.1	7	Portland, OR, USA	(Ligocki et al. 1985)
Rain water	0 - 0.43	56	Sweden	(Thuren and Larsson 1990)
Rain water	0.69 - 1.7	3	Amsterdam,	(Vethaak et al. 2005)
			Netherlands	
Rain water	0.42	72	Paris, France	(Teil et al. 2006)
Rain water	< LOQ – 3.25	92	France	(Dargnat 2008)
River water	180	1	Montréal, Canada	(Horn et al. 2004)
Storm water	< LOQ - 39	86	USA	(Cole et al. 1984)
Storm water	< 1 - 5	39	Stockholm, Sweden	(Björklund et al. 2009)
			Göteborg, Sweden	
Surface water	320	81	Netherlands	(Vethaak et al. 2005)
Surface water (several rivers)	9.3	14	Taiwan	(Yuan et al. 2002)
Surface water (Furu river)	8-25	17	Japan	(Yuwatini et al. 2006)
Surface water (Turano river)	0.3 - 31.2	14	Italy	(Vitali et al. 1997)

Type of sample	Concentration of DEHP	# of n	Country of origin	Source
	(µg/L) or (µg/kg)			
Sediment	600	19	Netherlands	(Vethaak et al. 2005)
Sediment (several rivers)	4600	14	Taiwan	(Yuan et al. 2002)
Sediment (Furu river)	1000 - 2000	18	Japan	(Yuwatini et al. 2006)
Sediment (Turano river)	3.2 - 487.3	12	Italy	(Vitali et al. 1997)
Sediment (North sea)	3390 (µg/kg dw)	10	Dutch coast	(Klamer et al. 2005)
Sediment (Gomti river)	30	30	India	(Srivastava et al. 2010)
Sediment in varying depth (Anacostia	0 – 7500 (µg/kg dw)	>40	Washington, D.C.,	(Velinsky et al. 2011)
river)			USA	
Sediment (around Auckland)	$< 550 - 4800 \; (\mu g/kg \; dw)$	14	New Zealand	(Stewart et al. 2014)
Wastewater treatment plant influent	70	2	Montréal, Canada	(Barnabe et al. 2008)
Untreated municipal wastewater	32	12	Netherlands	(Vethaak et al. 2005)
Untreated industrial wastewater	19	10	Netherlands	(Vethaak et al. 2005)
Wastewater treatment plant effluent	54	1	Montréal, Canada	(Barnabe et al. 2008)
Wastewater treatment plant effluent	1.5	7	Netherlands	(Vethaak et al. 2005)
Landfill leachate	62	1	Montréal, Canada	(Horn et al. 2004)
Tap water	0.06	3	Paris, France	(Martine et al. 2013)
Tap water	4.6	1	Montréal, Canada	(Horn et al. 2004)

4.7 Main Routes of Human Exposure to DEHP

Based on the results presented above, it is clear that the major route of exposure of the general population to DEHP is via food. DEHP is present in bottled water, but bottled water does not pose a major threat of exposure to DEHP (Fromme et al. 2007, Montuori et al. 2008, Schmid et al. 2008, Diana and Dimitra 2011). Several studies show that the ingestion of DEHP from food is far greater than from any other source (UBA 2012b) and, in the general population, can be as much as 1000 times higher than from water (Martine et al. 2013). Nonetheless, levels found even in food are below what is considered safe by the European Union and the US EPA (see Section 4.6.1). Given the omnipresent prevalence of DEHP in the environment, another way for human uptake is through a general environmental exposure (Wams 1987, Bauer and Herrmann 1997, Staples et al. 1997, EU 2008, Guo et al. 2011, Martine et al. 2013). Routes of exposure include, but are not limited to, house dust (Butte and Heinzow 2002, Becker et al. 2004), indoor air (Butte and Heinzow 2002), soil (Cartwright et al. 2000), and watersheds (see Table 4.2). However, the study by the German environmental agency UBA (2012b) suggests that these routes are minor compared to the uptake through food and only young infants are likely to be at increased risk through a combination of house dust and mouthing of toys.

The most intense degree of exposure of individuals to DEHP occurs in hospital patients. Table 4.1 shows a difference of three to six orders of magnitudes between the amounts of DEHP found in water and food samples compared to in liquids passed through hospital equipment. This is the only case where leaching can be observed directly from the container and linked to the specific hospital equipment. This is not surprising given that hospital equipment plastics such as tubing and IV bags are soft and frequently made using PVC and a plasticizer. However, the exposure experienced by an individual in a hospital environment is most likely to be of a short-term nature (i.e., acute exposure). Thus, it is difficult to compare to exposure through food and water, which would be more continuous and long-term over the life of the individual (i.e., chronic exposure). One also has to take into account the fact that DEHP is rapidly eliminated from the body, with between 65 % and 70 % of it being secreted in the first 24 hours after exposure (Koch et al. 2005). This means that the general population would likely not be at risk from infrequent hospital visits. However, people with certain medical conditions requiring regular treatment (i.e., neonates, dialysis patients, etc.) would be

at higher risk due to their more frequent exposure to high levels of DEHP. It has even been suggested to use DEHP and its metabolites in the human body as markers for blood transfusion misuse in sports (Monfort et al. 2010, Monfort et al. 2012). The article by Koch et al. (2005) provides an overview of the breakdown pathway of DEHP in the human body.

From the data presented, it is clear that one cannot avoid contact with DEHP due to its omnipresence. However, it is to be expected that regional differences in the amounts of DEHP present in the environment will result in different exposure risks. In fact, such a correlation has been observed for another contaminant, bisphenol A (BPA), which is a monomer used to make polyester and polycarbonate, i.e., hard plastics. Teuten et al. (2009) reported a correlation between the amount of BPA in leachates from waste disposal sites with the Gross Domestic Product (GDP) of the country where this leachate was collected: i.e., the higher the GDP, the more BPA was found in the leachates. BPA is a very different compound with different impacts altogether but, considering that production and use of plastics is expected to be different in developed countries producing more plastic waste, we can reasonably hypothesize that there is a higher risk of DEHP contamination in specific regions of the world. This is supported by a direct correlation between the industrial production of DEHP in Germany and the daily intake of DEHP by German university students (Helm 2007). This also suggests that there are likely many more routes of exposure than we can account for at present.

Although most studies suggest that levels of DEHP due to chronic exposure are below what is considered safe by the several governmental agencies (see end of Section 4.6.1), our understanding of the toxic effects of DEHP are still evolving (Fan et al. 2010, Piche et al. 2012, Martinez-Arguelles et al. 2013). Yet, there are arguments for the replacement of DEHP. Most of the established levels for daily intake are based on estimations and weighted risk factors, rendering these estimations prone to error. On the other hand, the levels established by the governmental agencies are based mainly on extrapolation of data acquired in experiments with high concentrations of DEHP in animals. There are reports of non-monotonic dose responses of endocrine-disrupting compounds, meaning that an extrapolation of these results at high concentrations of DEHP is not necessarily valid (Vandenberg et al. 2012). This of course adds to the uncertainty of what safe levels might be. Furthermore, there is little work looking at synergistic effects DEHP might have with other contaminants or compounds found in the environment and the human body.

4.8 DEHP Breakdown in the Environment

Owing to its heavy use in various applications, DEHP also finds its way into the environment, where it can be found in places such as soils (Bauer and Herrmann 1997, Cartwright et al. 2000), rivers (Taylor et al. 1981, Staples et al. 2000, Horn et al. 2004), snow (Horn et al. 2004), and air (Thuren and Larsson 1990). DEHP is considered to be a ubiquitous contaminant (Wams 1987). It is thus important to understand the interaction of organisms that are found in these diverse environments with DEHP. Extensive reviews on biodegradation of several kinds of phthalates have been published by Liang et al. (2008) and Staples et al. (1997). The nature of the environment such as the abundance of oxygen plays a critical role in the biodegradation rates of DEHP (Staples et al. 1997).

Biodegradation of DEHP has been observed in anaerobic environments (Staples et al. 1997), but only one bacterial species (i.e., *Clostridium* sp. NO9) capable of degrading DEHP anaerobically has been isolated from river sediment (Chang et al. 2005). The half-lives of hydrolysis of DEHP found for this isolated strain was of several weeks to months varying with temperature and carbon source (Chang et al. 2005). In aerobic environments, DEHP is known to be degraded rather quickly, with a half-life in the range of days to weeks in cell culture (Staples et al. 1997, Liang et al. 2008, Baek et al. 2009). In soils, the rate is slower and the half-life is on the order of months (Staples et al. 1997, Liang et al. 2008). Many bacteria are able to hydrolyse DEHP in aerobic environments (i.e., genomes *Rhodococcus, Bacillus, Micrococcus, Enterococcus, Pseudomonas, Acinetobacter, Mycobacterium, Corynebacterium, Brevibacterium, Gordonia*, etc.), but fungi can do the same (i.e., *Penicillium lilacinum, Aspergillus Niger, Aspergillus puniceus, Actinomucor elegans, Phanerochaete chrysosporium*, etc.) (Engelhardt et al. 1977, Staples et al. 1997, Nalli et al. 2006c, Liang et al. 2008, Baek et al. 2009). Abiotic degradation (i.e., UV radiation) does not seem to play an important role in the environmental fate of DEHP (Staples et al. 1997, Lertsirisopon et al. 2009).

The relevance of these biodegradation kinetics is directly related to the lipophilic nature of DEHP (ATSDR 2002): that is, it migrates preferentially to lipid-containing environments. An example of this is the migration of DEHP into the sediment of rivers (Vitali et al. 1997, Yuan et al. 2002, Vethaak et al. 2005, Yuwatini et al. 2006). In these studies, the ratio of DEHP found in sediment compared to surface water reaches values of up to 500, yet there seem to be other important contributing factors to this ratio such as proximity to urban centers and thus

wastewater influent and seasonal changes in water level. Several other studies (Klamer et al. 2005, Srivastava et al. 2010, Velinsky et al. 2011, Stewart et al. 2014) showed significantly higher concentrations of DEHP in river and ocean sediments compared to surface waters and in rain as shown in Table 4.2. These trends are consistent over several continents indicating this is a global problem. The result is bioaccumulation of DEHP in sediment due to the slower biodegradation kinetics in the anaerobic environment, as seen by the significantly higher levels of DEHP in sediment compared to surface water.

The general pathway of bacterial breakdown of DEHP (shown in Figure 4.2) is through de-esterification (i.e., hydrolysis of an ester bond) to its monoester (MEHP) followed by a second de-esterification step to its corresponding organic acid (phthalic acid). This is sometimes preceded by β-oxidation of the side chains, or *trans*-esterification of the molecule, but these latter pathways result only in different lengths of the side chains (Staples et al. 1997, Liang et al. 2008, Sauvageau et al. 2009). De-esterification is achieved by enzymatic degradation by esterases which are produced by many common microbes. The compound resulting from de-esterification of one of the two ester bonds of DEHP is its monoester MEHP (Figure 4.2), a compound that has been linked to anti-androgenic effects in humans (Richburg and Boekelheide 1996, Swan et al. 2005a, Pant et al. 2008, Fan et al. 2010, Piche et al. 2012). Several studies suggest that MEHP is more stable than DEHP in the environment (Ejlertsson et al. 1996, Jonsson et al. 2003), which suggests greater bioaccumulation of the problematic monoester in the environment. As a result, when designing a replacement plasticizer, great care needs to be taken to assure no buildup of a more recalcitrant and toxic metabolite (Erythropel et al. 2012, Erythropel et al. 2013). Another stable metabolite of DEHP is 2-ethylhexanoic acid. It is the oxidation product of the liberated 2-ethylhexanol during de-esterification, which is formed by a two-step oxidation, with the corresponding 2-ethylhexanal as intermediate as shown in Figure 4.2 (Nalli et al. 2006c). 2-Ethylhexanoic acid is also only slowly metabolized, which seems to be attributable to the ethyl-branch on the second carbon that interferes with β -oxidation (Nalli et al. 2006a, Nalli et al. 2006b, Erythropel et al. 2012). The pathways for further biodegradation of phthalic acid have been established previously and are well summarized by Liang et al. (2008) where ring-opening ultimately leads to oxaloacetate, pyruvate, acetate, hydrogen and carbon dioxide.
As DEHP is also found in wastewaters (Horn et al. 2004, Vethaak et al. 2005, Barnabe et al. 2008, Beauchesne et al. 2008, Liang et al. 2008, Luo et al. 2014, Zolfaghari et al. 2014), the question of its removal during wastewater treatment also arises. There exist extensive reviews on DEHP specifically by Zolfaghari et al. (2014), and more generally by Luo et al. (2014). Both reviews show that DEHP removal from wastewater is not complete. Table 4.2 shows two examples of untreated and treated waste water (Vethaak et al. 2005, Barnabe et al. 2008) where, although a reduction is achieved, levels of DEHP are still above what is found in surface water. This means that treated wastewater likely contributes to the contamination of surface waters with DEHP and ultimately to its bioaccumulation. On the other hand, it is not known what fraction of DEHP enters the environment through wastewater discharge.



Figure 4.2. De-esterification pathway of DEHP through esterases, and oxidation products of 2-ethylhexanol.

4.9 Perspectives

Despite this uncertainty, given the widespread use of DEHP in common materials and commercial products coupled with its established ubiquity in the environment, the accumulating evidence of negative impacts associated with this compounds - both on the environment and the health sides – and especially given the multiple routes of exposure of humans to this compound through water, food, indoor air and other avenues, it is suggested that, as per the precautionary principle (UNEP 1992, Kriebel et al. 2001, Gochfeld 2003), the replacement of DEHP with less problematic compounds should strongly be considered. Under this principle, there is a duty to take anticipatory action to prevent harm, and for government, industry and the general public to share in this responsibility. At present, there is a growing awareness of industry and government about the potential impacts of DEHP based on scientific findings. Furthermore, the general public is now expressing concerns over exposure to this compound and many are seeking ways to avoid such exposure in their day to day activities and purchases. Consumers also have a right to know about the potential impacts associated with the use of products and the burden to provide this information lies with the producer. In the case of DEHP, exposure of the public comes via many routes, most of which are unknown to the average consumer and are involuntary. The knowledge is in the hands of the producer to act upon and limit these routes of exposure. It is also in their hands to demonstrate that their products are safe to use.

Another critical element of the precautionary principle is the importance of examining alternatives to the compound of concern and the selection of alternatives that have the least potential impact on human health and the environment. In many instances, the application of the principle is confounded by a lack of alternatives. However, in the case of DEHP, alternative materials are available and can be engineered to be less harmful. When evaluating potential alternatives to DEHP, it is essential that consideration be given to all foreseeable costs and life cycle, including raw materials, the manufacturing, transportation, use, environmental remediation and eventual disposal of created products, as well as health costs, even when such costs are not reflected in the price of the material/product itself.

Therefore, replacement compounds for DEHP must be well designed and thoroughly studied prior to widespread commercialization to avoid the types of problems observed with DEHP, such as its persistence in the environment and accumulation of toxic metabolites. DEHP has especially been shown to be rather resistant to biodegradation by microbes, likely due to the positioning of the two ester groups to one another, as well as the branching on the side chains (Nalli et al. 2002, Gartshore et al. 2003, Sauvageau et al. 2009, Erythropel et al. 2012, Erythropel et al. 2013). Similarly, its most problematic metabolite MEHP is possibly even more recalcitrant than its parent compound (Ejlertsson et al. 1996, Jonsson et al. 2003, Amir et al. 2005).

To tackle the problems arising from the widespread use of DEHP, recent research aims at producing equally effective plasticizers that are more biodegradable and less toxic than DEHP and would thus be eliminated more quickly in the environment, an idea that can also be extended to the metabolites of this plasticizer (Firlotte et al. 2009, Kermanshahi pour et al. 2009a, Kermanshahi pour et al. 2009b, Stuart et al. 2010, Shi et al. 2011, Erythropel et al. 2012, Erythropel et al. 2013). There also exist many commercial efforts to create plasticizers that are less toxic and recalcitrant, however formulations of these compounds are often unknown. Among the proposed alternatives are vegetable-oil based plasticizers, citrate and succinate diesters, as well as hydrogenated DEHP-resembling compounds (DINCH®) (Rahman and Brazel 2004, Markarian 2010, Lanxess AG 2011). Much more testing is needed to validate these replacement compounds to not only ensure their utility as plasticizers but also to provide sufficient evidence that will either drive industry to adopt them or regulatory agencies to ban the use of DEHP to favour of these replacements.

4.10 Conclusion

Due to its widespread use and ubiquitous presence in the environment, DEHP ultimately finds its way into humans. It has been shown in this review that there is a broad consensus that this exposure is mainly due to ingestion through food and that bottled water in PET bottles only plays a very minor role in this exposure. In the latter case, it is highly unlikely that the contamination of the water stems from the container material itself, but it was more likely contaminated in an earlier production step or in the water source. However, there is one area of application in which the leaching of DEHP from a container to a liquid in contact with it does occur to a significant extent and that is in hospital equipment, which can result in a high acute exposure. This is especially problematic for those that need regular medical attention. Although is it difficult to accurately estimate the total chronic exposure of the general population to DEHP, the general consensus seems to be that this chronic exposure is below what government agencies judge as safe. However, given the prevalence of DEHP as contaminant worldwide, the uncertainty concerning what is considered a safe level of exposure, the possibility of synergistic effects with other compounds, and the possibility to engineer more biodegradable plasticizers, we suggest, following the precautionary principle, that DEHP be replaced with more suitable compounds. This would result in reduced human exposure to DEHP. However, before this can be achieved, a replacement plasticizer must be designed very carefully and tested thoroughly to ensure that its implementation avoids effects such as those observed with DEHP.

4.11 Acknowledgements

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5 Designing Green Plasticizers: Influence of Molecular Geometry on Biodegradation and Plasticization Properties

5.1 Preface

The following manuscript was published in 2012 in the Journal Chemosphere, Vol. 86(8): pp. 759-766 and the data presented was partly collected during research conducted during my Master's degree under the supervision of Prof. David G. Cooper and Prof. Milan Maric between May 2009 and April 2011. The manuscript outlines the synthesis of di (2-ethylhexyl) maleate (DEHM), -fumarate (DEHF), and -succinate (DEHS) from the corresponding anhydride or diacid (maleic anhydride, fumaric acid, succinic anhydride) with 2-ethylhexanol using the Dean-Stark setup. This technique takes advantage of the lower density of the solvents benzene or toluene to trap water, a by-product of the esterification reaction, thereby driving the reaction to completion.

The compounds were chosen due to their close resemblance to the important industrial plasticizers DEHP and DEHA. Earlier work had already established their compatibility with PVC (Würstlin and Klein 1956), as well as their good permanence (Kastner et al. 2012). Thus, the plasticizer efficiency as the third important aspect of a plasticizer according to Boyer (1951) was the principal aspect of interest. The three compounds were chosen to investigate the influence of the geometrical structure between the two ester groups (maleate: *cis*, fumarate: *trans*, succinate: saturated, thus freely rotatable) on both plasticizer and biodegradation behaviour in a model system. By using the same alcohol for esterification as used in DEHP and DEHA, a comparison of the central structures between the five compounds (i.e., three proposed, two commercial) was made possible.

The successfully synthesized compounds were tested for their plasticizer properties. First, they were extruded with unplasticized PVC (UPVC) at varying levels. The blending technique was based on previous work by Firlotte et al. (2009), and the technique for the production of the tensile test bars using a hot press had to be developed. After conditioning, plasticizer properties such as T_g reduction and tensile testing (following ASTM D-638 (2014) guidelines) were evaluated, and both the maleate and the succinate compound performed comparably to commercial plasticizers such as DEHP and DEHA. The blends containing the fumarate were significantly stiffer and this was related to its structure.

Biodegradation experiments of the pure compounds were carried out according to previously established protocols (Nalli et al. 2002). The common soil bacterium *Rhodococcus rhodocrous* was used because of its ability to break down hydrophobic compounds relatively quickly when grown with hexadecane as a carbon source. Samples were taken regularly, extracted with chloroform, appropriately concentrated, and analysed using a gas chromatograph with and FID detector (GC-FID). The same breakdown pathway as for similar diesters such as DEHP and DEHA was found (Nalli et al. 2002, Horn et al. 2004). Standards of the metabolites were purchased or synthesized, and quantification of biodegradation kinetics was done. By assuming first order kinetics (as discussed in Section 3.4.1), half lives for the first hydrolysis step were calculated. The spatial arrangement and flexibility of the central bond linking the two ester groups played an important role for the compounds' half-life, and the results also explain the slow hydrolysis of DEHP. The rate of the parent compound biodegraded also reflected in the degradability of the primary metabolite (i.e., the corresponding monoester), which did not build up in the cases where the parent compound was quickly degraded (i.e., succinate, fumarate, adipate). This is important, as the monoester of DEHP, MEHP, is believed to be the biologically active and problematic compound. The buildup of 2-ethylhexanoic acid, as an oxidation product of the liberated 2-ethylhexanol, was observed as expected, due to the alkyl chain on the β -carbon, thereby blocking β -oxidation (Schaeffer et al. 1979, Nalli et al. 2002, Nalli et al. 2006b).

Designing Green Plasticizers: Influence of Molecular Geometry on Biodegradation and Plasticization Properties

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5.2 Abstract

The plasticizer di (2-ethylhexyl) phthalate (DEHP) and its metabolites are considered ubiquitous contaminants, which have a range of implications on the environment and human health. This work considered several alternative compounds with structural features similar to DEHP. This added to the understanding of why DEHP is so poorly biodegraded once it enters the environment. These alternative compounds were based on 2-ethylhexyl diesters of maleic acid (*cis*-isomer), fumaric acid (*trans*-isomer) and succinic acid (saturated analogue). The rates of biodegradation by the common soil bacterium *Rhodococcus rhodocrous* were shown to be dependant on the structure of the central unit derived from the diacid used to make the ester. The diacid components of DEHP and the maleate both had a *cis* orientation and they were the two that were slow to biodegrade. Plasticizing properties were also compared and, because the ester of the saturated succinic acid was degraded quickly and also had good plasticizing properties, it was concluded that the succinic esters of straight chain alcohols would make the best green plasticizers. The maleate ester had excellent plasticizing properties but this is mitigated by a significant resistance to biodegradation.

Keywords: Green Plasticizer, Phthalates, Biodegradation, Succinate, Maleate

5.3 Introduction

Plasticizers have recently received significant negative attention. For example, several types of phthalate compounds were banned in children's toys in the European Union, the United States and Canada (EU 2005, CPSIA 2008, HPA 2010). Plasticizers are additives to polymers that serve to improve their workability and flexibility but, while they are mixed into

the polymer, they are not chemically bound, and can leach out during use or after disposal of the material (Sears and Darby 1982). The majority of plasticizers, about 80 % (Stevens 1999, Murphy 2001), are used for poly (vinyl chloride) (PVC). Worldwide, about 90 % of all PVC plasticizers (Murphy 2001) were based on phthalic acid and 51 % of the material used was di (2-ethylhexyl) phthalate (DEHP) (Murphy 2001), which is shown in Figure 5.1. In 2006, worldwide production of DEHP was approximately 6 Mt, accounting for USD 9.5x10⁹ (AgPU 2006). The majority of plasticized PVC is used in applications such as medical equipment (hospital tubing, blood bags, etc.), food wrapping, wire and cable insulation and automobile parts (Rahman and Brazel 2004).

Plasticizers can leach out of the PVC products over time after disposal. This is particularly noticeable at landfill sites (Öman and Hynning 1993, Marttinen et al. 2003). Twenty years ago, the presence of phthalate plasticizers was already considered to be ubiquitous in the environment (Wams 1987). Other studies have identified phthalates in soils (Bauer and Herrmann 1997, Cartwright et al. 2000), surface water (Taylor et al. 1981, Staples et al. 2000, Horn et al. 2004) and as pollutants in indoor air (Butte et al. 2001, Edwards et al. 2001, Becker et al. 2004) and in the atmosphere (Thuren and Larsson 1990). They were also found to accumulate in the tissue of mammals (Staples et al. 1997) as well as in several aquatic species such as oysters, brown shrimp and several types of fish (Wofford et al. 1981). DEHP is also believed to disrupt the endocrine system in laboratory rats (Fukuwatari et al. 2002) and has been proven to be weakly estrogenic (Jobling et al. 1995).

The breakdown pattern of DEHP and other plasticizers by bacteria (Nalli et al. 2002) has been studied and identified stable metabolites such as mono (2-ethylhexyl) maleate, 2-ethylhexanol, 2-ethylhexanal (as a volatile organic compound) and 2-ethylhexanoic acid (Nalli et al. 2006b, Nalli et al. 2006c). These compounds have been found to be more toxic than their parent compounds (Horn et al. 2004).

Because of all of the above problems, there is a strong incentive to develop "greener" plasticizers. Several workers have proposed alternative plasticizers such as those based on lactic esters and dibasic acids (Rehberg et al. 1952, van Veersen and Meulenberg 1967) as well as modified dibenzoate plasticizers (Firlotte et al. 2009). Recent work has focussed on succinic acid as a base for plasticizers because of the potential to produce it by fermentation (Stuart et al. 2010). Most of these studies have been concerned with the plasticization properties. There

has been less emphasis on biodegradability and toxicity of these compounds and/or their metabolites. To design greener plasticizers, emphasis must also be placed on the degradability of the plasticizer and on avoiding a build-up of concentrations of metabolites. The goal of this work has been to relate both plasticizing and environmental considerations to simple structural features of the plasticizers. Earlier work has determined that small diesters are particularly suitable as plasticizers (van Veersen and Meulenberg 1967). Our work considered diesters of three small organic acids. They were succinic acid and fumaric acid (both components of the citric acid cycle) and their structural isomer maleic acid. The properties of these compounds were compared to the two common conventional plasticizers DEHP and di (2-ethylhexyl) adipate (DEHA).



 $R = CH_2CH(CH_2CH_3)CH_2CH_2CH_2CH_3$

Figure 5.1. Structures of commercial phthalic and adipic diesters, and tested maleic, fumaric and succinic diesters.

5.4 Materials and Methods

The plasticizers DEHP and DEHA, both with 99 % purity, were purchased from Sigma-Aldrich. The other plasticizers studied were synthesized in our laboratories and the components used to make them are identified in the relevant sections below.

5.4.1 Synthesis of Di (2-Ethylhexyl) Maleate (DEHM)

A mixture of maleic anhydride (10.1 g, 77.4 mmol, 99%, Sigma Aldrich) and 2-ethylhexanol (18.4 g, 141.4 mmol, 99.6%, Sigma Aldrich) were dissolved in 200 mL of benzene (ACP Chemicals) with 1.0 mL (18.0 mmol) conc. sulphuric acid (Fisher Scientific) and heated at 95 °C over night in a 250 mL-flask fitted with a Dean-Stark trap attached to a reflux condenser. The white suspension in the flask gradually became clear and a total of 1.4 mL of water was collected in the Dean-Stark trap. After cooling this mixture, 50 mL of a concentrated aqueous solution of sodium bicarbonate (Fisher Scientific) was added while stirring. Once the evolution of carbon dioxide ceased the phases were separated using a separatory funnel and the aqueous phase was extracted with three aliquots of 50 mL dichloromethane (Fisher Scientific). The combined organic phases were washed with deionized water, dried with sodium sulphate (Anachemia) and then the solvents were removed on a rotatory evaporator at 95 °C and a pressure of 1.2 kPa (Büchi RE III with Heidolph Rotavac Valve Control). A colorless, oily liquid was obtained. Yield: 20.4 g (59.9 mmol) = 84.7 %.

¹H-NMR (500.1 MHz in CDCl₃): δ (ppm) = 0.92 [m, 12H, C<u>H</u>₃], δ (ppm) = 1.20 - 1.40 [m, 16H, CH(C<u>H</u>₂CH₃)((C<u>H</u>₂)₃CH₃)], δ (ppm) = 1.60 [m, 2H, OCH₂C<u>H</u>], δ (ppm) = 4.08 [t, 4H, OC<u>H</u>₂CH₂], δ (ppm) = 6.20 [s, 2H, CO(C<u>H</u>)₂CO].

5.4.2 Synthesis of Di (2-Ethylhexyl) Fumarate (DEHF)

Fumaric acid (10.0 g, 86.2 mmol, 99.5 %, Fisher Scientific) and 2-ethylhexanol (21.5 g, 165.1 mmol, 99.6 %, Sigma Aldrich) were dissolved in 150 mL of benzene (99 %, ACP Chemicals) with 1.0 mL (18.0 mmol) conc. sulphuric acid (Fisher Scientific) and heated at 95 °C for 24 h in a 250 mL-flask with a Dean-Stark trap attached to a reflux condenser. A total

of 2.8 mL of water was collected in the Dean-Stark trap. Following the same procedure as described above, a colorless, oily liquid was obtained. Yield: 13.8 g (40.6 mmol) = 49.2 %. ¹H-NMR (500.1 MHz in CDCl3): δ (ppm) = 0.92 [t, 12H, CH₂CH₃], δ (ppm) = 1.25 [m, 12H, CH(CH₂)₃CH₃], δ (ppm) = 1.40 [dd, 4H, CH(CH₂CH₃)], δ (ppm) = 1.60 [m, 2H, CH₂CH], δ (ppm) = 4.20 [m, 4H, OCH₂CH], δ (ppm) = 6.81 [s, 2H, CO(CH)₂CO].

5.4.3 Synthesis of Di (2-Ethylhexyl) Succinate (DEHS)

Succinic anhydride (10.0 g, 100.0 mmol, 99 %, Fisher Scientific) and 2-ethylhexanol (40.3 g, 309.1 mmol, 99.6 %, Sigma Aldrich) were dissolved in 150 mL of toluene (Sigma Aldrich) with 1.0 mL (18.0 mmol) conc. sulphuric acid (Fisher Scientific) and heated at 125 °C over night in a 250 mL-flask with a Dean-Stark trap attached to a reflux condenser. A total of 1.5 mL of water was collected in the Dean-Stark trap. Following the same procedure as described above, a colorless, oily liquid was obtained. Yield: 27.7 g (80.9 mmol) = 80.9 %. ¹H-NMR (500.1 MHz in CDCl₃): δ (ppm) = 0.92 [t, 12H, CH₂CH₃], δ (ppm) = 1.25 [m, 12H, CH(CH₂)₃CH₃], δ (ppm) = 1.40 [dd, 4H, CH(CH₂CH₃)], δ (ppm) = 1.60 [m, 2H, CH₂CH₁], δ (ppm) = 2.62 [s, 4H, CO(CH₂)₂CO], δ (ppm) = 4.00 [m, 4H, OCH₂CH]

5.4.4 Biodegradation Study: Microorganism, Growth and Sample Preparation

The microorganism used was *Rhodococcus rhodocrous*, American Type Culture Collection 13808. All biodegradation experiments were performed using 500 mL Erlenmeyer flasks with foam caps. Each flask contained 100 mL Minimum Mineral Salt Medium (MMSM), 10 mM plasticizer, 2 g/L hexadecane and 0.1 g/L yeast extract. The MMSM medium contained: 6 g/L Na₂HPO₄, 4 g/L NH₄NO₃, 4 g/L KH₂PO₄, 0.2 g/L MgSO₄•7H₂O, 0.014 g/L Na₂EDTA, 0.01 g/L CaCl₂•2H₂O, and 0.01 g/L FeSO₄•7H₂O (Fisher Scientific). The flasks were autoclaved at 121 °C and 100 kPa for 15 min (AMSCO, Model 3021-S), allowed to cool and then inoculated with 1 mL of cell broth from a previously grown culture in a laminar fume hood (Baker Company, Model VBM600) using sterile techniques. The flasks were then put into an incubator-shaker (innova44, New Brunswick Scientific or Multitron II, Infors AG) at 30 °C and 140 rpm for the duration of the experiment.

Because the plasticizers were only slightly soluble in water, it was impossible to take representative samples of the mixture. Thus, the entire contents of a flask were extracted for each set of measurements. Nine flasks were prepared for each compound. The first was extracted at day 0 as a control and the rest, one at a time, every third to fifth day. A tenth flask was also prepared, but not inoculated, as an abiotic control, and this was extracted at the same time as the last flask in the series.

Before a flask was extracted, the pH of its contents was adjusted to pH 2–3 using sulphuric acid. Then the flask was extracted using 20 mL of chloroform containing 2 g/L of pentadecane, which served as an internal standard for quantification of the GC analysis. The contents of each flask were added to a separatory funnel and the organic phase was recovered and stored at 4 °C until analysis.

5.4.5 Gas Chromatography

A GC was used to monitor plasticizer concentrations as well as to detect and quantify metabolites. The samples were diluted to an appropriate concentration for the GC (Trace GC Ultra with AI3000 Autosampler, Thermo Scientific) and then $1.0 \,\mu$ L was injected using a syringe. The column used was a Restek RTX-5 (length 30 m, id 0.32 mm, 0.25 μ m film), and a flame ionization detector. Calibration curves were prepared in order to calculate concentrations from the ratios of peak areas of the compounds to those of the internal standard pentadecane.

5.4.6 Extrusion of PVC Blends Containing Plasticizers

Unplasticized PVC was obtained from Solvay Benvic, France. A conical intermeshing twin-screw extruder (Haake Minilab, Thermo Electron Corporation, screw diameter 5/14 mm conical, screw length 109.5 mm) was used to create plasticized blends of PVC. The extruder was operated at a batch feed size of 3 g, a rotation speed of the screws of 60 rpm and an operating temperature between 110 and 130 °C, depending on the amount of plasticizer in the blend. Because unplasticized PVC is a solid and all of the compounds tested were liquids, the extrusions had to be carried out in several steps to ensure homogeneity of the resulting blends. In a first step, a blend of 20 phr was prepared, which also incorporated 4 phr of epoxidized soy

bean oil (Chemtura Corporation) as heat stabilizer and 5 phr of stearic acid (Fisher Scientific) as lubricant. The unit phr is often used in the polymer blends and additives industry and stands for parts per hundred rubber. After the desired number of batches was collected, all the material was chopped into small pieces and recycled through the extruder again to ensure homogeneity. This blend of 20 phr could then be used to prepare blends of higher plasticizer content by simply adding more of the plasticizer to the master batch.

5.4.7 Differential Scanning Calorimetry

A temperature modulated differential scanning calorimeter was used (TA Instruments Q2000). Thin slices of about 1-2 mg were cut from the blend while avoiding contamination of the surface of the freshly cut slices. Four or five of these freshly cut slices were placed in a standard DSC pan (TA Instruments, model #070221). The top was crimped on and the total weight of the loaded pan was recorded (Sartorius CP225D). The pan was then placed in the autosampler of the instrument, along with an empty pan for calibration. The samples were quenched at -90 °C, held at this temperature for 5 min, then heated with a rate of 2 °C/min to +100 °C and again held at this temperature for 5 min. The constant heating rate was superimposed by a sinusoidal modulation of 1.27 °C with a period of 60 s. A first cycle served to erase the sample's previous thermal history and a second cycle was used for the actual glass transition temperature (T_g) measurement. Using the software "TA Universal Analysis" the reversible heat flow of the second heating cycle was plotted against the temperature and, using the half height method, the glass transition temperature T_g was determined according to ASTM D-3418 (2015).

5.4.8 Production of Tensile Strength Test Bars

The plasticized PVC blends were pressed into tensile strength test bars using a hot press (Carver Manual Hydraulic Press with Watlow Temperature Controllers, Carver). The appropriate mold was filled with small cut pieces of PVC blend, wrapped in aluminum foil to prevent direct contact of the plates with the polymer, and inserted into the hot press between two steel plates. The applied pressure was calculated from the force of the press over the area of the mold. The apparatus was allowed to heat up to 180 °C for 10 min at an applied pressure

of 1 MPa, degassed three times and the mold was turned upside down. Following this, the samples were pressed at an applied pressure of 2 MPa for 10 min after which the mold was turned upside down once again. Finally, the samples were pressed for two times at an applied pressure of 3 MPa for 15 min from each side. The cooling water was turned on and the cooled test bars were carefully removed from the mold and placed in a desiccator until testing (Drierite, Fisher Scientific, Montréal, QC). The dimension of the test bars are shown in Figure 5.2 and correspond to those used for the method ASTM D-638 (2014).



Figure 5.2. Dimensions of tensile test bars. Top view shown.

5.4.9 Tensile Strength Testing

All tensile testing was done on a Yamazu Easy Test with a load cell of 500 N after the test bars had spent at least 2 d in the desiccator. The exact thickness and width of the middle section of the test bar were recorded (Electronic Outside Micrometer, Fowler Tools and Instruments) after which the test bars were clamped by their wider section into the apparatus and were then exposed to a strain rate of 5 mm/min. Both elongation distance and force imposed on the test bar were automatically recorded by a computer until rupture of the test bar. Using these data, a stress-strain curve was generated using Equations 5.1 and 5.2.

$$\varepsilon(\% EL) = \frac{(L - L_0)}{L_0} \times 100 \left(\frac{mm}{mm}\right)$$
(Eq.5.1)
$$\sigma(MPa) = \frac{F}{(T_0 \times W_0)} \left(\frac{N}{mm^2}\right)$$
(Eq.5.2)

Equation 5.1 was used to calculate the tensile strain, which is generally reported in percent elongation. L_0 represents the initial separation of the grips (32.5 mm) and L the elongation distance as recorded by the machine. Equation 5.2 was used to calculate the tensile

stress, which is usually reported in units of MPa. T_0 and W_0 represent the thickness and width, respectively, of the inner section of the test bar resulting in its cross sectional area. F is the force that is recorded at any moment by the machine (Callister 2005). Stress and strain were calculated for every recorded point and plotted to give a typical stress-strain curve.

The parameters extracted from the stress-strain curve were elongation at break, which is the strain at the point of rupture of the test bar, as well as the secant modulus, which is the slope of a straight line from the slack-corrected origin to a given point on a stress-strain curve. In this work, all secant moduli were calculated for a stress of 2 MPa. In tensile strength measurements, usually the modulus of elasticity is calculated, but if no Hookean behaviour was observed, the ASTM standard D-368 demands the calculation of the secant modulus as explained above. The only blend exhibiting Hookean behaviour was the fumarate samples, so that for these, the modulus of elasticity was reported. All reported data is the average of 3-5 samples. The procedure was adapted from the ASTM standard for tensile testing (ASTM D-638 2014).

5.5 Results

5.5.1 Biodegradation Properties

Figure 5.3 shows the results for a typical biodegradation experiment. The compound being tested here is di (2-ethylhexyl) succinate. This compound disappears within a period of approximately 14 d. Almost immediately, there is the appearance of the monoester, mono (2-ethylhexyl) succinate and the alcohol 2-ethylhexanol. The concentration of the monoester never increases to more than a trace amount but the concentration of the alcohol first increases and then starts to decrease. The metabolite with the highest observed concentration is 2-ethylhexanoic acid. This is the last of the metabolites to appear. It reaches a significant concentration just as the parent compound has disappeared and then degrades only very slowly by the end of the experiment.



Figure 5.3. Biodegradation of di (2-ethylhexyl) succinate by *Rhodococcus rhodocrous* in the presence of hexadecane as carbon source.

Table 5.1 shows the results for all of the biodegradation experiments. The table contains values for the half-life of each of these estimated using a first order, initial rate approximation. There was almost no biodegradation observed for either DEHP or di (2-ethylhexyl) maleate. All of the other compounds were biodegraded rapidly but none as quickly as the succinate. Table 5.1 also indicates the types of metabolites observed and the maximum amounts. It can be seen that, except for 2-ethylhexanoic acid, in some cases, none of these were observed in appreciable amounts.

	phthalate	maleate	fumarate	succinate	adipate
Position of ester group	"cis"	cis	trans	saturated	
Half-life	very large	very large	13.1 d	2.3 d	5.8 d
Maximum concentrations of metabolites (mM) ^a					
Monoester	trace amounts	trace amounts	trace amounts	trace amounts	trace amounts
2-ethyl- hexanol	trace amounts	0.5	0.8	1	1
2-ethyl- hexanoic	trace amounts	0.7	6	15	10

Table 5.1. Comparison of biodegradation rates of DEHM, DEHF, DEHS, DEHP and DEHA.

^a Highest concentration detected, with little change indicating slow degradation.

5.5.2 Plasticizer Properties

The glass transition temperatures of the PVC blends with the compounds of interest as well as with the commercial plasticizers are shown in Figure 5.4. Each of the compounds was tested at three different concentrations that covered the range of normal usage. Both the succinate and the maleate were shown to be more efficient in lowering the T_g compared to the commercial plasticizer DEHP, and as efficient as DEHA. The fumarate was the poorest at lowering the T_g at the lowest concentration of plasticizer added. It improved at higher concentrations but was not quite as good as the other compounds tested.

Further analyses done by tensile testing measurements show the succinate to be performing as well as the commercial plasticizers in terms of elongation at break once a threshold of about 30 wt.-% is reached, while also being more malleable than DEHP and as malleable as DEHA, using the secant modulus at 2 MPa (see Figure 5.5a) for graphs of strain at break ordered by central molecule and within these by concentration of plasticizer; and Figure 5.5b) for graphs of Young's modulus/secant modulus ordered in the same manner).

Neither the maleate nor the fumarate blends reach values as high as those of the commercial plasticizers in terms of elongation at break; especially the fumarate, which seems

to fracture at much lower strains. Meanwhile for a secant modulus at 2 MPa, the fumarate is even less malleable than the phthalate but the maleate is as malleable as the succinate and adipate.



Figure 5.4. Glass transition temperatures against plasticizer content for various di 2-ethylhexyl plasticizers (error bars representing standard deviation).



Figure 5.5. a) Strain at break and b) Young's modulus for the fumarate series and secant modulus for several blends containing different concentrations of various di (2-ethylhexyl)-terminated plasticizers (error bars representing standard deviation).

5.6 Discussion

Plasticizers are used in formulations to make many inexpensive plastic products. Usually this is with PVC, a relatively cheap polymer. Thus, the plasticizers must also be inexpensive. The general structure of diesters has been proven to be ideal for plasticizers for PVC (van Veersen and Meulenberg 1967, Stuart et al. 2010) and these can be easily synthesized using a Dean-Stark esterification that forces the reaction almost to completion by removing the water released from the reaction in a trap attached to a reflux condenser. A simple washing step with a solution of sodium bicarbonate was shown to remove excess catalyst and unreacted reagents as well as any monoester produced from incomplete esterification.

For both maleate and succinate esters, the starting material was an anhydride, which made the production even easier because only one equivalent of water needed to be removed instead of two from the diacid. Overall, the production of all of these compounds was simple, as the small amounts of contaminants could be removed cheaply and the overall process would be easy to scale up. However, it is also essential to show that any diester candidate has the appropriate plasticization properties. In order to design a green plasticizer, its biodegradation properties have to be considered because, inevitably, it will end up in the environment.

The biodegradation pattern for all of the diesters tested in this study is consistent with the pattern presented in Figure 5.6. This pattern is analogous to those reported for other diesters (Nalli et al. 2002, Horn et al. 2004, Nalli et al. 2006c). The first step was the hydrolysis of one of the ester groups yielding one equivalent each of alcohol and monoester. Both types of metabolites were observed for all of the compounds tested but often only in trace amounts. Subsequent steps were the hydrolysis of the second ester bond and the further biodegradation of both molecules of alcohol released after the two hydrolysis steps. The alcohols were oxidized to the corresponding carboxylic acids. The carboxylic acids were always observed in the highest concentrations of any of the metabolites and were only slowly removed from the media. This type of behaviour has been observed before (Nalli et al. 2006a, Nalli et al. 2006b, Kermanshahi pour et al. 2009a), and the slow degradation has been related to the pathway of β -oxidation being blocked due to the ethyl branch in the β -position to the acid function.

In these experiments, the release of succinic acid or fumaric acid can be inferred after the second hydrolysis step. However, both compounds are part of the Krebs cycle and would be expected to be quickly metabolized by the bacteria. This is an obvious example of the advantages of basing new compounds on natural products that can be considered as safe when entering the environment.



Figure 5.6. Observed pattern of degradation for all diester compounds tested.

The slow degradation of di (2-ethylhexyl) maleate is particularly interesting. Only very small amounts of even the initial metabolites released by the first hydrolysis step were observed in the time scale of the experiments. The geometry of the two ester functions with regard to the double bond are in the *cis* orientation and this is essentially the same orientation as the two ester functions in the commercial plasticizer, DEHP, which is well known to be difficult to biodegrade and persistent in the environment (Horn et al. 2004). The ester functions in the fumarate analogue are in the *trans* orientation. Thus, the stability of both the maleate and the phthalate could be attributed to steric hindrance interfering with the enzymatic

hydrolysis. Another contribution to the stability could be due to the delocalization of the ester functional groups with the aromatic ring of the phthalate or with the double bond of the planar core of the maleate ester. However, this same delocalization would be present for the analogous fumarate diester, which was biodegraded relatively quickly. Furthermore, the half-life of the fumarate diester was not that much longer than that of the unsaturated succinate diester. Therefore, a significant amount of the resistance to biodegradation in the commonly used phthalate esters must be due to these steric effects and not to the aromatic nature of these compounds. As rapid biodegradation is important for a green plasticizer, this makes the maleate less desirable even though it is lacking the aromatic component of the phthalates.

There is another possible factor in the relative rates of biodegradation and that is the solubility of the compounds in water. The more soluble compounds could be expected to degrade more quickly. However, this does not seem to be a factor in the data presented here. No data are available on the solubility of these diesters but the relative solubilities in water for a series of closely related compounds will be related to their relative polarities. For example, data is available for the solubility of the three diacids used to make diesters: maleic acid: 788 g/L (Sigma-Aldrich Canada 2010); succinic acid: 80 g/L (Fisher Scientific 2009); fumaric acid: 6 g/L (Acros Organics 2009). These values for solubility of the acids can be used to suggest the relative polarities of their esters. The *cis* isomer would be held in the most polar configuration and the *trans* isomer would be in the least polar orientation. The order of polarities for the diester. As this was by far the most stable structure with regard to biodegradation, it makes it clear that relative solubility in water is not a relevant factor in the stability of the esters with the *cis* orientation.

The two esters based on the saturated diacids (succinic and adipic acids) were both degraded very quickly. This is consistent with the above arguments because the freedom of rotation around the saturated bonds between the two ester groups would allow structures with the most favourable arrangements for interaction with the appropriate enzymes. However, the small differences in the rate of biodegradation of these two saturated compounds could show that, as expected, relative solubility does have some effect on biodegradation. The larger adipate ester would be expected to be less soluble in water than the succinate ester because it

has a longer central hydrocarbon chain. This would explain the fact that the half-life of the adipate ester is about twice that of the succinate ester.

An important consideration for a green plasticizer is that when it does start to biodegrade, there should not be an accumulation of metabolites. In all of the examples studied here, the only problematic compound was 2-ethylhexanoic acid. As discussed above, this is stable because of the ethyl branch (Nalli et al. 2002, Horn et al. 2004, Nalli et al. 2006a) but the problem is easily removed by using a straight chain alcohol to make the diesters.

In some of the earlier examples, the most toxic metabolites were considered to be the monoesters (Tomita et al. 1982, Richburg and Boekelheide 1996, Nalli et al. 2002, Horn et al. 2004). Small amounts of the various monoesters were observed (see Table 5.1) in this work but there were never more than trace amounts of any of the monoesters, which implies that they were removed relatively rapidly by a second hydrolysis step.

There were small amounts of 2-ethylhexanol in all of these biodegradation studies but, in every case, this was quickly oxidized to 2-ethylhexanal. The presence of this aldehyde has been shown in previous work (Nalli et al. 2002) but it was not observed in this work. This compound is quickly oxidized to the stable acid. Overall, there is little concern about an accumulation of toxic metabolites for any of these compounds except for 2-ethylhexanoic acid and earlier work has shown that this problem could be avoided by using a linear alcohol (Nalli et al. 2006a).

In general, all of the di (2-ethylhexyl) esters tested were found to be as good as or better at plasticizing PVC than the commercial plasticizers DEHP and DEHA. This can be seen in the trends for glass transition temperature, strain at break, Young's modulus and secant modulus. For example, at the highest concentrations tested, the succinate had the lowest T_g values –comparable to those for the adipate and these were both better than the data for the phthalate.

Again, there were noticeable affects due to the orientation of the two ester functions. The *trans* fumarate consistently had the highest T_g values as well as the poorest data for the tensile testing such as the lowest elongation and the highest stiffness, meaning that is was not a suitable candidate as a plasticizer. The other two had similar properties and both were good but the unsaturated succinate was slightly better than the *cis* maleate isomer.

Earlier work concluded that the polarity of a plasticizer is an important consideration in its effectiveness, improving the compatibility of the plasticizer with the PVC resin (Würstlin and Klein 1952b, Leuchs 1956). The *cis* orientation of the maleate diester would be significantly more polar than the *trans* fumarate and this would explain the significantly superior plasticizing properties of the former. The flexibility of the saturated succinate allows the two ester functions to be arranged in the most advantageous manner with respect to polarity of the compound in the polymer matrix. This results in the succinate having better properties than the maleate.

5.7 Conclusions

The esters made with either succinic acid or maleic acid would be suitable plasticizers for PVC because their plasticizing properties are comparable to DEHP. The fumarate esters had the poorest properties of all of the compounds studied. All of these compounds were made with simple procedures using relatively cheap starting materials.

The biodegradation of the various esters was very dependant on the structure of the central diacid used to make them. The succinate ester was rapidly removed from the growth medium and the only stable metabolite was 2-ethylhexanoic acid. This problem could be avoided by changing the alcohol used in the synthesis to a straight chain compound. The fumarate had a very similar biodegradation pattern but this took a bit longer to degrade.

The biodegradation of the maleate diester was as slow as that of the important plasticizer DEHP. Both compounds have a similar arrangement of the ester bonds. It was concluded that the *cis* orientation resulted in a stable compound that was resistant to enzymatic hydrolysis – probably due to unfavourable steric interactions. There were small amounts of metabolites observed for the maleate so it is reasonable to assume that this compound would slowly degrade in the environment in a manner similar to that of the phthalate.

Overall, the results for di-2-ethylhexyl succinate show the best potential for the development of a green plasticizer to replace the phthalate analogue. Despite its slow biodegradation, the di (2-ethylhexyl) maleate might also have potential. It was slow to biodegrade but this may be of less concern because it does not contain an aromatic group such as that found in DEHP.

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6 Designing Green Plasticizers: Influence of Alkyl Chain Length on Biodegradation and Plasticization Properties of Succinate Based Plasticizers

6.1 Preface

The following manuscript was published in 2013 in the Journal Chemosphere 91(3): pp. 358-365. The presented work focusses on the succinate compounds as the most promising plasticizer candidates from the previous study (Chapter 5). Compounds were again synthesized by the Dean-Stark method, but linear alcohols were used. They were chosen because earlier work had shown that such linear alcohols are readily biodegraded by soil microbes (Nalli et al. 2006a) once they are released during the biodegradation experiments. Additionally, compounds were synthesized that contained various substituents on the central alkyl chain of the succinate to hinder free rotation around the central bond of the molecule, in order to investigate the effect of this. The synthesized compounds were tested for T_g reduction potential and biodegradation rates by *Rhodococcus rhodocrous*.

The T_g measurements showed that the side chain length (and resulting overall molecule chain length) correlated with higher T_g reduction efficiency. Also, hindered rotatability around the central bond resulted in less efficient plasticizers.

Biodegradation studies resembled those previously described (Chapter 5) and it was found that the linear succinates were increasingly biodegradable with decreasing side chain length, yet half lives were all on the order of only several days. While buildup of some metabolites was found, this was transient. It could also be shown that microbes preferentially used the liberated alcohol as carbon source. When there was substitution on the central part of the succinate molecule, half lives were not affected when only a small methyl group was added and two different stereo-isomers were found in the broth. When the substitutions were larger, bulkier groups, very little biodegradation was observed, likely due to steric hindrance, thereby making it more difficult for the bacteria to reach the ester bond to be hydrolysed.

Designing Green Plasticizers: Influence of Alkyl Chain Length on Biodegradation and Plasticization Properties of Succinate Based Plasticizers

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6.2 Abstract

Phthalate diesters such as di (2-ethylhexyl) phthalate (DEHP) are considered ubiquitous contaminants and are poorly biodegraded in the environment. Moreover, both the parent compound and stable metabolites such as mono (2-ethylhexyl) phthalate (MEHP) are linked to several negative impacts on the environment and human health. Earlier work established that saturated diester compounds, such as succinates, showed better biodegradation characteristics and comparable plasticizer properties compared to DEHP. In this work we examine the effect of alkyl chain length of succinate molecules on plasticizer and biodegradation properties. This included both the side chains (n-ethyl to n-octyl) as well as substituents on the middle part of the succinate molecule. We showed that the common soil bacterium Rhodococcus rhodocrous could rapidly break down all unsubstituted succinates, without the appearance of stable metabolites. Furthermore, the organisms used the plasticizer metabolites as carbon source. The introduction of a large cyclohexyl substituent on the succinate resulted in a poorer degradation rate. Glass transition temperature (Tg) measurements were performed to evaluate plasticizer properties and showed that longer side chains reduced the T_g more efficiently, while large cyclohexyl substituents on the succinate decreased this effect. However, all compounds performed better or equal to DEHP at reducing the T_g. Keywords: Green Plasticizer, Phthalates, Biodegradation, Succinate

6.3 Introduction

Among the many additives to polymers that serve to improve certain properties, plasticizers account for a large fraction, as they can make up to 40 % of the overall material (Mersiowsky et al. 2001). These are usually relatively small compounds, which serve to render polymers flexible and workable (Sears and Darby 1982). Most plasticizers are not covalently bound to the polymer matrix which means that they can leach out of the material (Jaeger and Rubin 1970). Approximately 80 % of all plasticizers produced worldwide are used in poly(vinyl chloride) (PVC) formulations (Stevens 1999, Murphy 2001) and about 90 % of the plasticizers used for PVC are diesters of phthalic acid (Murphy 2001).

Di (2-ethylhexyl) phthalate (DEHP) is particularly well studied owing to its wide range of toxic effects (Lloyd and Foster 1988, Akingbemi et al. 2001, Foster et al. 2001, Gazouli et al. 2002, Akingbemi et al. 2004, Horn et al. 2004). Studies have found DEHP in samples from many different types of environments including the interior of buildings (Thuren and Larsson 1990, Bauer and Herrmann 1997, Cartwright et al. 2000, Staples et al. 2000, Butte et al. 2001, Becker et al. 2004, Horn et al. 2004). Due to this, DEHP and its metabolites are generally considered as ubiquitous environmental contaminants (Wams 1987). DEHP and its stable metabolites mono (2-ethylhexyl) phthalate (MEHP), 2-ethylhexanol and 2-ethylhexanoic acid have been shown to be toxic to microorganisms (Nalli et al. 2002, Horn et al. 2004) and linked to antiandrogenic activities in humans (Richburg and Boekelheide 1996, Swan et al. 2005a, Pant et al. 2008, Fan et al. 2010, Piche et al. 2012).

Several bans on the use of phthalates in various jurisdictions (EU 2005, CPSIA 2008, HPA 2010) have spurred interest in developing "greener plasticizers". Research has been done for example on modified dibenzoate esters (Firlotte et al. 2009, Kermanshahi pour et al. 2009b), oligo-esters made from ε -caprolactone (Shi et al. 2011) and small diesters based on four carbon diacids (Erythropel et al. 2012). Stuart et al. (2010) worked with diesters of succinic acid, a natural molecule involved in the citric acid cycle of mammals, and showed that it was compatible with PVC (Stuart et al. 2010). In this work, we present data demonstrating the influence of structural characteristics on plasticizing as well as biodegradation properties of various succinate-based plasticizers. Alkyl chain length was varied either by altering the alcohol used or the central diacid leading to several series of homologous compounds.

6.4 Materials and Methods

6.4.1 Plasticizers

Two of the plasticizers tested, diethyl succinate (DES; 99 %) and di (2-ethylhexyl) phthalate (DEHP; 99 %) were purchased from Sigma Aldrich. All others (Figure 6.1) were synthesized using Dean-Stark esterification as described earlier (Erythropel et al. 2012).

Dihexyl 2-methyl succinate (DHMS): 2-methyl succinic acid (4.45 g, 33.7 mmol, 99 %, Sigma Aldrich) and 1-hexanol (6.46 g, 63.2 mmol, 98 %, Sigma Aldrich) were dissolved in 100 ml of toluene (99 %, Sigma Aldrich) with catalytic amounts of concentrated sulphuric acid (Fisher Scientific). This mixture was refluxed at 120 °C over night in a 250 ml flask. A Dean-Stark trap was used between the flask and the reflux condenser to remove water. After cooling, the clear solution was washed with three aliquots of 50 ml of a saturated aqueous NaHCO₃ solution. The combined aqueous phases were set aside to recover any monoester produced and the combined organic phases were washed with deionized water, dried with sodium sulphate (Anachemia) and then the solvents were removed on a rotatory evaporator at 95 °C at a pressure of 1.2 kPa. (Büchi RE III with Heidolph Rotavac Valve Control). A colorless, oily liquid was obtained. Yield: 89 %.

All other diester syntheses were carried out in a similar manner, keeping the molar ratio of diacid to alcohol at 1 to 2. Whenever possible, the anhydride was used preferentially over the free diacid. The purity of each compund was verified by NMR spectroscopy, the spectra are shown in Appendix A. The yield of each reaction was obtained as follows: DBS: 90 %; DHS: 99 %; DOS: 82 %, DEHS: 81 % (Erythropel et al. 2012); DHMS: 89 %; DHCHS: 88 %; DHMCH: 85 %.

Monobutyl succinate (MBS): Succinic anhydride (1.0 g, 10.0 mmol, 99 %, Fisher Scientific) and 1-butanol (0.78 g, 10.5 mmol, 99 %, Fisher Scientific) were dissolved in 10 ml of chloroform (99 %, Fisher Scientific) and heated to 80 °C in a hermitically sealed vial. Once a clear solution was obtained, the vial was allowed to cool to room temperature and the chloroform was removed on a rotatory evaporator (Büchi RE III with Heidolph Rotavac Valve Control). The products were recrystallized from hexanes (99 %, Fisher Scientific).

All other succinate monoesters were prepared in a similar manner, keeping an equimolar ratio of succinic anhydride (or acid in some cases) to alcohol. The structures of the

products were verified using NMR and the spectra are shown in Appendix A. The yield of each reaction was obtained as follows: MBS: 85 %; MHS: 45 %; MOS: 70 %.

The synthesis of the mixture of monohexyl 2-methylsuccinate (MH2MS) and monohexyl 3-methylsuccinate (MH3MS) differed significantly as it was recovered as a byproduct from the synthesis of the corresponding diester, DHMS (as described above). The combined aqueous phases from the washing step were brought to pH 2 with hydrochloric acid (36 %, Sigma Aldrich), and extracted with three aliquots of 30 ml of chloroform. The combined organic phases were then washed with a saturated aqueous solution of NaHCO₃. Then this aqueous phase underwent a chloroform extraction followed by one more cycle of extractions. The solvent in the final organic phase was removed using a rotatory evaporator (Büchi RE III with Heidolph Rotavac Valve Control).



dihexyl 4-methyl cyclohexane-1,2-dicarboxylate (DHMCH)

Figure 6.1. Structures of compounds tested and selected metabolites.

6.4.2 Extrusion

A conical intermeshing twin-screw extruder (Haake Minilab, Thermo Electron Corporation, screw diameter 5/14 mm conical, screw length 109.5 mm) was used to incorporate the plasticizers into unplasticized PVC (UPVC, Solvay Benvic, France). The batch feed size was 3 g, rotation speed of the screws was set to 60 rpm and the operating temperature was between 110 °C and 130 °C. Due to the difference in viscosity between UPVC and the liquid plasticizers, the addition of plasticizer had to be carried out in a series of steps. The first step was to prepare a blend containing 20 phr of plasticizer, 4 phr of epoxidized soy bean oil (Chemtura Corporation) as heat stabilizer and 5 phr of stearic acid (Fisher Scientific) as lubricant. This was collected and recycled through the extruder a second time to ensure homogeneity. This blend of 20 phr served as master batch to prepare blends of higher plasticizer content in steps that added an additional 20 phr of the plasticizer each time. No additional amounts of heat stabilizer or lubricant were added.

6.4.3 Differential Scanning Calorimetry

Temperature modulated differential scanning calorimetry (TA Instruments Q2000) was carried out to determine glass transition temperatures. Samples were cut into slices of 1-2 mg each, which were placed into a standard DSC pan (TA Instruments, model #070221). A top was crimped on and the total weight and weight of the sample was recorded. Pans were loaded into the autosampler of the instrument, along with an empty pan for calibration. After quenching the sample at -90 °C for 5 min, a heating rate of 2 °C/min was applied, which was superimposed by a sinusoidal modulation of 1.27 °C with a period of 60 s. Once +100 °C was reached, the sample was held at this temperature for 5 min. A preliminary cycle was done to erase the thermal history of the sample. The data from the second cycle was analysed using the software TA Universal Analysis, plotting reversible heat flow against temperature to determine the glass transition temperature according to ASTM D-3418 (2015).

6.4.4 Biodegradation Study

The microorganism *Rhodococcus rhodocrous*, American Type Culture Collection (ATCC) 13808 was used to evaluate the biodegradation properties of the pure plasticizers. For

each measurement, it was necessary to extract the entire contents of a shake flask. Taking several samples from one flask led to errors because of the difficulty in obtaining a homogeneous sample. For each experiment, sets of 500 ml Erlenmeyer flasks fitted with foam caps were prepared, containing 100 ml Minimum Mineral Salt Medium, which contained: 6 g/L Na₂HPO₄, 4 g/L NH₄NO₃, 4 g/L KH₂PO₄, 0.2 g/L MgSO₄•7H₂O, 0.014 g/L Na₂EDTA, 0.01 g/L CaCl₂•2H₂O, and 0.01 g/L FeSO₄•7H₂O, (Fisher Scientific), 10 mM pure plasticizer and 0.1 g/L yeast extract. Other sets of flasks were prepared the same way, but these included 2 g/L hexadecane as carbon source. One flask per set was not inoculated and used as abiotic control. The flasks were autoclaved at 121 °C and 100 kPa for 15 minutes (Steris Amsco Lab 250), cooled and then inoculated with 1 ml of cell broth from a previously grown culture, using sterile techniques in a laminar fume hood (Baker Company, Model VBM600). The flasks were cultured in an incubator-shaker at 30 °C and 140 rpm (Multitron II, Infors AG).

The complete contents of one flask of each set were extracted on day 0. Subsequent flasks were extracted at intervals of 1 to 5 days depending on the degradation rate. All of these extractions were done in the same manner. First, the contents were adjusted to pH 2 with concentrated sulphuric acid and then extracted with 20 ml of chloroform containing 2 g/L pentadecane, as an internal standard. The isolated organic phase was stored at 4 °C until analysis.

In order to compare the results of the several biodegradation experiments, rate constants for the first hydrolysis step of the diesters were approximated using a first order fit. With these, values for half-lives were calculated (Tables 6.1 and 6.2).

6.4.5 Gas Chromatography

Determination of plasticizer concentrations in the extracts of the flasks, as well as detection and quantification of metabolites, was done using a gas chromatograph (Trace GC Ultra with AI3000 Autosampler, Thermo Scientific). After appropriate dilution of the samples, 1 μ L was injected into the GC, which was fitted with a Restek RTX-5 column (length 30 m, id 0.32 mm, 0.25 μ m film). A flame ionization detector was used. In order to calculate concentrations from the ratios of peak areas of the compounds to those of the internal standard pentadecane, calibration curves were prepared for each compound of interest.

6.4.6 NMR Spectroscopy

¹H - Nuclear Magnetic Resonance spectroscopy was carried out using one of two spectrometers: Varian Mercury-300 (¹H = 300 MHz) and Varian Unity-500 (¹H = 500 MHz), with an average of 8 repetitions. The solvent used for all measurements was deuterated chloroform (CDCl₃), with tetramethylsilane (TMS) as internal standard. The chemical shifts δ are indicated in ppm.

6.4.7 Statistics

Statistical Analysis was done using the software GraphPad Prism 5; one-sample *t*-tests and one-way ANOVA tests with Bonferroni post test were carried out as indicated. A p value less than 0.05 was taken as significant.

6.5 Results

6.5.1 Syntheses

The synthesis of the monoester of methylsuccinic acid resulted in two isomers, depending on which carboxylic function was esterified (Figure 6.1). Figure 6.2 contains the relevant parts of the gas chromatogram a) as well as the NMR spectrum b). By integrating the GC data, it can be seen that one of the monoesters was preferentially formed during synthesis. The identity of the major and minor isomers was resolved with NMR. This is shown in an enlargement of the region for the single proton on the optical centre of the methylated carbon atom between the two carboxylate groups of the diacid core of either isomer. As indicated in Figure 6.2b), the higher shifted signal belongs to the proton in MH3MS, as this proton is on the carbon in β -position to the COOH group, resulting in a slightly higher chemical shift, while the corresponding proton in MH2MS is in on the carbon in γ -position to the COOH group. These results suggest that the peak at 8.8 min belongs to MH2MS (smaller area), and the peak at 8.9 min to MH3MS (larger area). Since the pure compounds were not obtained, the calibration curve for the unsubstituted monohexyl succinate was used, assuming the effect of the added methyl group would have a negligible effect in gas chromatography.



Figure 6.2. a) Gas chromatogram of retention time 8.7 - 9.0 min, corresponding to MH2MS and MH3MS, respectively; b) NMR spectrum of the same sample from $\delta = 2.85 - 3.0$. Structures of MH3MS and MH2MS shown above corresponding signal.

6.5.2 Plasticizing Studies

Glass Transition Temperature (T_g) data for blends of 40 phr in PVC are shown in Figure 6.3. Comparing the data for the branched di (2-ethylhexyl) succinate and the unbranched dihexyl succinate there was no significant difference in T_g reduction (one sample *t*-test, p = 0.23). A significant statistical difference was found between DHS and DEHP, however (one sample *t*-test, p = 0.0002).

Figure 6.4 shows the glass transition temperatures for several succinates with varying substituents on the central part of the molecule, while the alcohol used to esterify all compounds was hexanol.

Amongst all data, a significant statistical difference was found between the means (oneway ANOVA, p < 0.0001). DHS was not statistically different from DHMS (addition of a methyl group), however it was statistically different compared to DHMCH and DHCHS (Bonferroni post test, both p < 0.0001), which both contain larger substituents. DHMS was also found to be statistically different from DHMCH and DHCHS (Bonferroni post test, both p < 0.0001). The results for both compounds with large substituents, DHCHS and DHMCH, were found to not be significantly different from each other.



Figure 6.3. Glass transition temperatures against molecule length at 40 phr for various succinate plasticizers with straight chains (Succinates), di (2-ethylhexyl) succinate (DEHS) and commercial di (2-ethylhexyl) phthalate (DEHP). Error bars representing standard deviation. \ddagger Significantly different than DEHS and DHS (p < 0.001).



Figure 6.4. Glass transition temperatures for several molecules with different substitutes on the central succinic part at 40 phr (error bars representing standard deviation). ***: p < 0.0001.

6.5.3 Biodegradation Studies

The abiotic controls showed no hydrolysis of any of the compounds. Figure 6.5 shows examples of typical biodegradation experiments. Figure 6.5A shows the biodegradation pattern for dihexyl succinate. The parent compound is quickly hydrolysed and a corresponding increase in monohexyl succinate and hexanol are observed, however by day 3 and day 7, respectively, the presence of these compounds have decreased to trace amounts. No significant build-up of any other metabolite, including the corresponding hexanoic acid, was detected. Figure 6.5A also shows the concentration of hexadecane in the solution and its concentration only starts decreasing once all of the other compounds have disappeared.

Figure 6.5B shows the biodegradation pattern for the dihexyl ester of the methylsubstituted succinate. An equally quick hydrolysis of the parent compound is observed, and there is only a temporary build-up of the metabolite hexanoic acid. As expected, two metabolites with very close retention times in GC could be detected. These corresponded to the two structurally different monoesters MH2MS and MH3MS. After day 3, the concentrations of the monoesters remained at a constant ratio of approximately 5:1 of MH3MS to MH2MS.
Table 6.1 shows data for the half-lives and time of maximum concentration during the biodegradation studies conducted with succinate esters of varying side chain length as well as results for several substituted succinates with constant side chain length. No data is presented for the small diethyl succinate and its metabolites because of their very quick removal from the broth, as well as for the bulky dihexyl 2-cyclohexylsuccinate, which showed almost no hydrolysis of one of the ester bonds, resulting in no significant amount of metabolites detected.

For the remaining compounds, a quick hydrolysis of the diester was observed, except for the compound in which R" was altered from a hydrogen to a cyclohexyl group (Figure 6.1). Table 6.1 summarizes the observed metabolites and their stability. Complete removal of the corresponding monoester within the timeframe of the degradation experiments was only observed if R' was six carbons or less and no hydrogen was replaced in the central structure of the succinate molecule (R"). Both corresponding alcohol and acid were quickly removed for all compounds with R' equal to or smaller than six.

Table 6.2 shows a comparison of half-lives of selected succinate compounds being biodegraded with and without hexadecane present. For all compounds, hexadecane did not affect the half-life and the onset of hexadecane use was correlated to the disappearance of the plasticizer compounds.



Figure 6.5. Biodegradation experiments including appearance of metabolites (A) of dihexyl succinate, (B) of dihexyl methylsuccinate.

Table 6.1. Estimated half-life of succinate based plasticizers, maximum concentration of monoester, alcohol and acid found in degradation broth (mM), time point of maximum concentration and time span observed until complete removal. Results for degradation experiments without added carbon source. d – days, h – hours.

	$R' - COO - CHR'' - CH_2 - COO - R'$							
R'	n-C ₂ H ₅	n-C ₄ H ₉	n-C ₆ H ₁₃				n-C ₈ H ₁₇	CH ₂ CH(C ₂ H ₅)(C ₄ H ₉)
R"	Н	Н	Н	CH ₃ ^a		$C_6 H_{11}{}^b$	Н	H ^b
Half-life	< 0.2d	0.2d ^c	0.5d ^c	0.8d		>> 30d	3.2d ^c	2.3d
Monoester				MH2MS	MH3MS			
Max [c] in mM		3.0	5.5	1.0	5.0		2.8	< 0.1
Time of max [c]		<16h	day 1	day 2	day 2		day 15	Only trace
Time span		2d	7d	>30d	>30d		>30d	>30d
Alcohol								
Max [c] in mM		4.3	6.3	0.8			3.2	1.1
Time of max [c]		<16h	day 1	<15h			day 2	day 4
Time span		2d	7d	7d			>30d	>30d
Acid								
Max [c] in mM		0.5	0.6	4.2			0.4	15.0
Time of max [c]		day 1	day 3	day 3			day 15	day 30
Time span		7d	7d	10d			>30d	>30d

^a Both possible monoesters detected and reported individually.

^b Experiment done with hexadecane as carbon source, because no growth could be observed without it.

^c Experiments with hexadecane showed comparable rate constants. The organisms grew preferentially on metabolites, as hexadecane was only used as carbon source once plasticizer and metabolite concentration was very low. Also see Table 6.2.

Table 6.2. Half-life and time of 95 % removal of succinate based plasticizers, assuming first order kinetics. Comparison of experiments with and without carbon source hexadecane.

R'	(CH ₂) ₃ CH ₃		(CH ₂))5CH3	(CH ₂)7CH ₃	
Carbon source	$C_{16}H_{34}$	none	C ₁₆ H ₃₄	none	C ₁₆ H ₃₄	none
Half-life	0.2d	0.3d	0.5d	0.5d	2.8d	3.2d
> 95 % removal of plasticizer compounds	day 3	day 3	day 7	day 7	Not observed ^a	Not observed ^a
Onset of hexadecane-use	day 3	-	day 7	-	_a	_a

 $R' - COO - CH_2 - CH_2 - COO - R'$

^a Over the course of 30 days, metabolites were always present in high enough concentrations. The concentration of hexadecane stayed constant over the whole course of the experiment.

6.6 Discussion

DEHP has been the object of intense scrutiny in North America and Europe due to its potential toxic effects. A safe replacement of DEHP requires the design of compounds that adequately plasticize PVC, biodegrade upon disposal, and avoid the buildup of stable and toxic metabolites. In this work we present a series of succinate plasticizers with good biodegradation kinetics and similar plasticizing properties compared to DEHP.

We used the glass transition temperature (T_g) as a measure of the plasticizing properties of our succinate plasticizers. Earlier work has shown that there is a strong correlation between the T_g of PVC mixtures and their tensile properties such as elongation at break and secant modulus (Erythropel et al. 2012). From Figure 6.3, it is clear that the length of the alcohol portion of the plasticizers is an important aspect of their plasticizing properties. It should be noted that increasing the length of the alcohol used in the esterification by one carbon atom must increase the length of the longest chain of atoms in these plasticizers by two carbon atoms. The longer the alcohol used to make the diester plasticizer, the more effective the diester is at lowering the T_g . A decrease in T_g indicated that the PVC was becoming more flexible. These data suggest that as the plasticizer molecule becomes longer the interaction with the polymer chains becomes more effective at reducing PVC chain-chain interactions.

The T_g data for the branched 2-ethylhexyl succinate is almost identical to the T_g of the succinate made with hexanol. While the 2-ethyl containing compound has a higher molecular weight, the lengths of the longest chains of atoms in the two plasticizers are identical (Figure 6.1). By comparison, the T_g of the succinate made with octanol (longest chain of C and O atoms of 22 atoms) is significantly lower than that for the compound made with 2-ethylhexanol, despite that these two compounds have an identical molecular weight. This supports the argument that the relative length of the plasticizer is the important factor in lowering T_g (Figure 6.3).

The T_g of the modified succinate plasticizer, in which one of the central hydrogen atoms has been replaced with a methyl group, is similar to the non-substituted succinate with the same alcohol (Figure 6.4). Again, this suggests the key factor is the length of the longest chain of atoms. However, adding a larger group to the central diacid significantly affects the overall plasticizing properties. When succinic acid is substituted with a cyclohexyl group (DHCHS) or when the central part of the diacid is part of a cyclohexane ring (DHMCH), T_g reduction is not as effective. These results can still be explained by considering the properties of these compounds relative to the plasticizer made with simple succinic acid. The cyclohexyl substituent would hinder rotation about the central bond of the plasticizer and no rotation is possible in case of the DHMCH. Rotational freedom is considered to play an important role in plasticizing abilities of an additive (Erythropel et al. 2012) and thus it is not surprising that these two compounds are poorer plasticizers than the compound made from the simpler succinic acid.

All of the above compounds are thermally stable. However, it has been shown that plasticizers inevitably leach out of the formulations and enter the environment (Jaeger and Rubin 1970, Thuren and Larsson 1990, Staples et al. 1997, Cartwright et al. 2000, Becker et al. 2004, Horn et al. 2004). In some cases, these released compounds can have long half-lives and therefore accumulate in the environment. It is therefore desirable to develop plasticizers that can be biodegraded quickly and not produce stable toxic metabolites.

Rhodococcus rhodocrous, a common soil bacterium, was the organism chosen because of its known ability to degrade a broad range of compounds, including aliphatic hydrocarbons (Jones and Goodfellow 2012). Previous studies have shown that *R.rhodocrous* has the capability to hydrolyse diester plasticizers rapidly in the timeframe of these experiments, thus allowing a comparison of relative hydrolysis rates between different compounds (Nalli et al. 2002, Nalli et al. 2006c, Sauvageau et al. 2009).

Similar to previous studies (Nalli et al. 2006b, Nalli et al. 2006c, Kermanshahi pour et al. 2009a), in the presence of bacteria, the diesters are first hydrolysed to the corresponding monoester and one equivalent of the alcohol used to synthesize the diester. The monoester is then hydrolysed to ultimately yield the diacid and two equivalents of the corresponding alcohol. The alcohols are then oxidized to yield the corresponding carboxylic acids and these will readily undergo β -oxidation (Nalli et al. 2006a). The intermediate aldehydes are inferred but were not observed, which can be explained by the volatility of these compounds as explained in earlier work (Nalli et al. 2006c).

As with the plasticizing properties in PVC mixtures, the type of alcohol used to make the succinate diesters influences the rate of biodegradation (Table 6.1). The longer the aliphatic chain of the alcohol – and thus of the overall length of the plasticizer – the slower is the overall degradation rate. This can be attributed to the increasing hydrophobicity of the compounds as the length of the hydrophobic portions of the plasticizers increase. This would lead to a decrease in the water-solubility of the compounds, which would make a plasticizer less available to the microbes in their aqueous environment. However, some recent studies have suggested that besides solubility of such organic compounds, other factors also influence bioavailability (Alexander 2000, Kickham et al. 2012, Segura et al. 2012, Smith et al. 2012).

Di (2-ethylhexyl) succinate is degraded more slowly than the equally long dihexyl succinate, yet only slightly more quickly than di-n-octyl succinate (Table 6.1). This could be attributed to its 2-ethyl branches adding to the hydrophobicity of the molecule but it is just as likely that the ethyl branches, located near the ester bond, are sterically hindering its interaction with the appropriate enzymes.

It is important to note that all of the succinate diesters were degraded in a few weeks, which is significantly faster than the rate for the commercial plasticizer DEHP. In earlier work with di (2-ethylhexyl) esters, we were able to show that one of the key parameters to hydrolysis of the ester bonds is the structure of the central part of the diester molecule (Erythropel et al. 2012). With unsaturated diesters that had their two ester groups in a *cis* orientation, similar to DEHP, the first hydrolysis step to the monoester was very slow. Yet when saturated compounds, such as a succinate diester were used, the first hydrolysis step was much faster. With the compounds in this study, the change in the alcohol used makes biodegradation even more facile.

While it is important that the first hydrolysis step is much quicker than that observed for DEHP, it is also essential to consider the possibility of the accumulation of stable metabolites. As expected, the use of straight-chained alcohols was advantageous with regard to the metabolites as reported earlier (Nalli et al. 2006a). None of these alcohols resulted in accumulation of stable, toxic metabolites (Table 6.1) as was observed with any of the di (2-ethylhexyl) compounds, namely 2-ethylhexanoic acid (Nalli et al. 2002, Nalli et al. 2006a, Nalli et al. 2006b, Erythropel et al. 2012).

The monoester of DEHP, MEHP, has been studied extensively due to its suspected effect on the endocrine system (Richburg and Boekelheide 1996, Swan et al. 2005a, Pant et al. 2008, Fan et al. 2010, Piche et al. 2012). MEHP has long been suspected as the active metabolite of DEHP, although more recent work has shown 2-ethylhexanal and 2-ethylhexanol to also be biologically active (Piche et al. 2012). We have previously shown that the stability of the monoesters is influenced by the choice of the central diacid (Erythropel et al. 2012), and in this study we show the influence of the side chain on the monoester stability. The monoesters were observed in the degradation experiments with all of the compounds (Table 6.1). The shortest diester, diethyl succinate, degraded quickly as did its monoester as it was not observed. The next two compounds in the series, dibutyl and dihexyl succinate (Figure 6.5A), did generate small amounts of their monoesters, however these were hydrolysed within a week. The exact time is obscured by the fact that monoester molecules continued to be released as the parent diesters were hydrolyzed. It was also found that the removal of this compound was even faster in the presence of a second carbon source; hexadecane. Future studies will evaluate the toxicity and potential antiandrogenic nature of these compounds.

The longest monoester in this series was monooctyl succinate and this was the slowest to biodegrade. This means that, as observed with the parent diesters, as the series of compounds becomes longer, the rate of biodegradation of the monoesters becomes slower. Again, this is probably related to the solubility of these compounds in water. However, none of these monoesters are as intractable as those observed with some of the compounds, such as MEHP, studied in earlier work (Nalli et al. 2002, Horn et al. 2004, Sauvageau et al. 2009). Another important consideration is that the earlier work was done in the presence of hexadecane. This work shows that while hydrolysis of the monoester is faster when hexadecane is present, it is not essential (Table 6.2).

The study's results with the compounds made with the branched alcohol, 2-ethylhexanol, show these to be stable as has been reported previously (Nalli et al. 2006c, Erythropel et al. 2012). It should be noted that without the presence of hexadecane as an easily used carbon source, no hydrolysis of the 2-ethylhexyl diester was observed during the course of the experiment (data not shown). However, it was not essential when the unbranched alcohols were used. Table 6.2 shows that there was no significant difference between any of the pairs of experiments with or without hexadecane in the media. In fact, in every case, there was a pattern of diauxic growth and there was no significant decrease in hexadecane concentration until the last of the plasticizer remnants had been removed (Table 6.2). Figure 6.5A shows this behaviour in an experiment with dihexyl succinate. This shows that the bacteria use the released alcohols and/or succinic acid as carbon sources for growth, which is not surprising as succinic acid is part of the TCA cycle (bacterial growth was observed, but no growth curve recorded). These results are important because rapid metabolism of the fragments released after the initial steps of biodegradation reduces concerns about their potential environmental impact. On the other hand, the ethyl branch in the branched compounds blocks β -oxidation, making this a poor carbon source to support growth of the organism and resulting in an accumulation of 2-ethylhexanoic acid.

The other metabolites are alcohols and acids and these were observed for every type of compound studied (Table 6.1). As stated above, the straight chain alcohols or the resulting organic acids can be metabolized and accumulation does not seem to be significant. The longest compounds, octanol and octanoic acid, are significantly slower to degrade than the others, but this is again attributed to relative solubility in water and these compounds are not expected to reach significant concentrations in the environment as many microbes would be expected to use all of the straight chain alcohols and acids as carbon sources.

The effects of modifying the central diacid on biodegradation were more complex. The addition of a methyl group to succinic acid did not prevent hydrolysis of the first ester bond (Figure 6.5B). However, the addition of a cyclohexyl group completely inhibited degradation of the diester (Table 6.1). This compound would be significantly less polar and hence less soluble in water. However, the dramatic change in biodegradation rate would seem to indicate that the bulky cyclohexyl substituent also interferes with the interaction with the enzyme to affect the first hydrolysis step. This can also be seen in the pattern of the first hydrolysis step with dihexyl methylsuccinate. There are two possible monoesters depending on which of the ester bonds was hydrolyzed, MH2MS and MH3MS (see Figure 6.1). These two monoesters had significantly different rates of production meaning that one ester bond was more susceptible to hydrolysis of the diester because this would cause a reduction in the rate of hydrolysis of the ester bond closer to the methyl substituent. In fact, the opposite is true so there may be a slight preferential stabilization of an intermediate in the preferred hydrolysis pathway.

However, there appears to be a steric inhibition of the biodegradation of the monoesters that are produced by this first step. Neither monoester is degraded quickly. This was not observed for the degradation of the monoester from the unsubstituted dihexyl succinate (Figure 6.5A).

6.7 Conclusion

Based on an analysis of both the plasticizing properties and the biodegradation properties of the new diesters, the majority of them are potential green plasticizers. All of the compounds showed plasticization properties comparable to DEHP but there were trends that showed the importance of structure. Within the range tested, an increasing overall molecule length positively influenced the plasticizing properties of unsubstituted succinate compounds, while a negative influence on plasticizer properties was found when alkyl substituents were added to the middle part of the succinate compounds. This negative influence became larger with increasing size of these substituents, which relates to the ability of these compounds to rotate around the axis connecting the ester functions. Structure was important to the biodegradation of these compounds: larger, less watersoluble compounds were slower to disappear. There is some evidence that steric hindrance near the ester bonds inhibited the rate of hydrolysis. However, the overall observation was that most of these compounds could be biodegraded and most of the metabolites were also removed quickly. Earlier work on biodegradation of some commercial plasticizers had shown that the presence of an easily degraded carbon source was important. The biodegradation of most of the diesters in this study was faster if hexadecane was included in the medium but it was not essential. The metabolites such as small diacids and unbranched alcohols were readily used as carbon sources by the bacterium.

Thus, a compromise has to be made between longer molecules favourable for plasticization and shorter molecules favourable for biodegradation. A good compromise found here would be dihexyl succinate. It was a comparable plasticizer to DEHP based on the T_g measurements and it was completely biodegraded in about one week, including all observed metabolites.

6.8 Acknowledgements

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7 Designing Greener Plasticizers: Effects of Alkyl Chain Length and Branching on the Biodegradation of Maleate Based Plasticizers

7.1 Preface

The following manuscript was published in 2015 in the Journal Chemosphere 134: pp. 106-112. The presented work is similar in nature to the previous study (Chapter 6) where, instead of the succinates, this research focussed on the maleate compounds. Di (2-ethylhexyl) maleate had been identified as good a plasticizer candidate earlier but with a poorer biodegradation rate (Chapter 5). The study aimed at improving biodegradation rates of maleate compounds in a similar fashion to the succinate compounds. Again, Dean-Stark esterifications were performed with linear alcohols and maleic anhydride and the synthesized compounds were tested for T_g reduction potential and biodegradation rates by *Rhodococcus rhodocrous*.

Similar to what was reported in the last section, the T_g measurements showed that the side chain length (and resulting overall molecule chain length) could be correlated to higher T_g reduction efficiency.

Biodegradation studies resembled those performed previously (Chapter 6) and a similar correlation between increasing side chain length and slower biodegradation rate was found. However, the recorded biodegradation rates were slower compared to rates observed in similar experiments with the succinates (Chapter 6), and, contrarily to the biodegradation of the succinates, the microbes preferentially grew on hexadecane. In the case of dioctyl maleate, growth inhibition was observed and this could successfully be linked to the build-up of the corresponding monoester monooctyl maleate (MOM) in separate experiments, for which MOM was successfully synthesized. It could be shown that the growth inhibiting properties of MOM on *Rhodococcus rhodocrous* were dependant on its concentration.

Designing Greener Plasticizers: Effects of Alkyl Chain Length and Branching on the Biodegradation of Maleate Based Plasticizers

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7.2 Abstract

The ubiquitous presence of the plasticizer di (2-ethylhexyl) phthalate (DEHP) in the environment is of concern due to negative biological effects associated with it and its metabolites. In particular, the metabolite mono (2-ethylhexyl) phthalate (MEHP) is a potential endocrine disruptor. Earlier work had identified the diester di (2-ethylhexyl) maleate (DEHM) as a potential greener candidate plasticizer to replace DEHP, yet its biodegradation rate was reported to be slow. In this study, we modified the side chains of maleate diesters to be linear (i.e., unbranched) alkyl chains that varied in length from ethyl to n-octyl. The plasticization efficiency of these compounds blended into PVC at 29 wt.-% increased with the overall length of the molecule, but all compounds performed as well as or better than comparable samples with DEHP. Tests conducted with the equally long DEHM and dihexyl maleate (DHM) showed that branching has no effect on glass transition temperature (T_g) reduction efficiency. Biodegradation experiments with the common soil bacterium *Rhodococcus rhodocrous* in the presence of the plasticizer showed acceptable hydrolysis rates of maleates with unbranched side chains, while the branched DEHM showed almost no degradation. The addition of hexadecane as auxiliary carbon source improved hydrolysis rates. Temporary buildup of the respective monoester of the compounds were observed, but only in the case of the longest molecule, dioctyl maleate (DOM), did this buildup lead to growth inhibition of the bacteria. Maleates with linear side chains, if designed and tested properly, show promise as potential candidate plasticizers as replacements for DEHP.

<u>Keywords:</u> Green Plasticizer, DEHP, Phthalate, Maleate, Biodegradation, Bacterial growth inhibition

7.3 Introduction

Plasticizers are low molecular weight compounds that are added in significant amounts (typically 30-40 wt.-%) to hard and brittle polymers, such as poly (vinyl chloride) (PVC), to render these flexible and malleable (Mersiowsky et al. 2001). About 80 % of all produced plasticizers used worldwide (Stevens 1999, Murphy 2001) are used in PVC formulations for which di (2-ethylhexyl) (DEHP) phthalate is the most widely used plasticizer (Murphy 2001). Plasticizers are generally not bound to the polymer matrix, which makes it possible for them to leach out of the material over time. DEHP is known to be rather slowly biodegraded (Nalli et al. 2002, Erythropel et al. 2012), and more importantly, once biodegraded leads to the production of stable metabolites which are of major concern because of their toxic nature (Horn et al. 2004, Nalli et al. 2006a). In particular, the monoester mono (2-ethylhexyl) phthalate (MEHP) is believed to be an endocrine disruptor (Gray and Gangolli 1986, Richburg and Boekelheide 1996, Lovekamp-Swan and Davis 2003, Swan et al. 2005a, Pant et al. 2008, Fan et al. 2010, Piche et al. 2012). Due to this and DEHP's large worldwide production volume of about 6 Mt per year (AgPU 2006), it has become a contaminant of concern to various organizations, such as the US Environmental Protection Agency (US EPA 2012) and the European Union (EU 2008), and has been banned in children's products throughout a large part of the Western world (EU 2005, CPSIA 2008, HPA 2010). Hence, there is great incentive to develop green replacement plasticizers that show improved biodegradation properties so as to avoid environmental build-up while simultaneously maintaining plasticizing efficiency and eliminating or reducing toxicity to various organisms.

Di (2-ethylhexyl) maleate (DEHM) and dibutyl maleate (DBM) are commercially available compounds, used as a secondary plasticizer and in applications such as coatings and adhesives in the case of DEHM and films in case of DBM (Wypych 2012, Wypych 2013). An earlier study had identified di (2-ethylhexyl) maleate (DEHM) as a possible replacement compound for DEHP with acceptable plasticization efficiency, however its biodegradation rates were rather poor (Erythropel et al. 2012). In this work, we show improved biodegradability of maleate diesters, while maintaining comparable reductions in glass transition temperature (T_g) compared to DEHP, through structural modification.

7.4 Materials and methods

7.4.1 Plasticizers

The tested compounds are shown in Figure 7.1. Diethyl maleate (DEM; 97 %) and dibutyl maleate (DBM; 96 %) were purchased from Sigma-Aldrich. Dihexyl maleate (DHM), dioctyl maleate (DOM) and di (2-ethylhexyl) maleate (DEHM) were synthesized in our laboratories, using the Dean-Stark esterification as described earlier (Erythropel et al. 2012).

Monoesters monobutyl maleate (MBM), monohexyl maleate (MHM) and monooctyl maleate (MOM) were synthesized by the same general scheme and their structure confirmed by NMR, shown below exemplarily for MBM:

Monobutyl maleate (MBM): maleic anhydride (10g, 102.0 mmol, 99 %, Sigma-Aldrich) and 1-butanol (7.56 g, 102.0 mmol, 99.9 %, Fisher Scientific) were dissolved in 25 mL of chloroform (99 %, Fisher Scientific) and heated to 80 °C in a hermetically sealed vial. The resulting mixture was recrystallized from hexanes (99 %, Fisher Scientific).

¹H-NMR (300.1 MHz in CDCl₃): δ (ppm) = 0.95 [t, 3H, CH₂C<u>H₃]</u>, δ (ppm) = 1.4 [m, 2H, CH₂C<u>H₂CH₃]</u>, δ (ppm) = 1.7 [m, 2H, CH₂C<u>H₂CH₂]</u>, δ (ppm) = 4.3 [t, 2H, COC<u>H₂CH₂]</u>, δ (ppm) = 6.35 [d, 1H, COC<u>H</u>CHCOOH], δ (ppm) = 6.45 [d, 1H, COCHC<u>H</u>COOH].



Figure 7.1. A: Structure of maleate diesters (left) and maleate monoesters (right). Abbreviations in order of R: diethyl maleate, DEM; dibutyl maleate, DBM; dihexyl maleate, DHM; dioctyl maleate, DOM; di (2-ethylhexyl) maleate, DEHM (sometimes misleadingly called dioctyl maleate, DOM); monoethyl maleate, MEM; monobutyl maleate, MBM; monohexyl maleate, MHM; monooctyl maleate, MOM; and mono (2-ethylhexyl) maleate, MEHM. B: Structure of commercial plasticizer di (2-ethyl hexyl) phthalate, DEHP (sometimes misleadingly called dioctyl phthalate, DOP).

7.4.2 NMR Spectroscopy

NMR spectroscopy was carried out at a frequency of ${}^{1}\text{H} = 300$ MHz (Varian Mercury-300) and ${}^{1}\text{H} = 500$ MHz (Varian Unity-500), with an average of 8 repetitions. Deuterated chloroform (CDCl₃) was used as solvent, and tetramethylsilane (TMS) as internal standard. The chemical shifts δ are indicated in ppm.

7.4.3 Biodegradation Studies

Biodegradation studies of the pure plasticizer were performed using the microorganism *Rhodococcus rhodocrous*, American Type Culture Collection (ATCC) 13808 as was used in previous studies (Nalli et al. 2002, Nalli et al. 2006a, Nalli et al. 2006b, Erythropel et al. 2012, Erythropel et al. 2013). The microorganism was grown in 500 ml Erlenmeyer flasks in 100 ml minimum mineral salt medium (MMSM) that also contained 0.1 g/L yeast extract and the pure

plasticizer at 10 mM or the metabolite in question at 1, 2 and 3 mM, plus 2 g/L hexadecane as carbon source for half of the experiments. The MMSM contained: 6 g/L Na₂HPO₄, 4 g/L NH₄NO₃, 4 g/L KH₂PO₄, 0.2 g/L MgSO₄•7H₂O, 0.014 g/L Na₂EDTA, 0.01 g/L CaCl₂·2H₂O, and 0.01 g/L FeSO₄•7H₂O (Fisher Scientific). Prepared flasks and their contents were autoclaved at 121 °C and 100 kPa for 15 min (Steris Amsco Lab 250) and subsequently inoculated with 1 ml of cell broth from a previously grown culture using sterile techniques in a laminar fume hood (Baker Company, Model VBM600). One flask per experiment was not inoculated and served as an abiotic control. The inoculated shake flasks were then cultured in an incubator-shaker at 30 °C and 140 rpm (Multitron II, Infors AG).

Due to the low aqueous solubility of the compounds in question, where much remained phase separated in the broth, liquid-liquid extractions of the entire contents of the flasks were performed. Extractions of flask contents were performed in regular intervals ranging from 1-5 d. Prior to the extraction, the content of each flask was brought to a pH of 2-3 using hydrochloric acid (37 %, Fisher Scientific). The solvent used for extraction was chloroform (99 %, Fisher Scientific), containing 2 g/L pentadecane (99 %, Acros Organics), which served as internal standard for the GC analysis, and each shake flask was extracted with 20 ml of the chloroform/pentadecane solution.

In order to provide a basis for the comparison of the degradation rates of the various compounds, rate constants for the first hydrolysis step were approximated using a best fit of a first-order expression and these values were subsequently used to calculate the values of half-life presented in Tables 7.1 and 7.2.

7.4.4 Gas Chromatography

Quantification of plasticizers as well as identification of metabolites was done using a GC (Trace GC Ultra/FID with AI3000 autosampler, Thermo Scientific). A 1 μ L aliquot of the appropriately diluted sample was injected via syringe into the instrument which was outfitted with a Restek RTX-5 column (length 30 m, id 0.32 mm, 0.25 μ m film). With the help of calibration curves established for all pure compounds of interest, quantities were calculated from the peak area ratios.

7.4.5 Extrusion

The plasticizers in question were blended with unplasticized PVC (Solvay Benvic, France) using a conical intermeshing twin-screw extruder (Haake Mini Lab, co-rotating mode, screw diameter 5/14 mm conical, screw length 109.5 mm, Thermo Electron Corporation). With a rotation speed set at 60 rpm and an operation temperature of 110 - 130 °C, at least 3 batches of 3 g were fed subsequently. Incorporation of the plasticizer had to be done in steps due to the viscosity differences between PVC and plasticizer. First, blends of 20 phr were prepared, which also contained 4 phr epoxidized soy bean oil (Chemtura Corporation) as a heat stabilizer, and 5 phr stearic acid (Fisher Scientific) as lubricant. All material was recycled through the extruder in a second pass in order to ensure homogeneity. The concentration was then increased to 40 phr by feeding the appropriate amounts of 20 phr blend and plasticizer, while no additional heat stabilizer or lubricant were added. As in the previous step, the resulting blend was recycled through the extruder for a second time to ensure homogeneity.

7.4.6 Differential Scanning Calorimetry

The glass transition temperature of the blends was determined using temperaturemodulated differential scanning calorimetry (TA Instruments Q2000). Several thin and freshly-cut slices of blend amounting to 4.6-17 mg were loaded into a standard DSC pan (TA Instruments, model #070221), and closed with a crimp-on top. The weight of the sample was determined (Sartorius CP225D) and it was loaded into the autosampler of the instrument, which also contained an empty pan for reference. After quenching at -90°C for 5 min, the sample was heated up to 100 °C at a rate of 2 °C/min, however this heating rate was superimposed with a sinusoidal modulation of 1.27 °C having a period of 60 s. After a first cycle to erase any thermal history, the same cycle was repeated. In two cases (DEHM), using all other conditions as described above, the sample was heated at 10 °C/min without superimposed modulation of the temperature. A first cycle was used to erase any thermal history, and the same cycle was repeated. Using the supplied software, the second heating cycle was used to determine the glass transition temperature from the chart of reversible heat flow or heat flow, respectively, versus temperature by the half height method (Appendix B). The procedure was done in accordance with the ASTM D-3418 (2015) standard test method.

7.4.7 Statistics

Statistical software (GraphPad Prism 5) was used for statistical analysis, such as twotailed *t*-tests and one-way ANOVA tests with a Bonferroni post-test. A p value less than 0.05 was interpreted as significant.

7.5 Results

7.5.1 T_g measurements

The glass transition temperature T_g was used as a good first indication of plasticizer properties as shown previously (Erythropel et al. 2012). Figure 7.2 contains a plot of the glass transition temperature (T_g) of blends of 40 phr versus the overall length of the maleate plasticizers. The T_g decreased linearly with increasing linear side chain length (p = 0.042, $R^2 = 0.92$). A comparison of the T_g data for the three compounds of equal overall molecule length (i.e., DEHP, DEHM and DHM) revealed a significant statistical difference between the means (one-way ANOVA, p < 0.0001). The T_g for DEHM did not statistically differ from that of DHM (Bonferroni post test, p > 0.05), however the T_g of DEHP was found to be significantly different than that of both DHM and DEHM (Bonferroni post test, p < 0.0001). Finally, the T_g data for the two compounds with equal molecular weight, DOM and DEHM, demonstrated that the T_g of the blend containing DOM was significantly lower (two-tailed *t*-test, p = 0.01). The DSC curves used to assemble Figure 7.2 are shown in Appendix B.



Figure 7.2. Glass transition temperatures against molecule length at 40 phr for various maleate plasticizers with straight chains ("Maleates"), di (2-ethylhexyl) maleate ("DEHM") and commercial di (2-ethylhexyl) phthalate ("DEHP"). Error bars represent standard deviations of two (DOM) or three (DHM, DEHM, DEHP) samples from one batch. \ddagger Significantly different than DEHM and DHM (p < 0.001).

7.5.2 Biodegradation Studies

Table 7.1 shows the results for the biodegradation studies of the various plasticizers conducted in the presence of hexadecane as an auxiliary carbon source. Values expressed as quantities denote the total amount of the respective organic compound, including both soluble and insoluble fractions in water, as analyzed by extraction with chloroform. The estimated half-life and final quantity of hexadecane are reported for each maleate diester. The side chains ranged in length from two to eight carbons in case of the unbranched maleates (i.e., DEM, DBM, DHM, DOM), and one maleate contained branched side chains (DEHM). The results show that an increase in molecule length results in an increase in half-life and, thus, resistance to hydrolysis by the microorganism *Rhodococcus rhodocrous*. Very little hydrolysis of the branched structure was observed, which is consistent with what has been reported previously

(Nalli et al. 2006a, Nalli et al. 2006b, Erythropel et al. 2012). In all cases, all hexadecane supplied was consumed by the microbes by the end of the experiments (day 30), with the exception of DOM. In this case, about 55 % of the initial quantity of hexadecane was still present at the end of the experiment (Figure 7.3). In abiotic controls of the biodegradation experiments, no degradation of any compound was observed over the full time course of the experiment.

Table 7.1 also reports the maximum quantity of the respective monoester for each plasticizer, and the corresponding time at which this maximum quantity was observed. Only trace amounts of monoester were observed for DEM and for the branched DEHM. The monoesters of DBM and DHM reached comparable maximum quantities of 4 mM, yet DBM reached this after 14 days while DHM took 26 days. The maximum quantity of MOM of only 2.5 mM was reached after about 7 days; however, this time also corresponded to an end to any bacterial activity. There was no further degradation or consumption of either DOM or hexadecane, respectively, as shown in Figure 7.3.

For all other linear compounds, the low molecular mass metabolites, such as the alcohol, appeared, but were subsequently consumed by the end of the experiment. In the case of the branched DEHM, only trace amounts of both the monoester and the corresponding alcohol were detected, but this is due to the extremely slow biodegradation kinetics of the parent compound, as reported previously (Erythropel et al. 2012).

Table 7.2 shows the results for the biodegradation of the maleate esters by *Rhodococcus rhodocrous* without the auxiliary carbon source hexadecane. The overall trends are consistent with the results shown in Table 7.1. Hydrolysis rates decreased with increasing molecule length; however, for each of these compounds the rate of hydrolysis was slower in the absence of hexadecane. Again, both DOM and DEHM were resistant to hydrolysis. No degradation of any compound was observed in abiotic controls of the biodegradation experiments.

Table 7.2 also includes data for the respective monoesters. Overall, there is less evidence of these metabolites. Only the monoesters MBM and MHM were detected in appreciable amounts and these were less than in the experiments with hexadecane present in the media (Table 7.1) Again, any other metabolites, such as the alcohols, were only detected at low levels and all disappeared before the end of the experiments.

The kinetics of DOM hydrolysis in the presence of hexadecane is shown in Figure 7.3. The quantity of its monoester, MOM, plateaus around day 7. This corresponds to a cessation of hexadecane consumption and DOM hydrolysis, which plateau around day 6 and day 10, respectively. Another experiment was done with a lower initial quantity of DOM (3 mM). Interestingly, all DOM was removed from the broth within 3 weeks, yet the hexadecane quantity dropped to only about 50 % of its initial value in the same time frame. An accumulation of MOM to approximately 1.5 mM was observed.

The inhibition of microbe activity by MOM was studied in separate experiments at MOM quantities of 1, 2 and 3 mM. When the initial quantity of MOM was 1 mM, both hexadecane and MOM were completely consumed or removed, respectively, from the broth. This took place within 3 and 2 weeks, respectively. However, when the initial quantity was 2 mM or greater, there was no observable degradation of either MOM or hexadecane. A similar experiment with MHM at an initial quantity of 2 mM showed no inhibition and both compounds were readily degraded.



Figure 7.3. Biodegradation of dioctyl maleate (DOM) in the presence of hexadecane and corresponding appearance of metabolites. ‡ Concentration values denote the total amount of organic compound (mainly phase separated) in the aqueous broth, as determined by extraction with chloroform.

Table 7.1. Results for biodegradation experiments including hexadecane as auxiliary carbon source: Estimated half-life of maleate-based plasticizers, quantity of hexadecane in broth after 30 days, maximum quantity of the corresponding monoester observed and the time at which the maximum occurred.

R'	n-C ₂ H ₅	n-C ₄ H ₉	n-C ₆ H ₁₃	n-C ₈ H ₁₇	$CH_2CH(C_2H_5)(C_4H_9)$	
Abbreviation	DEM	DBM	DHM	DOM	DEHM	
Half-life (d)	4.6	6.3	10.0	16.9	>> 30	
Final quantity of hexadecane (g/L) ^a	0	0	0	1.1	0	
Monoester ^b	MEM ^b	MBM ^b	MHM ^b	MOM ^b	MEHM ^b	
Maximum quantity (mM) ^a	Trace amounts	4	4	2.5°	Trace amounts	
Time of maximum quantity occurrence (d) ^d	-	14	26	7 ^b	-	

^a Quantity values denote the total amount of organic compound in the aqueous broth, as determined by extraction with chloroform.

^b MEM – monoethyl maleate; MBM – monobutyl maleate; MHM – monohexyl maleate; MOM – monooctyl maleate; MEHM – mono (2-ethylhexyl) maleate.

^c No change in quantity for any compound from d7, indicating inactivation of hydrolysis process by bacteria.

^d All experiments showed at least trace amounts of monoester left in the broth at the end of the experiment.

Table 7.2. Results for biodegradation experiments without auxiliary carbon source: Estimated half-life of maleate-based plasticizers, maximum quantity of the corresponding monoester observed and the time at which the maximum occurred.

R'	n-C ₂ H ₅	n-C ₄ H ₉	n-C ₆ H ₁₃	n-C ₈ H ₁₇	CH ₂ CH(C ₂ H ₅)(C ₄ H ₉)		
Abbreviation	DEM	DBM	DHM	DOM	DEHM		
Half-life (d)	11.6	8.9	39	>> 39	>> 39		
Monoester ^a	MEM ^a	MBM ^a	MHM ^a	MOM ^a	MEHM ^a		
Maximum quantity (mM) ^b	Trace amounts	2.4	1.3	Trace amounts	Not observed		
Time of maximum quantity occurrence (d) ^{b, c}	-	21	26	-	-		

^a MEM – monoethyl maleate; MBM – monobutyl maleate; MHM – monohexyl maleate; MOM – monooctyl maleate; MEHM – mono (2-ethylhexyl) maleate.

^b Quantity values denote the total amount of organic compound in the aqueous broth, as determined by extraction with chloroform.

^c All experiments but for DEHM showed at least trace amounts of monoester left in the broth at the end of the experiment.

7.6 Discussion

As DEHP is increasingly cited as a contaminant of concern, safer and more environmentally benign replacement compounds need to be designed and thoroughly tested to replace DEHP. Ideally, an adequate replacement plasticizer would be degraded rather quickly once exposed to the environment, and the same would hold true for the released metabolites. Here, we present a series of maleate plasticizers which, compared to DEHP, show similar plasticizing properties, yet better biodegradation properties. We show however, that replacement compounds need to be tested vigorously and diligently, as the longest maleate compound DOM causes bacterial growth inhibition.

7.6.1 Plasticizer Properties

The plasticizing properties of the maleate diesters were assessed using measurements of the glass transition temperature (T_g), which is generally a good indicator of the efficacy of a plasticizer. For example, earlier work has shown a good correlation between T_g data and tensile properties such as strain at break and elastic modulus (Erythropel et al. 2012). Figure 7.2 indicates that all maleate compounds tested performed better in terms of reducing the T_g than commercial DEHP when used at the same concentration.

Furthermore, Figure 7.2 shows that an increase in overall length (C- and O-atoms) of the maleate diester results in significantly lower values for T_g . In this series of compounds, the overall length of the molecule is determined by the length of the aliphatic chain of the alcohol used to make the diester, i.e., an increase of two carbon atoms in the alcohol would result in an increase of four atoms for the overall chain length of the maleate diester. A lower T_g value is desirable as it indicates a better efficiency of the plasticizer at disrupting the interactions of the long PVC chains, thereby rendering the material more flexible and malleable. As a direct result of this, a smaller absolute amount of the longer-chained plasticizers compared to the shorter-chained ones would be needed in order to produce a blend with a desired T_g .

Two of the maleates tested, namely DHM and DEHM, had the same number of units in the longest chain of C- and O-atoms, yet DEHM contained two ethyl branches in the side chains. Despite this difference in the overall size of the compound, these compounds had comparable plasticizing properties. This suggests again that the important factor in plasticizing is the overall length of the molecule. This is also supported by the fact that both DEHM and DOM have the same molecular weight, yet DOM is significantly more efficient at lowering the T_g (Figure 7.2). Again, this is related to the length of the major chain since DOM is appreciably longer. These findings are in agreement with an earlier study of succinate diesters in which similar effects of molecule chain length and molecular weight on T_g reduction were observed (Erythropel et al. 2013).

7.6.2 Biodegradation Properties

Plasticizers are not covalently bound to the polymers, so they have a tendency to leach out of the material. Strategies to stop leaching, such as a chemical attachment to the polymer matrix or coating of the surface of the material, have been assessed, however, these techniques are not yet fully developed for use in the general market, often increase the price of PVC products, and sometimes alter the functionality of the final product (Chiellini et al. 2013). Thus, it is important to develop plasticizers that are readily biodegradable to avoid problems with the leached plasticizers or their metabolites.

Biodegradation experiments of the pure plasticizers were done with the common soil bacterium *Rhodococcus rhodocrous*, which is known to be able to degrade a wide range of hydrophobic, organic compounds (Jones and Goodfellow 2012). The pathway of degradation of these diesters by *Rhodococcus rhodocrous* is well established and involves subsequent hydrolysis of one of the two ester bonds. This happens at varying rates, yielding the respective monoester, the respective alcohol, and maleic acid as metabolites. The alcohol is then further oxidized to the corresponding organic acid, and consumed by β -oxidation, except in the case of 2-ethylhexanol, which resists β -oxidation (Horn et al. 2004, Nalli et al. 2006b, Nalli et al. 2006c, Barnabe et al. 2008, Erythropel et al. 2012). Maleic acid was not further monitored, but is not expected to be problematic or toxic (Fitzhugh and Nelson 1947) and would likely be consumed by the microbes.

The results demonstrate the importance of the influence of length of the plasticizers on biodegradation but there are also other factors at play. As the overall length of the plasticizer increased, the compounds were less susceptible to biodegradation in the experiments including hexadecane (see Tables 7.1 and 7.2). This is likely related to the solubility of the compound, yet some other factors regarding bioavailability have recently been described (Alexander 2000,

Kickham et al. 2012, Segura et al. 2012, Smith et al. 2012). In terms of solubility, as these aliphatic molecules become longer, their solubility in water would decrease and, consequently, they would be less available to the microbes. This is supported by the importance of the cosubstrate hexadecane. When hexadecane was not present in the media, the rates of biodegradation were reduced (i.e., compare results in Tables 7.1 and 7.2). Hexadecane has been found to facilitate the biodegradation of these compounds. This is likely due to the fact that it is relatively hydrophobic, similar to the plasticizer molecules, and the microbes that grow on hexadecane would automatically be more exposed and conditioned to the plasticizer compounds dissolved in hexadecane (Nalli et al. 2002, Nalli et al. 2006c). A similar trend was found in a previous study on the biodegradation rate of a series of succinate diesters by the same microorganism (Erythropel et al. 2013).

In the above-mentioned study concerning succinate plasticizers, it was found that the addition of hexadecane did not change biodegradation rates because the bacteria grew preferentially on the metabolites from the degradation, such as the liberated alcohol (Erythropel et al. 2013). However, in this study with maleate diesters, which differ from succinate diesters only by a *cis*-double bond between the ester functionalities, the biodegradation rates did differ quite substantially between experiments with and without hexadecane (i.e., compare results in Tables 7.1 and Table 7.2). This exposes the relative difficulty encountered by the microbes to get to the ester bond and hydrolyse it, as growth is faster if a hydrophobic auxiliary carbon source is present. However, this would likely not cause a problem in terms of the environmental fate of such compounds, as it can be safely assumed that such a carbon source would be present in most environmental settings.

7.6.3 The Effect of Branching

Although branching of the side chain had little impact on the plasticizing properties, it did have a dramatic effect on the rate of biodegradation. Little or no biodegradation of the branched molecule, DEHM, was observed in any of the experiments. However, both the compound with the same length (DHM) and the compound with the same molecular weight (DOM) were biodegraded relatively quickly when hexadecane was present (Table 7.1); in the latter case at least until inhibition from a metabolite intervened (Figure 7.3). This is an important result because while the presence of the branch does not have a significant effect on

the plasticizing properties, it does make the use of these compounds problematic if they leach out of the polymer into the environment.

This raises concerns about several other commercial plasticizers, such as DEHP, di (2-ethylhexyl) adipate (DEHA) or trioctyl trimellitate (TOTM). These plasticizers all contain at least two of the branched 2-ethylhexyl side chains. In this study on maleate diesters it was demonstrated that the ethyl-branches add no benefit in terms of T_g reduction efficiency over non-branched side chains. Given that the resemblance of DEHM to DEHP, it is likely that the same would be true for DEHP. TOTM is analogous to DEHP in that it contains three organic acid functionalities from the central benzene ring (i.e., one in *ortho*-, one in *para*-position), all esterified with 2-ethylhexyl groups. Given its close resemblance to DEHP, it is hypothesized that in this case, as well, there would be no added benefit from the ethylbranches pose a major problem for hydrolysis by common soil bacteria, especially when the two ester groups are in *cis*-position relative to one another (Erythropel et al. 2012). Combining these findings, it is concluded that there is an environmental benefit associated with the removal of the ethyl branches without impairing the plasticizing properties.

7.6.4 Monoester Formation and Degradation

The consideration of the monoesters in the above experiments is important because the monoester, MEHP, of the commercial plasticizer DEHP is believed to exhibit toxicity and endocrine disrupting activity (Gray and Gangolli 1986, Richburg and Boekelheide 1996, Lovekamp-Swan and Davis 2003, Swan et al. 2005a, Pant et al. 2008, Fan et al. 2010, Piche et al. 2012). Thus, if any of the compounds being studied are to produce a very stable monoester that was not readily removed from the broth, this compound would have to be examined very thoroughly in order to avoid similar problems as with DEHP.

In an earlier study with succinate diesters (Erythropel et al. 2013), the respective monoesters were only observed in trace amounts, suggesting that these compounds were hydrolysed almost as soon as they were released. However, with the diesters in this study, it was apparent that the monoesters were more resistant to biodegradation. There were some exceptions in which very little of the monoester was observed but this is simply due to the fact

that, in these cases, little or no hydrolysis of the parent compound took place. This leads to the important question of whether or not the stability of the monoesters lasted for a significant period of time or was merely transitory. The trend in half-lives of the various monoester metabolites was the same as the trend in the half-lives of the parent compounds.

It should be noted that interpretations of the maximum quantities of each monoester and the times at which the peaks occur is complicated by the fact that these compounds are intermediates in the biodegradation process. They are created by the first hydrolysis step of the original compound and are subsequently degraded (Erythropel et al. 2013). The important observation, however, is that in most of the experiments, a maximum value was observed, thereby showing that the intermediates are transitory and, hence, are biodegradable.

The trends in the data for the degradation of the monoesters show that, as with the parent compounds, the smaller molecules are removed more quickly. When comparing the respective times at which these maximum quantities occur, it is once again apparent that molecules with smaller side chains are more easily hydrolysed by the microorganisms; for example in the experiments with hexadecane present, the maximum quantity of 4 mM of the metabolite MBM in the DBM experiment is reached after 14 days while for the molecule with only 4 extra carbon atoms added on both sides (i.e., DHM), the maximum quantity of 4 mM of these compounds; the slower hydrolysis of the parent diester would decrease the production rate of monoester and its own lower solubility would then also contribute to the slower removal rate of this compound compared to its smaller homologues such as MBM. An equivalent trend is found for the experiments without added hexadecane, but is less pronounced. This is explained by the overall reduced hydrolysis rates of the parent compounds in the absence of hexadecane (see above).

The most problematic of the monoesters was found to be MOM. This was observed during the degradation of DOM in the presence of hexadecane (see Figure 7.3). In fact, the production of this monoester by day 7 was to such an extent that the growth of the microorganism was halted and not even the hexadecane was consumed. This conclusion was supported by further experiments in which synthesized samples of the pure monoester were added to the microbe grown on only hexadecane. At the lowest quantity of MOM (i.e., 1 mM), bacterial growth was slightly hindered and the consumption of the hexadecane took longer than

in the control experiments (data not shown). However, when the initial quantity was either 2 mM or 3 mM, no bacterial growth was observed visually and the quantity of hexadecane remained unchanged. This result confirmed that the monoester, MOM, resulting from the biodegradation of DOM, has a growth-inhibiting effect on *Rhodococcus rhodocrous*. Interestingly, a small change in the molecule such as two linear CH₂-units, which is the only difference between MHM and MOM, seems to create this effect; that is, MHM at a quantity of 2 mM had no effect on bacterial growth: hexadecane was consumed in about 10 days, and removal of MHM occurred, thereby indicating no negative effect on bacterial growth.

On the other hand, the shortest maleate tested in this study, DEM, is known to deplete glutathione (GSH) levels in rat and human cell lines, thus increasing the concentration of oxidative free radicals (Adams et al. 1983, Russo et al. 1995, Tirmenstein et al. 2000). However, the bacteria used in this study do not produce GSH, but likely a similar thiol such as mycothiol (MSH), which is believed to play a similar role in prokaryotes as GSH in eukaryotes (Newton et al. 1996). No growth inhibition to *Rhodococcus rhodocrous* from DEM was found however. The exact mechanism of growth inhibition by MOM is beyond the scope of this article, and further studies are needed to understand it.

Other metabolites such as the alcohols and organic acids were usually only observed in trace amounts or not detected at all. This indicates that these compounds would likely not be a problem in the environment. From previous work (Nalli et al. 2006a, Erythropel et al. 2013), it was expected that these would be easily removed from the media.

7.7 Conclusion

Overall, it was shown that in terms of T_g reduction and biodegradation, maleate plasticizers have the potential to substitute phthalate-based plasticizers by changing the side chains from branched to straight aliphatic chains. No positive influence of branching in side chains of the compounds towards plasticization efficiency could be found, however removing the branching dramatically improved biodegradation rates. A balance between better plasticizing properties (longer side chains) and faster biodegradability of the compound (shorter side chains) and its principal metabolite, the monoester, needs to be struck. MOM should be avoided as it is inhibitory to *Rhodococcus rhodocrous*, although its mechanism of action still requires further investigation.

7.8 Acknowledgements

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8 Designing Green Plasticizers: Influence of Molecule Geometry and Alkyl Chain Length on the Plasticizing Effectiveness of Diester Plasticizers in PVC Blends

8.1 Preface

The following manuscript has been accepted for publication in the journal Polymer (DOI: 10.1016/j.polymer.2016.02.031). The presented work focussed on the linear maleate and succinate compounds discussed in Chapters 6 and 7, which were previously tested for their T_g reduction potential only. To further assess their effectiveness as plasticizers, they were incorporated into UPVC and tested by a variety of mechanical techniques, such as tensile testing, dynamic mechanical thermal analysis (DMTA), and surface hardness.

Overall, the results were in good agreement with the T_g data previously reported, except that the continuous improvement of T_g reduction efficiency with increasing side chain length was not observed past the dibutyl compounds, but the effectiveness by mechanical tests levelled off, and even slightly decreased for the dioctyl compound in some experiments. Also, the effect of the ethyl branches in the di (2-ethylhexyl) compounds was compared to the equally long dihexyl compounds and the dioctyl compounds, which are of equal molecular weight. No effect of the ethyl branches was found. When comparing maleates and succinates to each other, a comparable degree of plasticizing effectiveness was observed, suggesting that both are good plasticizer candidates. Compounds from both series also performed better than the commercial compounds DEHP and DINCH® in several tests.

Designing Green Plasticizers: Influence of Molecule Geometry and Alkyl Chain Length on the Plasticizing Effectiveness of Diester Plasticizers in PVC Blends

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8.2 Abstract

The influence of central structure and side chain length on plasticizer effectiveness of succinate and maleate diesters blended into poly(vinyl chloride) (PVC) was assessed by tensile testing, DMTA, and surface hardness measurements. While no significant differences between central structures with similar side chain lengths were found, the length of the side chain played an important role, and maximum effectiveness was found for the linear dihexyl esters. In comparison to commercial plasticizers such as DEHP and Hexamoll® DINCH®, succinates and maleates with linear side chains of four to eight carbons performed as well or better in several of the mechanical tests. Earlier work had established that the proposed succinate and maleate compounds exhibited higher biodegradation rates than DEHP, and pending toxicity studies, they seem to be viable replacement compounds for DEHP.

8.3 Introduction

Plasticizers are typically relatively small molecules that are incorporated into polymeric materials in order to increase the workability, flexibility, or distensibility of the matrix polymer (ASTM D-883 2012). Plasticizers are added to hard and brittle polymers such as poly(vinyl chloride) (PVC), resulting in lower melt viscosity, lower glass transition

temperature (T_g) , lower elastic modulus, and better elongation of the blend, or any combination of these properties (Sears and Darby 1982).

Due to the vast variety of polymers and plasticizers that exist, and the very different chemical moieties present in these materials, there are multiple theories on the mechanisms of plasticization that complement each other (i.e., the lubricity-, gel-, and free volume theories). In brief, there exist interactions between the polymer chains in the case of PVC due to the dipole present on each (δ^+) C-Cl (δ^-) bond. Due to this weak dipole, chains interact with neighbouring chains, thereby creating a network that, when below the T_g, does not permit significant chain mobility, resulting in macroscopic brittleness. Once the Tg is reached, there is sufficient energy to overcome the attractive forces between the chains, thereby allowing for more chain movement within the blend, and then the material becomes macroscopically flexible (Würstlin 1962, Sears and Darby 1982, Wypych 2012). The role of the plasticizer is to break up some of these chain-chain interactions, resulting in lowering the Tg which in turn influences the material properties of the final product. To achieve the separation of neighbouring polymer chains, the plasticizer should contain non-polar or slightly polarizable portions, yet a plasticizer that is too non-polar would be immiscible with PVC. For this reason, a plasticizer molecule will usually contain polar moieties (i.e., ester groups) that will interact with the PVC chains to assure compatibility, as well as non-polar groups (i.e., alkyl chains) that break up the chain-chain interactions and play the main role in the plasticizing effect (Leuchs 1956, Würstlin 1962, Sears and Darby 1982, Wypych 2012).

In 2008, 5.6 million metric tonnes of plasticizer were produced to plasticize PVC, with phthalate diesters accounting for 88 % of this production. Of these phthalate diesters, di (2-ethylhexyl) phthalate (DEHP; sometimes misleadingly called DOP) accounted for 54 % of production in 2008 (Bisig 2009), making DEHP the most important industrial plasticizer (see Figure 8.1 for the structure). Since added plasticizers (also called external plasticizers) are not chemically bound to the polymer matrix, they tend to leach out over time, ultimately ending up in the environment, where they preferentially accumulate in hydrophobic matter (Wams 1987, Staples et al. 1997, ATSDR 2002, Heinemeyer et al. 2013, Erythropel et al. 2014). DEHP and its breakdown products have been considered ubiquitous environmental contaminants as early as the 1980s, being found in soils, water, and air (Wams 1987) and in house dust (Butte et al. 2001, Becker et al. 2004). DEHP degradation happens primarily under aerobic conditions

and temperature plays an important role (Wams 1987, Erythropel et al. 2014). When DEHP is broken down by hydrolysis, it yields two stable metabolites; i.e., its monoester MEHP and 2-ethylhexanoic acid, which is the oxidation product of the liberated 2-ethylhexanol (Nalli et al. 2002, Horn et al. 2004). Extensive studies on these compounds have been carried out, which show a wide range of toxic effects (Lloyd and Foster 1988, Richburg and Boekelheide 1996, Akingbemi et al. 2001, Foster et al. 2001, Gazouli et al. 2002, Nalli et al. 2002, Horn et al. 2004, Fan et al. 2010, Martinez-Arguelles et al. 2011, Piche et al. 2012). The monoester MEHP has been particularly linked to antiandrogenic activities in humans (Swan et al. 2005a, Pant et al. 2008, Martinez-Arguelles et al. 2013).

Due to their environmental persistence and associated health concerns, several phthalates including DEHP have become increasingly subject to regulation (Health Canada 1996, EU 2005, CPSIA 2008, Bureau Veritas 2010), resulting in the need to develop safer, and more environmentally compatible alternatives. The choice of a suitable alternative would require an extensive screening process in order to assure comparable plasticizing properties, ideally faster biodegradation rates without the buildup of stable metabolites, reduced toxicity, and reduced leaching. DINCH® was introduced by BASF in the early 2000s as a plasticizer for "sensitive applications" (Wadey 2003). Recently, we proposed series of n-alkyl succinates and -maleates (see Figure 8.1 for structures) as possible alternatives to DEHP due to the faster biodegradation rates of the parent compound and their metabolites (Erythropel et al. 2012, Erythropel et al. 2013, Erythropel et al. 2015) and their reduced leaching rates (Kastner et al. 2012). In our previous studies, the plasticizing ability was assessed only in terms of T_g reduction.

The addition of plasticizer molecules to polymers results in the improvement of mechanical properties, and these improvements are a function of concentration but also of molecular structure (Wilkes et al. 2005). When comparing the effects of different molecular structures on material properties of polymers, both the term "plasticizer efficiency" (Boyer 1951, Wilkes et al. 2005) as well as "plasticizer effectiveness" (Koo 1974, Sinko and Amidon 1989) have been used. Some of the mechanical properties that are improved by the addition of plasticizer are, among others, increased strain at break, reduced tensile strength and modulus, reduced torsional modulus, and reduced surface hardness (Sears and Darby 1982, Wilkes et al. 2005).

In this work, we present more extensive material testing data of our candidate plasticizers to evaluate their plasticizer effectiveness when blended with PVC at equal weight-fractions (to ensure comparability) by tensile testing, dynamic-mechanical thermal analysis (DMTA), and surface hardness measurements. We investigated the effect of central structure, the overall molecule length as determined by the longest chain of oxygen- and carbon-atoms, and the influence of ethyl branches in the β -position to the ester moiety (as present in DEHP) on plasticizer properties. Finally, the results are compared to similar measurements conducted with commercially available DEHP and DINCH®.

8.4 Materials and Methods

8.4.1 PVC and Plasticizers

Unplasticized PVC (UPVC; K50) was provided by Solvay Benvic, France. Compounds commercially available were purchased through Sigma-Aldrich, and included di (2-ethylhexyl) phthalate (DEHP, 99 %), diethyl succinate (DES, 99 %), diethyl maleate (DEM, 97 %), and dibutyl maleate (DBM, 96%). The other commercially used, and marketed as "green", plasticizer DINCH® (99%) was provided by BASF Canada. The other compounds including dibutyl succinate (DBS), dihexyl succinate (DHS), dioctyl succinate (DOS), di (2-ethylhexy) succinate (DEHS), dihexyl maleate (DHM), dioctyl maleate (DOM), and di (2-ethylhexyl) maleate (DEHM) were synthesized in our laboratories using Dean-Stark esterification, as described previously (Erythropel et al. 2012). The structures of these plasticizers are indicated in Figure 8.1.


Figure 8.1. Structures of succinate and maleate diesters and the commercially-available plasticizers DINCH® and DEHP.

8.4.2 Extrusion of PVC/Plasticizer Blends

The blending of UPVC with the plasticizers was achieved with a conical intermeshing twinscrew extruder (Haake Minilab, Thermo Electron Corporation), with conical screws of 5/14 mm diameter and a length of 109.5 mm. The batch size was 3 g. Mixing of UPVC with the plasticizer was achieved in a two-step process. First, a blend of 20 parts per hundred rubber (phr; equivalent to 16.6 wt.-%) was prepared, then the plasticizer content was raised to 40 phr in a second extrusion step by addition of more plasticizer into the compounded blend. This two-step procedure was necessary to ensure the homogeneity of the resulting blends. The extruder was operated at 130 °C during the first step to 20 phr and at 110 °C in the second step to achieve 40 phr, with a screw speed of 30 rpm, which was raised to 60 rpm after 5 min of each 3 g batch to push out the remaining material. In the first step, 4 phr of epoxidized soy bean oil (Chemtura Corporation) as heat stabilizer and 5 phr of stearic acid (Fisher Scientific) as lubricant were added in addition to the appropriate amount of plasticizer. In the second step the remainder of the plasticizer was added (without other additives). Each extruded batch was recycled through the extruder a second time, meaning that each 40 phr blend tested had passed through the extruder for a total of 4 passes.

8.4.3 Production of Test Bars for Tensile Testing and Dynamic Mechanical Thermal Analysis

Test bars were produced by pressing the finely chopped blends containing 40 phr of plasticizer in a hot press (Carver manual Hydraulic Press, with Watlow temperature controllers). Type V test bars were used for tensile testing according to ASTM D-638 (2014), with the following dimensions: 1.5 mm thickness, 3.25 mm width of narrow section (W), 15.5 mm length of narrow section (L), 32.5 mm distance between grips (D), 63.5 mm overall length (LO), and 10mm width overall (WO). The rectangular test bars for DMTA were produced according to the ASTM D-4065 (2012) method, with the following dimensions: 1.5 mm thickness, 10 mm width, and 50 mm length. The rectangular DMTA test bars were also suitable for hardness testing by nanoindentation according to the ASTM E-2546 (2007) method. All test bars were conditioned in a desiccator at room temperature for a minimum of 48 h prior to testing.

8.4.4 Tensile Testing

Tensile testing was carried out on a Yamazu Easy Test with a load cell of 500 N according to ASTM D-638 (2003). The exact dimensions of the middle section were recorded (Electronic Outside Micrometer, Fowler Tools & Instruments), and n = 5 specimens (except for DBM, DOS with n = 4) per blend were tested at a strain rate of 5 mm/min. The stress-strain curves (see examples shown in Figure 8.2) were used to obtain values for strain and stress at break. To calculate the apparent modulus, the derivative of a polynomial curve fit to the experimental data (R^2 value of at least 0.98) was calculated to determine the apparent modulus at 15 % elongation (EL), 25 %EL, and 50 %EL as shown in Figure 8.2, but only the values for 25 %EL are reported for all blends in Table 8.1.

8.4.5 Dynamic Mechanical Thermal Analysis

DMTA was carried out on a rheometer with fixed bottom clamp and a moveable top clamp (Anton Paar MCR302 with SRF12 fixtures, CTD450 convection, Anton Paar GmbH). The convection oven was operated with nitrogen gas purge to avoid oxidation of the specimens. The exact dimensions of the middle section were recorded (Electronic Outside Micrometer, Fowler Tools & Instrument). DMTA was run in torsion mode, strain-controlled with an amplitude gamma of 0.01 %. A temperature sweep from 25 °C to 100 °C at a rate of 2 °C/min at a constant shear rate of 1Hz was carried out for each blend, with the machine controlling the normal force set to -0.5 N. The software RheoPlus (V3.61, Anton Paar GmbH) was used to obtain the storage modulus G', and the loss modulus G''.

8.4.6 Hardness Testing

The surface hardness of the various blends was determined using a micro-indenter (Nanovea PB1000 with Nano module, stainless steel ball tip of 1mm diameter). Measurements were done at room temperature and repeated three times on each test bar, with each test conducted in a different third of the length of the test bar. Once the indenter had reached the zero point, it subsequently built up a contact load of 0.3 mN, and then forced the tip into the sample at a rate of 30 mN/min until a load of 20 mN was reached. The tip was then removed at the same rate of 30 mN/min. During the experiment, indentation depth was recorded automatically by the software (Nanovea Nano Hardness Tester). A Matlab® program was used to calculate the surface hardness according to ASTM E-2546 (2007). In brief, this calculation is based on a stiffness term derived from the slope of the first third of the unloading curve, as well as the estimated contact area and indentation depth, with the result expressed in units of MPa.

8.4.7 Statistics

Statistical analysis was performed using GraphPad Prism 5 software, for one-way- and two-way ANOVA tests with Bonferroni post-tests. A *p* value less than 0.05 was interpreted as significant, with p < 0.05 represented by *, p < 0.01 by **, and p < 0.001 by ***.

8.5 Results

8.5.1 Tensile Strength

All samples exhibited strain softening during tensile testing, obtained stress strain curves were thus non-linear as shown, for example, in Figure 8.2 for blends with DEHP, DINCH®, DHM, and DHS. Averages of stress at break, strain at break, and apparent modulus at 25 %EL are reported in Table 8.1.

8.5.2 Strain at Break

The data for strain at break is reported in Table 8.1 and visualized in Figure 8.3, where results for blends with the commercial plasticizers DEHP and DINCH® are shown first. These are followed by blends with the series of succinate and maleate plasticizers, in ascending order of linear side chain length from C2 (diethyl; "DE") to n-C8 (dioctyl; "DO"). This is followed by the respective branched di (2-ethylhexyl) compound ("DEH"), which is not part of the series of unbranched alkyl side chains, but only compared to the equally-long dihexyl ("DH") compound, and the dioctyl ("DO") compound, which is of equal molecular weight.

There was a significant effect of chain length on strain at break (two-way ANOVA, p < 0.001), yet the central group structure had no effect (two-way ANOVA, p = 0.64) for our candidate plasticizers. DBS and DBM had the highest means of strain at break observed within their respective series, which were significantly higher than commercial DEHP (one-way ANOVA, Bonferroni post-test: DBS, p < 0.05; DBM, p < 0.01) and commercial DINCH® (one-way ANOVA, Bonferroni post-test: DBS, p < 0.001; DBM, p < 0.001). DOS and DOM had the lowest strain at break within their respective series, but were not significantly different from commercial DEHP (one-way ANOVA, Bonferroni post-test, p > 0.05, respectively) or DINCH® (one-way ANOVA, Bonferroni post-test, p > 0.05, respectively). Thus, we conclude that all compounds performed as well or better than the commercial plasticizers in terms of increasing strain at break.

While no significant difference between the equally long DHS and DEHS was observed (one-way ANOVA, Bonferroni post-test, p > 0.05), DOS was significantly lower than the compound with the same molecular weight, DEHS (one-way ANOVA, Bonferroni post-test, p < 0.05). No statistical difference between DHM and the equally-long DEHM was observed (one-way ANOVA, Bonferroni post-test, p > 0.05), nor between the compound of equal molecular weight, DOM and DEHM (one-way ANOVA, Bonferroni post-test, p > 0.05).

8.5.3 Stress at Break

The data for stress at break are presented in Table 8.1 and visualized in Figure 8.4. There was a significant effect of chain length on the stress at break (two-way ANOVA, p < 0.0001), as well as an effect of central group structure (two-way ANOVA, p < 0.0001) for our candidate plasticizers.

All tested compounds exhibited significantly lower means for stress at break compared to DEHP (one-way ANOVA, Bonferroni post-test, p < 0.001), for which the highest ultimate tensile stress was observed. Regarding the other commercial plasticizer, DINCH®, only DEM (the plasticizer blend with the highest stress at break among the maleates) showed a higher stress at break (one-way ANOVA, Bonferroni post-test, p < 0.01). The succinate with the highest stress at break recorded, DES, was shown to not differ significantly from DINCH® (one-way ANOVA, Bonferroni post-test, p > 0.05). Similar to DEHP, all other compounds showed significantly lower averages for stress at break compared to DINCH® (one-way ANOVA, Bonferroni post-test, p < 0.001).

When comparing the equally long DHS to DEHS and also DHM to DEHM, the unbranched DH-compounds showed significantly lower values than the DEH-compounds (one-way ANOVA, Bonferroni post-test, p < 0.001, respectively). However, when comparing the molecules of equal molecular weight, DOS to DEHS, and DOM to DEHM, no significant statistical difference between the means was observed (one-way ANOVA, Bonferroni post-test, p > 0.05, respectively).

8.5.4 Apparent Modulus at 25 % Elongation

According to ASTM D-638 (2014), the "Standard Test Method for Tensile Properties of Plastics", the modulus of elasticity should be reported preferably, however, no linear portion was present in the recorded curves (as shown in Figure 8.2). For non-linear behaviour, it is suggested in ASTM D638 that the secant modulus be reported, which is obtained by drawing a secant from the origin to a set strain (ASTM D-638 2014). Given the differences between the

obtained curves, especially in the first third of the curves (i.e., compare DEHP to DHS in Figure 8.2), reporting secant moduli would exaggerate the differences between the recorded curves and thus not accurately describe our observations (Figure 8.2 contains examples of stress-strain curves for four different compounds). Instead, we used the apparent modulus, which corresponds to the slope of the curve at a defined strain. As shown in Figure 8.2 for three different strains (15 %EL, 25 %EL, and 50 %EL), and for two curves (DEHP and DHS), the apparent modulus did not result in as large a difference between curves as the secant modulus did, while accounting for the change in slope as the elongation increases. In Table 8.1 we show the results for the apparent modulus at 25 %EL for all experiments.

The data for the apparent modulus are presented in Table 8.1 and visualized in Figure 8.5. A significant effect of chain length on the apparent modulus at 25 %EL was observed (two-way ANOVA, p < 0.0001), as well as an effect of central group structure (two-way ANOVA, p < 0.0001) for our candidate plasticizers.

All tested compounds exhibited significantly lower means for apparent modulus at 25 %EL compared to DEHP (one-way ANOVA, Bonferroni post-test, p < 0.001). This indicates that blends with DEHP were the stiffest specimens tested. The stiffest compounds within the series of succinates and maleates were DES and DEM, respectively, which were significantly less stiff than DEHP (one-way ANOVA, Bonferroni post-test, p < 0.001), but stiffer than DINCH® (one-way ANOVA, Bonferroni post-test, p < 0.001). The least stiff compounds within the series of succinates were DBS and DHS, which were not found to differ significantly (one-way ANOVA, Bonferroni post-test, p > 0.001). The least stiff to the longest compound in the series, DOS, which was significant compared to the aforementioned DBS and DHS (one-way ANOVA, Bonferroni post-test, p < 0.001). Within the series of maleates, DBM, DHM, and DOM showed the lowest stiffness and all three means were not significantly different from each other (one-way ANOVA, Bonferroni post-test, p > 0.05).

The unbranched DHS was found to exhibit a lower stiffness than the equally long but branched compound DEHS (one-way ANOVA, Bonferroni post-test, p < 0.001), yet for the compounds of equal molecular weight, DOS and DEHS, no significant difference between the means was found (one-way ANOVA, Bonferroni post-test, p > 0.05). For the maleates, a significant difference between both the equally long DHM and DEHM (one-way ANOVA, Bonferroni post-test, p < 0.001), as well as the molecules of equal molecular weight DOM and DEHM was observed (one-way ANOVA, Bonferroni post-test, p < 0.001).



Figure 8.2. Selected tensile stress—strain curves for 40 phr blends of PVC with DEHP, DINCH®, DHM and DHS. Estimated values of apparent moduli at 15 %, 25 % and 50 % elongation (in MPa) are shown for DEHP (top values), and DHS (bottom values).



Figure 8.3. Strain at break for blends of PVC/plasticizer at 40 phr. Averages and standard deviations of n = 5 specimens are shown, with the exception of DBM and DOS with n = 4. ‡ DINCH® strain at break results were statistically significantly different from: DES**, DBS***, DHS*, DEHS*, DEM***, DBM***. *: p < 0.05; **: p < 0.01; ***: p < 0.001.



Figure 8.4. Stress at break for blends of PVC/plasticizer at 40 phr. Averages and standard deviations of n = 5 specimens are shown, with the exception of DBM and DOS with n = 4. All results were statistically significantly different (***) except where shown (and except DES versus DEM **; DBS vs DBM **; DHS vs DHM *). *: p < 0.05; **: p < 0.01; ***: p < 0.001.



Figure 8.5. Apparent modulus at 25 %EL for blends of PVC/plasticizer at 40 phr. Averages and standard deviations of n = 5 specimens are shown, with the exception of DBM and DOS with n = 4. All results were statistically significantly different (***) except where shown (and except DBS versus DBM **). *: p < 0.05; **: p < 0.01; ***: p < 0.001.

8.5.5 Torsional DMTA

For all blends, DMTA was performed with a temperature sweep from 25 °C to 100 °C at a constant frequency of 1 Hz. Two exemplary curves of storage (G') and loss (G") moduli for blends of PVC/DEHP and PVC/DHS are shown in Figure 8.6, and the results for the other compounds can be found in Appendix C. Table 8.1 summarizes the results of G' and G" only at 25 °C since the general trends were the same for all tested blends. Values at 25 °C were chosen since this condition is closest to room temperature.

As shown by the torsional data in Table 8.1 in both series of succinates and maleates, the shortest compound, DES and DEM, had the highest torsional moduli (G' and G''). Moreover, as the chain length increased, torsional modulus decreased with a minimum reached at side chain lengths around four to six carbons (DBS, DHS, and DBM, DHM). The torsional modulus increased again slightly for the compounds with n-C8 side chains, DOS and DOM. All branched DEH compounds (DEHS, DEHM, DEHP) show values at similar levels, comparable to DOM, but higher than the DB and DH compounds, which showed the least torsional stiffness of all compounds. DINCH® and DOS showed slightly higher values than DEHP.



Figure 8.6. Selected plots of DMTA torsional temperature sweep for 40 phr blends of PVC with DEHP and DHS. Storage modulus G' and loss modulus G' are shown.

8.5.6 Surface Hardness by Nano-indentation

The results obtained for the surface hardness of the blends of PVC with the various plasticizers are presented in Table 8.1 and visualized in Figure 8.7. A significant effect of chain length on surface hardness was observed (two-way ANOVA, p = 0.0025), as well as an effect of central group structure (two-way ANOVA, p < 0.0001).

The blends with the highest surface hardness in the succinate and maleate series were those plasticized with the diethyl compounds, DES and DEM, respectively. While DEHP was significantly softer than DES and DEM (one-way ANOVA, Bonferroni post-test, p < 0.001), DINCH® was only softer than DES (one-way ANOVA, Bonferroni post-test, p < 0.001), while no significant difference in hardness was observed between DEM and DINCH® (one-way ANOVA, Bonferroni post-test, p > 0.05). All other members of the succinate and maleate series did produce hardness values slightly above that for DEHP, yet no significant difference was observed (one-way ANOVA, Bonferroni post-test, p > 0.05). This means that all plasticizer candidates rendered the surface of PVC softer than commercially-available DINCH®, and similarly soft as DEHP, with the exception of the diethyl compounds.

No significant differences in surface hardness were observed between the equally-long DHS and DEHS, and also DHM and DEHM, respectively (one-way ANOVA, Bonferroni posttest, p > 0.05), nor between the molecules of equal molecular weight, DOS and DEHS, and also DOM and DEHM (one-way ANOVA, Bonferroni post-test, p > 0.05).



Figure 8.7. Surface hardness (in MPa) for blends of PVC/compound at 40 phr. Averages and standard deviations are shown for n = 3 measurements, with the exception of DBM and DOS (n = 2) and DEHP (n = 5). Symbols above error bars indicate statistically significant differences (p < 0.05, Bonferroni post-test) with respect to DINCH® (+), DES (‡), and DEM (\$). *: p < 0.05; **: p < 0.01; ***: p < 0.001.

	Tensile strength			DMTA torsion		Surface hardness
Plasticizer	Strain at break	Max stress	Apparent modulus at	G' at 1Hz,	G" at 1Hz,	nanoindentation
	(%EL) ^a	(MPa) ^a	25 % EL (MPa) ^a	25 °C (MPa)	25 °C (MPa)	(MPa) ^b
DES	102 ± 6	9.3 ± 0.2	10.0 ± 0.3	34.0	12.0	2.59 ± 0.24
DBS	107 ± 4	5.7 ± 0.2	6.3 ± 0.3	8.7	2.1	0.69 ± 0.13
DHS	99 ± 6	5.5 ± 0.2	6.4 ± 0.3	7.5	2.1	0.54 ± 0.07
DOS	87 ± 4^{c}	6.5 ± 0.3^{c}	7.4 ± 0.2^{c}	31.0	9.2	$1.02\pm0.09^{\rm d}$
DEM	105 ± 3	9.9 ± 0.2	10.3 ± 0.3	35.9	14.2	1.70 ± 0.31
DBM	109 ± 5^{c}	6.3 ± 0.4^{c}	$7.2\pm0.1^{ m c}$	6.7	1.4	0.74 ± 0.17^{d}
DHM	92 ± 6	5.9 ± 0.2	6.8 ± 0.2	6.5	2.0	0.51 ± 0.05
DOM	92 ± 4	6.6 ± 0.2	7.4 ± 0.3	23.8	7.3	0.93 ± 0.04
DEHS	99 ± 6	6.7 ± 0.2	7.9 ± 0.2	17.0	5.9	0.78 ± 0.07
DEHM	96 ± 5	7.0 ± 0.2	8.4 ± 0.2	17.0	6.4	1.08 ± 0.18
DEHP	96 ± 4	11.8 ± 0.1	12.1 ± 0.6	7.6	3.1	0.44 ± 0.05^{a}
DINCH®	87 ± 5	8.8 ± 0.4	9.1 ± 0.2	32.9	11.8	1.64 ± 0.53

Table 8.1. Properties of 40 phr blends of PVC and different plasticizers. Averages and standard deviations are reported for n specimens, as indicated. Structures of plasticizers are shown in Figure 8.1.

^a n=5; ^b n=3; ^c n=4; ^d n=2

8.6 Discussion

As DEHP and phthalates are increasingly considered to be compounds of concern, alternative plasticizers that are less problematic for human health and environmental stability must be developed. However, if these compounds are to be viable replacements for DEHP and other commercially-available phthalates, they should be as effective, or more effective in plasticizing PVC. Here, we tested a series of maleate and succinate diesters with linear side chains, varying from two to eight carbons, including one branched compound with the same branching as present in commercial DEHP. Note that the side chain is incorporated into the diester by the appropriate choice of alcohol in the esterification reaction. Previously, we have shown that the succinates and maleates are readily biodegraded and that metabolites produced were only transient (Erythropel et al. 2013, Erythropel et al. 2015). In the present study, these greener compounds and, for comparison, the commercial plasticizers DEHP and DINCH®, were extruded with PVC at 40 phr and these blends were then subjected to a series of tests in order to compare their effectiveness as plasticizer in blends with PVC. We present the results of some standard tests to evaluate the blends regarding their formability (elongation at break), their strength (tensile strength), their stiffness (tensile and torsional moduli), and their durability (surface hardness).

The performance of the plasticizer candidates improved with increasing size, from the smallest compounds (C2 side chains) to the dihexyl compounds (C6), and then decreased slightly for the longest molecules (C8 side chains). No apparent effect was visible from the addition of ethyl branches in di (2-ethylhexyl) maleate (DEHM) and succinate (DEHS), when compared to the equally-long, but unbranched dihexyl compounds. Particularly, the dihexyl compounds of the maleate and the succinate showed comparable plasticizing effectiveness to DEHP in terms of strain at break and hardness, and better performance in terms of stress at break, and torsional and tensile moduli. The mentioned dihexyl compounds were also more effective in all tested properties when compared to DINCH®.

8.6.1 Influence of Side Chains

In diester plasticizers, the side chains are often made up of alkyl chains, with their length and the degree of branching imparted through the alcohol used in the esterification reaction. Their primary role is to separate the PVC polymer chains and reduce chain-chain interactions. These result from the weak but permanent dipole present on each carbon-chlorine bond ($C(\delta+) - Cl(\delta-)$). The large amount of attractive chain-chain interactions along the backbone of PVC are the cause for the hard and brittle nature of PVC when not plasticized. Alkyl chains are non-polar and almost non-polarizable and therefore, can disrupt the interactions between PVC chains. We wanted to determine whether the length of these side chains had an effect on plasticizing efficiency beyond the data previously reported (Leuchs 1956, Würstlin and Klein 1956).

We observed an improvement in plasticizing effectiveness as the (unbranched) side chain length was increased (Table 8.1). From side chain lengths of two to six carbons, the stress at break, apparent modulus, torsional modulus, and hardness improved with increasing side chain length. When the side chain length is increased, which occurs on both sides of the diester, more non-polar groups are added to both sides of the molecule, which in turn can disrupt more PVC chain-chain interactions. This explains the increased plasticizer effectiveness of the succinates and maleates with longer side chains. Curiously, this effect seems to level off once six carbons in the side chain are reached. In fact, for some experiments, there was a deterioration in plasticizing performance of the n-dioctyl compounds (i.e., stress at break, torsional modulus, and surface hardness, see Table 8.1), meaning that the most effective compounds were those with a side chain length of around six carbons. This deterioration is partly due to the amount of plasticizer within the blends, which is determined by weight, and not by moles, which is standard procedure in the polymer industry. With increasing side chain length, the molecular weight (MW) of the overall compound increases and therefore, given that the same mass of plasticizer is used in every blend (i.e., 40 phr), the corresponding absolute amount of plasticizer-molecules in the blend decreases (i.e., on molar basis, there would be 14 % more DOS molecules compared to DEHP, or 70 % more DBM molecules compared to DEHP). It has been previously shown that addition of dialkyl phthalates on a molar basis to PVC, rather than a mass basis, did indeed result in a linear increase in T_g reduction efficiency with increasing side chain length, and not the above-described maximum efficiency near the

six-carbon compounds (original in German by Würstlin and Klein (1955), described in English by Immergut and Mark (1965)).

The differing number of moles between samples is not the only explanation why the data in Figures 8.4, 8.5, and 8.7 exhibit the afore-mentioned effectiveness maximum near dihexyl compounds. The compatibility of the plasticizers with the PVC resin also plays a role (Immergut and Mark 1965). Several studies have been conducted to investigate the effect of alkyl chain length of diesters on their compatibility with PVC, and similar maxima for compatibility around side chain lengths of six carbons were observed. Compatibility measurements were based on Flory-Huggins theory (Flory 1941, Huggins 1942). Doty and Zable (1946) reported such compatibility maxima for dialkyl phthalates and sebacates using osmotic pressure measurements. Anagnostopoulos et al. (1960, 1962) found compatibility maxima for phthalates (ortho-substituted), isophthalates (meta-substituted), terephthalates (para-substituted), maleates, and fumarates when measuring solid-to-gel temperatures of PVC particles in plasticizers. In a methodologically similar study, Luther et al. (1962) also reported such compatibility maxima for phthalates, adipates, and citrates. Note that sebacates and adipates differ from succinates only in the length of the central chain that connects the two ester functions. Similarly, Würstlin and Klein (1952b), Würstlin and Klein (1956) reported similar curves for phthalates and a matrix of saturated diesters varying both in central chain length (C2-C10) and alkyl side chain (C1-C8), through the measurement of the cloud point (i.e., determined by cooling a dilute solution of PVC dissolved in the plasticizer). In fact, the authors found succinates with side chains from four to six carbons to be most compatible with PVC (Würstlin and Klein 1956, Würstlin 1962).

In all of the above-mentioned studies, the recorded curves went through a local maximum of compatibility between a side chain length of four to six carbons. Stuart et al. (2010) focussed on the T_g reduction efficiency of dialkyl succinates blended with PVC, as measured by differential scanning calorimetry (DSC), and also found the best performing compound to be dihexyl succinate. The above observations match very well with our own observations as shown in Figures 8.4 and 8.5, as well as Table 8.1.

There also exist other studies that focussed on plasticizer effectiveness by material properties measurements rather than compatibility based on fundamental properties as described in the above paragraph. These further support our findings that diesters with unbranched alkyl chains between four and six carbons were the most effective plasticizers. For example, Walter (1954) measured the modulus of elasticity of soft PVC gels plasticized with dialkyl phthalates and observed that blends with dibutyl phthalate had the lowest modulus (i.e., and, hence, the best performance), which is similar to the results reported here in Figure 8.5 and Table 8.1.

8.6.2 Influence of Branching

For each maleate and succinate, one branched diester was tested, in which the side chains resembled those of DEHP, meaning that the alcohol used to esterify the diacid was 2-ethylhexanol. We compared the results of these compounds specifically to the corresponding dihexyl compound as these are similar in overall molecular length (i.e., the longest chain of carbon- and oxygen atoms), with the only difference being the ethyl-branch on the β -carbon in the side chain of the di (2-ethylhexyl) (DEH) compounds. Additionally, we compared the DEH-succinate and -maleate to the corresponding di-n-octyl compounds as these are of similar molecular weight.

Results indicate that there were no added benefits in terms of plasticizer effectiveness for either the succinates or the maleates when the ethyl-branch was present, which has previously been described for phthalates (Rahman and Brazel 2004). In fact, for stress at break and apparent modulus at 25 %EL, the ethyl branches seemed to have a negative effect on these properties when compared to the equally long, linear dihexyl compounds. No statistically significant differences between the compounds of equal molecular weight, DEHS/DOS and DEHM/DOM were observed, except for strain at break (DEHS/DOS) and apparent modulus at 25%EL (DEHM/DOM). Overall, this suggests that the more important factor in plasticizer efficiency is the overall molecule length rather than the molecular weight. Similar observations were reported previously by Würstlin and Klein (1955) for dialkyl phthalates, where in every case the branched phthalates showed a poorer T_g reduction efficiency compared to their unbranched counterparts of equal molecular weight.

8.6.3 Influence of Central Structure

The central structure of diesters played an equally important role as the side chains with respect to plasticizer effectiveness. Purely non-polar compounds containing only alkyl chains would not be compatible with PVC due to the inherent polar nature of the PVC chains. This means that in order to retain the non-polar part of the plasticizer - which plays the more active role in plasticization (i.e., the alkyl chain part) - within the PVC matrix, one or more polar parts that interact with PVC are also needed. However, the polarity of these groups must be of the same order of magnitude as the PVC polarity, otherwise there would not be a sufficient interaction between the two and phase separation would occur. Diesters have been shown to have the required degree of polarity (Smyth 1955, Leuchs 1956), thus rendering diester molecules good candidates for PVC plasticizers.

As indicated in Figure 8.1, the maleate contains a double bond between the two central carbon atoms, thereby locking the orientation of the two ester groups towards one another in the shown *cis* position. Both dipoles inherent to the ester groups would be pointing essentially in the same general direction (i.e., δ - on the carbonyl oxygen, and δ + on the carbonyl carbon), allowing this compound to interact with one or two PVC chains at two different locations. The same would apply to DEHP, where the ester groups are also in a similar position to one another. The specific orientation of the diester molecules towards one another has also been shown to play an important role in its plasticizer effectiveness: that is, in previous work, we were able to show that the fumarate molecule, a structural isomer to the maleate except for the positioning of the ester bonds towards one another, did not reduce the Tg in blends with PVC as effectively as the maleate (Erythropel et al. 2012). In the fumarate, the ester groups are in a trans position relative to one another, resulting in the dipoles of the ester groups pointing in different directions. Finally, in the saturated succinate molecule, there is no double bond present, with the result that the succinate is free to rotate around the central bond. Because of this, the succinates would be free to take the most advantageous position for interaction with PVC chains within the polymer matrix. In earlier work, we showed that Tg reduction efficiency of succinates and maleates were at comparable levels (Erythropel et al. 2013, Erythropel et al. 2015), and similar observations were made in this study for several other material properties.

The structure of DINCH[®] is slightly more complicated, due to presence of the cyclohexane ring, which unlike the phthalate is not planar and can take on a range of spatial

conformations. The "chair"-conformation is generally the most stable conformation, especially when bulky substituents are present (see Figure 8.8) (Ouellette and Rawn 2014). In a 1,2-disubstituted cyclohexane such as DINCH®, these substitute groups are on neighbouring carbons of the ring and can be in *cis*- and in *trans* positions relative to each other, as shown in Figure 8.8 (a ,b: *trans*; c, d: *cis*) (Ouellette and Rawn 2014). Additionally, due to the cyclic structure of cyclohexane, substituents can be in an equatorial position and in an axial position, resulting in the four possibilities shown in Figure 8.8; the first row (a) and (b) shows the *trans*-configuration, in which both substituents can either be in the equatorial (Figure 8.8a) or the axial position (Figure 8.8b). In a *trans*-DINCH®, the favourable conformation would be the one in which both bulky substituents are positioned equatorially (Figure 8.8a), due to the reduced steric interaction with neighbouring atoms, as compared to the axial position (Figure 8.8b) (Ouellette and Rawn 2014). For a *cis*-DINCH®, one substituent has to be in axial and one in equatorial position, which means that there is no preferred conformation between those shown in Figure 8.8c and 8.8d when both substituents are similar, as is the case for DINCH®.



Figure 8.8. Cyclohexane in the "chair" conformation with equatorial substituents indicated in bold: a) R-substituents *trans* and in equatorial position; b) R-substituents *trans* and in axial position; c) and d) R-substituents *cis* with one substituent in axial and one substituent in equatorial position.

No detailed information was provided by the supplier on the composition of the DINCH® sample tested (which also contains structural isomers stemming from the mixture of alcohols used to create the ester bond), so it is assumed that the sample likely consisted of a mixture of *cis/trans* isomers. In such a mixture, one would thus expect the conformations shown in Fig. 8.8a), 8.8c), and 8.8d) to be predominantly present. Upon examination, it can be seen that all 3 are more closely related to the maleate in terms of the ester positioning than the fumarate (i.e., compare Figure 8.1 to Figure 8.8a, 8.8c, 8.8d). Only the axial conformation of the *trans*-DINCH® (Figure 8.8b) would resemble the fumarate rather than the maleate but, as explained above, this configuration is sterically unfavourable. Taken together, this explains why DINCH® is an effective plasticizer.

However, for some of the experiments carried out in this study, the plasticizer performance of DINCH[®] was lower compared to DEHP and the maleates with chain lengths of four carbons (i.e., when comparing stress at break, apparent modulus, torsional modulus, and surface hardness). This suggests that the positioning of the ester groups relative to one another is not as advantageous in DINCH® as in DEHP or the maleates with side chains of at least four carbons. Given that DINCH® is likely a mixture of isomers, this is not surprising and similar experiments conducted with pure cis- and trans-DINCH® would bring more insight. Comparing plasticizer effectiveness of DINCH® to the succinates with side chains of at least four carbons, similar results were found: that is, values for stress at break, apparent modulus, torsional modulus, and surface hardness were lower for the succinates, indicating higher plasticizer effectiveness. In both DINCH® and the succinate, the carbonyl groups are linked by two saturated carbons, but rotation around the central bond is only possible in the succinate. In DINCH® rotation cannot occur due to the presence of the cyclohexyl-ring (see Figure 8.1). In earlier work, we demonstrated that the ability of the central chain to rotate plays an important role in T_g reduction effectiveness (Erythropel et al. 2013), and the results for the mechanical properties tested in this study further support this.

Overall, whenever a side chain length of at least 4 carbons was present in our candidate plasticizers, members of both series of compounds showed comparable or higher plasticizer effectiveness than the commercial plasticizers. In comparison to DEHP, better performance was found for stress at break and apparent modulus. However, in comparison with DINCH®, the mentioned succinates and maleates with side chains of at least four carbons showed better

performance with respect to stress at break, apparent modulus, torsional modulus, and surface hardness. Differences between the succinate and maleate series seemed rather minor, from which we conclude that from a plasticizing effectiveness standpoint, both central structures make such molecules feasible alternatives to DEHP and DINCH®.

8.6.4 Global Structural Considerations

A credible alternative plasticizer must be one that is not only an effective plasticizer in comparison with current commercially-available compounds, but it must also be less hazardous to the environment and human health. In this work, we focussed primarily on evaluating the plasticizing effectiveness of compounds in terms of final PVC formulation material properties for which green characteristics had already been observed (Erythropel et al. 2013, Erythropel et al. 2015).

From Table 8.1, the dibutyl- and dihexyl compounds DBS, DBM, DHS, and DHM stand out as the compounds with the highest plasticizer effectiveness within their respective series, but also when compared to the commercial plasticizers DEHP (with lower stress at break, apparent modulus, and torsional modulus, and comparable strain at break and surface hardness) and DINCH[®] (with higher strain at break and lower stress at break, apparent modulus, surface hardness). Additionally, these compounds also lowered the T_g more efficiently than DEHP (Erythropel et al. 2013, Erythropel et al. 2015).

Higher plasticizer efficiency is important as it means that a lower concentration (resulting in a smaller mass) of plasticizer would need to be incorporated into PVC to obtain the desired product properties. Such an effect could possibly overcome the higher cost premium that these compounds may have relative to DEHP or other current commercial plasticizers.

As alluded to above, the mechanical properties alone are not the only important characteristics that need to be considered when designing alternative plasticizers, but important factors including biodegradation, environmental persistence and toxicity must also be given due consideration. Earlier work focussed on assessing the biodegradability of the succinate and maleate plasticizer candidates compared to DEHP (Erythropel et al. 2013, Erythropel et al. 2015) and additional studies with respect to toxicity are ongoing. An added important advantage that one alternative plasticizer may have over another would be if the compound

could be produced from renewable resources. In this respect, succinate diesters could be of particular interest because of the increased fermentative production capacities of succinic acid (Debabov 2015), which could lead to a product that is more fully in line with the principles of green chemistry (Anastas and Warner 2000).

8.7 Conclusion

Our results lend further support to earlier findings that: (1) within the diesters of a central-chain length of four carbons, an unsaturated compound in *cis* position is about as effective as the saturated compound (Erythropel et al. 2012); (2) between such compounds, the length of the side chain plays a more important role than the central structure (Erythropel et al. 2013, Erythropel et al. 2015); and (3) free rotation around the central bond plays an important role in the plasticizer effectiveness of saturated compounds (Erythropel et al. 2013).

We have demonstrated that diester plasticizers based on succinic and maleic acid with linear side chains between four and eight carbons in length are as effective as commerciallyavailable DEHP and DINCH[®] in terms of plasticizing PVC resin, and for some tests even improved mechanical properties were apparent. The length of the alcohol used to create the ester bonds, which determines the alkyl side chain length of the diester, had a strong influence on plasticizer properties: for both succinates and maleates, the compounds with a side chain length of four to six carbons showed the highest plasticizer effectiveness. In terms of alkyl branching within the molecule, adding an ethyl-branch (such as that present in DEHP) did not improve plasticizer performance, but instead deteriorated some mechanical properties. When comparing the two central groups (i.e., saturated, and non-saturated in *cis* configuration), some differences were observed, but these were minor. Taken together, this suggests that the right choice of alcohol to produce diesters with a certain side chain length is as important as the choice of the central structure to produce effective plasticizers.

8.8 Acknowledgements

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9 Conclusion and Future Work

9.1 Conclusion

The long-term goal of this research is ultimately focussed on developing green alternatives to the problematic phthalate plasticizers. In light of this goal, the global research questions of this thesis were to establish the main routes of human exposure to phthalates, and to assess how the central group structure and the side chain length and branching of replacement candidates affect plasticizer performance in blends with PVC and the biodegradation rate and the buildup of metabolites. Specific research objectives have been addressed through the work presented here in a series of papers that collectively showed good potential for succinate and possibly maleate diesters with side chain lengths of around six carbons.

In the first manuscript presented in Chapter 4, literature sources were used to establish the main routes of human exposure to the most important and increasingly critically viewed commercial plasticizer DEHP (Objective 1). While an increasing amount of studies suggests that DEHP levels in bottled water are a source of concern, it was shown that two other routes lead to human exposure at several orders of magnitude higher levels: i.e., through fatty foodstuff and through hospital equipment. Although exposure levels of the general population to phthalates are probably below the safe limits used by several jurisdictions, certain vulnerable groups may still be at risk (i.e., children in the pre- and perinatal period, and individuals with high hospitalization frequency). It is concluded that focus on exposure through bottled water should be of low priority if one wants to reduce exposure levels to DEHP. Furthermore, the review advocates a phasing-out of phthalate diesters based on the precautionary principle.

The remainder of the thesis focussed on the investigation of several synthesized small diesters that structurally resemble DEHP. The work addressed questions on how the central group structure and the side chain length and branching affect plasticizer performance and biodegradation behaviour and it was found that the central structure played a key role and that the length of the alkyl side chains was also important.

The second manuscript presented in Chapter 5 focussed entirely on the influence of the central structure through the study of compounds that contained 2-ethyhexyl side chains, including DEHP. It was shown that the spatial orientation of the carbonyl groups of the diester

molecule is a very important factor for both T_g reduction potential (Objective 4) and biodegradation rate and metabolite buildup (Objectives 2 and 3). The T_g reduction efficiency was comparably high for compounds in which the ester groups stood in *cis* position towards each other, as in the maleate, or if free rotation around the central bond was possible, as in the succinate. This observation was also made in mechanical tests of plasticized PVC presented in Chapter 8, where no significant differences between similarly long maleate and succinate plasticizers were observed (Objective 5). In Chapter 6, T_g reduction measurements showed that hindering the rotation around the central bond decreased the T_g reduction efficiency of the succinate diesters (Objective 4). Taken together, the spatial arrangement of the diesters as present in the maleate or the phthalate (i.e., both carbonyl-oxygens on the same side of the diester molecule) is advantageous and leads to good plasticizer efficiency. However, if the molecule is saturated and can freely rotate, it is able to rotate to a similarly advantageous position, explaining the good plasticizer performance of both the maleate and succinate.

The biodegradation studies using the common soil bacterium *Rhodococcus rhodocrous* also showed that the molecular geometry of the central bond was very important in determining the biodegradation rates of the compound. Saturated compounds were hydrolysed more quickly (Chapter 5) and any steric hindrance affecting the rotatability around the central bond in turn affected the biodegradation rate negatively (Chapter 6). For unsaturated structural isomers such as the fumarate (*trans*) and the maleate (*cis*), it was shown the molecule in *trans* orientation was broken down more quickly than the one in *cis* orientation (Chapter 5; Objective 2). The *cis* compound di (2-ethylhexyl) maleate, which significantly resembles DEHP in terms of its structure, showed little degradation over the course of the 30 days timeframe of the experiments. This helps in explaining why DEHP is rather slowly biodegraded in the environment, as the ester groups in DEHP are in a position to each other that resembles the *cis*-maleate (Chapter 5). Taken together, it is apparent that, similar to the results concerning plasticizer performance, the ability of a molecule to freely rotate around the central bond is the key factor to achieve higher biodegradation rates, as it is likely easier for the bacteria to reach the ester bonds to hydrolyse them.

In terms of metabolite production and removal (Objective 3), the described differences in hydrolysis rates between the plasticizer candidates were also observed for the rate of the second hydrolysis step from the monoester to the diacid (Chapters 6 and 7). Owing to this, it can be concluded that saturated diesters are better plasticizer candidates in terms of biodegradability, as only a transient buildup of the corresponding monoester was found. Again, avoiding metabolite buildup altogether, and particularly that of the corresponding monoester, would be beneficial from an environmental standpoint. In the case of DEHP, it is its monoester MEHP that is believed to be the bioactive endocrine disruptor.

The other important factor examined in this work was the effect of the length of the side chain on plasticizer performance and biodegradation behaviour. The effect of chain length on succinate and maleate diesters was examined in Chapters 6 and 7, respectively. In both studies it was shown that T_g reduction efficiencies increase from the shortest to the longest compound (Objective 4), yet the mechanical tests performed in Chapter 8 only partially confirmed this, as plasticizer performance levelled off once a side chain length of four carbons was reached (Objective 5). The best performance was generally seen for the compounds with side chain lengths of around six carbons. Furthermore, it was shown that the ethyl-branch present in the di (2-ethylhexyl) diesters did not enhance plasticizer performance, but that the determining factor was the overall length of the molecule (Objectives 4 and 5).

The buildup of the potentially problematic compound 2-ethylhexanoic acid, which is the stable oxidation product of 2-ethylhexanol and thus a metabolite of DEHP, could be avoided when unbranched, linear side chains were added by using the appropriate alcohol for the esterification reaction (Chapters 6 and 7). It was further shown that longer molecules were slower to biodegrade than shorter ones (Objective 2), independent of the central structure, and this behaviour is probably related to the decreased solubility of these in water, resulting in lower bioavailability. The liberated alcohols were quickly removed from the biodegradation broth and, if liberated sufficiently quickly, were consumed preferentially over hexadecane as carbon source by the microbes (i.e., for the succinates in Chapter 6, but not for the maleates in Chapter 7; Objective 3). On the other hand, the study in Chapter 7 revealed that one metabolite had a negative effect on the bacteria: monooctyl maleate was shown to have a growthinhibiting effect in separate experiments (Objective 3). This behaviour provides a good example as to why all compounds must be thoroughly investigated for many ecological and toxicological endpoints and further testing of these particular compounds is needed. Another advantage of the presented maleate and succinate diesters is that they can be produced through a similar process as phthalate diesters. That is, the starting point is usually the anhydride (or the diacid), which is subsequently esterified with two equivalents of an alcohol. Phthalates are usually synthesized through reactive distillation (Berman et al. 1948) and it is likely that a facility producing phthalate diesters could be modified in such a way that maleates or succinates could be produced instead. An additional reason to pursue the succinate diesters is the recent commercialisation of succinic acid production by way of fermentation (Debabov 2015), which could lead to a renewably-sourced plasticizer.

In conclusion, this work showed the potential for several maleate and succinate compounds to be used as an alternative to phthalate plasticizers. However, toxicity studies of these compounds are still required to get a more complete picture. Combining the summary above with a study regarding leaching into water (Kastner et al. 2012), it seems that the ideal compounds for further testing are especially dihexyl- and dioctyl succinate, as well as dihexyl maleate.

9.2 Future Work

While the general suitability of succinate and maleate diesters to be used as plasticizers for PVC was established in the present work, in order to truly assess how "green" these compounds are, general and specific toxicity studies need to be conducted to ensure that the use of these compounds do not lead to adverse effects when they enter the humans, animals, and the environment.

As outlined by the US EPA, specific toxicity endpoints to be investigated should include carcinogenicity, skin sensitization, and reproductive-, developmental-, neurological-, systemic- and genotoxicity (US EPA 2005) with respect to human health, as well as acute and chronic eco-toxicity. However, the degree to which such tests are required varies with the intended use of the material and projected final exposure of such products. For example, PVC plasticized with the proposed green plasticizer candidates used in sensitive applications (e.g., hospital equipment) would require extensive toxicology testing. Other tests should include more specific mechanical testing such as puncture resistance, leaching into matrices other than RO-water and processing parameters as determined for example through rheology. Such tests would be necessary depending on the area of intended use of these compounds.

Further focus should also be placed on exploring the potential of the succinate diesters due to the increased microbial production and thus renewable sourcing of succinic acid (Debabov 2015). If the alcohol used to make the diester were also to be renewably sourced, the whole plasticizer molecule would be a more sustainable alternative to petroleum-derived products. Such products could be of immense commercial importance, especially as phthalate plasticizers are gradually driven from the market due to evolving government regulations.

10 Original Contributions to knowledge

Most significantly, this research has established that some succinate and maleate diesters may be feasible candidates for the replacement of phthalates because of their comparable or better plasticizing properties combined with higher biodegradability. However, final judgement as to their suitability as alternative plasticizers must be reserved in the absence of thorough toxicity testing. That being said, through the work presented in this thesis, important significant advances in the development of green plasticizers have been made. In particular, it was demonstrated that:

- (1) The physicochemical properties of DEHP and the knowledge of its predominant use in PVC products are the amongst the most important factors in understanding which kinds of products can lead to increased human DEHP exposure. Due to its lipophilic nature, DEHP accumulates in materials with high lipid content. The source of DEHP contamination in different products can be either direct leaching of DEHP if the material does in fact contain DEHP, but also through environmental accumulation of DEHP in the mentioned high-lipid foods and materials.
- (2) The molecular geometry of diesters plays an important role in the plasticization efficiency of these in blends with PVC. More specifically, saturated diesters guaranteeing unrestricted rotation around the central bond, or those where the ester moieties point in the same general direction (as opposed to being on opposite ends of a carbon chain such as the fumarate molecule), make more effective plasticizers.
- (3) The determining factor for plasticization effectiveness in any series is the overall molecule length, rather than the degree of branching or the molecular weight, as seen with chain lengths between two and eight carbons. Further tests with longer alkyl side chains would have to be carried out to see whether the described trends follow the same pattern, or if the deciding factor is a ratio between non-polar and polar parts, as has been suggested by van Veersen and Meulenberg (1964, 1966, 1967, 1972)

- (4) The structural feature that led to the most effective PVC plasticizers in this work (i.e., side chain length of around six carbons) had been previously shown to also lead to the most compatible PVC plasticizers. This further confirms the proposed interdependence of the two properties (i.e., plasticizer efficiency and compatibility) proposed by Boyer (1951).
- (5) The dibutyl and dihexyl diesters of maleic and succinic acid exhibited better plasticization effectiveness in several of the mechanical tests carried out in this work, when compared to commercially used DEHP and DINCH plasticizers.
- (6) Hydrolysis rates of small diesters by common soil bacteria such as *Rhodococcus rhodocrous* are strongly dependant on the structural features of these compounds. Those compounds that are free to rotate around the central bond were most easily hydrolysed, likely because access to the ester-bond was most facile for the microbes. Hydrolysis rates decreased as bulky groups on the central chain hindered this free rotation, although steric hindrance from the attached group itself probably also plays a role in the slowed rates. When no rotation was possible, such as in the maleate (*cis*) and fumarate (*trans*) compounds containing a double bond, hydrolysis rates between the two. This might be linked to the distribution of charge in the overall molecule, which depends on the *cis/trans*-positioning of the ester functionalities.
- (7) The ethyl-branches on the β-carbon in the di (2-ethylhexyl) compounds caused a significant decrease in plasticizer biodegradation rates by *Rhodococcus rhodocrous*, when compared to compounds with linear side chains. Within the linear compounds, those with smaller side chains generally showed higher biodegradation rates.
- (8) Monooctyl maleate showed growth-inhibiting effects on *Rhodococcus rhodocrous*. However, the exact mechanism remains unclear and further experiments are necessary to investigate this effect.

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Appendix A

NMR spectra for Chapter 6: Designing Green Plasticizers: Influence of Alkyl Chain Length on Biodegradation and Plasticization Properties of Succinate Based Plasticizers.

Dibutyl succinate (DBS):

¹H-NMR (300.1 MHz in CDCl₃): δ (ppm) = 0.90 [t, 6H, CH₂C<u>H₃]</u>, δ (ppm) = 1.30 [m, 4H, CH₂C<u>H₂CH₃]</u>, δ (ppm) = 1.60 [m, 4H, CH₂C<u>H₂CH₂]</u>, δ (ppm) = 2.60 [s, 4H, CO(C<u>H₂)₂CO]</u>, δ (ppm) = 4.10 [t, 4H, COOC<u>H₂CH₂]</u>

Dihexyl succinate (DHS):

¹H-NMR (500.1 MHz in CDCl₃): δ (ppm) = 0.90 [t, 6H, CH₂C<u>H₃]</u>, δ (ppm) = 1.35 [m, 12H, CH₂(C<u>H₂</u>)₃CH₃], δ (ppm) = 1.60 [m, 4H, COOCH₂C<u>H₂</u>CH₂], δ (ppm) = 2.60 [s, 4H, CO(C<u>H₂</u>)₂CO], δ (ppm) = 4.05 [t, 4H, COOC<u>H₂</u>CH₂]

Dioctyl succinate (DOS):

¹H-NMR (400.1 MHz in CDCl₃): δ (ppm) = 0.85 [t, 6H, CH₂C<u>H₃]</u>, δ (ppm) = 1.30 [m, 20H, CH₂(C<u>H₂)₅CH₃]</u>, δ (ppm) = 1.60 [m, 4H, COOCH₂C<u>H₂</u>CH₂], δ (ppm) = 2.60 [s, 4H, CO(C<u>H₂)₂CO]</u>, δ (ppm) = 4.05 [t, 4H, COOC<u>H₂</u>CH₂]

Di (2-ethylhexyl) succinate (DEHS):

¹H-NMR (500.1 MHz in CDCl₃): δ (ppm) = 0.92 [t, 12H, CH₂C<u>H₃]</u>, δ (ppm) = 1.25 [m, 12H, CH₂(C<u>H₂</u>)₃CH₃], δ (ppm) = 1.40 [dd, 4H, CH(C<u>H₂</u>CH₃)], δ (ppm) = 1.60 [m, 2H, CH₂C<u>H</u>], δ (ppm) = 2.62 [s, 4H, CO(C<u>H₂</u>)₂CO], δ (ppm) = 4.00 [m, 4H, OC<u>H₂</u>CH]

Dihexyl 2-methyl succinate (DHMS):

¹H-NMR (300.1 MHz in CDCl₃): δ (ppm) = 0.90 [t, 6H, CH₂C<u>H₃]</u>, δ (ppm) = 1.20 [d, 3H, COCH(C<u>H</u>₃)CH₂CO], δ (ppm) = 1.30 [m, 12H, CH₂(C<u>H</u>₂)₃CH₃)], δ (ppm) = 1.60 [m, 4H, COOCH₂C<u>H</u>₂], δ (ppm) = 2.39 [dd, ²J = 16.5 Hz, ³J = 8.1 Hz, 1H, COCH(CH₃)C<u>H</u>₂CO], δ (ppm) = 2.69 [dd, ²J = 16.2 Hz, ³J = 6.0 Hz, 1H, COCH(CH₃)C<u>H</u>₂CO], δ (ppm) = 2.90 [m, 1H, COC<u>H</u>(CH₃)CH₂CO], δ (ppm) = 4.05 [m, 4H, COOC<u>H</u>₂CH₂]

Dihexyl 2-cyclohexyl succinate (DHCHS):

¹H-NMR (300.1 MHz in CDCl₃): δ (ppm) = 0.90 [t, 6H, CH₂C<u>H₃]</u>, δ (ppm) = 1.00 – 1.80 [m, 27H, CH₂(C<u>H</u>₂)₄CH₃), COCH(C₆<u>H</u>₁₁)CH₂CO], δ (ppm) = 2.40 [m, 1H, COCH(C₆H₁₁)C<u>H</u>₂CO], δ (ppm) = 2.70 [m, 2H, COC<u>H</u>(C₆H₁₁)C<u>H</u>₂CO], δ (ppm) = 4.05 [m, 4H, COOC<u>H</u>₂CH₂]

Dihexyl 4-methyl cyclohexyl-1,2-dicarboxylate (DHMCH):

¹H-NMR (500.1 MHz in CDCl₃): δ (ppm) = 0.90 [m, 6H, CH₂C<u>H₃]</u>, δ (ppm) = 0.95 [d, 3H, CHC<u>H₃]</u>, δ (ppm) = 1.15 - 1.65 [m, 20H, CH₂(C<u>H₂)4</u>CH₃, COCH(C<u>H₂CH(CH₃)CH₂C<u>H₂</u>)CHCO], δ (ppm) = 1.75 - 1.90 [m, 1H, COCH(C<u>H₂CH(CH₃)CH₂CH₂</u>)CHCO], δ (ppm) = 1.95 - 2.05 [m, 1H, COCH(C<u>H₂CH(CH₃)CH₂C<u>H₂</u>)CHCO], δ (ppm) = 2.15 - 2.25 [m, 1H, COCH(C<u>H₂CH(CH₃)CH₂C<u>H₂</u>)CHCO], δ (ppm) = 2.35 - 2.45 [m, 1H, COC<u>H(C₅H₁₀)CH</u>CO], δ (ppm) = 3.15 - 3.25 [m, 1H, m, 1H, COC<u>H</u>(C₅H₁₀)C<u>H</u>CO], δ (ppm) = 4.05 [m, 4H, COOC<u>H₂CH₂</u>]</u></u></u>

Monobutyl succinate (MBS):

¹H-NMR (500.1 MHz in CDCl₃): δ (ppm) = 0.9 [t, 3H, CH₂C<u>H₃]</u>, δ (ppm) = 1.35 [m, 2H, CH₂C<u>H₂CH₃</u>], δ (ppm) = 1.6 [m, 2H, CH₂C<u>H₂CH₂</u>], δ (ppm) = 2.63 [t, 2H, CH₂OOCC<u>H₂CH₂CH₂COOH</u>], δ (ppm) = 2.69 [t, 2H, CH₂OOCCH₂C<u>H₂COOH</u>], δ (ppm) = 4.1 [t, 2H, COOC<u>H₂CH₂</u>]

Monohexyl succinate (MHS):

¹H-NMR (500.1 MHz in CDCl₃): δ (ppm) = 0.9 [t, 3H, CH₂C<u>H₃]</u>, δ (ppm) = 1.3 [m, 6H, CH₂(C<u>H₂</u>)₃CH₃)], δ (ppm) = 1.6 [m, 2H, CH₂C<u>H₂</u>CH₂], δ (ppm) = 2.63 [t, 2H, CH₂OOCC<u>H₂</u>CH₂COOH], δ (ppm) = 2.69 [t, 2H, CH₂OOCCH₂C<u>H₂</u>COOH], δ (ppm) = 4.1 [t, 2H, COOC<u>H₂</u>CH₂]

Monooctyl succinate (MOS):

¹H-NMR (500.1 MHz in CDCl₃): δ (ppm) = 0.9 [t, 3H, CH₂C<u>H₃]</u>, δ (ppm) = 1.3 [m, 10H, CH₂(C<u>H₂)₅CH₃</u>], δ (ppm) = 1.6 [m, 2H, CH₂C<u>H₂CH₂</u>], δ (ppm) = 2.63 [t, 2H, CH₂OOCC<u>H₂CH₂CH₂COOH</u>], δ (ppm) = 2.69 [t, 2H, CH₂OOCCH₂C<u>H₂COOH</u>], δ (ppm) = 4.1 [t, 2H, COOC<u>H₂CH₂</u>]

Appendix B

DSC data for Chapter 7: Designing Greener Plasticizers: Effects of Alkyl Chain Length and Branching on the Biodegradation of Maleate Based Plasticizers

Table B1 - File name, page number in Appendix A, corresponding compound, and extracted $T_{ei,g}$, $T_{m,g}$, and $T_{ef,g}$. $T_{ei,g}$: extrapolated onset temperature, $T_{m,g}$: midpoint temperature (T_g), $T_{ef,g}$: extrapolated end temperature (ASTM D-3418).

File Name	Appendix Page #	Compound	T _{ei,g} (°C)	$T_{m,g}(^{\circ}C)$	$T_{ef,g}(^{\circ}C)$
40etmal.001	2	DEM	-35.16	-15.14	4.92
40butmal.003	3	DBM	-41.49	-19.85	1.82
40hexmal.001	4	DHM	-50.66	-21.65	7.48
40hexmal_oct.004	5	DHM	-48.95	-23.51	1.93
DHM_40phr_mod.002	6	DHM	-44.25	-19.07	6.06
40octmal_modulated.001	7	DOM	-57.42	-32.06	-7.88
40octmal.001	8	DOM	-61.58	-28.56	4.52
40b2ehmal_2.001	9	DEHM (non-mod)	-56.74	-25.14	5.98
40b2ehmal_3.001	10	DEHM (non-mod)	-58.70	-24.01	9.37
DEHM_40phr_mod.001	11	DEHM	-51.01	-20.23	10.37
40DEHPmod0_5SA5.001	12	DEHP	-24.26	-2.16	20.07
40DEHPmod0_5SA4.001	13	DEHP	-28.36	-4.25	20.14
40DEHP.001	14	DEHP	-25.19	-2.92	19.32



























Appendix C

DMTA data for Chapter 8: Designing Green Plasticizers: Influence of Molecule Geometry and Alkyl Chain Length on the Plasticizing Effectiveness of Diester Plasticizers in PVC Blends.




















