Stakeholder Technical Workshop Document

Approach for Using Chemical Categories and Read-Across to Address Data Gaps for Effects on the Developing Male Reproductive System

Phthalate Substance Grouping

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1. Introduction

1.1 Phthalate Substance Grouping under the Chemicals Management Plan (CMP)

The Phthalate Substance Grouping is a group of substances identified as a priority for assessment pursuant to the *Canadian Environmental Protection Act, 1999* (CEPA 1999) under the CMP (Government of Canada 2012).

This grouping of substances is based on chemical similarity and common use in plasticizers, adhesives, sealants, paints and coatings, plastic and rubber materials, and automotive parts in Canada that could result in exposure to the general population, including children. The selection of the 14 substances covered under the Phthalate Substance Grouping was based on the categorization process completed in 2006, and new information received as part of the first phase of the CMP (Health Canada 2009).

An additional 14 phthalates on the *Domestic Substance List* (DSL) are being considered because they may inform the assessment being conducted under the CMP, including a potential cumulative risk assessment (CRA).

This document will refer to the 14 phthalates in the CMP Phthalate Substance Grouping and the additional 14 phthalates collectively as “phthalates of interest”.

1.2 Background

The purpose of this document is to describe the approach used to generate chemical categories for read-across to primarily address data gaps for effects of certain phthalates on the developing male reproductive system in rats.

Adverse effects on the development of the male reproductive system of laboratory animals are a sensitive health outcome following administration of certain phthalates (see Section 3 for details). These effects will inform both the health effects assessments of individual phthalates as well as a potential CRA being considered under the CMP. This document provides a general overview of some of the adverse effects observed for certain phthalates on the developing male reproductive system following oral administration (diet and/or gavage) during the gestational period in rats. The current understanding for the proposed mode of action for these effects is also summarized (Section 3). The scope of developmental toxicity studies considered within this document is limited to those where phthalates are administered during the critical window of male reproductive development in rats. Multi-generation reproductive toxicity studies are also considered as dosing typically covers the relevant developmental window. Certain phthalates of interest are considered to have ‘data gaps’ as they do

not have studies available to assess effects on the developing male reproductive system during this critical window. To facilitate addressing these data gaps, a structure activity relationship (SAR) analysis across the 28 phthalates of interest using available studies has been developed and presented in this document (Section 5.4). Based on the SAR analysis, subcategories of phthalates are proposed relating to effects on the developing male reproductive system in rats (Section 6). The subcategories, along with other considerations, are subsequently used to facilitate read-across for effects on the developing male reproductive system for phthalates lacking relevant health effects studies (Section 7).

This proposed approach was developed by staff in the Existing Substances Risk Assessment Bureau (ESRAB) at Health Canada. The data presented in this document was collected as part of Health Canada's scientific review of hazard data identified from a targeted search of publicly available sources of information. The information presented is a summary of a selection of studies related to phthalate developmental effects of the male reproductive system in rats and the proposed mode of action that were available up to December 2013.

1.3 Chemical Category and Read-Across Approach

The chemical category approach is an assessment methodology that considers closely-related chemicals as a group, or category, rather than as individual chemicals. In the category approach, the overall data for a category can provide relevant information to inform a health effects assessment (OECD 2007).

It is recognized that chemicals whose physical-chemical and toxicological properties are likely to be similar or follow a regular pattern as a result of structural similarity may be considered as a group or 'category' of chemicals in order to characterize potential health effects. A strength of this methodology is that it supports health effects characterization when one or more substances in a category lack data for one or more endpoint(s), or when there are challenges with data adequacy for some substances in a category (e.g., low quality studies). Additionally, for some categories there is a basis for establishing subcategories.

The methodology of using data from (a) similar chemical(s) to predict endpoint or property information for one or more substances that lack empirical data is generally referred to as the 'read-across' approach. Determining similarity of a group of substances is described in the OECD Guidance Document on Chemical Groupings (OECD 2007). General elements that should be considered when justifying the read-across approach between 'similar' chemicals to fill data gaps include chemical structure, composition, toxicokinetics, physical-chemical properties, mechanism/mode of action and responses found in alternative assays (e.g., toxicogenomics, *in vitro* cell systems,

or other screening assays). These elements should be considered within the context of the endpoint for which the approach is used (OECD 2007; ECHA 2008).

The read-across of data can be qualitative or quantitative. In qualitative read-across, the presence (or absence) of toxicity for the untested chemical is inferred from the presence (or absence) of the same toxicity for one or more similar chemicals with data. Qualitative read-across gives a “yes/no” extrapolation for an effect. In quantitative read-across, the known value(s) of a property for one or more similar chemicals is used to estimate the unknown value of the same property for the untested chemical. Quantitative read-across is used to obtain a quantitative value for an endpoint, such as a dose-response relationship [e.g., No Observed (Adverse) Effect Level (NO(A)EL), Lowest Observed (Adverse) Effect Level (LO(A)EL)] (OECD 2007).

1.3.1 Phthalate-specific chemical category approaches

The proposed approach takes into consideration the Guidance on Grouping of Chemicals provided by the Task Force on Hazard Assessment of the Organisation for Economic Co-operation and Development (OECD) (OECD 2007).

In addition, there have been initiatives focused on developing phthalate-specific categories for characterizing male developmental reproductive toxicity (and other endpoints) in laboratory animals and examples are presented in Table 1-1 below. These were considered when developing the proposed approach and adapted to reflect newly available information and the phthalates of interest under the CMP.

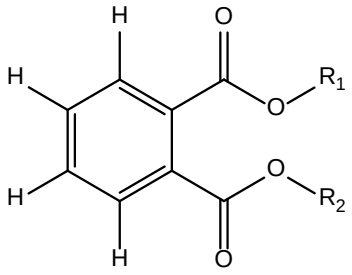
Table 1-1: Examples of uses of the category approach to assess adverse developmental and reproductive effects of phthalates

Report	Category Definition(s)	Summary	Reference
Proposal for Harmonised Classification and Labelling of Diisohexyl Phthalate (DIHP)	*Ortho* phthalate diesters with an alkyl chain of at least 3 carbon atoms, but not more than 6 carbon atoms long (C3-C6).	Swedish Chemicals Agency submitted proposal. No mammalian fertility or developmental toxicity studies were available for DIHP. For the purpose of filling data gaps of reproductive toxicity for the harmonized classification and labelling of DIHP, a chemical category was established according to OECD recommendations. The category comprised of *ortho*-phthalates with side chain lengths of 3 to 6 carbons. Read-across was used to characterize the endpoint.	(ECHA, 2012)
A Category Approach for Reproductive Effects of Phthalates	*Ortho* phthalate diesters with an alkyl chain of at least 4 carbon atoms, but not more than 6 carbon atoms long (C4-C6). Both linear and branched alkyl carbon chains are included.	The review examined the assessments of phthalates of various alkyl chain lengths (C3 – C11). The authors conclude that phthalates with a carbon range of 4-6 produce similar severe reproductive effects in experimental animals. It was noted that for somewhat smaller and longer alkyl chains, reproductive effects are expected but needed further consideration prior to including in the category. The authors propose using the C4-C6 category for classification and labelling under the Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH).	(Fabjan et al. 2006)

Report	Category Definition(s)	Summary	Reference
High Production Volume Program Test Plan: Phthalate Esters Category	*Ortho* phthalate diesters with alkyl chains in various ranges (subcategories). Subcategory I: Low Molecular Weight Phthalate Esters (C1-C3). Subcategory II: Transitional Phthalate Esters (C4-C6). Subcategory III: High Molecular Weight Phthalate Esters (C≥7).	The HPV Sponsor proposed subdividing the *ortho* phthalates into three subcategories based on similar physicochemical and toxicological properties. The (sub)category approach was used for read-across between members for both environmental and human health endpoints (including developmental and reproductive toxicity).	(ACC 2006)
OECD Screening Information Dataset (SIDS) Initial Assessment Profile (SIAP) – Hazard Assessment	*Ortho* phthalate diesters High Molecular Weight Phthalate Esters (HMWPE) Category consisting of esters with an alkyl carbon backbone of greater than 7.	The SIAP used the high molecular weight phthalate ester category to apply read-across between members for both environmental and human health effects endpoints (including developmental and reproductive toxicity).	(OECD 2004)

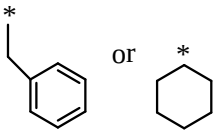
2. Substance Identity

The *ortho* phthalate diesters (phthalates) are a group of chemicals in which phthalic acid is esterified to alkyl or aryl chains that may vary in carbon chain length and structure. The phthalates discussed in this document share common structural features. The general structure is presented in Figure 2-1.



R1 and/or R2 = saturated linear or branched alkyl chain of carbon atoms

OR



attachment occurs at * carbon

Figure 2-1: General structure common to the phthalates of interest

2.1 Phthalates of Interest

2.1.1 Substance identities

Substance identity information for the 28 phthalates of interest considered within this document is presented in Table 2-1 below. There are 15 discrete substances (single compound), and 13 mixture substances [isomer mixtures and Unknown or Variable Composition, Complex Reaction Products or Biological (UVCB) phthalates].

Table 2-1: Substance identity for phthalates of interest ordered by length of the main alkyl carbon chain on the ester linkage

CAS RN	Chemical Name (DSL)[a]	Acronym	Substance Type
131-11-3†	1,2-Benzenedicarboxylic acid, dimethyl ester	DMP	Discrete
84-66-2^	1,2-Benzenedicarboxylic acid, diethyl ester	DEP	Discrete
131-16-8^	1,2-Benzenedicarboxylic acid, dipropyl ester	DPrP	Discrete
84-69-5†	1,2-Benzenedicarboxylic acid, bis(2-methylpropyl) ester	DIBP	Discrete
5334-09-8†	1,2-Benzenedicarboxylic acid, cyclohexyl 2-methylpropyl ester	CHIBP	Discrete
84-64-0†	1,2-Benzenedicarboxylic acid, butyl	BCHP	Discrete

CAS RN	Chemical Name (DSL)[a]	Acronym	Substance Type
	cyclohexyl ester		
84-74-2^	1,2-Benzenedicarboxylic acid, dibutyl ester	DBP	Discrete
85-68-7^	1,2-Benzenedicarboxylic acid, butyl phenylmethyl ester	BBP	Discrete
84-61-7†	1,2-Benzenedicarboxylic acid, dicyclohexyl ester	DCHP	Discrete
27987-25-3†	1,2-Benzenedicarboxylic acid, bis(methylcyclohexyl) ester	DMCHP	Discrete
71888-89-6†	1,2-Benzenedicarboxylic acid, di-C_{6-8}-branched alkyl esters, C_7-rich	DIHepP	Isomer Mixture
27554-26-3^	1,2-Benzenedicarboxylic acid, diisooctyl ester	DIOP	Isomer Mixture
27215-22-1†	1,2-Benzenedicarboxylic acid, isooctyl phenylmethyl ester	BIOP	Isomer Mixture
117-81-7^	1,2-Benzenedicarboxylic acid, bis(2-ethylhexyl) ester	DEHP	Discrete
84-75-3^	1,2-Benzenedicarboxylic acid, dihexyl ester	DnHP	Discrete
111381-89-6^	1,2-Benzenedicarboxylic acid, heptyl nonyl ester, branched and linear	79P	UVCB
68515-48-0^; 28553-12-0†	1,2-Benzenedicarboxylic acid, di-C8-10-branched alkyl esters, C9-rich; 1,2-Benzenedicarboxylic acid, diisononyl ester	DINP1,2	Isomer Mixture
68515-40-2†	1,2-Benzenedicarboxylic acid, benzyl C7-9-branched and linear alkyl esters	B79P	UVCB
68648-93-1^	1,2-Benzenedicarboxylic acid, mixed decyl and hexyl and octyl diesters	610P	UVCB
26761-40-0[b]†	1,2-Benzenedicarboxylic acid, diisodecyl ester	DIDP	Isomer Mixture
117-84-0^	1,2-Benzenedicarboxylic acid, dioctyl ester	DnOP	Discrete
3648-20-2†	1,2-Benzenedicarboxylic acid, diundecyl ester	DUP	Isomer Mixture
68515-43-5^	1,2-Benzenedicarboxylic acid, di-C9-11-branched and linear alkyl esters	D911P	UVCB
111381-91-0^	1,2-Benzenedicarboxylic acid, nonyl undecyl ester, branched and linear	D911P-2	UVCB
85507-79-5^	1,2-Benzenedicarboxylic acid, diundecyl ester, branched and linear	DIUP	UVCB

CAS RN	Chemical Name (DSL)[a]	Acronym	Substance Type
68515-47-9^	1,2-Benzenedicarboxylic acid, di-C11-14-branched alkyl esters, C13-rich	DTDP	UVCB
16883-83-3†	1,2-Benzenedicarboxylic acid, 2,2-dimethyl-1-(1-methylethyl)-3-(2-methyl-1-oxopropoxy)propyl phenylmethyl ester	B84P	Discrete
523-31-9†	1,2-Benzenedicarboxylic acid, bis(phenylmethyl) ester	DBzP	Discrete

[a] DSL – Domestic Substance List;
[b] Due to international variations in the nomenclature used the substance 1,2-Benzenedicarboxylic acid, diisodecyl ester (DIDP - CAS RN 26761-40-0) may also be represented by the following substance: 1,2-Benzenedicarboxylic acid, di-C9-11-branched alkyl esters, C10-rich (CAS RN 68515-49-1)
† Substances that are part of the Phthalate Substance Grouping (Government of Canada 2012);
^Additional DSL phthalates being considered to inform risk assessment and considered for potential cumulative risk assessment.

2.1.2 Composition

Many of the phthalates of interest are mixtures, reflecting the constitution of the feedstock alcohols from which they are derived.

To derive best estimates of composition for the phthalates of interest, the following sources of information were considered:

- Information obtained through searching publicly available risk assessments and hazard reviews from other organizations (eg. ECHA REACH, EPA HPV, US CPSC, etc); and
- Information obtained through publicly available product monographs that reference a given CAS RN.

Appendix A provides an overview of the composition of the 28 phthalates of interest.[1]

2.1.3 Chemical structures

As discussed above, the phthalates of interest share common structural features. As a result of similar starting materials and processes used during their synthesis, these substances are all *ortho* phthalate diesters with various degrees of linear or branched alkyl or aryl ester chains.

[1] For the purposes of this document, only information obtained through public sources of information are presented. This may be revised after examination of the information submitted by stakeholders through survey efforts and other voluntary submissions.

The following were considered in the determination of a representative structure:

- Information obtained through searching publicly available risk assessments and hazard reviews from other organizations (eg. ECHA REACH, EPA HPV, US CPSC, etc);
- Open scientific literature; and
- Where ester chain length is variable in the mixture (isomer mixture and UVCB phthalates), the representative structures were selected by examining the available compositional information and selecting structures that represent individual components.

Appendix B presents representative structures for the phthalates of interest.

2.1.4 Physicochemical properties

Preliminary information regarding physicochemical properties was collected as part of the search of publicly available data for the phthalates of interest. Where data was unavailable, appropriate models were used to estimate the missing property. This information was considered when forming subcategories (Section 6) as well as when selecting closest analogues within the subcategories to apply read-across (Section 7).

Appendix C presents physicochemical properties for the 28 phthalates of interest.

2.1.5 Toxicokinetics

Preliminary toxicokinetic information was collected as part of the search of publicly available data for the phthalates of interest. This information was considered when forming subcategories (Section 6) as well as when selecting closest analogues within the subcategories to apply read-across (Section 7).

Appendix D presents an overview of select toxicokinetic data for the phthalates of interest. A more in-depth analysis of phthalate toxicokinetics will be presented in the state of the science documents to be published at a future date.

3. Health Effects and Proposed Mode of Action for Phthalate-Induced Toxicity on the Developing Male Reproductive System in Rats

A critical effect for risk assessments of certain phthalates is adverse effects on the development of the male reproductive system as a result of gestational exposure. In laboratory animals, administration of certain phthalates has been demonstrated to cause a variety of effects; however, adverse effects on the development of the reproductive system of male animals occur at lower doses than doses causing adverse effects in mature animals (NAS 2008). Consideration of effects on the development of the male reproductive system following prenatal exposure is relevant to both individual health effects assessment of the phthalates of interest as well as to a CRA.

Prenatal exposures to DEHP and DBP have been well studied. These phthalates have been shown to cause adverse effects on specific developmental parameters and in the postnatal development of the male rat reproductive system (Foster et al. 2006; Makris et al. 2013). The effects on male laboratory rats that are detected in early postnatal life as a result of fetal exposure (during the critical development window of GD15-17) to certain phthalates include altered feminization parameters such as decreased anogenital distance (AGD) and areolar/nipple retention in juveniles (Gray et al., 2000). Other effects observed include reproductive tract malformations (cryptorchidism, hypospadias, and testicular pathological changes) and effects on sperm counts, motility, and quality at adulthood (Gray et al. 2006). This spectrum of effects on male reproductive development has been described as the “phthalate syndrome”. Although primarily studied in rats, it has also been demonstrated in other species (reviewed in NAS 2008).

Male reproductive tract development in rats, and humans, is a complex process requiring the production and regulatory activity of hormones at specific timeframes during prenatal and neonatal development. Reviews of male reproductive tract development are available (Tilman and Capel 2002; Viger et al. 2005). Briefly, expression of the sex determining gene SRY initiates gonadal differentiation, which in males is the testis. Subsequent development of internal and external reproductive organs and acquisition of male secondary sex characteristics are dependent on hormones produced in the testis. The key hormones produced by the testis are: Müllerian inhibiting substance (MIS), testosterone, and insulin-like factor 3 (insl3). These hormones are required at the correct time, tissue location and concentrations during development (NAS 2008; Tilman and Capel 2002). The critical period for male reproductive tract development in the rat occurs during gestational days 15-21 where production of testicular testosterone is initiated (NAS 2008). In the developing testis, Sertoli and Leydig cells play important roles in the development of the reproductive tract

and testis. Sertoli cells are considered to direct other cell types into their respective lineages and Leydig cells are responsible for the production of hormones including testosterone and Insl3. Abnormal functioning of Sertolli cells is likely to affect the physiology of adjacent Leydig cells and *vice versa* (Martino-Andrade and Chahoud 2010).

The testis appears to be the target organ in the fetal rat resulting in life stage-specific effects (Foster 2005; Howdeshell et al. 2008). Proposed mode(s) of action for the rat "phthalate syndrome" has been reviewed elsewhere (NAS 2008). Conceptually, the effects associated with the rat phthalate syndrome can be divided into three subsets with different key event considerations. The first subset of effects is related to androgen insufficiency (decreased testicular testosterone production) in the fetal rat and is caused by altered functioning of Leydig cells. The second subset of phthalate syndrome effects has also been attributed to altered functioning of Leydig cells; however, the effects are separate from testosterone's role in development. Insl3 gene expression is reduced and is a second proposed mechanism involved in causing cryptorchidism (McKinnell et al., 2005; Wilson et al., 2004). Finally, the third subset of phthalate syndrome effects has been linked to altered functioning of the Sertoli cells in the fetal testes. Certain phthalates can affect the Sertoli cell *in utero* and may result in altered Sertoli-germ cell interactions leading to multi-nucleated gonocytes (MNG) (Kleymenova 2005). Figure 3-1 is a representation of the proposed mode of action of the "phthalate syndrome" with associated hormonal and phenotypic alterations (adapted from Martino-Andrade and Chahoud 2010 and Sharpe 2001).

The SAR analysis (Section 5.4) used to develop subcategories of phthalates is based on important events relating to androgen insufficiency in the fetal rat (highlighted in red in Figure 3-1). First, fetal Leydig cell function is altered by phthalates. A molecular target responsible for altered Leydig cell function has not been established. However, several authors have shown that certain phthalates can cause reduced gene expression critical for normal testosterone production in the fetal testes (Hannas et. al 2012; Barlow et al. 2003a). Hannas et al. (2012) postulates that certain phthalates act through a similar mode of action through the downregulation of genes critical for steroid biosynthesis. This is considered as an important event for the androgen-dependent effects on the developing male reproductive tract. Data relating to expression of genes critical for the steroid biosynthesis (Section 5.1) pathway are considered in the SAR analysis. It is biologically plausible that downregulation of genes critical for steroid biosynthesis results in a subsequent decrease in fetal testicular testosterone production (Hannas et al. 2012). Decreased fetal testicular testosterone production is considered to be the second important event for the effects on the developing male reproductive tract. As a result, data relating to fetal testicular testosterone production are also considered in the SAR analysis (Section 5.2). Decreased testosterone production by

fetal Leydig cells is critical to the subsequent reproductive track malformations in rats exposed to certain phthalates during gestation. Decreased testosterone production during the development of the male reproductive system results in androgen-dependent adverse outcomes including failure of Wolffian duct to develop into the epididymis (Mylchreest et al. 2002; Barlow and Foster 2003b); abnormalities in the development of the prostate and testes; decreased testes weight; hypospadias, crytorchisdism, nipple retention; and decreased sperm counts at adulthood (Makris et al 2013; NAS 2008). AGD of newborn male rats is considered an important biomarker of testosterone levels during development. In normal development, testosterone secreted by the fetal testes acts to lengthen the AGD in males relative to females [indirectly through the action of an androgen derived from testosterone, dihydrotestosterone (DHT)]. Reduced testosterone levels disturb DHT levels by reducing the amount of DHT that can be produced from testosterone by 5α-reductase. DHT is required for the growth of the perineum to produce the normal male AGD. As a result, fetal rats exposed to substances that reduce testicular testosterone synthesis during development show decreased AGD at birth (NAS 2008). AGD is a well studied parameter in new born male rats exposed to certain phthalates during gestation. These data are available across multiple phthalates of interest and the magnitude of decreased AGD seen in male rats following prenatal phthalate exposure has been shown to be predictive of other reproductive tract malformations in adulthood (Barlow et al., 2004; Hotchkiss et al., 2004). Therefore, decreased AGD at birth is an observable effect related to the mode of action for phthalate-induced toxicity to the developing male reproductive system; and therefore, is used in the SAR analysis (Section 5.3).

Following gestational exposure, testosterone levels in the testes can return to normal after the metabolites of phthalates are cleared from the circulation (Thomspson et al. 2004). However, the malformations induced by exposure during the key developmental window may persist into adulthood (Barlow et al., 2004; Barlow and Foster, 2003b).

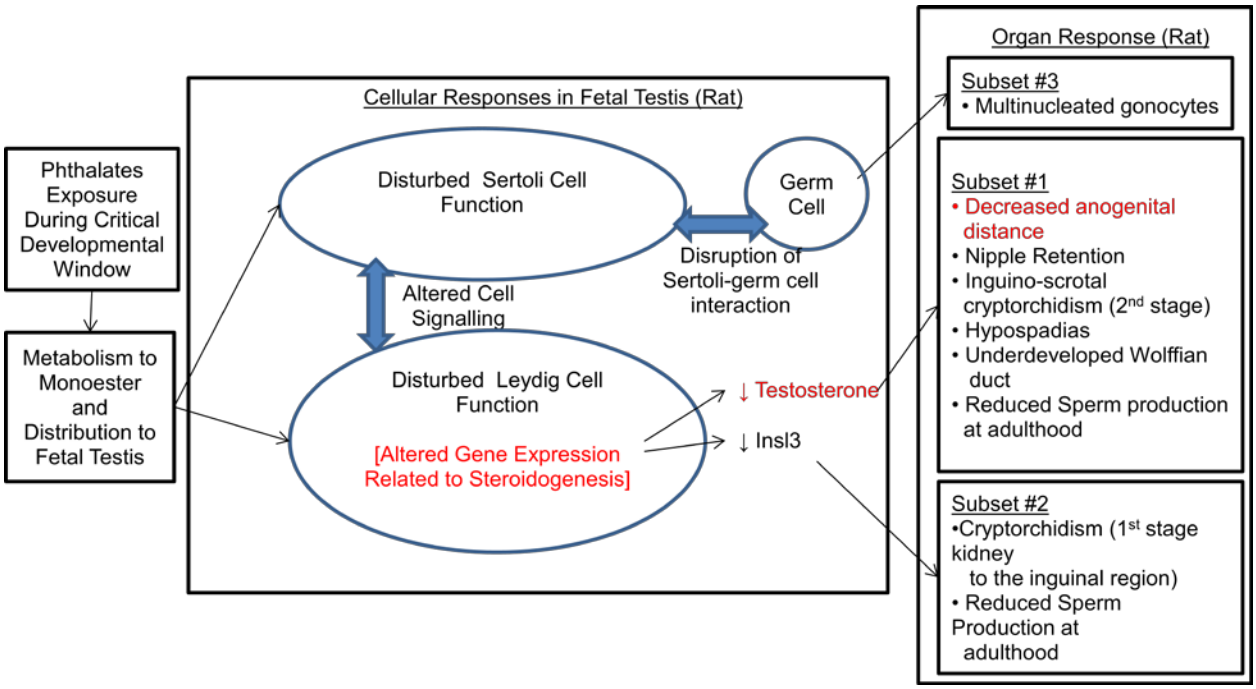


Figure 3-1: Representation of the cellular targets for the "phthalate syndrome" with associated changes in gene expression and subsequent hormonal and organ responses.
The important events highlighted in red form the basis of the SAR analysis in Section 5 (with considerations from NAS 2008; Sharpe 2001; Martino-Andrade and Chahoud 2010; Foster 2005).

4. Study Availability for Effects on the Developing Male Reproductive System across the Phthalates of Interest

A targeted literature search examining effects related to the phthalate syndrome in the fetal rat was conducted for the purpose of the category approach for the 28 phthalates of interest. For this document, particular interest is paid to a subset of effects within the phthalate syndrome related to androgen insufficiency (reduced fetal testicular testosterone production) during development as described in Section 3. Therefore, developmental toxicity and multigenerational reproductive studies that examine effects related to androgen insufficiency during male development in fetal rats were collected. In these studies, pregnant rats were orally administered certain phthalates during the sensitive male development window. Androgen insufficiency during development was typically assessed through examination of apical endpoints related to feminization parameters (decrease in AGD, nipple retention), reproductive tract malformations (crytorchidism, hypospadias, testicular defects, etc.) and functional fertility parameters

at adulthood (sperm number, motility, morphology, viability, etc.) of male rat offspring. Collected studies also included *in vivo s*tudies where certain phthalates were investigated for their potential to alter gene expression (mRNA) related to steroidogenesis and studies where certain phthalates were tested for their ability to alter testicular testosterone production in the fetal rat testes (*ex vivo*). These were identified as important events in the mode of action for phthalate-induced androgen insufficiency during male reproductive development (Figure 3-1, Section 3) and are subsequently used to develop a SAR analysis in Section 5.

Data availability for gene expression assays, testicular testorsterone production assays and toxicity studies examining androgen-dependent endpoints in the fetal rat for each of the 28 phthalates is presented in Table 4-1. Of the 28 phthalates of interest, there are 11 substances for which no relevant studies have been identified, i.e., no developmental toxicity or multigenerational reproductive studies that examine effects related to androgen insufficiency during development in fetal rats were found in the literature. As this is a key consideration for the health effects characterization of all 28 phthalates and may be considered in a potential CRA, an approach for filling data gaps is required (discussed in Section 6 and 7).

Table 4-1 Data availability for fetal rat testicular gene expression, testosterone production and studies observing androgen-dependent effects in male rat offspring for the phthalates of interest

Phthalate	Steroidogenic related gene expression	Testicular testosterone production (*ex vivo)*	Developmental study with dosing during the critical developmental window	Reproductive study with dosing during the critical developmental window
DMP†	√	X	√	X
DEP^	√	√	√	√
DPrP^	X	X	√	X
DIBP†	√	√	√	X
CHIBP†	X	X	X	X
BCHP†	X	X	X	X
DBP^	√	√	√	√
BBP^	√	√	√	√
DCHP†	X	X	√	√
DMCP†	X	X	X	X
DIHepP†	√	√	√	√
DIOP^	X	√	√	X
BIOP†	X	X	X	X
DEHP^	√	√	√	√
DnHP^	√	√	√	X
79P^	X	X	X	X

Phthalate	Steroidogenic related gene expression	Testicular testosterone production (*ex vivo)*	Developmental study with dosing during the critical developmental window	Reproductive study with dosing during the critical developmental window
DINP-1/2†^	√	√	√	√
B79P†	X	X	√	X
610P^	X	X	X	X
DIDP†	√	√	√	√
DnOP^	X	X	√	X
DUP†	X	X	√	X
D911P^	X	X	X	√
D911P -2^	X	X	X	X
DIUP^	X	X	X	X
DTDP^	X	X	√	X
B84P†	X	X	X	X
DBzP†	X	X	X	X

† Substances that are part of the Phthalate Substance Grouping (Government of Canada 2012);
^Additional phthalates being considered to inform risk assessment and considered for potential cumulative risk assessment;
√ - Data Available; X – No Data Identified

5. Structure Activity Relationship (SAR) Analysis Related to Mode of Action of Androgen Insufficiency for Phthalate-Induced Toxicity to the Male Reproductive System

The 10 phthalates noted in the previous section for which no relevant health effects information is available are considered to have data gaps for effects related to androgen insufficiency during development, a key consideration for the health effects characterization of the phthalates of interest. The approach for filling data gaps for phthalates without relevant health effects information involves two steps: (1) available data from other phthalates is used to develop a SAR analysis with respect to the nature of the phthalate ester group and relation to induction of androgen insufficiency during development; (2) the SAR analysis is used to form subcategories of phthalates (presented in Section 6). The subcategories are subsequently used to fill data gaps with read-across for data poor phthalates with respect to androgen insufficiency during development (presented in Section 7).

The SAR analysis (Section 5.4) was based on examining whether the phthalates of interest caused changes in important events related to the mechanism of action for developmental effects on the male reproductive system.

Changes in important events examined are:

- effects on gene expression related to the steroid biosynthesis pathway in the fetal rat testis
- effects on testicular testosterone production in the fetal rat
- decreases in AGD at birth after gestational exposure

AGD was selected for the SAR analysis as it is a biomarker of testosterone action during the critical developmental window in male offspring. The magnitude of decreased AGD seen in male rats following prenatal phthalate ester exposure was also predictive of reproductive tract malformations in adulthood (Barlow et al., 2004; Hotchkiss et al., 2004). Furthermore, AGD is a well-studied endpoint across the phthalates of interest with multiple ester carbon lengths and chain types being represented.

5.1 Effects on Gene Expression Related to the Steroid Biosynthesis Pathway in the Fetal Rat Testes

Selection of Studies / Selection of Genes

To inform the approach, a report summarizing the genomic research published in peer-reviewed scientific literature between 2000 and January 2013 that relates to the phthalates of interest was prepared (Intrinsik 2013). This report summarizes publicly available data for *in vivo* and *in vitro* health effect studies on the phthalates of interest that use genomics as a tool for measuring effects. One hundred and eight (108) *in vivo* studies and 57 *in vitro* studies were identified. Several microarray studies were identified but the majority of studies used real-time polymerase chain reaction (RT-PCR) as the method for measurement.

Four studies were selected for the SAR analysis covering 9 of the 28 phthalates of interest. Across the phthalates considered in these studies, the number of carbons in the longest alkyl chain varies between 2-8 carbons. The remaining phthalates have no data regarding relevant gene expression changes in the testes. The studies for the SAR analysis were selected using the following criteria: 1) *in utero* rat studies that have at least three biological replicates (dams) per dose; 2) maternal exposure occurring during the critical development window (GD 15-17 at minimum) and; 3) where the authors analyzed the appropriate tissue at the appropriate time (fetal rat testes on GD18/19)) and expression for biologically relevant genes related to the mechanism for decreased androgen synthesis. The genes selected for the SAR analysis are scavenger receptor class B member 1 (SR-B1), steroidogenic acute regulatory protein (StAR), cytochrome P450 side-chain cleavage enzyme (Cyp11A), beta-hydroxysteroid dehydrogenase (3bHSD), and cytochrome P450 17A1 (Cyp17A1) as these proteins are involved in the steroid biosynthesis pathway of the testes. As illustrated in Figure 5-1, it is biologically plausible to consider that decreased gene expression would be linked to

altered production of testicular testosterone (Hannas et al. 2012) given the respective functions of these proteins. Specifically, SR-B1 protein facilitates cholesterol uptake into steroidogenic cells. StAR transports cholesterol across mitochondrial membranes, which is the rate-limiting step in gonadal steriodogenesis (Petrescu et al. 2001). Cyp11A then cleaves the cholesterol side chain (the first enzymatic step in steroidogenic pathway). 3bHSD protein converts pregnenolone to progesterone which is subsequently converted to androstenedione by Cyp17a1 followed by conversion to testosterone by another enzyme. Decreased testosterone synthesis during the critical period of androgen-dependent male reproductive tract development is a key event in the mechanism of action for phthalate toxicity to the male reproductive tract.

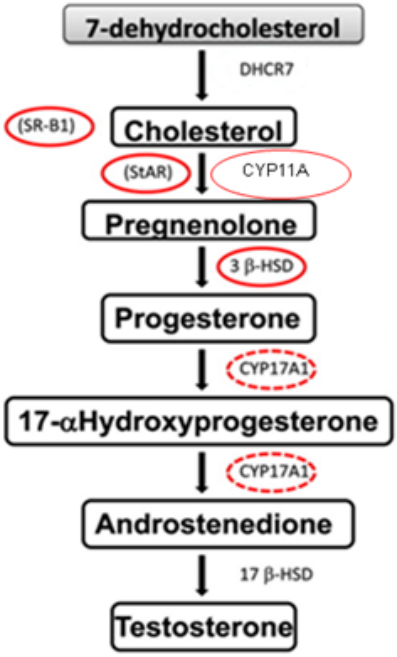


Figure 5-1: Steroid biosynthesis pathway of the testes.
Steroidogenesis-related enzymes and transport proteins (noted by parentheses) affected by in utero phthalate exposure are circled. Dashed lines around CYP17A1 signify this enzyme acting on multiple steps. (Adapted from Hannas et al. 2012)

Study Details and Relative Potency Interpretation between Phthalates

Study details (e.g., strain, route, dose levels, and duration of dosing) and results (LOEL and ED50 values) for gene expression changes for the genes used in the phthalate SAR analysis are presented in Table 5-1.

Liu et al. (2005) examined global gene expression in the fetal testis of the rat following *in utero* exposure to DMP, DEP and other phthalates. Pregnant Sprague-Dawley rats

were treated by gavage daily during GD 12 - 19 with vehicle control or phthalate at a single dose of 500 mg/kg/day. Testes were isolated on GD 19, and global changes in gene expression were determined through a microarray analysis. Gene pathways examined by microarray included cholesterol transport and steroidogenesis covering the genes selected for the SAR analysis. The authors state that no significant changes in gene expression were detected for DMP or DEP at the dose tested. Gene expression of select genes was further examined by real-time RT-PCR (results did not include the genes selected for the SAR analysis). This study also examined other phthalates including DBP and BBP which did show decreased gene expression related to cholesterol transport and steroidogenesis; however, there were more recent studies available for these phthalates that administered more than a single dose which were considered more relevant for the SAR analysis.

Hannas et al. (2011, 2012) examined changes in gene expression for the steroid biosynthesis pathway including those identified as relevant for the SAR analysis. Pregnant Sprague-Dawley rats were treated by gavage daily during GD 14 - 18 with vehicle control or DIBP, DnHP, DEHP, DnHepP, DINP or DIDP over a dose range (see Table 5-1 for selected doses). Testes were isolated on GD 18, and gene expression was determined through RT-PCR. DIBP, DnHP, DEHP, DnHepP and DINP all reduced expression of the relevant genes in the steroid biosynthesis pathway in a dose dependent manor while DIDP had no effect up to the highest dose tested (1500 mg/kg/day). The order of most potent to least potent phthalate for reducing gene expression for this particular study was found to be DnHP > DIBP > DEHP ≈ DnHepP > DINP (see Table 5-1).

Lehmann et al. (2004) and Saillenfait et al. (2013a) conducted a similar gene expression analysis but at lower doses for the steroid biosynthesis pathway for DBP and DnHP, respectively. Pregnant Sprague-Dawley rats were treated by gavage daily during GD 12 - 19 with vehicle control, DBP or DnHP over a dose range (see Table 5-1 and 5-2 for selected doses). In both studies, testes were isolated on GD 19, and gene expression was determined through RT-PCR. Comparing the two studies, DBP was found to be the most potent phthalates for inducing gene expression changes with statistically significant changes from control noted at 1mg/kg/day for SR-B1 while DnHP reduced expression of the same gene starting at 20 mg/kg/bw. Both phthalates reduced StAR expression starting at 50 mg/kg/day.

Based on the results of all four studies, the order of most potent to least potent phthalate for reducing steroid biosynthesis gene expression is DBP > DnHP > DIBP > DEHP ≈ DnHepP > DINP.

Table 5-1: Selected studies for phthalate SAR analysis for gene expression related to cholesterol transport in the steroid biosynthesis pathway in the fetal rat testes

Phthalate	Strain and Species: Doses [mg/kg/day] (Route) Duration {Testes Removed for Analysis}	[Measured Gene(s)]: Lowest Observed Effect Level (LOEL) (min p<0.05) / Dose resulting in 50% Effect (ED50) for ↓Expression (mg/kg/day)*	Reference (method)
DMP	SD Rats: 500 (Gavage): GD 12-19 {GD19}	[SR-B1]: NE [StAR]: NE	Liu et al. 2005 (microarray)
DEP	SD Rats: 500 (Gavage): GD 12-19 {GD19}	[SR-B1]: NE [StAR]: NE	Liu et al. 2005 (microarray)
DIBP	SD Rats: 0, 100, 300, 600, 900 (Gavage) GD 14-18 {GD18}	[SR-B1]: 300 / 302 [StAR]: 300 / 295	Hannas et al. 2011 Hannas et al. 2012 (RT-PCR)
DBP	SD Rats: 0, 0.1, 1, 10, 50, 100, 500 (Gavage): GD 12-19 {GD19}	[SR-B1]: 1 / - [StAR]: 50 / -	Lehmann et al. 2004 (RT-PCR)
DnHP	SD Rats: 0, 100, 300, 600, 900 (Gavage): GD 14-18 {GD18}	[SR-B1]: 100 / 86 [StAR]: 100 / 54	Hannas et al. 2012 (RT-PCR)
DEHP	SD Rats: 0, 100, 300, 500, 625, 750, 875 (Gavage) GD 14-18 {GD18}	[SR-B1]: NM [StAR]: 500 / 443	Hannas et al. 2011 (RT-PCR)
DnHepP^	SD Rats: 0, 100, 300, 600, 900 (Gavage): GD 14-18 {GD18}	[SR-B1]: 300 / 372 [StAR]: 600 / 360	Hannas et al. 2012 (RT-PCR)

Phthalate	Strain and Species: Doses [mg/kg/day] (Route) Duration {Testes Removed for Analysis}	[Measured Gene(s)]: Lowest Observed Effect Level (LOEL) (min p<0.05) / Dose resulting in 50% Effect (ED50) for ↓Expression (mg/kg/day)*	Reference (method)
DINP-1/2	SD Rats: 0, 500, 750, 1000, 1500 (Gavage) GD 14-18 {GD18}	[SR-B1]: 500 / 602 [StAR]: 500 / 597	Hannas et al. 2011 Hannas et al. 2012 (RT-PCR)
DIDP	SD Rats: 0, 500, 750, 1000, 1500 (Gavage) GD 14-18 {GD18}	[SR-B1]: NE [StAR]: NE	Hannas et al. 2012 (RT-PCR)

^ Di-n-heptyl Phthalate (DnHepP) is not a phthalate of interest but is considered for the SAR analysis

*NE is presented when no effect observed at highest dose tested; NM is presented when gene was not measured;
- is presented when ED50 not calculated by study authors

Table 5-2: Selected studies for phthalate SAR analysis for gene expression related to steroidogenesis in the steroid biosynthesis pathway in the fetal rat testes

Phthalate	Strain and Species: Doses [mg/kg/day] (Route) Duration {Testes Removed for Analysis}	[Measured Steroidogenic Related Gene(s)]: Lowest Observed Effect Level (LOEL)(min p<0.05) / Dose resulting in 50% Effect (ED50) for ↓Expression (mg/kg/day)*	Reference (method)
DMP	SD Rats: 500 (Gavage): GD 12-19 {GD19}	[Cyp11a]: NE [3bHSD]: NE [Cyp17a1]: NE	Liu et al. 2005 (microarray)
DEP	SD Rats: 500 (Gavage): GD 12-19 {GD19}	[Cyp11a]: NE [3bHSD]: NE [Cyp17a1]: NE	Liu et al. 2005 (microarray)
DIBP	SD Rats: 0, 100, 300, 600, 900 (Gavage) GD 14-18 {GD18}	[Cyp11a]: 300 / 339 [3bHSD]: 300 / 538 [Cyp17a1]: 300 / 325	Hannas et al. 2011 Hannas et al. 2012 (RT-PCR)

Phthalate	Strain and Species: Doses [mg/kg/day] (Route) Duration {Testes Removed for Analysis}	[Measured Steroidogenic Related Gene(s)]: Lowest Observed Effect Level (LOEL)(min p<0.05) / Dose resulting in 50% Effect (ED50) for ↓Expression (mg/kg/day)*	Reference (method)
DBP	SD Rats: 0, 0.1, 1, 10, 50, 100, 500 (Gavage): GD 12-19 {GD19}	[Cyp11a]: 50 / - [3bHSD]: 0.1 / - [Cyp17a1]: 500/ -	Lehmann et al. 2004 (RT-PCR)
DnHP	SD Rats: 0, 100, 300, 600, 900 (Gavage): GD 14-18 {GD18}	[Cyp11a]: 300 / 267 [3bHSD]: 100 / 185 [Cyp17a1]: 100 / 119	Hannas et al. 2012 (RT-PCR)
DEHP	SD Rats: 0, 100, 300, 500, 625, 750, 875 (Gavage) GD 14-18 {GD18}	[Cyp11a]: 500 / 574 [3bHSD]: NM [Cyp17a1]: NM	Hannas et al. 2011 (RT-PCR)
DnHepP^	SD Rats: 0, 100, 300, 600, 900 (Gavage): GD 14-18 {GD18}	[Cyp11a]: 600 / 488 [3bHSD]: 600 / 656 [Cyp17a1]: 600 / 406	Hannas et al. 2012 (RT-PCR)
DINP-1/2	SD Rats: 0, 500, 750, 1000, 1500 (Gavage) GD 14-18 {GD18}	[Cyp11a]: 500 / 1148 [3bHSD]: 500 / 963 [Cyp17a1]: 500 / 797	Hannas et al. 2011 Hannas et al. 2012 (RT-PCR)
DIDP	SD Rats: 0, 500, 750, 1000, 1500 (Gavage) GD 14-18 {GD18}	[Cyp11a]: NE [3bHSD]: NE [Cyp17a1]: NE	Hannas et al. 2012 (RT-PCR)

^ Di-n-heptyl Phthalate (DnHepP) is not a phthalate of interest but is considered for the SAR analysis

*NE is presented when no effect observed at highest dose tested; NM is presented when gene was not measured;
- is presented when ED50 not calculated by study authors

5.2 Effects on Testicular Testosterone Production in the Fetal Rat

Selection of Studies

Decreased testosterone production within the fetal testes during the male development window was proposed as a critical part of the mode of action for phthalate-induced toxicity to the developing male reproductive system (See Section 5.0). Studies were collected for the phthalates of interest where pregnant rats were administered phthalates by gavage during the critical male development window and where the testes of fetal males were collected for the *ex vivo* testicular testosterone production assay. Typically for these studies, the fetal testes of offspring are collected on GD18 or 19 and incubated in media (*ex vivo*) for a period of two to three hours. Testosterone is then measured in the media either through radioimmunoassay (RIA) or turbulent flow liquid chromatography coupled with tandem mass spectrometry (TFC–MS/MS). It is recognized that there may be sensitivity differences between the methods used for testosterone measurement which could impact the potency comparison of the phthalates across these studies. Both types of studies are presented below; however, for the potency interpretation and SAR analysis (Section 5.4), only studies using RIA were considered. RIA was selected over TFC-MS/MS since more phthalates have been tested using this method and it therefore supports a more robust SAR analysis.

Three studies were selected for the SAR analysis covering 10 of the phthalates of interest where the number of carbons in the longest alkyl chain varies between 2-8 carbons in length. The remaining phthalates of interest have no data regarding *ex vivo* testicular testosterone production in the testes. In order to develop a SAR based on similar studies, those selected for the SAR analysis met the following criteria: (1) *in utero* rat studies that have at least three biological replicates (dams) per dose; (2) maternal exposure occurring during the critical development window (GD 15-17 at minimum) and testes isolated between GD18 and 19; (3) testes from male offspring incubated between 2-3 hours and incubation media tested for testosterone.

Study Details and Relative Potency Interpretation between Phthalates

Study details (e.g., strain, route, dose levels, and duration of dosing) and results (LOEL and ED50 values) for *ex vivo* testicular testosterone production in the fetal testes used for the phthalate SAR analysis are presented in Table 5-3.

Howdeshell et al. (2008) administered DEP, DIBP, DBP, BBP, and DEHP by gavage to pregnant Sprague–Dawley rats on GD 8–18 at doses of 0, 100, 300, 600 or 900 mg/kg/day. The testes of male pups were removed on GD 18 and incubated for three hours. *Ex vivo* fetal testicular testosterone production was measured using the incubation media and radioimmunoassay (RIA). DEP did not reduced fetal testicular

testosterone production compared to controls at any of the doses tested. DIBP, DBP, BBP, and DEHP significantly reduced fetal testicular testosterone production compared to controls starting at 300mg/kg/day with magnitude of change increasing with dose. To compare relative potency for decreasing fetal testicular testosterone production, logistic regression analysis was used by the authors to determine ED50 values. The ED50 values derived for DIBP, DBP, BBP and DEHP were 466, 399, 464, and 383 mg/kg/day respectively. Therefore, the order of most potent to least potent phthalate for reducing fetal testicular testosterone production (*ex vivo*) for this particular study was found to be DEHP > DBP > BBP ≈ DIBP.

In similarly conducted experiments, Hannas et al. (2011, 2012) administered vehicle control, DIBP, DnHP, DEHP, DIHepP, DnHepP, DINP or DIDP over a dose range (see Table 5-2 for selected doses for each phthalate) by gavage daily to pregnant Sprague-Dawley rats during GD 14 – 18. DIDP did not reduce fetal testicular testosterone production compared to controls at any of the doses tested (up to 1500mg/kg/day). DnHP, reduced fetal testicular testosterone production compared to controls starting at 100mg/kg/day (lowest dose tested) with magnitude of change increasing with dose. DIHepP and DEHP reduced fetal testicular testosterone production compared to controls starting at 300mg/kg/day with magnitude of change increasing with dose. DnHepP and DINP reduced testicular testosterone production compared to controls starting at 600 and 500 mg/kg/day, respectively (lowest dose tested for DINP). As with Howedshell et al. (2008), to compare relative potency for decreasing fetal testicular testosterone production, regression analysis was used to determine ED50 values. The ED50 values calculated for DIBP, DnHP, DEHP, DIHepP, DnHepP, DINP were 305, 75, 383, 410, 444, and 852 mg/kg/day respectively. Therefore, the order of most potent to least potent phthalate for reducing fetal testicular testosterone production (*ex* vivo) for this study was DnHP > DIBP > DEHP > DIHepP > DnHepP >> DINP.

Based on the results of Howdeshell et al. 2008 and Hannas et al. (2011, 2012) the order of most potent to least potent phthalate for reducing fetal testicular testosterone production (*ex* vivo) is DnHP > DIBP > DEHP > DBP > DIHepP > DnHepP > BBP >> DINP.

In separate experiments, Saillenfait et al. (2013a, 2013b) administered vehicle control, DIOP, DnHP, or DEHP over a dose range by gavage daily to pregnant Sprague-Dawley rats during GD 12 – 19. The testes of male pups were removed on GD 19 and incubated for three hours. *Ex vivo* fetal testicular testosterone production was measured using the incubation media and turbulent flow liquid chromatography coupled with tandem mass spectrometry (TFC–MS/MS). DIOP, DnHP, and DEHP significantly reduced fetal testicular testosterone production compared to controls starting at 100, 20 and 50 mg/kg/day respectively (lowest dose tested for DEHP), with magnitude of change increasing with dose for all three phthalates. The ED50 values were only

calculated for DIOP and DnHP which were 145 and 67 mg/kg/day, respectively. From these results, DEHP and DnHP appear more potent than DIOP. As an ED50 value was not derived for DEHP by the study authors, the relative potency of DEHP to DnHP from these studies could not be determined.

Table 5-3: Selected studies for phthalate SAR analysis for *ex vivo* testicular testosterone production in the fetal testes following gestational exposure.

Phthalate	Strain and Species: Doses [mg/kg/day] (Route): Duration {Testes Removed for Analysis}	Lowest Observed Effect Level (LOEL)(min p<0.05) / Dose resulting in 50% Effect (ED50) for ↓Testicular Testosterone Production (*ex vivo*) (mg/kg/day)*	Reference (method for testosterone measurement)
DEP	SD Rats: 0, 100, 300, 600, 900 (Gavage): GD 8-18 {GD18}	NE	Howdeshell et al. 2008 (RIA)
DIBP	SD Rats: 0, 100, 300, 600, 900 (Gavage): GD 8-18 {GD18}	300 / 305 (similar effect levels in Howdeshell et al. 2008)	Hannas et al. 2011, 2012 (RIA)
DBP	SD Rats: 0, 100, 300, 600, 900 (Gavage): GD 8-18 {GD18}	300 / 399	Howdeshell et al. 2008 (RIA)
BBP	SD Rats: 0, 100, 300, 600, 900 (Gavage): GD 8-18 {GD18}	300 / 464	Howdeshell et al. 2008 (RIA)
DIHepP	SD Rats: 0, 100, 300, 600, 900 (Gavage) GD 14-18 {GD18}	300 / 410	Hannas et al. 2011 (RIA)
DnHP	SD Rats: 0, 100, 300, 600, 900 (Gavage): GD 14-18 {GD18}	100[#] / 75 [#] Lowest dose tested.	Hannas et al. 2012 (RIA)
DEHP	SD Rats: 0, 100, 300, 600, 900 (Gavage): GD 8-18 {GD18}	300 / 383 (similar effect levels in Hannas et al. 2011)	Howdeshell et al. 2008 (RIA)

Phthalate	Strain and Species: Doses [mg/kg/day] (Route): Duration {Testes Removed for Analysis}	Lowest Observed Effect Level (LOEL)(min p<0.05) / Dose resulting in 50% Effect (ED50) for ↓Testicular Testosterone Production (*ex vivo*) (mg/kg/day)*	Reference (method for testosterone measurement)
DnHepP^	SD Rats: 0, 100, 300, 600, 900 (Gavage): GD 14-18 {GD18}	600 / 444	Hannas et al. 2012 (RIA)
DINP-1/2	SD Rats: 0, 500, 750, 1000, 1500 (Gavage) GD 14-18 {GD18}	500[#] / 852 [#]Lowest dose tested.	Hannas et al. 2012 (RIA)
DIDP	SD Rats: 0, 500, 750, 1000, 1500 (Gavage) GD 14-18 {GD18}	NE	Hannas et al. 2012 (RIA)

^ Di-n-heptyl Phthalate (DnHepP) is not a phthalate of interest but is considered for the SAR analysis
*NE is presented when no effect observed at highest dose tested; - is presented when ED50 not calculated

5.3 Decreased Anogenital Distance (AGD) as an Indicator of Androgen Insufficiency during the Critical Developmental Window in Male Rat Offspring

Selection of Studies

AGD in newborn male rats is a biomarker of androgen action during development. In normal development, testosterone secreted by the fetal testes acts to lengthen the AGD in males relative to females (indirectly through the action of dihydrotestosterone (DHT) an androgen derived from testosterone).Fetal rats exposed to substances that reduce testicular testosterone synthesis during development show decreased AGD at birth (NAS 2008). The magnitude of decreased AGD seen in male rats following prenatal administration of certain phthalate esters were predictive of reproductive tract malformations in adulthood (Barlow et al., 2004; Hotchkiss et al., 2004). Therefore, decreased AGD at birth is an observable effect related to the mode of action for phthalate-induced effects on the developing male reproductive system (See Section 3.0) and is a useful parameter for informing a SAR analysis. Studies were collected for the phthalates of interest where pregnant rats were administered phthalates orally during the critical male development window (GD15-17 at minimum) and where the AGD of male offspring were measured close to birth (from GD 21 to PND 2). Approximately 45 *in vivo* studies were identified.

Fourteen studies were selected for the SAR analysis covering 16 phthalates of interest (plus one additional substance useful for the SAR analysis - DnHepP) where the number of carbons in the longest alkyl chain varies between 1 to 12 carbons in length. The remaining phthalates of interest have no data for AGD measurements. It is recognized that there is uncertainty in conducting a comparison across multiple studies for AGD as authors use different measurement methodologies and body weight correction metrics. For the SAR analysis, studies were selected meeting the following criteria: (1) where multiple studies were available, those that used a dose range (opposed to single dose) were selected to facilitate dose-response comparison. Preference was also given to studies were the data was suitable for benchmark dose (BMD) calculation and; (2) where multiple generations where evaluated only the results for the F1 males were considered.

In order to have an appropriate metric to compare the potency of phthalates for decreasing the AGD of male offspring across multiple studies, BMDs were calculated where appropriate. BMD analysis was conducted using EPA software (US EPA, BMDS version 2.4). The AGD datasets are considered continuous data (i.e. has an exact numerical measure of some biological effect), presented by mean and standard deviation (SD). The choice of the best fitting model for each AGD dataset was based on default test p-values ($p > 0.1$), and lowest Akaike's Information Criterion (AIC) value. To avoid possible wavy or infinite slopes in the curve fitting, restricted power was selected for modelling. Generally, models were run using the constant variance setting to start by hypothesizing a homogenous variance in the data. A calculation of a BMD requires a selection of a benchmark response (BMR). The selection of a BMR value for the BMD calculations used for the potency and SAR analysis is not intended to establish a change in AGD that constitutes toxicological significance but rather is selected to establish a common metric for potency comparison across different studies. For the purposes of the SAR analysis, a 10% deviation from control mean (BMR=10%) was deemed appropriate and is based on findings of Barlow et al. (2004) where an 8-12% decrease in AGD at birth for male rats exposed to DBP during gestation were associated with a 50% incidence of lesions in the testes and seminal vesicles, respectively. A lower confidence limit is placed on the BMD to obtain a dose (BMDL) that assures with high confidence (95%) that the BMR is not exceeded at a given dose and depends on study specific data. The BMDL is used for the SAR analysis.

Study Details and Relative Potency Interpretation between Phthalates

Study details (e.g., strain, route, dose levels, duration of dosing) and results for AGD are presented in Table 5-4. Summaries of the studies are presented in Appendix E.

DMP, DEP, DnOP, DIDP, DUP and DTDP did not decrease AGD of male rat offspring compared to controls when exposure occurred during gestation (Gray et al. 2000; Saillenfait et al. 2011b; Hushka et al. 2001; Saillenfait et al. 2013c).

DPrP, DIBP, DBP, BBP, DnHP, DCHP, DEHP, DIHepP, DnHepP, DINP and MBzP significantly decreased AGD of male rat offspring compared to controls when exposure occurred during gestation (Saillenfait et al. 2011a; Saillenfait 2008; Mylchreest et al. 1999; Tyl et al. 2004; Saillenfait et al. 2009a; Saillenfait et al. 2009b; Gray et al. 2009; McKee et al. 2006; Saillenfait et al. 2011b; Boberg et al. 2011, Ema et al. 2003). As mentioned, to compare relative potency across these phthalates for decreasing the AGD of male rat offspring, BMDL values corresponding to a 10% decrease in the AGD from control were calculated. The $BMDL_{10\%}$ values estimated for DPrP, DIBP, DBP, BBP, DnHP, DCHP, DEHP, DIHepP, DnHepP and DINP are 1180, 204, 208, 205, 98, 225,161, 414, 887 and 909 mg/kg/day, respectively. Therefore, the order of most potent to least potent phthalate for decreasing AGD of male offspring when exposure occurs during gestation is estimated to be DnHP > DEHP > DIBP ≈ BBP ≈ DBP ≈ DCHP > DIHepP >DnHepP > DINP > DPrP.

Table 5-4: Selected studies for phthalate SAR analysis for decreased AGD in male rat offspring

Phthalate	Strain and Species: Maternal Doses [mg/kg/day] (Route) Duration {Day of AGD measure}	Control AGD (mean ± SD)	Observations [Dose]: (mean ± SD) / (% change of control)	AGD Correction Metric	Ref.
DMP	SD Rats: 0, 750 (Gavage): GD 14- PND3 {PND 2}	NR	[750]: NE	body weight	Gray et al. 2000
DEP	SD Rats: 0, 750 (Gavage): GD 14- PND3 {PND 2}	NR	[750]: NE	body weight	Gray et al. 2000

Phthalate	Strain and Species: Maternal Doses [mg/kg/day] (Route) Duration {Day of AGD measure}	Control AGD (mean ± SD)	Observations [Dose]: (mean ± SD) / (% change of control)	AGD Correction Metric	Ref.
DPrP	SD Rats: 0, 500, 1000, 1500 (Gavage): GD 6-20 {GD 21}	1.67 ± 0.07	[500]: 1.65 ± 0.06 [1000]: 1.56 ± 0.07* (6.59%) [1500]: 1.47 ± 0.08* (11.98%) $BMDL_{10\%}$: [1180]	cubic root of body weight	Saillenfait et al. 2011a
DIBP	SD Rats: 0, 125, 250, 500, 625 (Gavage) GD 12-21 {PND 1}	2.55 ± 0.17	[125]: 2.44 ± 0.15 [250]: 2.28 ± 0.30* (10.59%) [500]: 2.02 ± 0.13** (20.78%) [625]: 1.98 ± 0.16** (22.35%) $BMDL_{10\%}$: [204]	body weight	Saillenfait et al. 2008
DBP	SD Rats: 0, 100, 250, 500 (Gavage): GD 12-21 {PND 1}	~3.0[#]	[100]: ~2.9[c] [250]: ~2.75[c]* (9%) [500]: ~2.25[c]* (24%) $BMDL_{10\%}$: [208]	none body weight not affected	Mylchreest et al. 1999
BBP	SD Rats: 0, 50, 250, 750 est. (Diet): 2-gen {F1- PND 0}	2.06 ± 0.03	[50]: 2.01 ± 0.04 [250]: 1.89 ± 0.02*** (8.25%) [750]: 1.71 ± 0.03*** (16.99%) $BMDL_{10\%}$: [205][a]	body weight	Tyl et al. 2004

Phthalate	Strain and Species: Maternal Doses [mg/kg/day] (Route) Duration {Day of AGD measure}	Control AGD (mean ± SD)	Observations [Dose]: (mean ± SD) / (% change of control)	AGD Correction Metric	Ref.
DnHP	SD Rats: 0, 50, 125, 250, 500 (Gavage): GD 12-21 {PND 1}	1.32 ± 0.08	[50]: 1.30 ± 0.04 [125]: 1.21 ± 0.10* (8.33%) [250]: 1.14 ± 0.07** (13.64%) [500]: 1.08 ± 0.08** (18.18%) $BMDL_{10\%}$: [98][a]	cubic root of body weight	Saillenfait et al. 2009a
DCHP	SD Rats: 0, 250, 500, 750 (Gavage): GD 6-20 {GD21}	1.66 ± 0.07	[250]: 1.52 ± 0.09** (8.43%) [500]: 1.47 ± 0.09** (11.45%) [750]: 1.43 ± 0.08** (13.86%) $BMDL_{10\%}$: [225][a]	cubic root of body weight	Saillenfait et al. 2009b
DEHP	SD Rats: 0, 11, 33, 100, 300 (Gavage): GD 8 - PND 17 {PND 2}	3.25 ± 0.11	[11]: 3.21 ± 0.05 [33]: 3.17 ± 0.09 [100]: 3.17 ± 0.05 [300]: 2.74 ± 0.08** (14.64%) $BMDL_{10\%}$: [161][a]	none	Gray et al. 2009
DIHepP	SD Rats: 0, 64, 304, 532 (Diet): 2-gen {F1- PND 1}	4.34 ± 0.34	[64]: 4.32 ± 0.36 [304]: 4.25 ± 0.47 [532]: 3.71 ± 0.41* (14.52%) BMD_{10}: [414][a]	with (shown) and without cubic root of body weight	McKee et al. 2006

Phthalate	Strain and Species: Maternal Doses [mg/kg/day] (Route) Duration {Day of AGD measure}	Control AGD (mean ± SD)	Observations [Dose]: (mean ± SD) / (% change of control)	AGD Correction Metric	Ref.
DnHepP[b]	SD Rats: 0, 250, 500, 1000 (Gavage): GD 6-20 {GD21}	1.64 ± 0.08	[250]: 1.61 ± 0.07 [500]: 1.59 ± 0.09 [1000]:1.48 ± 0.09** (10%) $BMDL_{10\%}$: [887][a]	cubic root of body weight	Saillenfait et al. 2011b
DINP	Wistar Rats: 0, 300, 600, 750, 900 (Gavage) GD 7-PND 17 {PND 1}	11.60 ± 1.04	[300]: 11.43 ± 0.82 [600]: 11.31 ± 0.20 [750]: 11.29 ± 0.75 [900]: 11.01 ± 0.88* (5.09%) $BMDL_{10\%}$: [909][a]	cubic root of body weight	Boberg et al. 2011
DBzP (MBzP)	SD Rats: 0, 167, 250, 375 (Wistar): GD 15-17 {GD21}	2.4[c] ± NR	[167]: 2.3[c] ± NR [250]: 2.0*[c] ± NR (17%) [375]: 1.8*[c] ± NR (25%) $BMDL_{10\%}$: Data not suitable	cubic root of body weight	Ema et al. 2003
DnOP	SD Rats: 0, 250, 500, 1000 (Gavage): GD 6-20 {GD21}	1.61 ± 0.07	[250]: 1.61 ± 0.07 [500]: 1.58 ± 0.06 [1000]: 1.57 ± 0.07 NE	cubic root of body weight	Saillenfait et al. 2011b
DIDP	SD Rats: 0, 13-15, 39-43, 127-147, 254-295 (Diet): 2-gen {F1- PND0}	1.98 ± 0.39	[15-13]: 2.02 ± 0.36 [43-39]: 1.98 ± 0.35 [147-127]: 2.05 ± 0.34 [295-254]: 2.00 ± 0.35 NE	none	Hushka et al. 2001

Phthalate	Strain and Species: Maternal Doses [mg/kg/day] (Route) Duration {Day of AGD measure}	Control AGD (mean ± SD)	Observations [Dose]: (mean ± SD) / (% change of control)	AGD Correction Metric	Ref.
DUP	SD Rats: 0, 250, 500, 1000 (Gavage): GD 6-20 {GD21}	1.65 ± 0.08	[250]: 1.65 ± 0.08 [500]: 1.59 ± 0.05*(3.6%) [1000]: 1.60 ± 0.09 Not	cubic root of body weight	Saillenfait et al. 2013c
DTDP	SD Rats: 0, 250, 500, 1000 (Gavage): GD 6-20 {GD21}	1.58 ± 0.06	[250]: 1.56 ± 0.05 [500]: 1.56 ± 0.06 [1000]: 1.55 ± 0.08 NE	cubic root of body weight	Saillenfait et al. 2013c

stat. sig. change *(p<0.05);**(p<0.01); ***(p<0.001)
[a] The selected bench mark response is a 10% reduction of AGD relative to the AGD of control animals.
[b] Di-n-heptyl Phthalate (DnHepP) is not a phthalate of interest but is considered for the SAR analysis
NE is presented when no effect observed at highest dose tested; NR when value was not reported in the study
[c] Exact value not reported in paper. Value extracted from graph.

5.4 SAR Analysis

By examining the nature of the ester alkyl chains of the tested phthalates and the data presented in Sections 5.1-5.3, a SAR can be established for the important events for the mechanism of action for androgen-dependent effects on the developing male reproductive system. The activities in the following important events are considered: (1) effects on gene expression related to the steroid biosynthesis pathway in the fetal rat tests; (2) effects on testicular testosterone production in the fetal rat; and (3) decreases in AGD at birth after gestational exposure. The SAR across these important events are depicted in Figure 5-2 to 5-5.

Short-Chain Phthalate Esters (longest carbon backbone length equal to 1 or 2)

Short chain phthalates do not show activity in assays for important events in the mode of action for androgen-dependent effects on the developing male reproductive system. DMP and DEP (phthalates with 1 and 2 carbon backbone esters) do not alter gene expression related to the steroid biosynthesis pathway in the fetal testes (Liu et al. 2005) (Figure 5-2 and 5-3). DEP does not decrease testicular testosterone production in the fetal testes up to a high dose of 900 mg/kg/day when administered to pregnant

rats during the critical male developmental window (Howdeshell et al. 2008) (Figure 5-4). Finally, both DMP and DEP do not decrease AGD in male offspring exposed to phthalates during gestation up to doses of 750 mg/kg/day (Grey et al. 2000) (Figure 5-5).

Medium Chain Phthalate Esters (longest carbon backbone length 3 to 7)

Phthalates where the longest carbon backbone of the ester group is between 3 and 7 show activity in assays for important events in the mode of action for androgen-dependent effects on the developing male reproductive system.

DIBP, DBP, DnHP, DEHP, DnHepP (number of carbon atoms within longest chain on the ester linkage are 3, 4, 6, 6 and 7, respectively) all reduced gene expression related to the steroid biosynthesis pathway (Hannas et al. 2011; Hannas et al. 2012; Lehmann et al. 2004; Saillenfait et al. 2013a). DINP[2] also reduced gene expression (Hannas et al. 2012). Potency trends between phthalates and gene expression were discussed previously in Section 5.1 and are depicted in Figure 5-2 below. The order of most potent to least potent phthalate for reducing steroid biosynthesis gene expression is DBP > DnHP > DIBP > DEHP > DnHepP > DINP. Phthalates where the number of carbons atoms within the longest carbon backbone on the ester linkage is between 3 (DIBP) and 7 (DnHepP) reduce gene expression related to the steroid biosynthesis pathway. Phthalates that have 4 to 6 carbon backbones without branching are the most potent (DBP, DnHP) while phthalates that have 7 carbon backbones without branching where the least potent (DnHepP). DINP is active but its potency is roughly half of DnHepP.

DIBP, DBP, BBP, DnHP, DEHP, and DnHepP (number of carbon atoms within longest chain on the ester linkage is 3, 4, 4 (other chain is a benzyl group), 6, 6 and 7, respectively) all reduced *ex vivo* testicular testosterone production in the fetal testes following gestational exposure (Hannas et al. 2011; Hannas et al. 2012; Howdeshell et al. 2008) Mixture phthalates DINP[2] and DIHepP[3] also reduced *ex vivo* testicular testosterone production in the fetal testes following gestational exposure (Hannas et al. 2012). Potency trends between phthalates and fetal testicular testosterone production are discussed in Section 5.2 and are depicted in Figure 5-4. The order of most potent to least potent phthalate for this effect is DnHP > DIBP > DEHP > DBP > DIHepP > DnHepP > BBP > DINP. Phthalates where the number of carbons atoms within the longest carbon backbone on the ester linkage is between 3 (DIBP) and 7 (DnHepP)

[2] DINP is a mixture where due to branching the number of carbon atoms within longest backbone on the ester linkage varies between six (5%), seven (50%), and over eight (45%) (ECHA 2013k).
[3] DIHepP is a mixture phthalate mixtures where due to methyl branching the number of carbon atoms within longest chain on the ester linkage varies between six (80%) and seven (20%) (CPSC 2011a; ACC 2006)

reduce gene expression related to the steroid biosynthesis pathway. Phthalates that have 3 to 6 carbon backbones are the most potent (DIBP, DBP, DnHP, DEHP) while phthalates that have 7 carbon backbones are less potent (DnHepP). DINP is active but again its potency is approximately half of DnHepP.

Finally, administration of DPrP, DIBP, DBP, BBP, DnHP, DEHP and DnHepP (number of carbon atoms within longest chain on the ester linkage is 3, 3, 4, 4(other chain is a benzyl group), 6, 6 and 7, respectively) decreased the AGD of male rat offspring (Saillenfait et al. 2011a; Saillenfait et al. 2008; Mylchreest et al 1999; Ty et al. 2004; Saillenfait et al 2009a; Gray et al. 2010; Saillenfait et al. 2011b). Mixture phthalates DINP[4] and DIHepP[5] also decreased the AGD of male rat offspring (Boberg et al. 2011; McKee et al. 2006). Potency trends for the AGD effects are discussed in Section 5.3 and are depicted in Figure 5-5. The order of most potent to least potent phthalate for decreasing AGD of male rat offspring when administration of the phthalate occurs during gestation is estimated to be DnHP > DEHP > DIBP ≈ BBP ≈ DBP ≈ > DIHepP > DnHepP > DINP > DPrP.

Cyclohexyl and Benzyl based Phthalate Esters

Phthalates where the ester group is cyclohexyl or benzyl based show activity in some studies for androgen-dependent effects on the developing male reproductive system. DCHP, a phthalate where the ester group is based on a cyclohexyl alkyl chain, decreased the AGD of male rat offspring as a result of gestational exposure (Saillenfait et al. 2009b). DCHP was not as potent as its straight alkyl chain analogue DnHP for decreasing AGD (see Section 5.3 and Figure 5-5). No gene expression or testicular testosterone production data were available for DCHP.

DBzP (a phthalate where the ester groups are benzyl based) has not been studied for steroid biosynthesis gene expression changes, testosterone production, or for effects on AGD of male offspring. However, monobenzyl phthalate [MBzP] (1,2-benzenedicarboxylicacid, mono(phenylmethyl)ester: CAS # 2528-16-7) is thought to be an appropriate surrogate substance to examine the possible effects of DBzP during gestation. Upon oral administration, phthalate diesters are hydrolysed to their respective monoesters within the gastrointestinal tract prior to absorption and it is predominantly the monoester that is taken up systemically. The monoester metabolites are also considered to be responsible for reducing testosterone synthesis as shown in

[4] DINP is a mixture where due to branching the number of carbon atoms within longest backbone on the ester linkage varies between six (5%), seven (50%), and over eight (45%) (ECHA 2013k).
[5] DIHepP is a mixture phthalate mixtures where due to methyl branching the number of carbon atoms within longest chain on the ester linkage varies between six (80%) and seven (20%) (CPSC 2011a; ACC 2006).

cultured Leydig cells (Clewell et al. 2010). MBzP is the monoester hydrolysis product of DBzP and therefore suitable for inferring toxicity in oral developmental studies. MBzP decreased the AGD of male rat offspring as a result of gestational exposure (Ema et al. 2003). The data presented in Ema et al. (2003) were not suitable for BMD modeling so a potency comparison with other phthalates is difficult. No gene expression or testicular testosterone production data were available for MBzP.

Long Chain Phthalate Esters (carbon backbone length equal to or greater than 8)

Long chain phthalates do not show activity in assays for important events in the mechanism of action for androgen-dependent effects on the developing male reproductive system. DIDP[6] did not alter gene expression related to the steroid biosynthesis pathway or decrease fetal testicular testosterone production (up to the highest dose tested 1500mg/kg/day) (Hannas et al. 2012) (Figure 5-2, 5-3 and 5-4). Furthermore, DnOP and DUP (carbon atoms within longest chain on the ester linkage is 8 and 11, respectively) did not decrease AGD of male rat offspring compared to controls when exposure occurred during gestation (at the highest dose tested 1000 mg/kg/day) (Saillenfait et al. 2011b; Saillenfait et al. 2013c). Likewise, DTDP[7] did not decrease AGD of male rat offspring (up to the highest dose tested 1000 mg/kg/day) (Saillenfait et al. 2013c) (Figure 5-5).

[6] DIDP is a mixture where due to methyl branching the number of carbon atoms within longest chain on the ester linkage varies between seven (0-10%), eight (80-90%), and nine (0-10%) (ECHA 2013k).
[7] DTDP is a mixture where the number of carbon atoms within longest chain on the ester linkage varies but are all over eight (ECHA 2013j).

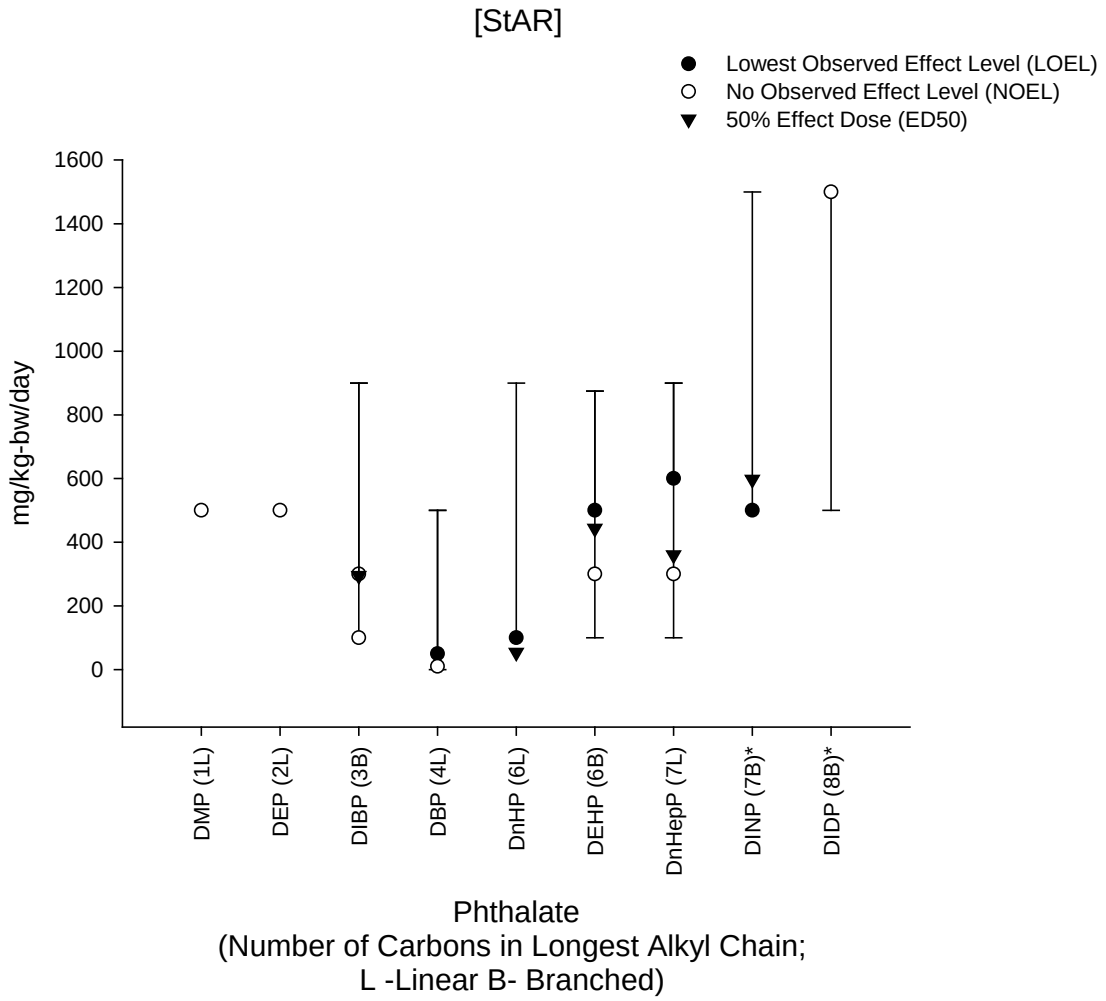


Figure 5-2: Exposure response array showing the structure activity relationship (SAR) between number of carbons in the longest alkyl chain on the ester linkage and the effect levels for reducing gene expression for the cholesterol transport gene StAR.

The vertical and horizontal lines indicate the dose range tested and lowest and highest doses, respectively. Phthalates where the number of carbons in the longest alkyl chain is 1, 2 or 8+ carbons in length (DMP, DEP and DIDP) do not alter gene expression in the fetal tests at the doses tested. Phthalates where the number of carbons atoms in the longest alkyl chain are between 3 and 7 (DIBP, DBP, DnHP, DEHP, DnHepP, DINP) alter gene expression in the fetal tests at the doses tested. See Table 5-1 for study details. *Indicates mixture phthalates where the major constituent is described in brackets.

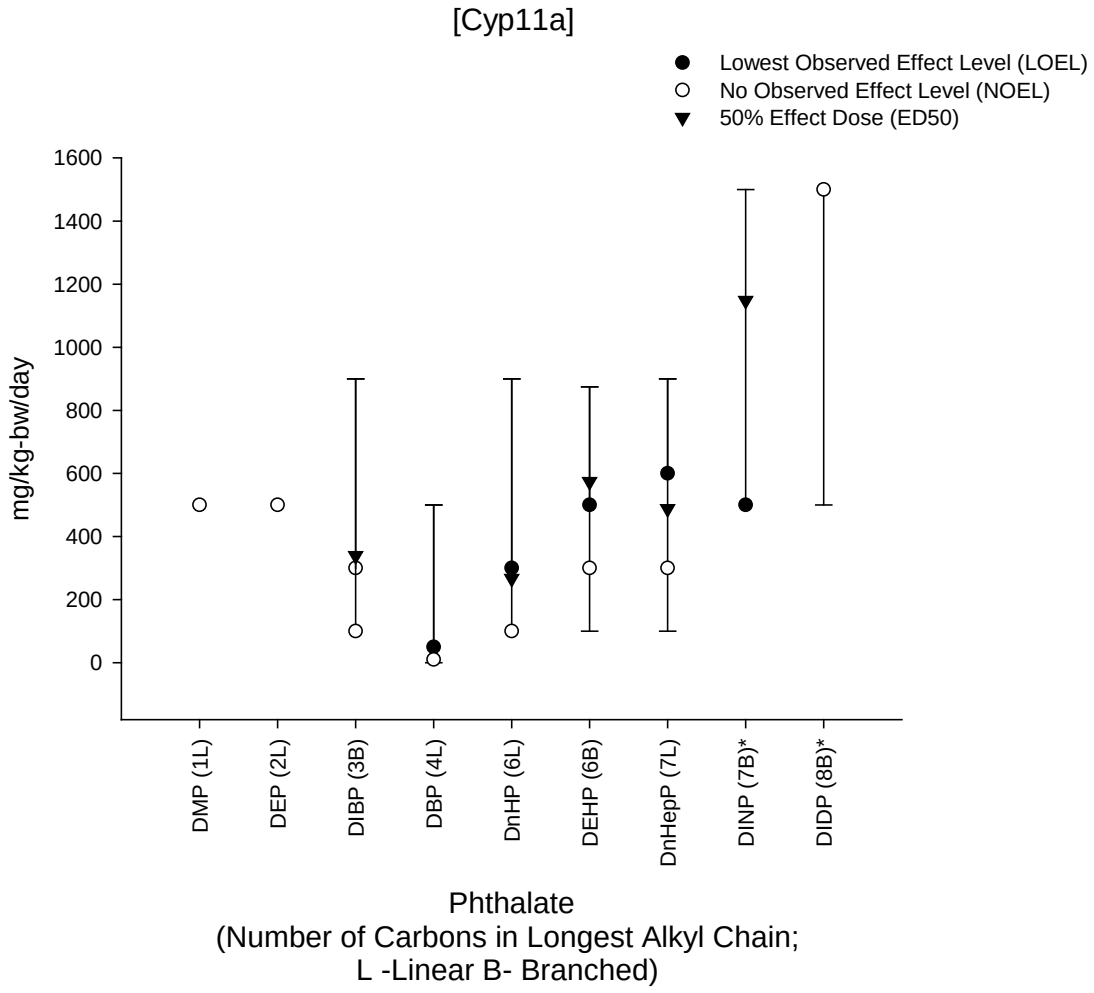


Figure 5-3: Exposure response array showing the structure activity relationship (SAR) between number of carbons in the longest alkyl chain on the ester linkage and the effect levels for reducing gene expression for the steroidogenic gene Cyp11a.

The vertical and horizontal lines indicate the dose range tested and lowest and highest doses, respectively. Phthalates where the number of carbons in the longest alkyl chain is 1, 2 or 8+ carbons in length (DMP, DEP and DIDP) do not alter gene expression in the fetal tests at the doses tested. Phthalates where the number of carbons atoms in the longest alkyl chain are between 3 and 7 (DIBP, DBP, DnHP, DEHP, DnHepP, DINP) alter gene expression in the fetal tests at the doses tested. See Table 5-2 for study details. *Indicates mixture phthalates where the major constituent is described in brackets.

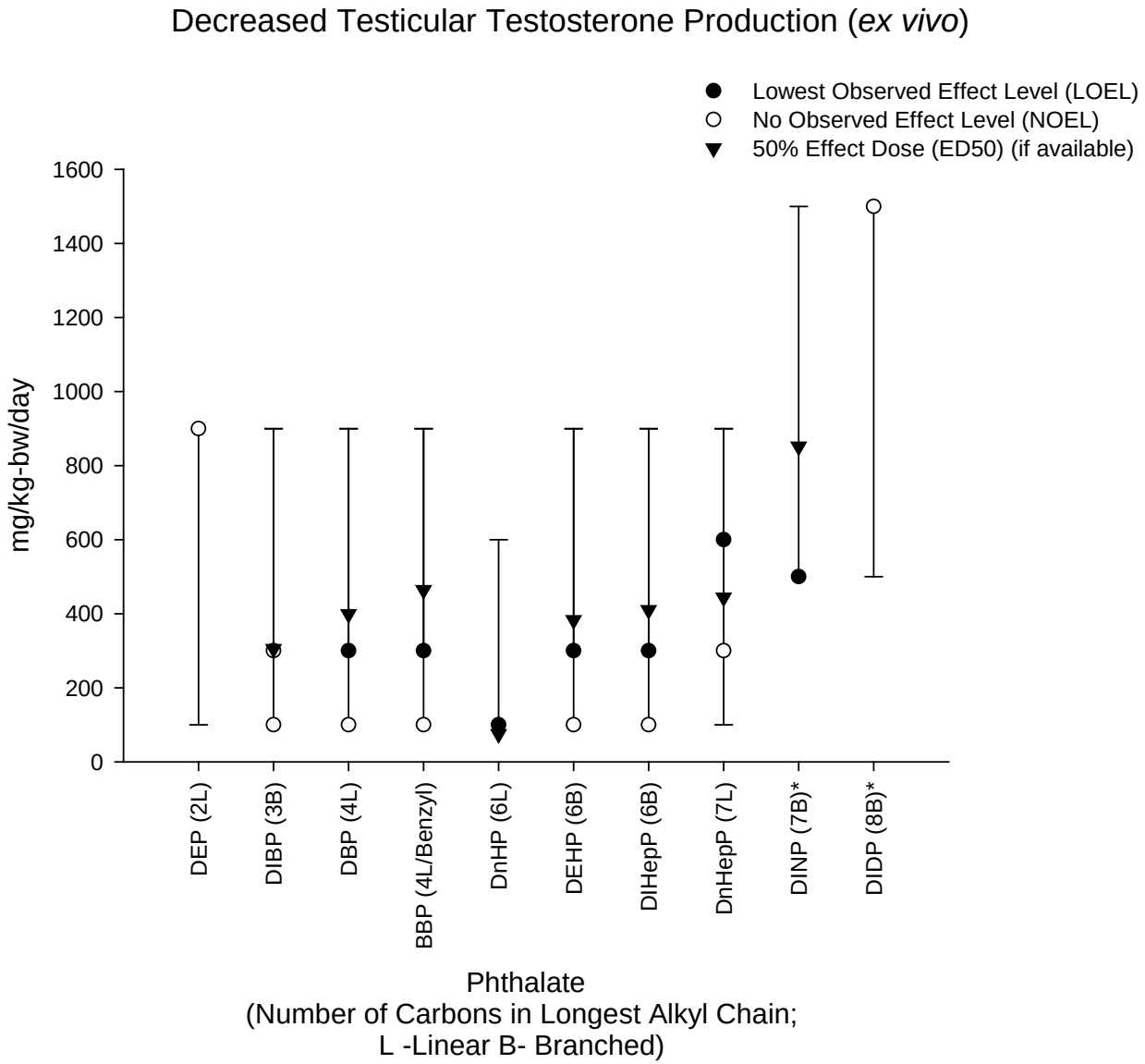


Figure 5-4: Exposure response array showing structure activity relationship (SAR) between number of carbons in the longest alkyl chain on the ester linkage and the effect levels for reducing fetal testicular testosterone production (ex vivo).

The vertical and horizontal lines indicate the dose range tested and lowest and highest doses, respectively. Phthalates where the number of carbons in the longest alkyl chain is 1, 2 or 8+ carbons in length (DMP, DEP and DIDP) do not alter testicular testosterone production (ex vivo) in the fetal tests at the doses tested. Phthalates where the number of carbons atoms in the longest alkyl chain are between 3 and 7 (DIBP, DBP, BBP, DnHP, DIOP, DiHepP, DEHP, DnHepP, and DINP) decrease testosterone production (ex vivo) in the fetal tests. See Table 5-3 for study details. *Indicates mixture phthalates where the major constituent is described in brackets.

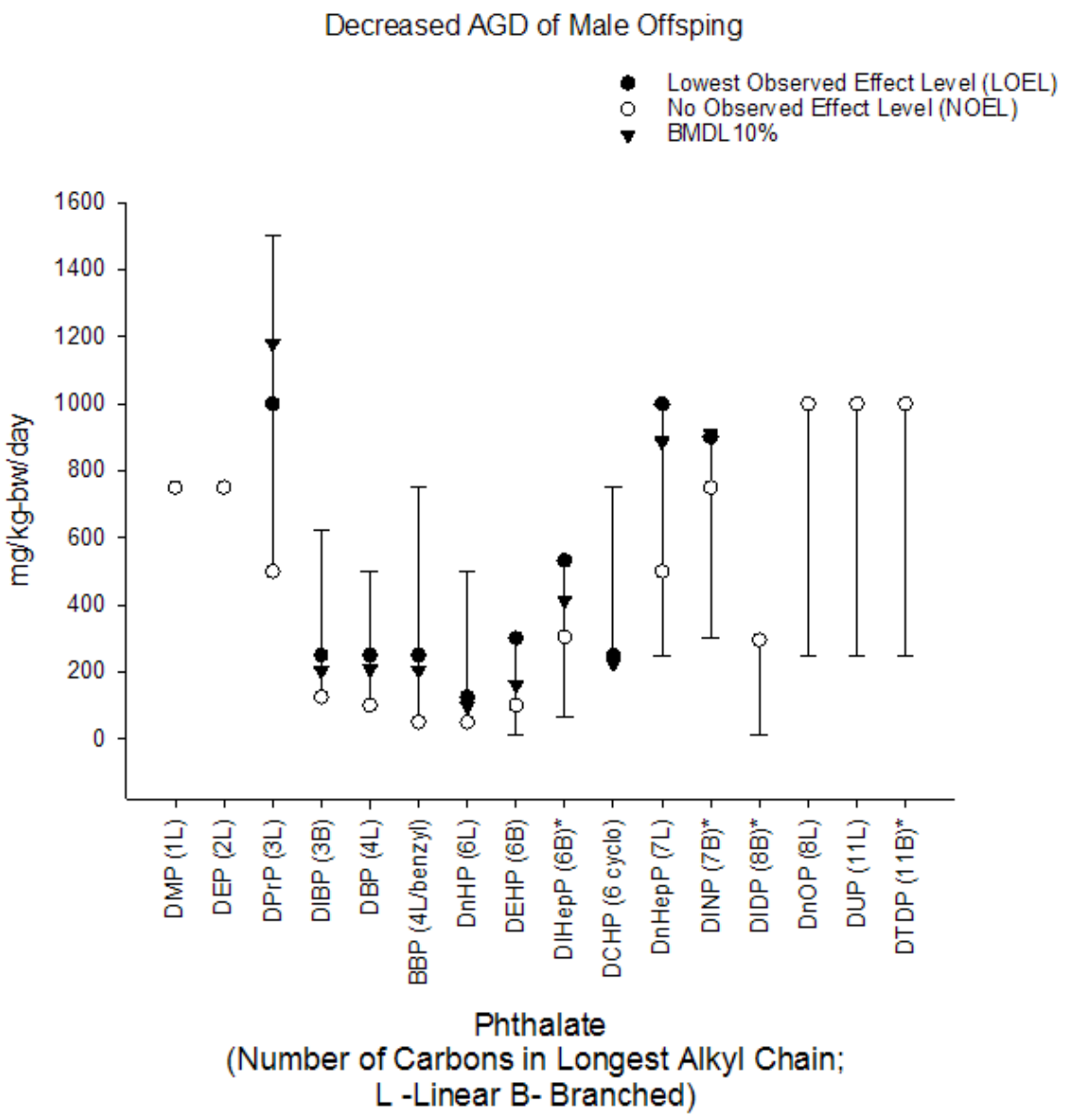


Figure 5-5: Exposure response array showing structure activity relationship (SAR) between number of carbons in the longest alkyl chain on the ester linkage and the maternal doses for decreasing AGD in male pups measured close to birth (from GD 21 to PND 2).

The vertical and horizontal lines indicate the dose range tested and lowest and highest doses, respectively. Phthalates where the number of carbons in the longest alkyl chain is 1, 2 or 8+ carbons in length (DMP, DEP, DIDP, DnOP, DUP and DTDP) do not decrease AGD in male pups compared with controls at the doses tested. Phthalates where the number of carbons atoms in the longest alkyl chain are between 3 and 7 (DIBP, DBP, BBP, DnHP, DEHP, DIHepP, DCHP, DnHepP, and DINP) decrease AGD in male pups compared with controls at the doses tested. See Table 5-4 for study details. *Indicates mixture phthalates where the major constituent is described in brackets.

6. Justification for Subcategories of Phthalates for Assessing Effects Related to Androgen Insufficiency during the Critical Developmental Window in Male Rat Offspring

6.1 SAR Analysis and Mechanistic Considerations

The primary justification for developing the subcategories of phthalates is derived from the structure SAR analysis (Section 5.4) using studies related to important mechanistic events for phthalate-induced androgen insufficiency during male reproductive development in the rat. The effects of phthalate esters for these important events appear to be structure-dependent, and highly related to the length and nature of their alkyl chain.

The SAR analysis indicates that phthalates with 1 to 2 carbon backbones are not active in studies related to important mechanistic events for phthalate-induced androgen insufficiency during male reproductive development in the rat. Similarly, phthalates where the number of carbons in the longest alkyl chain is greater than or equal to 8, are also not considered active in these studies.

Phthalates where the longest carbon backbone is between 3 and 7 are active in studies related to important mechanistic events for phthalate-induced androgen insufficiency during male reproductive development in the rat. However, within this category there are potency differences observed depending on the nature of the ester side chain of the phthalate (Section 5.4 for details).

6.2 Other Considerations

6.2.1 Physicochemical properties

The subcategories of the phthalates of interest are primarily based on the differences observed for effects related to important mechanistic events for phthalate-induced androgen insufficiency during male reproductive development in the rat that are associated with incremental changes in chemical structure of the alkyl side chains on the ester linkage. Differences in the physicochemical properties across the subcategories alone could not explain the differences seen in the mechanistic events. For example, DEHP and DnOP are both produced from phthalic acid and octanol (branched and linear types, respectively) and as a consequence, their physicochemical properties are similar but effects on the developing male reproductive system differ. For example, DEHP decreases AGD in male rats at birth after gestational exposure, while

DnOP does not (See Appendix C for physical-chemical properties and Section 5.0 for study details and SAR analysis). No clear cut-off values could be determined for log K_{ow}, water solubility or molecular weight that were associated with the boundaries of the subcategories. This was also the observation of others when defining structural categories for the reproductive effects of phthalates (Fabjan et al. 2006). Nevertheless, there are broad trends that can be observed across all phthalates of interest. In general, more carbons on the ester chain of the phthalate results in higher hydrophobicity and as a consequence higher log K_{ow} values and lower water solubility.

6.2.2 Toxicokinetics

There is evidence that phthalates, regardless of chain length, are absorbed from the gastrointestinal (GI) tract after oral exposure (Appendix D). However, several studies have shown that absorption of phthalates in the GI tract of rats was not linear with increasing dose, probably due to saturation of the mechanism of uptake or of the diester hydrolysis, particularly for the phthalates with long carbon chains on the ester linkage. There are similarities and differences seen across the phthalates with respect to metabolism and chain length. The smaller phthalates such as DMP and DEP undergo hydrolysis to their respective monoester in the GI tract and are excreted without further metabolism. Larger phthalates undergo hydrolysis in the GI tract to their respective monoester but can also undergo further oxidative metabolism to other metabolites and be excreted as such or as conjugates. There were also differences with respect to the concentration of phthalates distributed to the fetal testes. Monoester metabolites of smaller phthalates such as DMP and DEP were found in the highest concentrations in the fetal testes after maternal dosing. Monoester metabolites of DEHP were found in the lowest concentrations (Clewell et al. 2010).

As with the physicochemical properties, differences in the kinetics relative to the fetal testes alone could not explain the differences seen in studies related to androgen insufficiency in the fetal rat. For example, monoester metabolites of DINP were found at a higher concentration in the fetal testes after maternal exposure during gestation compared with DEHP (~100 and 12 uM, respectively) (Appendix D). This is inversely related to the potency seen for the important events of the mode of action (gene expression for the steroid biosynthesis pathway, testicular testosterone production and AGD) related to androgen insufficiency during the development of the male reproductive system (Section 5.0). It appears that structural differences of the alkyl chain are more important than differences in kinetics. This is supported by Clewell et al. (2010, 2013) who show that the difference in potency between phthalates with long-term effects on the male reproductive tract (DBP, DEHP) and those with reduced (DINP) or no effects (DEP) are not from differential kinetics (i.e., monoester dose to the fetal testes).

6.3 Conclusion

Based on the above considerations, the proposed subcategory definitions for the phthalates of interest are as follows:

Subcategory 1- Short Chain Phthalate Esters (carbon backbone length equal to 1 or 2)

- These phthalates are produced from alcohols with carbon backbones of less than or equal to 2. The resulting products are *ortho* phthalates where the carbon length of the ester side chain is between 1 and 2 carbons in length.

Subcategory 2- Medium Chain Phthalate Esters (carbon backbone length 3 to 7)

- These phthalates are produced from alcohols where the length of the longest carbon backbone is between 3 and 7. The carbon chains on the ester linkage can be linear or branched provided the main chain length is between 3 and 7 carbons in length. For example, di(2-ehtylhexyl) phthalate (DEHP) is made from an alcohol with 8 carbons. However, due to branching the longest chain length is 6 carbons long. Therefore, it is placed in the Medium Chain Phthalate Ester Subcategory. This subcategory also includes phthalates produced from benzyl alcohol or cyclohexanol since these ester groups were also found to be similar for effects (decreased AGD) on the developing male reproductive system.

Subcategory 3 – Long Chain Phthalate Esters (carbon backbone length equal to or greater than 8)

- These phthalates are produced from alcohols where the longest carbon backbones are equal to or greater than 8. The carbon chain lengths on the ester linkage can be linear or branched provided the main chain length is equal to or greater than 8 in length.

7. Filling Data Gaps for Androgen-Related Effects on the Developing Male Reproductive System for Data Poor Phthalates

Out of the 28 phthalates within this document, 10 do not have developmental toxicity or reproductive studies that examine effects related to androgen insufficiency during male development in fetal rats. These phthalates are considered to have a data gap for these effects. As the availability of effects data for this critical endpoint is a key consideration for the health effects characterization of the phthalates of interest and may be considered for a potential CRA, an approach for filling data gaps is required.

7.1 Methodology for Filling Data Gaps

7.1.1 Placing phthalates of interest into the defined categories

The subcategories of phthalates (Section 6) were developed in order to facilitate read-across of data from tested phthalates to phthalates that have data gaps for androgen-dependent endpoints for developmental and reproductive toxicity to the male reproductive system in rats. The 28 phthalates of interest were placed in one of the three subcategories based on an analysis of the ester side chain for each given phthalate against the subcategory definitions presented in Section 6.3 (Table 7-1 to 7-3).

Categorizing Phthalate Mixtures

Certain phthalates covered under this initiative are mixtures. These substances fall under two types:

1) Isomer mixtures - Phthalates synthesized using a mixture of alcohol isomers with a certain carbon number. For example, DIDP (CAS 26761-40-0) is a mixture containing mainly C10 methyl branched isomers. Best estimates indicated DIDP is comprised of 0-10% tri-methyl heptyl chains, 70-80% di-methyl octyl chains, and 0-10% methyl nonyl chains (ECHA 2013).

2) UVCB (Unknown or Variable Composition, Complex Reaction Products, or Biological) - Substances that are a mixture of *ortho* phthalates with carbon chains on the ester linkage of varying lengths. An example of a UVCB phthalate mixture is, 1,2-Benzenedicarboxylic acid, mixed decyl, hexyl and octyl diesters (610P) (CAS 68648-93-1).

To place these two types of mixture phthalates into an appropriate subcategory, information on general composition was collected where available (Appendix A).

Following the cut-off presented in the EPA HPV phthalate test plan presented by the American Chemistry Council Phthalate Ester Panel (ACC 2006), mixtures where the composition indicates that >10% of the phthalates have the longest carbon chain between 3 and 7 have been conservatively placed in the Medium Chain Phthalate Ester Category.

Table 7-1: Subcategory 1- Short Chain Phthalate Esters (carbon backbone length equal to 1 or 2)

CAS RN	Chemical Name (DSL)	Acronym	Substance Type
131-11-3	1,2-Benzenedicarboxylic acid, dimethyl ester	DMP	Discrete
84-66-2	1,2-Benzenedicarboxylic acid, diethyl ester	DEP	Discrete

Table 7-2: Subcategory 2- Medium Chain Phthalate Esters (carbon backbone length 3 to 7)

CAS RN	Chemical Name (DSL)	Acronym	Substance Type
131-16-8	1,2-Benzenedicarboxylic acid, dipropyl ester	DPrP	Discrete
84-69-5	1,2-Benzenedicarboxylic acid, bis(2-methylpropyl) ester	DIBP	Discrete
5334-09-8	1,2-Benzenedicarboxylic acid, cyclohexyl 2-methylpropyl ester	CHIBP	Discrete
84-64-0	1,2-Benzenedicarboxylic acid, butyl cyclohexyl ester	BCHP	Discrete
84-74-2	1,2-Benzenedicarboxylic acid, dibutyl ester	DBP	Discrete
85-68-7	1,2-Benzenedicarboxylic acid, butyl phenylmethyl ester	BBP	Discrete
84-61-7	1,2-Benzenedicarboxylic acid, dicyclohexyl ester	DCHP	Discrete
27987-25-3	1,2-Benzenedicarboxylic acid, bis(methylcyclohexyl) ester	DMCHP	Discrete
71888-89-6	1,2-Benzenedicarboxylic acid, di-C_{6-8}-branched alkyl esters, C_7-rich	DIHepP	Isomer Mixture
27554-26-3	1,2-Benzenedicarboxylic acid, diisooctyl ester	DIOP	Isomer Mixture
27215-22-1	1,2-Benzenedicarboxylic acid, isooctyl phenylmethyl ester	BIOP	Isomer Mixture

CAS RN	Chemical Name (DSL)	Acronym	Substance Type
117-81-7	1,2-Benzenedicarboxylic acid, bis(2-ethylhexyl) ester	DEHP	Discrete
84-75-3	1,2-Benzenedicarboxylic acid, dihexyl ester	DnHP	Discrete
111381-89-6	1,2-Benzenedicarboxylic acid, heptyl nonyl ester, branched and linear	79P	UVCB
68515-48-0; 28553-12-0	1,2-Benzenedicarboxylic acid, di-C8-10-branched alkyl esters, C9-rich; 1,2-Benzenedicarboxylic acid, diisononyl ester	DINP1,2	Isomer Mixture
68515-40-2	1,2-Benzenedicarboxylic acid, benzyl C7-9-branched and linear alkyl esters	B79P	UVCB
16883-83-3	1,2-Benzenedicarboxylic acid, 2,2-dimethyl-1-(1-methylethyl)-3-(2-methyl-1-oxopropoxy)propyl phenylmethyl ester	B84P	Discrete
523-31-9	1,2-Benzenedicarboxylic acid, bis(phenylmethyl) ester	DBzP	Discrete

Table 7-3: Subcategory 3 – Long Chain Phthalate Esters (carbon backbone length equal to or greater than 8)

CAS RN	Chemical Name (DSL)	Acronym	Substance Type
68648-93-1	1,2-Benzenedicarboxylic acid, mixed decyl and hexyl and octyl diesters	610P	UVCB
26761-40-0 (68515-49-1)	1,2-Benzenedicarboxylic acid, diisodecyl ester	DIDP	Isomer Mixture
117-84-0	1,2-Benzenedicarboxylic acid, dioctyl ester	DnOP	Discrete
68515-43-5	1,2-Benzenedicarboxylic acid, di-C9-11-branched and linear alkyl esters	D911P	UVCB
3648-20-2	1,2-Benzenedicarboxylic acid, diundecyl ester	DUP	Isomer Mixture
111381-91-0	1,2-Benzenedicarboxylic acid, nonyl undecyl ester, branched and linear	D911P-2	UVCB
68515-47-9	1,2-Benzenedicarboxylic acid, di-C11-14-branched alkyl esters, C13-rich	DTDP	UVCB
85507-79-5	1,2-Benzenedicarboxylic acid, diundecyl ester, branched and linear	DIUP	UVCB

7.1.2 Selecting the closest analogue within subcategory for read-across

With the phthalates of interest placed in the subcategories, read-across can be used to apply values for dose-response relationships and effect levels from well-studied phthalates to phthalates where there is a data gap for androgen-dependent effects on the developing male reproductive system. When applying quantitative read-across, there are four general ways of estimating the missing data point (OECD 2007):

- Using the endpoint value of a similar chemical [e.g., the closest analogue within a (sub)category)];
- Using a trend to mathematically scale the available experimental results from two or more similar chemicals to the target chemical (e.g., trend analysis with an internal model);
- Processing the endpoint values from two or more source chemicals (e.g., by averaging, by taking the most representative value); or
- Taking the most conservative value of the closest analogues or the most conservative value in the whole (sub)category.

For the 28 phthalates of interest, it is proposed to use the endpoint value of the 'closest analogue' phthalate within the subcategory. This was considered appropriate based on the empirical evidence outlined throughout this approach document which indicates that there are differences in potency across members of the Medium Chain Phthalate Ester Category for important events related to the mode of action for androgen insufficiency during male reproductive development (See SAR analysis Section 5.4).

The effects of phthalate esters on male reproductive development appear to be structure dependent, and highly related to the alkyl length and nature of the ester chain. Therefore the 'closest analogue' phthalate within the subcategory is determined by the length and nature of the ester chain. Upon oral administration, phthalate diesters are hydrolysed to their respective monoesters within the GI tract before absorption and it is predominantly the monoester that is taken up systemically. The monoester metabolites are also considered to be responsible for reducing testosterone synthesis as shown in cultured Leydig cells (Clewell et al. 2010). Therefore, metabolism to common monoester metabolites is also considered when assessing closest analogues. For mixture phthalates, compositional similarity is also considered.

Selection of the most similar phthalate for each of the phthalates where there is a data gap is discussed below.

Subcategory 1- Short Chain Phthalate Esters (Carbon backbone length equal to 1 or 2)

Dimethyl Phthalate (DMP) and Diethyl Phthalate (DEP)

Dimethyl phthalate (DMP) and diethyl phthalate (DEP) were placed in this subcategory. Both of these phthalates have rat studies to evaluate androgen-related effects on the developing male reproductive system when administration occurs during gestation. Available data indicates that these phthalates are not active for altering testicular testosterone production during the critical *in utero* window of male reproductive system development. No read-across for androgen-sensitive endpoints will be required for these phthalates.

Subcategory 2- Medium Chain Phthalate Esters (Carbon backbone length 3 to 7)

Cyclohexyl Isobutyl Phthalate (CHIBP)

Cyclohexyl Isobutyl Phthalate (CHIBP) is a mono constituent *ortho* phthalate with asymmetric ester groups. One ester group consists of an isobutyl alkyl chain while the other consists of a cyclohexyl chain. Based on the ester groups and the SAR analysis, CHIBP has been placed in the Medium Chain Phthalate Subcategory and is expected to result in androgen-related effects on the developing male reproductive system in rats when exposure occurs during gestation. The most similar phthalates within the subcategory based on the ester groups of CHIBP are Diisobutyl Phthalate (DIBP) and Dicyclohexyl Phthalate (DCHP). CHIBP when given orally to rats is expected to yield monoester metabolites identical to the monoester metabolites of DIBP and DCHP. Due to structural similarities, the physicochemical properties for DIBP and DCHP are also within a similar range of CHIBP. Therefore, for evaluating androgen-dependent effects to the male reproductive system after gestational exposure, it is proposed to use reported effect levels from DIBP and DCHP to characterize the health effects for CHIBP.

Butyl Cyclohexyl Phthalate (BCHP)

Butyl Cyclohexyl Phthalate (BCHP) is a mono constituent *ortho* phthalate with asymmetric ester groups. One ester group consists of an n-butyl alkyl chain while the other consists of a cyclohexyl chain. Based on the ester groups and the SAR analysis, BCHP has been placed in the Medium Chain Phthalate Subcategory and is expected to result in androgen-related effects on the developing male reproductive system in rats when exposure occurs during gestation. The most similar phthalates within the subcategory based on the ester groups of BCHP are Dibutyl Phthalate (DBP) and Dicyclohexyl Phthalate (DCHP). BCHP when given orally to rats is expected to yield monoester metabolites identical to the monoester metabolites of DBP and DCHP. Due

to structural similarities, the physicochemical properties for DBP and DCHP are also within a similar range of BCHP. Therefore, for evaluating androgen-dependent effects to the male reproductive system after gestational exposure, it is proposed to use reported effect levels from DBP and DCHP to characterize the health effects for BCHP.

Dimethylcyclohexyl Phthalate (DMCHP)

Dimethylcyclohexyl Phthalate (DMCHP) is a mono constituent *ortho* phthalate with symmetric ester groups. Both ester chains consist of a methylcyclohexyl group. Based on the ester groups and the SAR analysis, DMCHP has been placed in the Medium Chain Phthalate Subcategory and is expected to result in androgen-related effects on the developing male reproductive system in rats when exposure occurs during gestation. The most similar phthalate within the subcategory based on the ester groups of DMCHP is Dicyclohexyl Phthalate (DCHP). DMCHP and DCHP are closely-related structurally as the ester groups consist of cyclohexyl chains with DMCHP having an additional methyl group on the cyclohexyl ring. Due to structural similarities, the physicochemical properties for DMCHP and DCHP are also similar. Therefore, for evaluating androgen-dependent effects to the male reproductive system after gestational exposure, it is proposed to use reported effect levels from DCHP to characterize the hazard effects for DMCHP.

Benzyl Isooctyl Phthalate (BIOP)

Benzyl Isooctyl Phthalate is a mixture of *ortho* phthalates with asymmetric ester groups. One ester group consists of isomer dimethyl hexyl chains while the other consists of a benzyl group (EPA 2006). (See Appendix A for composition information). Based on the composition of the ester groups and the SAR analysis, BIOP has been placed in the Medium Chain Phthalate Subcategory and is expected to result in androgen-related effects on the developing male reproductive system in rats when exposure occurs during gestation. The most similar phthalates within the subcategory based on the composition of the ester groups of BIOP are Diisooctyl Phthalate (DIOP) and Monobenzyl Phthalate (MBzP). BIOP when given orally to rats is expected to yield similar monoester metabolites to the monoester metabolites of DIOP. BIOP is also expected to yield the monoester metabolite MBzP and therefore, MBzP is also appropriate as an analogue of BIOP. Due to structural similarities, the physicochemical properties for DIOP are also within a similar range of BIOP. Therefore, for evaluating androgen-dependent effects to the male reproductive system after gestational exposure, it is proposed to use reported effect levels from DIOP and MBzP to characterize the health effects for BIOP.

79P [UVCB]

Phthalate Mixture (79P) is a mixture of *ortho* phthalates with branched and linear alkyl esters with seven to nine carbons. Available composition information suggests that 79P typically consists of 15% C4-C6 content (due to methyl branching) and 85% ≥C7 ester groups (EPA 2006). Based on the composition of the ester groups and the SAR analysis, 79P has been placed in the Medium Chain Phthalate Subcategory and is expected to result in androgen-related effects on the developing male reproductive system in rats when exposure occurs during gestation. Based on the composition of the ester groups, the most similar phthalate within the subcategory is Diisononyl Phthalate (DINP). Therefore, for evaluating androgen-dependent effects to the male reproductive system after gestational exposure, it is proposed to use reported effect levels from DINP to characterize the health effects for 79P.

B79P [UVCB]

Phthalate Mixture (B79P) is a mixture of *ortho* phthalates with asymmetric ester groups. One ester group consists of isomers of branched and linear alkyl esters with seven to nine carbons. Available composition information suggests that B79P typically consists of 2% C4-C6 content (due to methyl branching) and 48% ≥C7 ester groups. The remaining 50% of the ester groups are benzyl-based (EPA 2006). Based on the composition of the ester groups and the SAR analysis, B79P has been placed in the Medium Chain Phthalate Subcategory and is expected to result in androgen-related effects on the developing male reproductive system in rats when exposure occurs during gestation. Based on the composition of the ester groups, the most similar phthalates within the subcategory are Diisononyl Phthalate (DINP) and Monobenzyl Phthalate (MBzP). B79P when given orally to rats is expected to yield some monoester metabolites similar to the monoester metabolites of DINP. B79P is also expected to yield the monoester metabolite MBzP and therefore, MBzP is also appropriate as an analogue of B79P. Due to structural similarities, the physicochemical properties for DINP are also within a similar range of B79P. Therefore, for evaluating androgen-dependent effects to the male reproductive system after gestational exposure, it is proposed to use reported effect levels from DINP and MBzP to characterize the health effects for B79P. The available study summary data from the REACH dossier for B79P will also be used to characterize the health effects for B79P.

B84P [UVCB]

Phthalate B84P is an *ortho* phthalate with asymmetric ester groups. One ester group is based on branched isooctylbutyrate. This alkyl group is different from the other phthalates of interest as it has an ester linkage within the longest alkyl chain. The second ester group is benzyl based. A hydrolysis metabolic simulator (Oasis 2013)

indicates that potential monoester metabolites of B84P are MBzP and a monoester with a carbon chain length of five within the longest backbone (2-(((1-hydroxy-2,2,4-trimethylpentan-3-yl)oxy)carbonyl)benzoic acid). This is due to the ester linkage present in the middle of the alkyl chain of the parent compound being subject to hydrolysis. Based on this potential metabolite it is conservatively placed in the Medium Chain Phthalate Ester Category and is expected to result in androgen-related effects on the developing male reproductive system in rats when exposure occurs during gestation. There are three potential analogues that can be used for read-across for androgen related effects for B84P. DIBP is a similar phthalate within the category based on similarities of monoester metabolites [five carbon branched monoester metabolite (B84P) vs. three carbon branched monoester metabolite (DIBP)]. B84P is also expected to yield the monoester metabolite MBzP and therefore, MBzP is also appropriate as an analogue of B84P. Finally, BBP is also a suitable analogue based on similarities in monoester metabolites [(five carbon branched monoester and benzyl monoester metabolites (B84P) vs. four carbon monoester and benzyl monoester metabolites (BBP)]. Therefore, for evaluating androgen-dependent effects to the male reproductive system after gestational exposure, it is proposed to use reported effect levels from BBP, DIBP and MBzP to characterize androgen-related effects for B84P. Recognizing the uncertainty with respect to B84P metabolism, there is also more uncertainty for the proposed read-across compared to others in the category and the proposed approach is considered conservative.

Dibenzyl Phthalate (DBzP)

Dibenzyl Phthalate (DBzP) is a mono constituent *ortho* phthalate with symmetric ester groups. Both ester chains consist of a benzyl group. Monobenzyl phthalate [MBzP] is thought to be an appropriate analogue to examine the possible effects of DBzP during gestation since it is the monoester metabolite of DBzP.

Subcategory 3 – Long Chain Phthalate Esters (Carbon backbone equal to or greater than 8)

610P [UVCB]

Phthalate Mixture (610P) is a mixture of *ortho* phthalates with predominately linear alkyl esters with six to ten carbons. Available composition information suggests that 610P (68648-93-1) typically consists of 8% hexyl (C6); 42% octyl (C8); and 50% decyl (C10) phthalates (BASF 2011; BASF 2004). The major components in this mixture belong to the Long Chain Phthalate Ester Category (Longest Carbon backbone ≥8) and based on the SAR analysis these phthalates are not expected to result in androgen-related effects on the developing male reproductive system in rats when exposure occurs during gestation. There is a small fraction of this mixture (~8%) that would belong in the

Medium Chain Phthalate Ester Category; however, following the recommendation of the American Chemistry Council Phthalate Ester Panel (ACC 2006), the fraction is below 10%; and therefore, this mixture is not placed in the medium chain category. As the major components in the mixture are linear eight and ten carbon phthalate esters, DnOP and DUP (eight and eleven carbon linear esters, respectively) are considered the most similar phthalates in the grouping. Therefore, for evaluating androgen-dependent effects to the male reproductive system after gestational exposure, it is proposed to use reported effect levels from DnOP and DUP to characterize the health effects for 610P.

Diisoundecyl Phthalate (DIUP)

Diisoundecyl Phthalate is a mixture of *ortho* phthalates with predominately branched alkyl esters with eight to ten carbons in the longest chain (due to methyl branching) (ECHA 2013i). Based on the composition of the ester groups and the SAR analysis, DIUP has been placed in the Long Chain Phthalate Subcategory and is not expected to result in androgen-related effects on the developing male reproductive system in rats when exposure occurs during gestation. The most similar phthalate within the subcategory based on the ester groups of DIUP is Diisodecyl Phthalate (DIDP). Due to structural and compositional similarities, the physicochemical properties for DIDP are also within a similar range of DIUP. Therefore, for evaluating androgen-dependent effects to the male reproductive system after gestational exposure, it is proposed to use reported effect levels from DIDP to characterize the hazard potential for DIUP.

D911P-2 [UVCB]

Phthalate Mixture (D911P-2) is a mixture of *ortho* phthalates with branched and linear alkyl esters with nine and eleven carbons. Available composition information suggests that D911P-2 (CAS RN 111381-91-0) was formerly the major component of Palatinol® 11-9P-I (BASF 2011). Product monographs referring to Palatinol® 11-9P-I (containing CAS RN 111381-91-0) is a mixture of linear phthalate esters based on C9 and C11 alcohols (BASF 2001). Based on the composition of the ester groups and the SAR analysis, D911P-2 has been placed in the Long Chain Phthalate Subcategory and is not expected to result in androgen-related effects on the developing male reproductive system in rats when exposure occurs during gestation. The most similar phthalate within the subcategory based on the ester groups of D911P-2 (CAS RN 111381-91-0) is D911P (CAS RN 68515-43-5). In CPSC publicly available meeting notes, BASF indicates that D911P (CAS RN 68515-43-5) is a phthalate produced from 18% Nonanols (C9); 42% Decanols (C10); and 40% Undecanol (C11) where the alcohols are >80% linear (BASF 2009). Therefore, for evaluating androgen-dependent effects to the male reproductive system after gestational exposure, it is proposed to use reported effect levels from D911P (CAS RN 68515-43-5) to characterize the health effects for D911P-2 (CAS RN 111381-91-0).

7.1.3 Summary of proposed analogues for read-across of androgen-related effect levels for data poor phthalates

Table 7-4: Proposed analogues for read-across of androgen-related effect levels for data-poor medium chain phthalates

Phthalate	Proposed Analogue(s) for Androgen Related Endpoints (*in utero* exposure)
CHIBP (5334-09-8)†	DIBP / DCHP
BCHP (84-64-0)†	DBP / DCHP
DMCHP (27987-25-3)†	DCHP
BIOP (27215-22-1)†	DIOP / MBzP
79P (111381-89-6)^	DINP
B79P (68515-40-2)†*	DINP / MBzP
B84P (16883-83-3)†	BBP / DIBP / MBzP
DBzP (523-31-9)†	MBzP

† Substances that are part of the Phthalate Substance Grouping (Government of Canada 2012);
^Additional DSL phthalates being considered to inform risk assessment and considered for potential cumulative risk assessment.
* A summary for the extended one-generation reproductive and developmental toxicity study was in a REACH dossier for B79P. This summary, as well as read-across, will be considered for evaluating androgen related effects.

Table 7-5 Proposed analogues for read-across of androgen-related effect levels for data-poor long chain phthalates

Phthalate	Proposed Analogue(s) for Androgen Related Endpoints (*in utero* exposure)
610P (68648-93-1)^	DnOP^ / DUP*
D911P -2 (111381-91-0)^	D911P^*
DIUP (85507-79-5)^	DIDP*

† Substances that are part of the Phthalate Substance Grouping (Government of Canada 2012);
^Additional DSL phthalates being considered to inform risk assessment and considered for potential cumulative risk assessment (if applicable);
* Read-across of 'no effect' since these phthalates do not show androgen related effects or effects in important events related to the mode of action for the rat phthalate syndrome.

8. Read-Across for Systemic Effects of Phthalates

The categories and selected analogues were primarily developed for addressing data gaps for androgen-related effects when exposure occurs *in utero* for male rat offspring. However, it is anticipated that the presented analogue approach may also be appropriate for assessing other systemic effects related to phthalate oral exposures. Since the overall justification for the read-across for the androgen related endpoints accounts for similarities in structure, composition, physicochemical properties and metabolism, the analogues may also be appropriate for assessing systemic effects based on these considerations. However, without mode of action information underpinning the commonalities in effects, there is a greater degree of uncertainty associated with the read-across for systemic effects.

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Appendix A

Estimates of the Typical Compositions of Phthalates of Interest

Table A- 1 Estimates of the typical composition (percent) for ester carbon backbones for the short chain phthalate ester category

Phthalate (CAS #)	Ester Groups branching	≤C2	C3	C4	C5	C6	C7	≥C8	Other	Ref.
DMP (131-11-3)	methyl linear	100	-	-	-	-	-	-	-	(ACC 2006)
DEP (84-66-2)	ethyl linear	100	-	-	-	-	-	-	-	(ECHA 2013b)

Table A- 2 Estimates of the typical composition (percent) for ester carbon backbones for the medium chain phthalate ester category

Phthalate (CAS #)	Ester Groups branching	≤C2	C3	C4	C5	C6	C7	≥C8	Other	Ref.
DPrP (131-16-8)	propyl linear	-	100	-	-	-	-	-	-	(Saillenfait et al. 2011a)
DIBP (84-69-5)	isobutyl branched	-	100	-	-	-	-	-	-	(ECHA 2013c)
CHIBP (5334-09-8)	isobutyl; and cyclo-hexyl branched / cyclo	-	50	-	-	-	-	-	50 cyclo-hexyl	Limited data[a]
BCHP (84-64-0)	n-butyl; and cyclo-hexyl branched; cyclo	-	-	50	-	-	-	-	50 cyclo-hexyl	Limited data[a]

DBP (84-74-2)	n-butyl linear	-	-	100	-	-	-	-	-	(ECHA 2013d)
BBP (85-68-7)	n-butyl; and benzyl linear / benzyl	-	-	50	-	-	-	-	50 benzyl	(ECHA 2013e)
DCHP (84-61-7)	cyclohexyl cyclo	-	-	-	-	-	-	-	100 cyclo-hexyl	(ECHA 2013f)
DMCHP (27987-25-3)	methyl cyclohexyl cyclo	-	-	-	-	-	-	-	100 cyclo-hexyl	Limited data[a]
DIHepP (71888-89-6)	isoheptyl; and n-heptyl linear and branched	-	-	-	-	80	20	-	-	(CPSC 2011a) (ACC 2006)
DIOP (27554-26-3)	dimethyl hexyl; and methyl heptyl branched	-	-	-	-	70 - 75	25 - 30	-	-	(CPSC 2011b) (ACC 2006)
BIOP (27215-22-1)	dimethyl hexyl; methyl heptyl; and benzyl branched and benzyl	-	-	-	-	45-50	0-5	-	50	Limited data[a] extrapolated from DIOP composition
DEHP (117-81-7)	ethylhexyl branched	-	-	-	-	100	-	-	-	(ECHA 2013g)
DnHP (84-75-3)	n-hexyl linear	-	-	-	-	100	-	-	-	(ECHA 2011)

79P [UVCB mix C7 C9] (111381-89-6)	methyl heptyl; and nonyl linear and branched			15			85			(ACC 2006)
DINP-1 (68515-48-0)	methyl ethylhexyl; dimethyl heptyl; methyl octyl; n-nonyl; and isodecyl linear and branched	-	-	-	-	5-10	45-55	20-45	-	(ECHA 2013k)
DINP-2 (28553-12-0)	methyl-ethylhexyl; dimethyl heptyl; methyl octyl; and n-nonyls linear and branched	-	-	-	-	5-10	40-45	35-50	-	(ECHA 2013k)
B79P (68515-40-2)	methyl branched heptyl / nonyl; and benzyl linear, branched and benzyl	-	-	2			48		50 benzyl	(ACC 2006)
B84P (16883-83-3)	benzyl, isooctylbutyrate branched and benzyl	-	-	-	50[b]	-	-	-	50 benzyl	(ACC 2006)

DBzP (523-31-9)	benzyl	-	-	-	-	-	-	-	100 benzyl	Limited data[a]

[a] No composition information was found. The composition was estimated based on DSL name or expert judgement.
[b] B84P contains a benzyl ester group and an isooctylbutyrate ester group. The hydrolysis simulator within the OECD Toolbox indicates that a potential metabolite of B84P has a carbon chain length of 5 in longest backbone. This is due to the ester linkage present in the middle of the alkyl chain. B84P is conservatively placed in the medium chain category due to this potential metabolite.

Table A- 3 Estimates of the typical composition (percent) for ester carbon backbones for the long chain phthalate ester category

Phthalate (CAS #)	Ester Groups branching	≤C2	C3	C4	C5	C6	C7	≥C8	Other	Ref.
610P [UVCB mix C6, 8 10] (68648-93-1)	hexyl, octyl, decyl linear	-	-	-	-	8	-	92	-	(ACC 2006) (BASF 2011; BASF 2004)
DIDP (26761-40-0) (68515-49-1)	methyl heptyl; methyl octyl; and methyl nonyl branched	-	-	-	-	-	0-10	70-90	-	(ECHA 2013k)
DnOP (117-84-0)	n-octyl linear	-	-	-	-	-	-	100	-	(CPSC 2010) (ECHA 2010)
D911P [UVCB Mix C9-C11] (68515-43-5)	nonyl; decyl; and undecyl mostly linear	-	-	-	-	-	-	>97	-	(CPSC 2011c) (BASF 2009)
DUP (3648-20-2)	undecyl mostly linear	-	-	-	-	-	-	100	-	(ECHA 2013h) (BASF 2013)

D911P-2 [UVCB Mix nonyl, undecyl] (111381-91-0)	nonyl; undecyl mostly linear	-	-	-	-	-	-	100	-	(BASF 2011) (BASF 2001)
DIUP (85507-79-5)	methyl decyl; methyl undecyl; and methyl dodecyl branched	-	-	-	-	-	-	100	-	(ECHA 2013i)
DTDP [UVCB Mix] (68515-47-9)	undecyl; dodecyl; tridecyl; tetra-decyl branched	-	-	-	-	-	-	100	-	(ECHA 2013j)

Appendix B

Representative structures of the phthalates of interest

Table B- 1 Representative structures for the short chain phthalates

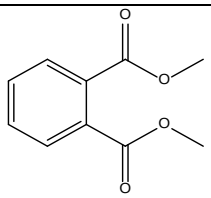
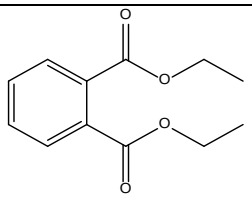
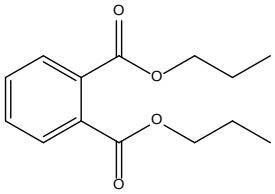
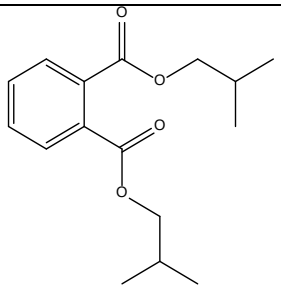
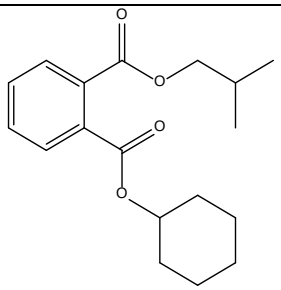
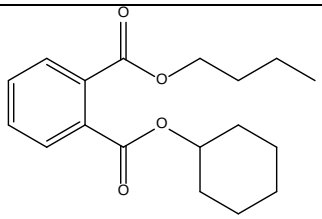
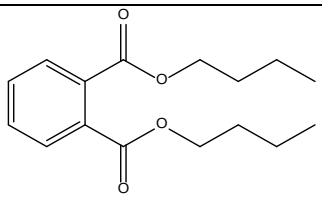
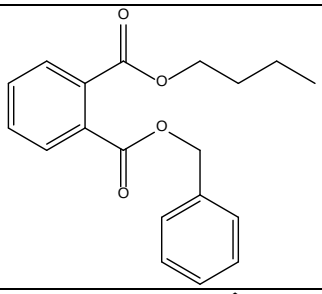
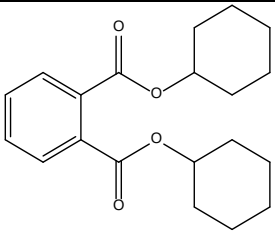
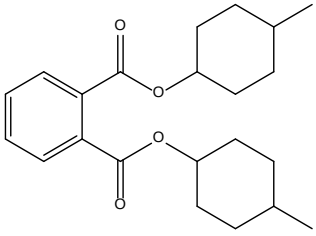
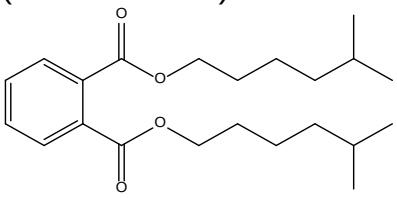
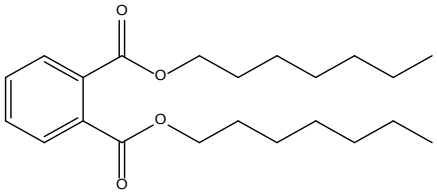
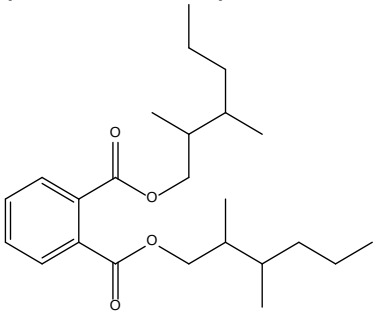
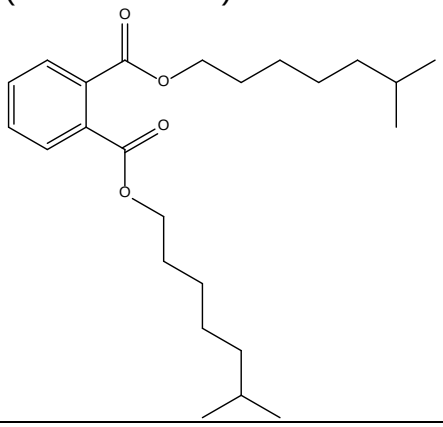
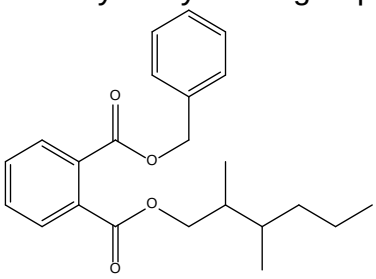
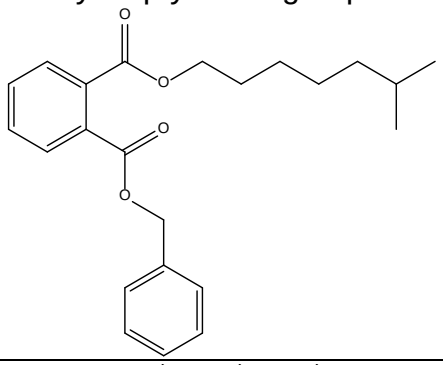
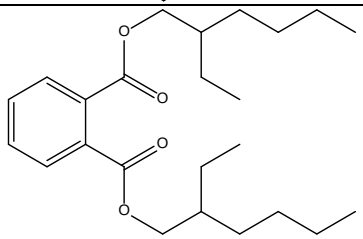
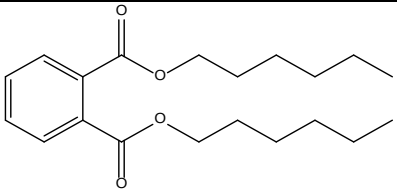
Phthalate (CAS #) [Substance Type]	Branching	Number of Carbons in Alkyl Chain	Number of Carbons in Longest Backbone	Representative Structure(s)
DMP (131-11-3) [Discrete]	Linear	1	1	
DEP (84-66-2) [Discrete]	Linear	2	2	

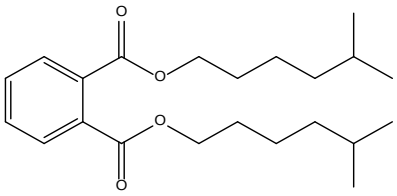
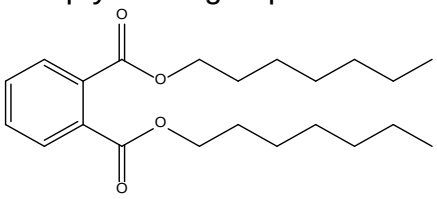
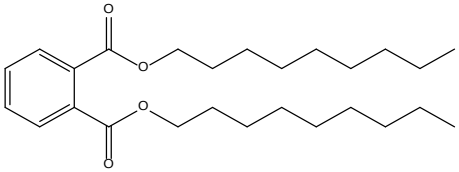
Table B- 2 Representative structures for the medium chain phthalates

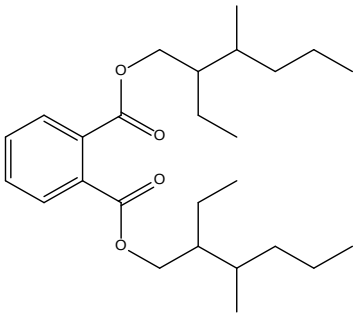
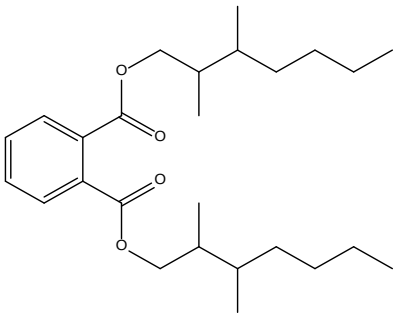
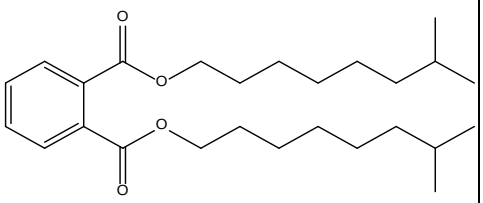
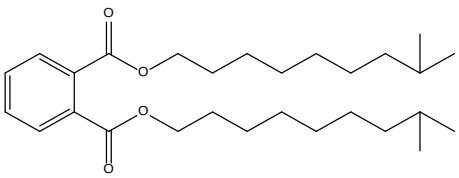
Phthalate (CAS #) [Substance Type]	Branching	Number of Carbons in Alkyl Chain	Number of Carbons in Longest Backbone	Representative Structure(s)
DPrP (131-16-8) [Discrete]	Linear	3	3	
DIBP (84-69-5) [Discrete]	Branched	4	3	

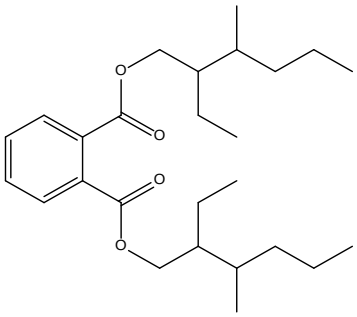
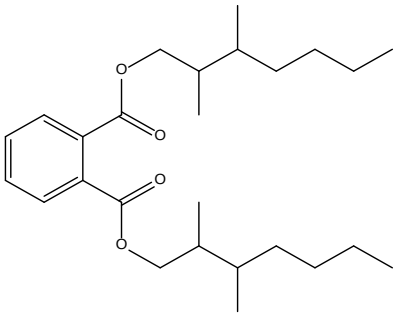
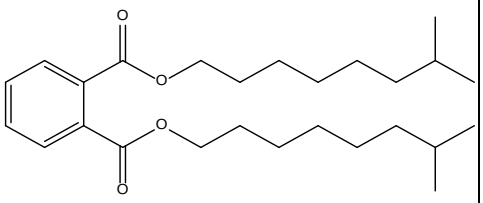
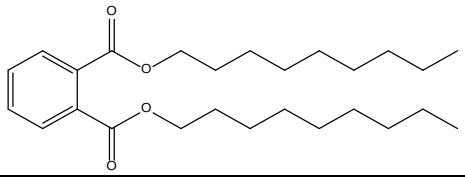
CHIBP (5334-09-8) [Discrete]	Branched / Cyclo	4 6 (cyclo)	3 6 (cyclo)	
BCHP (84-64-0) [Discrete]	Linear / Cyclo	4 6 (cyclo)	4 6 (cyclo)	
DBP (84-74-2) [Discrete]	Linear	4	4	
BBP (85-68-7) [Discrete]	Linear / Benzyl	4 Benzyl	4 Benzyl	
DCHP (84-61-7) [Discrete]	Cyclo	6 (cyclo)	6 (cyclo)	
DMCHP (27987-25-3) [Discrete]	Cyclo	7	6 (cyclo)	

DIHepP (71888-89-6) [Isomer Mixture]	Linear / Mostly Branched	7	6 - 7	methyl hexyl ester groups (mixed isomers)  n-heptyl ester groups 
DIOP (27554-26-3) [Isomer Mixture]	Branched	8	6 – 7	dimethyl hexyl ester groups (mixed isomers)  methyl heptyl ester groups (mixed isomers) 

BIOP (27215-22-1) [Isomer Mixture]	Branched	8 Benzyl	6 – 7 Benzyl	dimethyl hexyl ester groups  methyl heptyl ester groups 
DEHP (117-81-7) [Discrete]	Branched	8	6	
DnHP (84-75-3) [Discrete]	Linear	6	6	

79P (111381-89-6) [UVCB]	Linear and Branched	7 - 9	6 - 9	methyl hexyl ester group  n-heptyl ester group  n-nonyl ester group 

DINP-1 (68515-48-0) [Isomer Mixture]	Branched	9-10	6* - 9	methylethyl hexyl ester groups  dimethyl heptyl ester groups  methyl octyl ester groups  isodecyl ester groups 

DINP-2 (28553-12-0) [Isomer Mixture]	Mostly Branched	9	6*- 9	methylethyl hexyl ester groups  dimethyl heptyl ester groups  methyl octyl ester groups  n-nonyl ester groups 

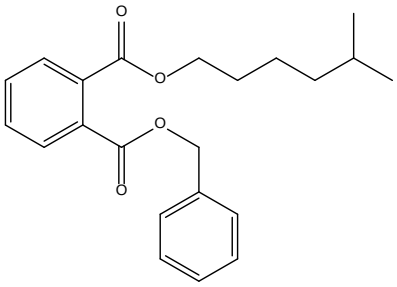
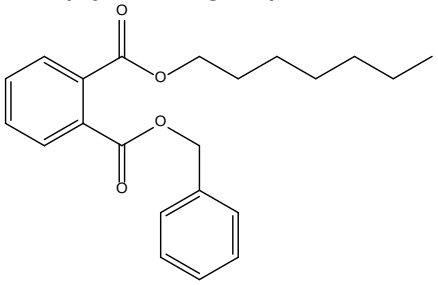
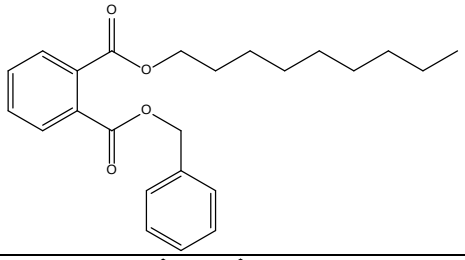
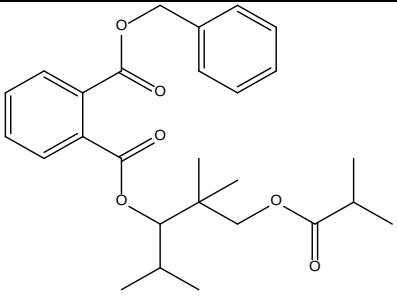
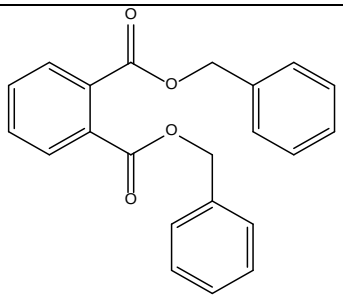
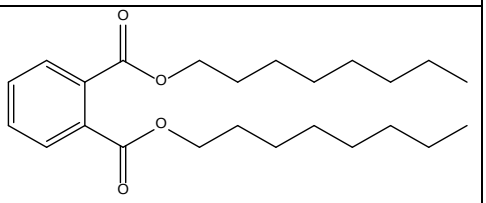
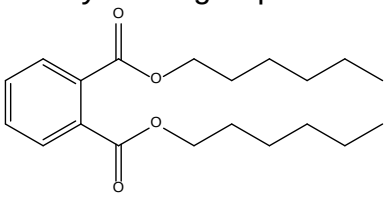
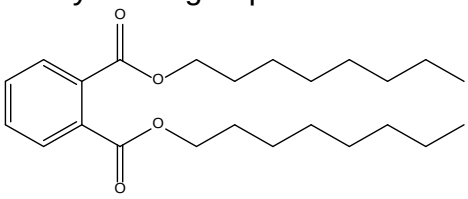
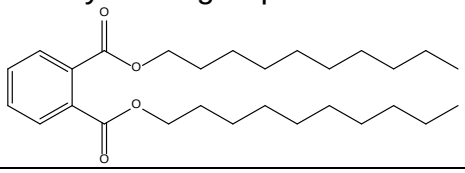
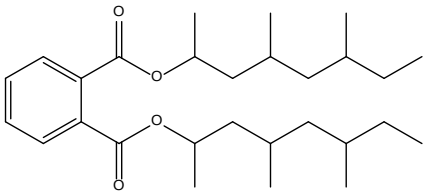
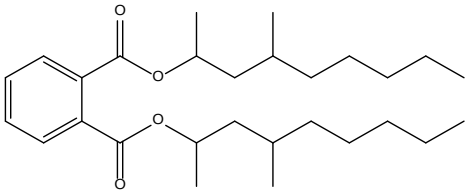
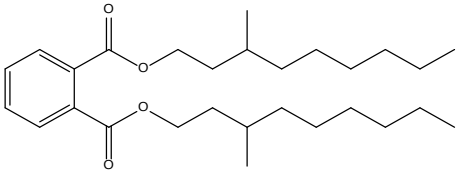
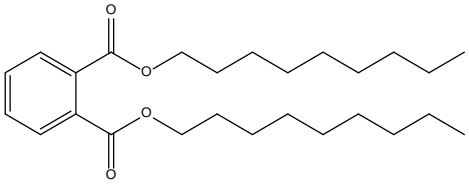
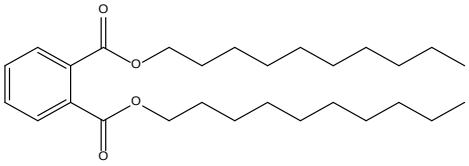
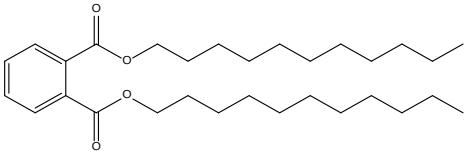
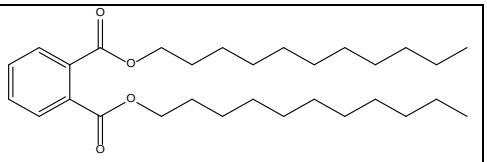
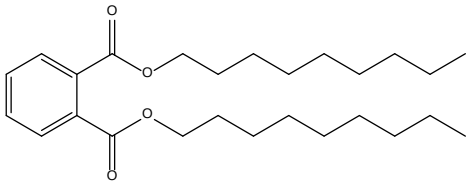
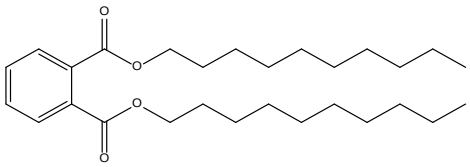
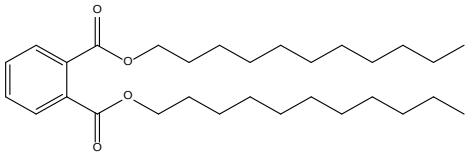
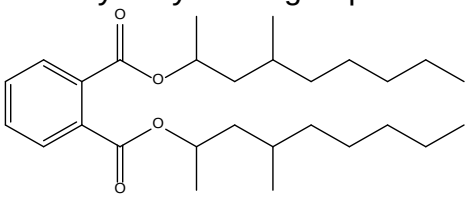
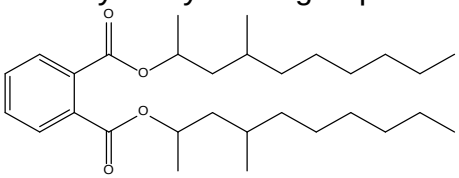
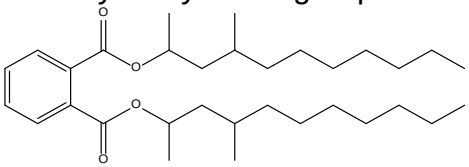
B79P (68515-40-2) [UVCB]	Linear and Branched	7 – 9 Benzyl	6 – 9 Benzyl	methyl hexyl ester group  n-heptyl ester group  n-nonyl ester group 
B84P (16883-83-3) [Discrete]	Branched	12	8 (parent) (ester linkage mid chain)	
DBzP (523-31-9) [Discrete]	--	Benzyl	Benzyl	

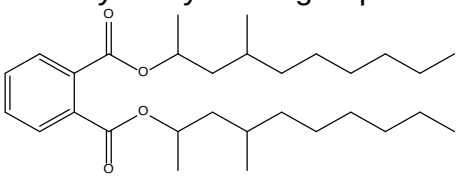
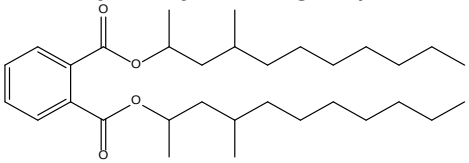
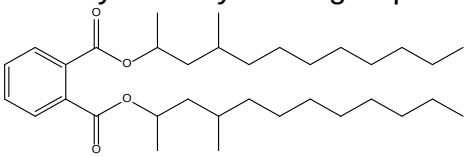
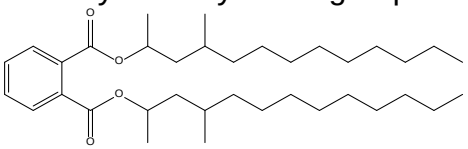
Table B- 3 Representative structures for the long chain phthalates

Phthalate (CAS #) [Substance Type]	Linear or Branched Alkyl Chain	Number of Carbons in Alkyl Chain	Number of Carbons in Longest Backbone	Representative Structure(s)
DnOP (117-84-0) [Discrete]	Linear	8	8	
610P (68648-93-1) [UVCB]	Linear	6, 8, 10	6, 8, 10	n-hexyl ester groups  n-octyl ester groups  n-decyl ester groups 

DIDP (26761-40-0) (68515-49-1) [Isomer Mixture]	Branched	10	7* – 9	trimethyl heptyl ester groups*  dimethyl octyl ester groups  methyl nonyl ester groups 
D911P (68515-43-5) [UVCB]	Mostly Linear (>80%) Branched (20%)	9-11	9-11	n-nonyl ester groups  n-decyl ester groups  n-undecyl ester groups 

DUP (3648-20-2) [Isomer Mixture]	Mostly Linear	11	11	
D911P-2 (111381-91-0) [UVCB]	Mostly Linear	9-11	9-11	n-nonyl ester groups  n-decyl ester groups  n-undecyl ester groups 

DIUP (85507-79-5) [UVCB]	Mostly Branched	10-12	8-10	dimethyl octyl ester groups  dimethyl nonyl ester groups  dimethyl decyl ester groups 

DTDP (68515-47-9) [UVCB]	Branched	11-14	9-12	dimethyl nonyl ester groups  dimethyl decyl ester groups  dimethyl undecyl ester groups  dimethyl dodecyl ester groups 

Appendix C

Physicochemical Properties for the Phthalates of Interest

Table C- 1 Select physicochemical properties for the short chain phthalates

CAS RN Acronym	Mol. Weight (Da)	Melting point (ºC)	Boiling point (ºC)	Vapour pressure (Pa)	Water solubility (mg/L)	Log K_{ow}
131-11-3 DMP	194	-42 (Exp)[a]	283.1 (Exp)[a]	0.23 (25°C) (Exp)[a]	4.0×10^3 (25°C) (Exp)[a]	1.54 (Exp)[a]
84-66-2 DEP	222	-60 (Exp)[a]	297.3 (Exp)[a]	0.22 (25°C) (Exp)[b]	9.3×10^2 (25°C) (Exp)[a]	2.47 (Exp)[c]

(Exp) – Experimental values: [a] ECHA 2013; [b] NICNAS 2008; [c] HSDB 2013

Table C- 2 Select physicochemical properties for the medium chain phthalates

CAS RN Acronym	MW (Da)	Melting point (ºC)	Boiling point (ºC)	Vapour pressure (Pa)	Water solubility (mg/L)	Log K_{ow}
131-16-8 DPrP	250	No Data	317.5 (Exp)[d]	0.02 (25°C) (Exp)[c]	108 (20°C) (Exp)[c]	3.27 (Exp)[c]
84-69-5 DIBP	278	-64 (Exp)[h] -52 (Exp)[a]	296.5 (Exp)[i] 320 (Exp)[a]	0.01 (20°C) (Exp)[a]	20.3 (20°C) (Exp)[a] 6.2 (24°C) (Exp)[g]	4.11 (Exp)[a]
5334-09-8 CHIBP	304	No Data	359.48 (Mod)[j]	1.05×10^{-2} (25°C) (Mod)[j]	0.323 (Mod)[K] 6.66 (Mod)[M] 3.04 (Mod)[N]	5.33 (Mod)[L] 4.92 (Mod)[M] 4.28 (Mod)[N]

CAS RN Acronym	MW (Da)	Melting point (ºC)	Boiling point (ºC)	Vapour pressure (Pa)	Water solubility (mg/L)	Log K_{ow}
84-64-0 BCHP	304	<25 (Exp)[c]	366.48 (Mod)[j]	6.4×10^{-4} (25°C) (Exp)[a]	1.073 (Mod)[K] 4.4 (Mod)[M] 3.4 (Mod)[N]	5.41 (Mod)[L] 5.02 (Mod)[M] 4.56 (Mod)[N]
84-74-2 DBP	278	< -70 (Exp)[a]	340 (Exp)[a]	9.7×10^{-3} (25°C) (Exp)[a]	11.4 (25°C) (Exp)[a]	4.46 (Exp)[a]
85-68-7 BBP	312	< -35 (Exp)[a]	370 (Exp)[a]	1.1 (25°C) (Exp)[a]	2.69 (25°C) (Exp)[a]	4.91 (Exp)[a]
84-61-7 DCHP	330	65.6 (Exp)[a]	322 (Exp)[a]	1.2×10^{-4} (25°C) (Exp)[a]	1.01 (20°C) (Exp)[a]	4.82 (Exp)[a]
27987-25-3 DMCHP	358	No Data	411.33 (Mod)[j]	1.98×10^{-4} (25°C) (Mod)[j]	5.38 × 10-3 (Mod)[K] 0.46 (Mod)[M] 0.31 (Mod)[N]	7.04 (Mod)[L] 6.46 (Mod)[M] 5.47 (Mod)[N]
71888-89-6 DIHepP	362	-40 (Exp)[e]	393.74 – 407.73 (Mod)[j]	2.8×10^{-4} – 1.1×10^{-3} † (25°C) (Mod)[j]	0.017 (22°C) (Exp)[f]	7.41 – 7.56† (Mod)[L] 6.42 – 7.92† (Mod)[M] 6.15 – 7.26† (Mod)[N]

CAS RN Acronym	MW (Da)	Melting point (ºC)	Boiling point (ºC)	Vapour pressure (Pa)	Water solubility (mg/L)	Log K_{ow}
27554-26-3 DIOP	391	No Data	370 (Exp)[b]	7.3×10^{-4} (25°C) (Exp)[b]	9.0×10^{-2} (25°C) (Exp)[b]	8.24 – 8.39[†] (Mod)[L] 7.52 – 7.96[†] (Mod)[M] 6.62 – 7.02[†] (Mod)[N]
27215-22-1 BIOP	368	No Data	420 – 426[†] (Mod)[j]	5.78×10^{-5} - 1×10^{-4} [†] (25°C) (Mod)[j]	8.47×10^{-3} - 9.8×10^{-3} (Mod)[K] 0.48 – 0.52 (Mod)[M] 0.28 - 0.42 (Mod)[N]	6.66 – 6.74[†] (Mod)[L] 5.81 – 6.55[†] (Mod)[M] 5.87 – 6.31[†] (Mod)[N]
117-81-7 DEHP	391	-50 (Exp)[a]	374 (Exp)[a]	3.0×10^{-5} (25°C) (Exp)[a]	3.0×10^{-3} (20°C) (Exp)[a]	7.14 (Exp)[a]
84-75-3 DnHP	334	-58 (Exp)[c]	384.52 (Mod)[j]	1.8×10^{-3} (25°C) (Exp)[b]	3.0×10^{-2} (25°C) (Exp)[b]	6.82 (Exp)[b]
111381-89-6 79P	362 - 418[†]	-45 (Exp)[d]	394 - 454[†] (Mod)[j]	2.5×10^{-5} (25°C) (Exp)[d]	1.7×10^{-5} - 2.5×10^{-3}[†] (Mod)[K] 0.02 – 0.40 (Mod)[M] 2.8×10^{-2} - 3.3×10^{-1} (Mod)[N]	7.41 – 9.52 (Mod)[L] 6.41 – 10.23 (Mod)[M] 6.15 – 8.46 (Mod)[N]
68515-48-0 DINP-1	419 - 447[†]	< -50 (Exp)[a]	331 - 341 (Exp)[a^]	5.17×10^{-5} (25°C) (Mod)[j]	6.0×10^{-4} (21°C) (Exp)[a]	8.8 (Exp)[a^]

CAS RN Acronym	MW (Da)	Melting point (ºC)	Boiling point (ºC)	Vapour pressure (Pa)	Water solubility (mg/L)	Log K_{ow}
28553-12-0 DINP-2	419	-54 (Exp)[a]	331 - 341 (Exp)[a]	6×10^{-5} (20°C) (Exp)[a]	6.0×10^{-4} (21°C) (Exp)[a]	8.8 – 9.7 (Exp)[a]
68515-40-2 B79P	354-382[†]	No Data	390 (Exp)[a]	1.04×10^{-5} 1.42×10^{-4}[†] (25°C) (Mod)[j]	0.3 (25°C)[†] (Exp)[a]	5.5 (Exp)[a]
16883-83-3 B84P	455	No Data	>300 (Exp)[a]	8.0×10^{-7} (25°C) (Mod)[j]	0.81 (22°C) (Exp)[j]	7 (Mod)[L] 6.52 (Mod)[M] 5.61 (Mod)[N]
523-31-9 DBzP	346	44 (Exp)[d]	437 (Mod)[j]	9.3×10^{-5} (25°C) (Mod)[j]	0.30 (Mod)[K] 2.82 (Mod)[M] 0.72 (Mod)[N]	5.08 (Mod)[L] 5.09 (Mod)[M] 4.63 (Mod)[N]

(Exp) – Experimental values: [a] ECHA 2013; [b] HSDB 2013; [c] PhysProp 2006; [d] ACC 2006; [e] European Commission 2000a; [f] Letinski et al. 2002; [g] Leyder and Boulanger 1983; [h] Clayton and Clayton 1981-1982; [i] Haynes and Lide 2010

(Mod) – Modelled values: [j] MPBPVPWIN 2010; [K] WSKOWWIN 2010; [L] KOWWIN 2010; [M] ACD/Percepta 2012; [N] VCC 2005

†Calculated based on representative structures for mixture (Appendix B)

Table C- 3 Select physicochemical properties for the long chain phthalates

CAS RN Acronym	MW (Da)	Melting point (ºC)	Boiling point (ºC)	Vapour pressure (Pa)	Water solubility (mg/L)	Log K_{ow}
68648-93-1 610P	334 - 446[†]	-45 (Exp)[c]	384 - 477[†] (Mod)[e]	1.3×10^{-5} (25°C) (Exp)[c]	3.0×10^{-2} (25°C) (Exp)[c]	8.17 (Exp)[c]
117-84-0 DnOP	391	No Data	431 (Mod)[e]	1.3×10^{-5} (25°C) (Exp)[b]	2.2×10^{-2} (25°C) (Exp)[b]	8.10 (Exp)[b]

CAS RN Acronym	MW (Da)	Melting point (ºC)	Boiling point (ºC)	Vapour pressure (Pa)	Water solubility (mg/L)	Log K_{ow}
26761-40-0; 68515-49-1 DIDP	446	-50 (Exp)[d]	435-463[†] (Mod)[e]	7.0×10^{-5} (25°C) (Exp)[b]	0.28 (21°C) (Exp)[b]	10.06-10.36[†] (Mod)[g] 8.31 – 8.62[†] (Mod)[f] 9.72 – 9.84[†] (Mod)[h]
68515-43-5 D911P	418-475[†]	-50 (Exp)[a]	337 (Exp)[a]	1.6×10^{-7} - 5.2×10^{-5} (25°C)[†] (Mod)[e]	1.6×10^{-7} - 1.7×10^{-5}[†] (Mod)[f] 1.9×10^{-3} - 2.3×10^{-2}[†] (Mod)[h] 1.7×10^{-2} - 2.8×10^{-2}[†] (Mod)[i]	8.3 (Exp)[a]
111381-91-0 D911P-2	418-475[†]	-50 (Exp)[a] RA from D911P	337 (Exp)[a] RA from D911P	1.6×10^{-7} - 5.2×10^{-5} (25°C)[†] (Mod)[e]	1.6×10^{-7} - 1.7×10^{-5}[†] (Mod)[f] 1.9×10^{-3} - 2.3×10^{-2}[†] (Mod)[h] 1.7×10^{-2} - 2.8×10^{-2}[†] (Mod)[i]	8.3 (Exp)[a] RA from D911P

CAS RN Acronym	MW (Da)	Melting point (ºC)	Boiling point (ºC)	Vapour pressure (Pa)	Water solubility (mg/L)	Log K_{ow}
3648-20-2 DUP	475	-40 (Exp)[a]	336 (Exp)[a]	1.01×10^{-4} (25°C) (Mod)[e]	1.6×10^{-7} (Mod)[f] 1.9×10^{-3} (Mod)[h] 1.7×10^{-2} (Mod)[i]	8.7 (Exp)[a]
85507-79-5 DIUP	446 - 502†	<-39 (Exp)[a]	449-495† (Mod)[e]	3.3×10^{-6} - 2.0×10^{-5} † 25°C) (Mod)[e]	2.8×10^{-8} - 2.9×10^{-7}† (Mod)[f] 8.9×10^{-4} - 2.9×10^{-3}† (Mod)[h] 9.9×10^{-3} - 1.2×10^{-2}† (Mod)[i]	11.29 - 12.17† (Mod)[g] 10.48 - 11.66† (Mod)[h] 8.89 - 9.36† (Mod)[i]
68515-47-9 DTDP	474 - 502†	< -36 (Exp)[a]	473-559† (Mod)[e]	5.8×10^{-10} - 3.3×10^{-6} † (25°C) (Mod)[e]	2.5×10^{-10} - 2.9×10^{-7}† (Mod)[f] 9.9×10^{-5} - 2.9×10^{-3}† (Mod)[h] 9.9×10^{-3} - 1.2×10^{-2}† (Mod)[i]	11.19 - 14.14† (Mod)[g] 10.48 - 14.3† (Mod)[h] 8.89 - 10.17† (Mod)[i]

(Exp) – Experimental values: [a] ECHA 2013; [b] HSDB 2013; [c] ACC 2006; [d] European Commission 2000b;
(Mod) – Modelled values: [e] MPBPVPWIN 2010; [f] WSKOWWIN 2010; [g] KOWWIN 2010; [h] ACD/Percepta 2012; [i] VCC 2005
†Calculated based on representative structures for mixture (Appendix B)

Appendix D

Toxicokinetics Data in Rats for the Phthalates of Interest

Table D- 1 Selected toxicokinetic data for the short chain phthalates

Phthalate (CAS #)	Oral Absorption (Rat)	Identified metabolites in urine after oral administration (Rat)	Distribution to the Fetal Testes (Rat)
DMP (131-11-3)	No Data	monomethyl phthalate (78%); phthalic acid (14.4%) and unchanged dimethyl phthalate (8.1%) (Albro and Moore 1974)	Pregnant Rats Repeat dose: 500 mg/kg/day (GD12-19) Conc. of monoester in fetal testes: [GD 19]: 396 uM @ 2hr post dose (Clewell et al. 2010)
DEP (84-66-2)	Dose: Single Dose: 10 or 100mg Rate: Absorption >75% for 10 and 100 mg over 24h. (Kawano 1980)	Monoethyl phthalate (67–70%), phthalic acid (8- 9%), or unchanged diethyl phthalate EP (0.1– 0.4%). (Kawano 1980)	Pregnant Rats Repeat dose: 500 mg/kg/day (GD12-19) Conc. of monoester in fetal testes: [GD 19]: 409 uM @ 2hr post dose (Clewell et al. 2010)

Table D- 2 Select toxicokinetic data for the medium chain phthalates

Phthalate (CAS #)	Oral Absorption (Rat)	Identified metabolites in urine after oral administration (Rat)	Distribution to the Fetal Testes (Rat)
DBP (84-74-2)	Dose: Single dose: 500-1500 mg/kg Rate: Absorption 60% for 500 mg/kg over 48h Absorption 48% for 1500 mg/kg over 48h. (Saillenfait et al. 1998)	Monobutyl phthalate, monobutyl phthalate-glucuronide, monohydroxybutyl phthalate, monohydroxybutyl phthalate glucuronide, mono(3-carboxypropyl) phthalate, phthalic acid, butanoic acid phthalate, and butanoic acid phthalate- glucuronide. (Silva et al. 2007) (Albro and Moore 1974) (Calafat et al 2006a) (Fennell et al 2004) (Foster et al 1983) (Kaneshima et al 1978) (Saillenfait et al 1998) (Williams and Blanchfield 1975a)	Pregnant Rats Repeat dose: 500 mg/kg/day (GD12-19) Conc. of monoester in fetal testes: [GD 19]: 189 uM @ 2hr post dose (Clewell et al. 2010)
BBP (85-68-7)	Dose: Single dose: 2-2000 mg/kg Rate: Absorption 70-80% for 2-200mg/kg over 96h. Absorption 22% for 2000mg/kg over 96h. (Eigenberg et al. 1986)	Monobutyl phthalate (29-34%), monobenzyl phthalate (7-12%), hippuric acid (51-56%) (Nativelle, et al. 1999)	Pregnant Rats Repeat dose: 500 mg/kg/day (GD12-19) Conc. of monoester in fetal testes: [GD 19]: MBP: 124 uM; MBzP: 21 uM @ 2hr post dose (Clewell et al. 2010)

DIHepP (71888-89-6)	Dose: Single dose: 250 mg/kg Rate: Absorption 75% bile over 96h and urine over 7 days. (Sato et al. 1984)	5-hydroxy-5-methylhexyl phthalate; 6-hydroxy-5-methylhexyl phthalate; 5-carboxyhexyl phthalate; 3-carboxypropyl phthalate (Sato et al. 1984)	No Data
DEHP (117-81-7)	Dose: Single dose studies: 1000-12000 ppm Rate: Absorption >50% over 96h. (Short et al. 1987)	Mono(2-ethyl hexyl)phthalate, mono(2-ethyl-5-oxohexyl) phthalate, mono(2-ethyl-5-hydroxyhexyl) phthalate, mono(2-ethyl-5-carboxypentyl) phthalate, mono[2-carboxymethyl)hexyl] phthalate, mono-(3-carboxypropyl) phthalate, phthalic acid, glucuronidated secondary metabolites (Calafat et al 2006a) (Daniel and Bratt 1974) (Sjoberg et al 1985) (rat) (Albro et al 1982) (Lhuguenot et al 1985)	Pregnant Rats Repeat dose: 500 mg/kg/day (GD12-19) Conc. of monoester in fetal testes: [GD 19]: 12 uM (Clewell et al. 2010)
DIOP (27554-26-3)	No Data	The metabolites identified in the urine of rats after oral administration were: mono-(3-carboxypropyl) phthalate; mono-*n*-octyl phthalate; monoisononyl phthalate (Calafat et al. 2006b)	No Data

DINP (68515-48-0; 28553-12-0)	Dose: Single dose studies: 50-500 mg/kg Repeat Dose Studies : Daily 50, 150 or 500 mg/kg Rate: Absorption 49-75% over 72h in the single-dose studies and 62-90% over 72h in the repeated-dose studies. (McKee et al. 2002)	Monoisononyl phthalate, mono(hydroxyisononyl) phthalate, mono(oxoisononyl) phthalate, mono-carboxy-isooctyl phthalate, mono(carboxy-isoheptyl) phthalate, mono-(3-carboxypropyl) phthalate, mono-n-octyl phthalate, phthalic acid (Calafat et al. 2006b) (Silvia et al. 2006) (McKee, et al. 2002)	Pregnant Rats Repeat dose: 250 mg/kg/day (GD12-19) Conc. of monoester in fetal testes: [GD 19]: ~100 uM @ 1hr post dose (Clewell et al. 2013)

Table D- 3 Select toxicokinetic data for the long chain phthalates

Phthalate (CAS #)	**Oral Absorption (Rat)**	**Metabolism (Rat)**	**Distribution to the Fetal Testes (Rat)**
DIDP (26761-40-0)	Dose: Single dose: 0.1, 11.2, 1000 mg/kg Rate: Absorption 46-56% for 0.1 and 11.2 mg/kg over 72h. Absorption 17% for 1000 mg/kg over 72h (General Motors Research Laboratories 1983 cited in European Chemicals Bureau 2004)	Monoisodecyl phthalate, mono(carboxyisodecyl) phthalate, mono(hydroxyisodecyl) phthalate, mono(hydroxyisononyl) phthalate, mono(oxyisodecyl) phthalate, mono(carboxyisononyl) phthalate, mono-n-octyl phthalate, monoisononyl phthalates, mono-(3-carboxypropyl) phthalate, phthalic acid (Calafat et al 2006b) (Kato et al 2007) (General Motors Research Laboratories 1983 in European Chemicals Bureau 2004)	No Data

DnOP (117-84-0)	Dose: 0.2 ml x2 @ 24h interval Rate: Absorption 31% over 48h. (Albro and Moore 1974)	Mono-n-octyl phthalate, mono(3-carboxypropyl) phthalate, mono-hydroxy- *n*-octyl phthalate, mono-oxo-*n*-octyl phthalate, mono-(7-carboxy-*n*-heptyl) phthalate, mono-(5-carboxy-*n*-pentyl) phthalate, mono-carboxymethyl phthalate, phthalic acid (Albro and Moore 1974) (Calafat et al. 2006b) (Silva et al. 2005).	No Data

Appendix E

Studies Used for Potency and SAR Analysis for Phthalate Effects on the AGD of Male Rat Offspring when Exposure Occurs During Gestation

Dimethyl Phthalate (DMP)

Gray et al. (2000) administered DMP orally to pregnant SD rats at 750 mg/kg from gestational day (GD) 14 to postnatal day (PND) 3. Treatment did not induce overt maternal toxicity or reduced litter sizes. At PND 2, DMP had no effect on AGD compared with controls. Other androgenic dependent parameters (nipple retention, cryptorchidism, hypospadias, testes/epididymis weight or testicular pathological changes) were also not affected by DMP in male offspring at the dose tested.

Diethyl Phthalate (DEP)

In the same study as DMP, Gray et al. (2000) administered DEP orally to pregnant SD rats at 750 mg/kg from gestational day (GD) 14 to postnatal day (PND) 3. Treatment did not induce overt maternal toxicity or reduced litter sizes. At PND 2, DEP had no effect on AGD compared with controls. Other androgenic dependent parameters (nipple retention, cryptorchidism, hypospadias, testes/epididymis weight or testicular pathological changes) were also not affected by DEP in male offspring at the dose tested.

Dipropyl Phthalate (DPrP)

Saillenfait et al. (2011) administered DPrP to pregnant SD rats at doses of 0, 500,1000 or 1500mg/kg/day, by gavage, on GD 6-20. A significant but transient reduction in maternal body weight gain was noted at the beginning of treatment of the high dose group. The fetal body weight of both sexes was decreased at 1000 mg/kg/day and was significantly lower at 1500 mg/kg/day. *In utero* exposure to DPrP in rats appears to cause some androgen-dependent effects in the male fetus at high doses. The AGD was significantly decreased in male fetuses at 1000 and 1500 mg/kg/day at GD21. Other androgenic dependent effects were also observed. Undescended testis occurred in three males (from three different litters) out of the 75 male fetuses from the highest dose group.

Diisobutyl Phthalate (DIBP)

Saillenfait et al. (2008) administered 125, 250, 500, and 625 mg/kg-bw per day of DIBP by gavage to pregnant SD rats on gestation days (GD) 12–21. Decreased AGD at PND1 in male offspring was observed at 250mg/kg-bw/day and above. Other

androgen-dependent effects were also observed including nipple retention at PND12-14, and effects in sperm at maturity (postnatal weeks 11, 16) in the absence of maternal effects. At higher doses, the onset of puberty (preputial separation, PPS) was delayed along with other reproductive tract malformations such as undescended testes (cryptorchidism, CRY), hypospadias (HYP), exposed os penis, cleft prepuce, and reduced testis weight. Histopathological lesions were also present in testes of these males at maturity which mainly consisted of seminiferous tubule degeneration.

Dibutyl Phthalate (DBP)

Mylchreest et al. (1999) administered DBP to pregnant SD rats at doses of 0, 100, 250 or 500mg/kg/day, by gavage, on GD 6-20. DBP had no effect on maternal body weight or other parameters at 100 or 250 mg/kg/day. Fetal body weight of offspring was unaffected at all doses. The AGD was significantly decreased in male fetuses at 250 and 500 mg/kg/day at PND 1. Other androgenic dependent effects were also observed. Exposure to DBP resulted in a dose-dependent increase in the incidence of retained thoracic nipples, hypospadias, under developed epididymis and cryptorchidism.

Butyl Benzyl Phthalate (BBP)

In a rat two-generation reproductive toxicity study, Ty et al. (2004) administered 0, 750, 3750, or 11250 ppm via the diet (estimated maternal intake stated by authors: 50, 250, 750 mg/kg/day). Decreased AGD, on PND 0, was observed for F1 males compared to controls at the mid and high dose tested (250 and 750 mg/kg/day). Other androgen-dependent effects were also observed in F1 males at the high dose, including increase in incidence of retained nipples, delayed acquisition of puberty and reproductive system malformations.

Dicyclohexyl Phthalate (DCHP)

Saillenfait et al. (2009) administered DCHP at 0, 250, 500, and 750 mg/kg-bw per day by gavage to pregnant SD rats on gestation days (GD) 12–20. A dose-dependent decrease in AGD in male offspring was observed at the lowest dose tested and above (250 to 750 mg/kg-bw per day) on GD21. No effects on testicular descent (ie. cryptorchidism) were identified. The body weights of males were decreased at the highest dose (750 mg/kg bw/day), but this dose was also associated with maternal toxicity and decreased food consumption.

Diethylhexyl Phthalate (DEHP)

Gray et al. (2009) administered DEHP at 0, 11, 33, 100 and 300 mg/kg-bw per day by gavage to pregnant SD rats on gestation days (GD) 7 to PND 17. A decrease in AGD and a reduction in body weight on PND 2 was observed in male offspring at the highest

dose tested (300 mg/kg-bw per day). Other androgenic dependent effects were also observed at this dose. When examined on PND13 retained nipples were in male offspring were noted. Decreases in reproductive organ weights and sperm counts were observed for F1 males at adulthood.

Diisoheptyl Phthalate (DIHepP)

In a rat two-generation reproductive toxicity study, McKee et al. (2006) administered 0, 1000, 4500, or 8000 ppm via the diet (estimated maternal intake during gestation: 64, 304, 532 mg/kg/day and lactation: 162, 716, 1289 mg/kg/day). Parental effects observed included significant increases in liver and kidney weights in F0 and F1 animals of both sexes. These increases were accompanied with histological changes in liver (minimal centrilobular hypertrophy in males at mid and high dose and females at the highest dose, hepatocellular vacuolation in male at the highest dose) and histological changes in kidney (chronic progressive nephropathy in males at the highest dose). Decreased AGD, on PND 1, was observed for F1 males compared to controls at the highest dose tested (532 mg/kg/day during gestation). Other androgen-dependent effects were also observed in F1 males at the high dose, including increase in incidence of retained nipples and testicular abnormalities, significant reduction in weights of testes, ovaries and male accessory reproductive organs, significant decrease in testicular sperm counts and daily sperm production, significant delays in balanopreputial separation and decrease in fertility.

Di-n-Hexyl Phthalate (DnHP)

Saillenfait et al. (2008) administered 0, 50, 125, 250, and 500 mg/kg-bw per day of DnHP by gavage to pregnant SD rats on gestation days (GD) 12-21. DnHP had no significant effects on maternal body weight gain and pup weight during lactation. Decreased AGD on PND 1 in male offspring was observed at 125 mg/kg-bw/day and above. At this dose, other androgen-dependent effects were also observed including nipple retention at weaning and adulthood. At adulthood, reproductive tract malformation were also observed at this dose and above, including undescended testes, underdeveloped testes, and hypospadias. Histopathological examinations showed seminiferous tubule degeneration at the two high doses as well.

Di-n-Heptyl Phthalate (DnHepP)

Saillenfait et al. (2011) administered DnHepP to pregnant SD at doses of 0, 250, 500 or 1000mg/kg/day, by gavage, on GD 6-20. DnHepP had no adverse effect on maternal feed consumption and body weight gain, or on the incidence of post-implantation loss and fetal body weight. A decrease in AGD of male offspring was significantly different from controls at 1000 mg/kg/day (10% change from control). However, internal

examinations did not reveal any malformations of the male reproductive tract at this dose.

Diisononyl Phthalate (DINP)

Boberg et al. (2011) administered DINP-2 to pregnant Wistar rats at doses of 0, 300, 600, 750 or 900 mg/kg/day, by gavage, on GD7 to PND17. No differences were noted for maternal body weight and gain, gestation length, number of fetuses or litters in pregnant rats. The AGD was significantly decreased in male fetuses at 900 mg/kg/day at birth. Other androgenic dependent effects were also observed including increased nipple retention (at PND 13) starting at 750 mg/kg/day.

B79P [UVCB]

B79P is a registered substance under REACH in the EU. The original report for the extended one-generation reproductive and developmental toxicity study was not available and the limited data in the summary was not useful for the SAR analysis. Nevertheless, the study summary results are consistent with other structurally similar phthalates within the category. In the study, parental female SD rats were administered 0, 750, 3750 or 7500 ppm of B79P in the diet from GD6 through lactation to PND21. Estimated dose for the maternal rats was estimated to be 0, 50, 250 or 500 mg/kg bw/day based on food consumption. In F1 males, a statistically significant reduction in anogenital distance (AGD) on GD 21 was seen at 250 mg/kg bw/day and above. It should be noted that a statistically significant reduction in AGD was seen in all treatment groups in both males and females at birth. Also in the male pups, a statistically significant reduction in AGD on PND 21 was observed at 250 mg/kg bw/d and above. No differences in AGD, epispadias or areolae were observed in the F1 males of any of the dose groups assessed at PND 75 suggesting transient effects. A dose-related and statistically significant increase in the percentage of males pups with a defect of the penis (epispadias) was observed on PND 21 at mid-dose and above (0, 1.5, 14 and 21% at 0, 50, 250 or 500 mg/kg bw/d, respectively). Also the percentage of male pups with one or more retained areolae at PND 11-13 was statistically significantly increased at 500 mg/kg bw/d only (27% compared with 2.8% in the controls). Treatment-related histopathological lesions of the left testis (dilated seminiferous tubule lumina) were observed in the 500 mg/kg bw/d group at PND 75 (no histopathology performed on other groups). A slight increase in the incidence of undescended testes (cryptorchidism, CRY) was also observed at PND 21, but not at PND 75, in F1 males from all treated groups. During the lactational period only, F1 males and females showed reduced body-weight gain in all treatment groups (ECHA 2013j).

Dibenzyl Phthalate (DBzP)

A literature search did not identify any studies examining the potential toxicity of DBzP during gestation. However, monobenzyl phthalate [MBzP] (1,2-benzenedicarboxylicacid, mono(phenylmethyl)ester: CAS # 2528-16-7) is thought to be an appropriate surrogate substance to examine the possible effects of DBzP during gestation. Upon oral administration, phthalate diesters are hydrolysed to their respective monoesters within the gastrointestinal tract before absorption and it is predominantly the monoester that is taken up systemically. The monoester metabolites are also believed to be responsible for reducing testosterone synthesis as shown in cultured Leydig cells. MBzP is the monoester hydrolysis product of DBzP and therefore suitable for inferring toxicity in oral developmental studies.

Ema et al. (2003) administered MBzP via gavage to pregnant Wistar rats at doses of 0, 167, 250, or 375 mg/kg-bw/ day on GD 15-17 and offspring were examined on GD 21. AGD was decreased in male offspring starting at 250mg/kg-bw/day. Other androgen-dependent developmental effects were noted including significant increases in the incidence of undescended testes (cryptorchidism) in male fetuses at 250 mg/kg-bw/day and higher as well as significantly decreased fetal weight at 375 mg/kg-bw / day. However, a significant and dose-dependent decrease in maternal body weight gain (22%) and food consumption (8-15%) was also noted at the lowest dose (167 mg/kg-bw per day) and higher.

Diisodecyl Phthalate (DIDP)

In a multi-generation reproductive toxicity study, Hushka et al. (2001) administered DIDP to SD rats at doses of 0, 0.02, 0.06, 0.2 or 0.4% (equivalent to approximately 0, 13-15, 39-44, 127-150 and 254-295 mg/kg-bw per day). In the F1 and F2 pups, there were no effects on AGD up to the highest dose tested (254-295 mg/kg-bw/day). No effects were observed for other androgen-dependent endpoints including, nipple retention or histological lesions or weight changes in the reproductive organs of male offspring in both generations. In the F1 and F2 pups, there were no effects on body weight gain or organ weight,. In the F2 pups there was significantly decreased pup survival on PND 1 and 4 at 0.2 and 0.4% DIDP. No difference in survival was observed for the F1 generation. Age of PPS was increased by 1.2 days in high dose F2, but not F1 pups, but was not considered adverse.

Di-n-Octyl Phthalate (DnOP)

Saillenfait et al. (2011) administered DnoP to pregnant SD rats at doses of 0, 250, 500 or 1000mg/kg/day, by gavage, on GD 6-20. DnOP had no adverse effect on maternal feed consumption and body weight gain, or fetal body weight. Maternal liver weight was slightly but significantly higher than control at 1000 mg/kg/day. No histopathological changes related to DnOP administration were observed in the maternal liver at any of

the doses tested. The AGD of the male fetuses was not significantly different from controls in any of the treatment groups. The incidence of supernumerary lumbar ribs in foetuses or litters was reported to be significantly higher than controls in all treated groups. The number of implants and live foetuses, and the incidence of post-implantation loss, resorptions and foetal deaths were similar across all groups including control. There was no increase in the incidence of fetal malformations or external and visceral variations in any of the treatment groups.

Di-n-undecyl Phthalate (DUP)

Saillenfait et al. (2013) administered DUP to pregnant SD rats at doses of 0, 250, 500 or 1000mg/kg/day, by gavage, on GD 6-20. DUP had no adverse effects on maternal body weight and food consumption. The number of live fetuses, percent of post-implantation loss and of resorptions, fetal sex, and fetal body weights were not affected. AGD was slightly reduced at the mid dose (500 mg/kg-bw/day, 3% change) but not at the high dose (1000 mg/kg/day) in male fetuses of dams exposed to DUP during gestation. Since statistical significance is not noted at the high dose for DUP and the change noted at the mid-dose is within a comparable control presented for the another phthalate test group (DTDP) in the same study, the association between decreased AGD and the mid dose in the DUP experiment is uncertain and not used for the SAR analysis. Furthermore, no other effects were noted for testicular descent (cryptorchidism) another androgen-dependent outcome in this study.

Ditridecyl Phthalate (DTDP)

Saillenfait et al. (2013) administered DTDP to pregnant SD rats at doses of 0, 250, 500 or 1000mg/kg/day, by gavage, on GD 6-20. DTDP had no adverse effects on maternal body weight and food consumption. The number of live fetuses, percent of post-implantation loss and of resorptions, fetal sex, and fetal body weights were not affected. DTDP had no effect on AGD of male offspring at all doses tested.