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프탈레이트류와 그 대체물질의 내분비계 교란독성: 에스트로겐성과 안드로겐성을 중심으로

Estrogenic and Androgenic Potential of Phthalates and Their Alternatives

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ABSTRACT

Objectives: Although information on the toxicity of phthalate diesters is readily available, little is known about phthalate alternatives. The present article provides a summary of available information on the toxicity of phthalate diesters and their alternatives, with a special focus on estrogenicity and androgenicity.

Methods: We collected a battery of *in vitro* and *in vivo* assay data from the literature to assess the estrogenicity/anti-estrogenicity and androgenicity/anti-androgenicity of 15 phthalate diesters and 21 phthalate alternatives.

Results: A number of *in vitro* studies show that certain phthalate diesters can bind to estrogen receptors and have a weak estrogenic potential. However, this potential was not seen in *in vivo* studies. Phthalate diesters produced anti-androgenic effects in animals by reducing testosterone production. Among them, di-(2-ethyl-hexyl) phthalate (DEHP) was the most potent. While almost all phthalate alternatives have a lower toxic potential than does DEHP, evidence of reproductive toxicity and estrogenic potential were found in several substances.

Conclusion: Significant data gaps exist for phthalate alternatives regarding reproductive endocrine disruption, requiring further investigation.

Keywords: Alternatives, anti-androgenicity, endocrine disruption, estrogenicity, phthalate

I. Introduction

Endocrine disrupting chemicals (EDCs) consist of a broad class of compounds including industrial chemicals, pesticides, plastics and plasticizers, and many other chemicals that are present in the environment. Among them, phthalates in particular pose a major concern due to their widespread use. Phthalate diesters are high production volume chemicals, with over 470 million pounds (approximately 2.13 million tons) produced per year in the United States.[1] These chemicals have been used in children's toys, cosmetics, medical devices, and household products.[2,3] Their main usage is dependent on their alkyl chain length. The long-chain phthalates, ones with 7-13 carbon atoms in their chemical backbone, are primarily used in polyvinyl chloride (PVC) polymers such as food packaging, flooring, and medical devices; the short-chain phthalates are often used for non-PVC applications, including personal care products, paints, or adhesives.[4] Recently, di-iso-butyl phthalate (DIBP) has replaced di-*n*-butyl phthalate (DBP) in usage, due to their similar properties.[5]

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Additionally, di-iso-nonyl phthalate (DINP) and di-iso-decyl phthalate (DIDP) have replaced di-(2-ethyl-hexyl) phthalate (DEHP) due to their durability and availability.[6]

Because of their potential toxicity, in particular their reproductive and developmental toxicity, the US Environmental Protection Agency (EPA) has placed eight phthalate diesters (DBP, DIBP, butyl benzyl phthalate [BBP], di-n-pentyl phthalate [DPP], di-n-octyl phthalate [DOP], DINP, DIDP, and DEHP) on their list of priority substances with potential endocrine disruption.[1] The European Union (EU) has banned the use of certain phthalates in toys and childcare products intended to be placed in the mouth by children[7] and in plastic materials that contact food (i.e., food contact materials).[8] In Korea, phthalate content in products are self-regulated; for example, the content standards (0.1% wt) of DEHP, DBP, BBP, and DOP were established for toys and childcare articles by their respective industries.[9] DBP and BBP are banned from baby bottles, while DEHP is not permitted in food packaging.[10] DEHP and DBP cannot be used in cosmetics.[11] For food contact and building materials (e.g., PVC floor covering and wall paper), further regulatory standards are also applied.[12-14]

Table 1. Chemical structure of phthalate alternatives that are commonly used in consumer products

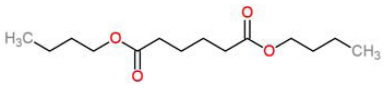
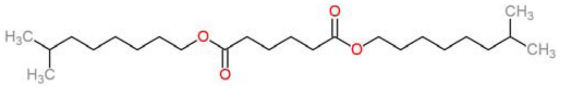
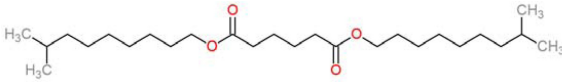
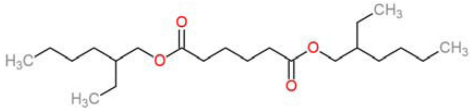
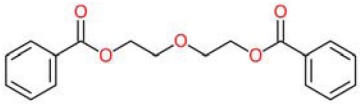
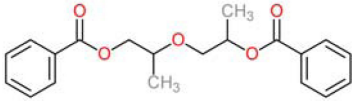
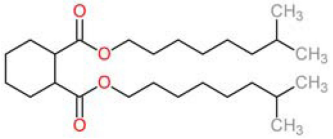
Category	Alternatives	Abbreviation	CAS number	Chemical structure
Adipates	Di-butyl adipate	DBA	105-99-7	
	Di-iso-nonyl adipate	DINA	33703-08-1	
	Di-iso-decyl adipate	DIDA	27178-16-1	
	Di-(2-ethylhexyl) adipate	DEHA	103-23-1	
Benzoates	Di-(ethylene glycol) dibenzoate	DEGDB	120-55-8	
	Di-(propylene glycol) dibenzoate	DPGDB	27138-31-4	
Carboxylates	1,2-cyclohexane dicarboxylic acid, diisononyl ester	DINCH	474919-59-0	

Table 1. Continued

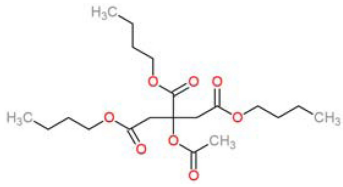
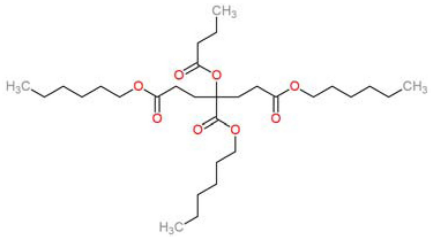
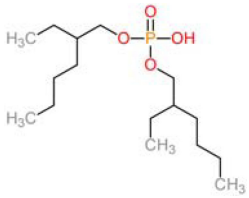
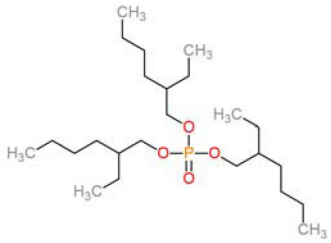
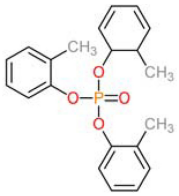
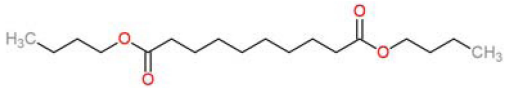
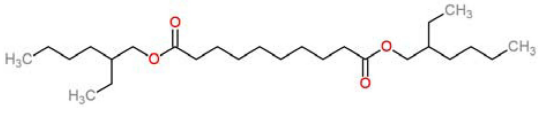
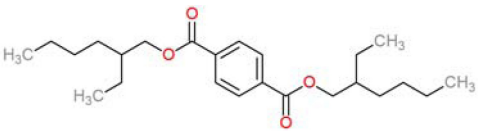
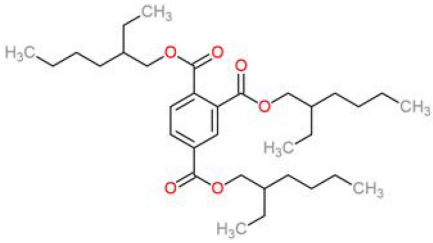
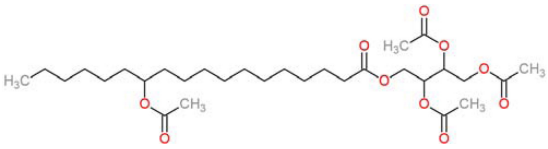
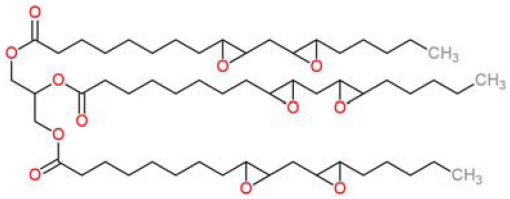
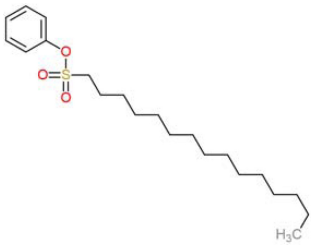
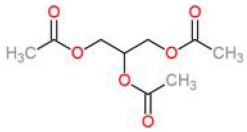
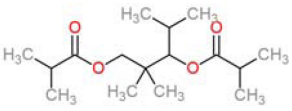
Category	Alternatives	Abbreviation	CAS number	Chemical structure
Citrates	Acetyl tri-n-butyl citrate	ATBC	77-90-7	
	n-butyl tri-n-hexyl citrate	BTHC	82469-79-2	
Organophosphates	Bis(2-ethylhexyl) phosphate	DEHPA	298-07-7	
	Tris-(2-ethylhexyl) phosphate	TEHPA	78-42-2	
Organophosphates	Tri-cresyl phosphate	TCP	1330-78-5	
Sebacates	Di-butyl sebacates	DBS	109-43-3	
	Di-(2-ethylhexyl) sebacates	DOS	122-62-3	

Table 1. Continued

Category	Alternatives	Abbreviation	CAS number	Chemical structure
Terephthalates	Di-(2-ethyl-hexyl) terephthalate	DEHT	6422-86-2	
Trimellitates	Tris-(2-ethylhexyl) trimellitate	TETM	3319-31-1	
Vegetable oil derivatives	Glycerides, castor oil-mono, hydrogenated, acetates	COMGHA	736150-63-3	
	Epoxidized soybean oil	ESBO	8013-07-8	
Others	Alkylsulfonic phenyl ester	ASE	91082-17-6	
	Glycerin triacetate	GTA	102-76-1	
	2,2,4-trimetyl-1,3-pentanediol diisobutyrate	TXIB	6846-50-0	

In response to these restrictions, a number of alternative compounds, such as adipates, benzoates, carboxylates, citrates, organophosphates, sebacates, terephthalates, and trimellitates, have appeared. An overview of 21 substances in total that are used as alternatives is given in Table 1. The adipates including di-butyl adipate (DBA), di-iso-nonyl adipate (DINA), di-iso-decyl adipate (DIDA), and di-(2-ethyl-hexyl) adipate (DEHA) are extensively used in cosmetics, floor and wall coverings, food contact materials, and medical products.[15,16] DEHA, most prominent adipate compounds, is a high production volume chemical, with an annual production volume above 1 million pounds in the US,[17] and is similar in structure and metabolism to DEHP.[18] The benzoates such as di-(ethylene glycol) dibenzoate (DEGDB) and di-(propylene glycol) dibenzoate (DPGDB) are used mainly as additives in PVC flooring.[19] Di(isononyl)cyclohexane-1,2-dicarboxylate (DINCH), trademarked as Hexamoll® DINCH® by BASF, Ltd (Cheshire, US), was recently introduced as an alternative to DEHP for more sensitive applications such as toys, food contact materials, and medical devices.[17,20] The citrates such as acetyl tri-n-butyl citrate (ATBC) and n-butyl tri-n-hexyl citrate (BTHC) are used in children's products, food contact substances, cosmetics and medical products[17] because of its performance, low cost, and lack of odor.[21] Organophosphates including bis(2-ethylhexyl) phosphate (DEHPA), tris-(2-ethylhexyl) phosphate (TEHPA), and tri-cresyl phosphate (TCP) are another group of alternatives mainly used in cables due to their high resistance to burning[16] as well as flame retardant substances.[22] The sebacates such as di-butyl sebacates (DBS) and di-(2-ethylhexyl) sebacates (DOS) are commonly used for flexible PVC applications requiring lower plasticizer volatility.[19] The terephthalates are structural isomers to phthalates having two adjacent ring substitutions occupying para-positions instead of ortho-positions.[23] Among them, di(2-ethylhexyl) terephthalate (DEHT) is a structural isomer of DEHP and is widely used in various application such as in plastic toys, childcare items, and medical devices.[19,23] Tris-(2-ethylhexyl) trimellitate (TETM) is a group of esters of trimellitic acid and is used in high temperature applications such as PVC cables with significantly improved migration resistance relative to other DEHP alternatives.[19] The vegetable oil derivatives derived from castor oil and soybean, i.e., glycerides, castor oil-mono, hydrogenated, acetates (COMGHA) and epoxidized soybean oil (ESBO), respectively, are used as a common additive in PVC gaskets, lacquer, and painting inks.[16,19] Other alternatives include alkylsulfonic phenyl ester (ASE, trademarked as Mesamoll® II), glycerin triacetate (GTA, trademarked as Triacetin), and 2,2,4-trimetyl-1,3-pentanediol diisobutyrate (TXIB).

As their production and discharge into the environment are estimated to increase in Korea, the health risk potentials of phthalates and their alternatives are of growing concern. Unlike phthalate diesters, whose endocrine toxicity has received thorough investigation, limited attention has been paid to the toxicity of phthalate alternatives until now. For most phthalate alternatives, toxicological information was not easily available to the public. This review focuses on the endocrine disruption potentials of 15 phthalate diesters and 21 alternatives to identify knowledge gaps as for future research.

II. Methods

For phthalate diesters, a summary of the available information on their estrogenicity and androgenicity is provided. The selected 15 compounds are as follows: di-methyl phthalate (DMP, CAS no. 131-11-3), di-ethyl phthalate (DEP, CAS no. 84-66-2), di-*n*-propyl phthalate (DPrP, CAS no. 131-16-8), DBP (CAS no. 84-74-2), DIBP (CAS no. 84-69-5), BBP (CAS no. 85-68-7), DPP (CAS no. 131-18-0), di-*n*-hexyl phthalate (DHP, CAS no. 84-75-3), di-iso-hexyl phthalate (DIHP, CAS no. 68515-50-4),

di-iso-heptyl phthalate (DIHepP, CAS no. 41451-28-9), DOP (CAS no. 117-84-0), DINP (CAS no. 28553-12-0), DIDP (CAS no. 26761-40-0), DEHP (CAS no. 117-81-7), and di-cyclohexyl phthalate (DCHP, CAS no. 84-61-7). Only toxicity data that measured estrogenicity/anti-estrogenicity and androgenicity/anti-androgenicity in *in vitro* and *in vivo* assays were summarized. Specifically, the following studies were summarized:

- *In vitro* estrogen receptor (ER) binding assay, estrogen transactivation assay, ER reporter gene assay, ER recombinant gene assay, E-screen assay
- *In vitro* androgen receptor (AR) binding assay, androgen transactivation assay, AR reporter gene assay
- *In vivo* estrogenic assay (Uterotrophic assay and pubertal assay)
- *In vivo* androgenic assay (Hershberger assay and testicular assay)

For phthalate alternatives, the available information on their reproductive toxicity in rodents (Klimisch score 1 and 2)[24] and endocrine disrupting effects (estrogenicity and androgenicity) was summarized.

III. Results and Discussion

1. Estrogenicity and androgenicity of phthalate diesters

Table 2. Estrogenicity/anti-estrogenicity and androgenicity/anti-androgenicity of phthalates in *in vitro* studies

Compound	Test type	Endpoint	Toxicity data	Ref.
DEP	ER recombinant gene assay in yeast	Estrogenic activity	Yes	36
	ER transactivation assay in human MCF-7 cell	LOEC, estrogenic activity	10^{-6} M	46
	ER transactivation assay in hamster CHO cell			
DBP	hER binding assay based on rabbit uterine tissue	IC_{50}, estrogenic activity	$>1\times10^{-5}$ M	27
	hER recombinant gene assay in yeast	IC_{50}, estrogenic activity	$>2\times10^{-4}$ M	
	E-screen assay in human MCF-7 cell	LOEC, estrogenic activity	10^{-8} M	38
	ER transactivation assay in human MVLN cell	LOEC, estrogenic activity	5×10^{-6} M	31
	ER transactivation assay in human MVLN cell	LOEC, anti-estrogenic activity (at 25×10^{-12} M 17β-estradiol)	5×10^{-6} M	
	ER recombinant gene assay in yeast	Estrogenic activity	Yes	36
	ER binding assay in human MCF-7 cell	LOEC, estrogenic activity	10^{-6}~10^{-5} M	28
	hERα binding assay	IC_{50}, estrogenic activity	27.5×10^{-6} M	29
	E-screen assay in human MCF-7 cell	RPE[c], estrogenic activity	109	
	ERα binding assay	IC_{50}, estrogenic activity	2.7×10^{-4} M	30
	ER reporter gene assay in human MVLN cell	NOEC, estrogenic activity	10^{-4} M	
	ER reporter gene assay in human MVLN cell	NOEC, anti-estrogenic activity (at 1.0×10^{-10} M 17β-estradiol)	10^{-4} M	
	AR binding assay	IC_{50}, androgenic activity	6.0×10^{-5} M	
	AR reporter gene assay in hamster CHO-K1 cell	NOEC, androgenic activity	10^{-4} M	
	AR reporter gene assay in hamster CHO-K1 cell	IC_{50}, anti-androgenic activity (at 1.7×10^{-10} M 5α-dihydrotestosterone)	4.1×10^{-5} M	
	ER reporter gene assay in monkey CV-1 cell	LOEC, estrogenic activity	10^{-5} M	33
	AR reporter gene assay in MDA-kb2 cell	LOEC, androgenic activity	10^{-4} M	
	AR reporter gene assay in MDA-kb2 cells	LOEC, anti-androgenic activity	10^{-7} M	

Table 2. Continued

Compound	Test type	Endpoint	Toxicity data	Ref.
DBP	hERα transactivation assay in hamster CHO cell	REC_{20}[a], estrogenic activity	6.0×10^{-6} M	32
	AR transactivation assay in hamster CHO cell	RIC_{20}[b], anti-androgenic activity	4.8×10^{-6} M	
	ER reporter gene assay in human MCF-7 cell	LOEC, estrogenic activity	10^{-5} M	34
DiBP	ER recombinant gene assay in yeast	Estrogenic activity	Yes	36
	hERα transactivation assay in hamster CHO cell	REC_{20}[a], estrogenic activity	6.1×10^{-6} M	32
	AR transactivation assay in hamster CHO cell	RIC_{20}[b], anti-androgenic activity	6.2×10^{-6} M	
BBP	hER binding assay based on rabbit uterine tissue	IC_{50}, estrogenic activity	$>1\times10^{-5}$ M	27
	hER recombinant gene assay in yeast	IC_{50}, estrogenic activity	1.2×10^{-5} M	
	E-screen assay in human MCF-7 cell	RPE[c], estrogenic activity	10^{-6}~10^{-7} M	
	E-screen assay in human MCF-7 cell	LOEC, estrogenic activity	10^{-8} M	38
	ER recombinant gene assay in yeast	RPE[c], estrogenic activity	0.0004	35
	ER transactivation assay in human MVLN cell	LOEC, estrogenic activity	5×10^{-6} M	31
	ER transactivation assay in human MVLN cell	LOEC, anti-estrogenic activity (at 25×10^{-12} M 17β-estradiol)	5×10^{-6} M	
	ER recombinant gene assay in yeast	Estrogenic activity	Yes	36
	ER binding assay in human MCF-7 cell	LOEC, estrogenic activity	10^{-6}~10^{-5} M	28
	hERα binding assay	IC_{50}, estrogenic activity	5.67×10^{-6} M	29
	E-screen assay in human MCF-7 cell	RPE[c], estrogenic activity	106	
	E-screen assay in human MCF-7 cell	Estrogenic activity	Yes	39
	Anti-estrogen assay in human MCF-7 cell	Anti-estrogenic activity	Yes	
	hERα transactivation assay in CHO cells	REC_{20}[a], estrogenic activity	1.7×10^{-6} M	32
	hERβ transactivation assay in CHO cells	REC_{20}[a], estrogenic activity	3.8×10^{-6} M	
	hERβ transactivation assay in hamster CHO cell	RIC_{20}[b], anti-estrogenic activity	9.4×10^{-6} M	
	AR transactivation assay in hamster CHO cell	RIC_{20}[b], anti-androgenic activity	2.9×10^{-6} M	
	ER-reporter gene assay in human MCF-7 cell	LOEC, estrogenic activity	10^{-5} M	34
	ER-reporter gene assay in human HeLa cell	LOEC, estrogenic activity	10^{-5} M	
DPP	hERα transactivation assay in hamster CHO cell	REC_{20}[a], estrogenic activity	5.7×10^{-6} M	32
	hERβ transactivation assay in hamster CHO cell	RIC_{20}[b], anti-estrogenic activity		
	AR transactivation assay in hamster CHO cells	RIC_{20}[b], anti-androgenic activity		
DHP	hERα transactivation assay in hamster CHO cell	REC_{20}[a], estrogenic activity	5.6×10^{-6} M	32
	hERβ transactivation assay in hamster CHO cell	RIC_{20}[b], anti-estrogenic activity	4.0×10^{-6} M	
	AR transactivation assay in hamster CHO cell	RIC_{20}[b], anti-androgenic activity	3.5×10^{-6} M	
DiHP	hERα transactivation assay in hamster CHO cell	REC_{20}[a], estrogenic activity	2.8×10^{-6} M	32
	hERβ transactivation assay in hamster CHO cell	RIC_{20}[b], anti-estrogenic activity	3.2×10^{-6} M	
	AR transactivation assay in hamster CHO cell	RIC_{20}[b], anti-androgenic activity	3.4×10^{-6} M	

Table 2. Continued

Compound	Test type	Endpoint	Toxicity data	Ref.
DiHepP	hERα transactivation assay in hamster CHO cell	REC_{20}[a], estrogenic activity	6.3×10^{-6} M	32
	hERβ transactivation assay in hamster CHO cell	RIC_{20}[b], anti-estrogenic activity	5.3×10^{-6} M	
	AR transactivation assay in hamster CHO cell	RIC_{20}[b], anti-androgenic activity	3.8×10^{-6} M	
DiNP	Recombinant gene assay in yeast	Estrogenic activity	Yes	36
DEHP	E-screen assay in human MCF-7 cell	LOEC, estrogenic activity	10^{-5} M	37
	E-screen assay in human MCF-7 cell	LOEC, estrogenic activity	10^{-8} M	38
	ER transactivation assay in human MVLN cell	LOEC, anti-estrogenic activity (at 25×10^{-12} M 17β-estradiol)	5×10^{-5} M	31
	E-screen assay in human MCF-7 cell	Estrogenic activity	Yes	39
	hAR binding assay in monkey COS cell	NOEC, anti-androgenic activity	10^{-6} M	53
	ER reporter gene assay in monkey CV-1 cell	NOEC, estrogenic activity	10^{-6} M	33
	AR reporter gene assay in human MDA-kb2 cells	LOEC, androgenic activity	10^{-4} M	
	AR reporter gene assay in human MDA-kb2 cells	LOEC, anti-androgenic activity	10^{-6} M	
	AR reporter gene assay in human MDA-MB453 cell	NOEC, anti-androgenic activity (at 4×10^{-10} M dihydroestosterone)	10^{-6} M	52
DEHP	hERα transactivation assay in hamster CHO cell	REC_{20}[a], estrogenic activity	5.5×10^{-6} M	32
	hERβ transactivation assay in hamster CHO cell	RIC_{20}[b], anti-estrogenic activity	3.4×10^{-6} M	
	E-screen assay in human MCF-7 cell	EC_{50}, estrogenic activity	1.34×10^{-5} M	40
DCHP	E-screen assay in human MCF-7 cell	Estrogenic activity	Yes	39
	hERα transactivation assay in hamster CHO cell	REC_{20}[a], estrogenic activity	2.8×10^{-6} M	32
	hERβ transactivation assay in hamster CHO cell	RIC_{20}[b], anti-estrogenic activity	2.5×10^{-6} M	
	AR transactivation assay in hamster CHO cell	RIC_{20}[b], anti-androgenic activity	3.8×10^{-6} M	

[a]: 20% relative effective concentration, the concentration of the test compound showing 20% of the agonistic activity of 10^{-9} M E2.
[b]: 20% relative inhibitory concentration, the concentration of the test compound showing 20% of the antagonistic activity of 10^{-10} M E2 via ERβ or 10^{-10} M DHT via AR, respectively.
[c]: Relative proliferative effect is calculated as 100×proliferative effect.

Several studies have been published confirming the estrogenic and anti-androgenic activity of phthalates in diverse *in vitro* and *in vivo* assays (Tables 2-3). Phthalate esters can act as endocrine disruptors, resulting in reproductive toxicity. The structure and position of the paired ester groups are important factors for endocrine disruption. Phthalate esters of a specific chain length (4-6 carbons) with an ester moiety in the ortho position induced anti-androgenic effects such as testicular atrophy and decreased testicular zinc content in pubertal Sprague-Dawley rats following 4 days of oral administration.[25] In contrast, phthalates with ester group of shorter or longer than 4-6 carbons or with an ester moiety in the para position were not active.[26] These results were supported by a number

Table 3. Summary of *in vivo* studies published on the estrogenic and androgenic activity of phthalates

Compound	Test type	Test organisms (age and sex)	Exposure duration	Endpoint	Toxicity data	Ref.
DEP	Uterotrophic assay	Wistar rat (immature female)	24 h	LOED, estrogenic effects (uterine epithelial cell height)	50 mg/kg	46
DPrP	Uterotrophic assay	Sprague-Dawley Crj:CD rat (immature female)	3 d	LOED, estrogenic effects (uterus weight)	300 mg/kg	45
	Hershberger assay	Brl Han:WIST Jcl (GALAS) rat (male)	10 d	LOED, anti-androgenic effects (Cowper's gland weight)	200 mg/kg	47
DBP	Uterotrophic assay	rat (immature female)	3 d	NOED, estrogenic effects (uterus weight)	10 mg/kg	2
	Uterotrophic assay	Sprague-Dawley rat (immature female)	3 d	NOED, estrogenic effects (uterus weight)	2,000 mg/kg	29
	Uterotrophic assay	Sprague-Dawley Crl:CD rat (immature female)	3 d	NOED, estrogenic effects (uterus and vagina weight)	500 mg/kg	41
	Uterotrophic assay	B6C3F1 mice (ovariectomized female)	4 d	NOED, estrogenic effects (uterus weight)	5,000 mg/kg	43
	Uterotrophic assay	Sprague-Dawley Crj:CD rat (immature female)	3 d	NOEC, estrogenic effects (uterus weight)	200 mg/kg	42
	Uterotrophic assay	Sprague-Dawley rat (immature female)	5 d	NOED, estrogenic effects (uterus weight)	2,000 mg/kg	34
	Uterotrophic assay	CFLP mice (female)	3 d	NOED, estrogenic effects (uterus weight)	5 mg/kg	35
	Pubertal assay	rat (immature female)	20 d	LOED, estrogenic effects (uterus and ovary weight)	10 mg/kg	2
	Hershberger assay	Sprague-Dawley Crl:CD rat (male)	10 d	LOED, anti-androgenic effects (ventral prostate weight)	20 mg/kg	50
DBP	Peripubertal male rat assay	Alpk:APfSD rat (immature male)	14 d	LOED, anti-androgenic effects (testes weight)	500 mg/kg	49
	Hershberger assay	Sprague-Dawley Crl:CD rat	10 d	LOED, anti-androgenic effect (testes weight)	500 mg/kg/d	48
DIBP	Uterotrophic assay	Wistar Albino rat (immature female)	3 d	NOED, estrogenic effects (uterus and ovary weight)	1,250 mg/kg	44
	Pubertal assay	Wistar Albino rat (immature female)	20 d	NOED, estrogenic effects (uterus, ovary, and vagina weight)	1,250 mg/kg	44
BBP	Uterotrophic assay	rat (immature female)	3 d	LOED, estrogenic effects (uterus weight)	200 mg/kg	2
	Uterotrophic assay	Sprague-Dawley rat (immature female)	3 d	NOED, estrogenic effects (uterus weight)	2,000 mg/kg	29
	Uterotrophic assay	CFLP mice (female)	3 d	NOED, estrogenic effects (uterus weight)	5 mg/kg	35
	Pubertal assay	(immature female)	20 d	LOED, estrogenic effects (uterus weight)	20 mg/kg	2
	Hershberger assay	Sprague-Dawley Crl:CD rat (male)	10 d	NOED, anti-androgenic effects (ventral prostate weight)	500 mg/kg	50

Table 3. Continued

Compound	Test type	Test organisms (age and sex)	Exposure duration	Endpoint	Toxicity data	Ref.
	Peripubertal male rat assay	Alpk:APfSD rat (immature male)	14 d	NOED, anti-androgenic effects (testes weight)	500 mg/kg	49
DPP	Uterotrophic assay	Sprague-Dawley Crj:CD rat (immature female)	3 d	LOED, estrogenic effects (uterus weight)	1,000 mg/kg	45
DPP	Hershberger assay	Brl Han:WIST Jcl (GALAS) rat (male)	10 d	LOED, anti-androgenic effects (BC/LA weight)	200 mg/kg	47
DHP	Uterotrophic assay	Sprague-Dawley Crj:CD rat (immature female)	3 d	NOED, estrogenic effects (uterus weight)	1,000 mg/kg	45
	Hershberger assay	Brl Han:WIST Jcl (GALAS) rat (male)	10 d	NOED, anti-androgenic effects	1,000 mg/kg	47
DOP	Uterotrophic assay	Sprague-Dawley rat (immature female)	5 d	NOED, estrogenic effects (uterus weight)	2,000 mg/kg	34
	Hershberger assay	Sprague-Dawley Crl:CD rat (male)	10 d	NOED, anti-androgenic effects (ventral prostate weight)	500 mg/kg	50
DINP	Uterotrophic assay	Wistar Albino rat (immature female)	3 d	NOED, estrogenic effect (uterus and ovary weight)	1,380 mg/kg	44
	Uterotrophic assay	Sprague-Dawley rat (immature female)	5 d	NOED, estrogenic effects (uterus weight)	200 mg/kg	34
	Pubertal assay	Wistar Albino rat (immature female)	20 d	LOED, estrogenic effect (ovary weight)	276 mg/kg	44
	Hershberger assay	Sprague-Dawley Crl:CD rat (male)	10 d	LOED, anti-androgenic effects (ventral prostate weight)	20 mg/kg	50
DIDP	Uterotrophic assay	Sprague-Dawley rat (immature female)	5 d	NOED, estrogenic effects (uterus weight)	200 mg/kg	34
	Hershberger assay	Sprague-Dawley Crl:CD rat (male)	10 d	LOED, anti-androgenic effects (ventral prostate weight)	500 mg/kg	50
DEHP	Uterotrophic assay	Sprague-Dawley rat (immature female)	5 d	NOED, estrogenic effects (uterus weight)	20 mg/kg	34
	Hershberger assay	Sprague-Dawley Crl:CD rat (male)	10 d	LOED, anti-androgenic effect (ventral prostate weight)	20 mg/kg	50
DEHP	Hershberger assay	Wistar rat (male)	10 d	LOED, anti-androgenic effect (BC/LA relative weight)	100 mg/kg	52
	Hershberger assay	Wistar rat (male)	10 d	LOED, anti-androgenic effect (testes weight)	300 mg/kg/d	51
DCHP	Uterotrophic assay	Sprague-Dawley Crj:CD rat (immature female)	3 d	NOED, estrogenic effects (uterus weight)	200 mg/kg	42

of published *in vivo* and *in vitro* studies (Tables 2-3); additionally, the toxic potencies of DBP and BBP were found to be greater than other phthalate diesters.

A number of *in vitro* studies have demonstrated estrogenic activity by three major phthalate diesters, DBP, BBP, and DEHP, through an ER binding assay,[27-30] ER transactivation assay,[31-32] ER reporter

gene assay,[30,33-34] ER recombinant gene assay,[27,35-36] and E-screen assay.[27,29,37-40] The results of these studies demonstrate that phthalates are capable of binding to ERα, inducing ERα-mediated gene expression, and enhancing the proliferation of MCF-7 cells expressing abundant ER. The relative estrogenic potencies of phthalate diesters range from, in descending order (BBP, DBP, DIBP, DEP, DINP), and their estrogenic potencies ranged from approximately 10^6 to 5×10^7 times lower than 17β-estradiol in a recombinant yeast screen assay.[36]

While a number of phthalates have been reported to possess estrogenic potential *in vitro*, some phthalate esters do not appear to exert estrogenic effects *in vivo*. For example, DBP was shown to exert weak estrogenic activity in an *in vitro* assay, but the uterotrophic effects of DBP were not observed in immature rats[2,29,34,41-42] and mice.[35,43] Other studies have also shown that some phthalate esters (DIBP, BBP, DHP, DOP, DINP, DIDP, DEHP, and DCHP) were unable to induce a significant increase in uterine weight compared to control in a 3 day Uterotrophic assay and 20 day pubertal assay.[29,34-35,42,44-45] In fact, some studies demonstrated the adverse effects on reproductive systems (e.g., augmentation of uterine weight and delayed vaginal opening) in rodents exposed to DEP,[46] DPrP,[45] DBP,[2] BBP,[2] DPP,[45] and DINP.[44]

Phthalate exposure produce adverse effects on the male reproductive system via an anti-androgenic pathway. For example, the weight of sex organs was significantly changed in male rats exposed to DPrP,[47] DBP,[48-50] DPP,[47] DINP,[50] DIDP,[50] and DEHP.[50-52] Among them, DEHP was more potent than the other phthalate diesters.[50] Phthalate esters DBP and DEHP did not bind the AR *in vitro*.[52-54]

2. Estrogenicity and androgenicity of phthalate alternatives

For all selected phthalate alternatives, toxicological information, while sparse, is available to a certain extent. Since some of these substances are produced in high volumes, toxicological data are required in the EU and thus could be found in the ECHA database for registered substances.[55] An overview of the available information and data gaps can be found in Table 4. Overall, the endpoints of reproductive toxicity are well-covered, although some areas need further investigation. For the alternatives DEGDB, DPGDB, DINCH, ATBC, DEHT, and COMGHA, a reproductive toxicity study over two generations (Organization for Economic Cooperation and Development [OECD] test guideline [TG] 416) was available.[17,55,59,62,63] For four of the alternatives (DEHA, DINCH, ESBO, and ASE), reproductive toxicity over one generation (OECD TG 415) was investigated.[17,55,57,59,62] For DBA, TETM, GTA, and TXIB, the data set was available from a reproduction and developmental toxicity screening assay (OECD TG 421) or combined repeated dose toxicity study with the reproduction and developmental toxicity screening assay (OECD TG 422).[17,55,56,62] For DINA, DIDA, BTHC, TEHPA, TCP, DBS, and DOS, no information on reproductive toxicity could be found.

Almost all phthalate alternatives have a lower toxic potential than DEHP, and more data gaps related to endocrine disruption were identified. For instance, no information for DBA, DINA, DIDA, BTHC, DEHPA, DOS, ASE, GTA, and TXIB exist. In many cases, evidence of the endocrine disruption potential on the gonadal system was found, notably for DEHA, DINCH, ATBC, TEHPA, TCP, and TETM.[21,22,61,65,67,71] However, a firm conclusion was not possible, since the underlying mechanism of all types of effects has not been fully investigated.

1) Adipates

Among the selected adipates, DBA and DEHA have demonstrated adverse effects on reproductive toxicity. The key no observed adverse effect levels (NOAELs) were 300 mg/kg bw/d for DBA[55-56] and 170-200 mg/kg bw/d for DEHA.[55,57-59]

DBA was studied in rats according to the OECD

Table 4. Toxicity data for reproduction and endocrine disruption of phthalate alternatives

Category	Alternatives	Reproductive toxicity [a]			Endocrine disruption	
		Endpoint	Klimisch score	Ref.	Endpoint	Ref.
Adipates	DBA	+ NOAEL 300 mg/kg/d, LOAEL 1,000 mg/kg/d for pup weight in F1 rat - NOAEL 1,000 mg/kg/d for reproduction in F0 rat (OECD TG 421)	1	55,56	?	
	DINA	?			?	
	DIDA	?			?	
	DEHA	+ NOAEL 170 mg/kg/d, LOAEL 1,080 mg/kg/d for reduced body weight gain in F0 rat + NOAEL 170 mg/kg/d, LOAEL 1,080 mg/kg/d for reduced mean pup weight in F1 rat (OECD TG 415)	1	55 (study report in 1988), 57,59	- estrogenic effect (MVLN luciferase assay)	31
					- anti-androgenic effect in male rat	18,51,60
		+ NOAEL 200 mg/kg/d, LOAEL 1,000 mg/kg/d for increase of follicle atresia in ovaries in F0 rat (OECD TG 407)	2	55,58	+ steroidogenic effect (↓ sex steroid-regulated gene expression and sexual behavior) in male rat	61
Benzoates	DEGDB	- NOAEL 10,000 ppm (500 mg/kg/d) for reproduction in P rat + NOAEL 3,300 ppm (165 mg/kg/d), LOAEL 10,000 ppm (500 mg/kg/d) for DE in F1 rat (OECD TG 416)	1	55 (study report in 2001),62	- estrogenic effect, NOAEL 2,000 mg/kg/d (uterotrophic assay)	62
	DPGDB	- NOEL 10,000 ppm (500 mg/kg/d) in F0, F1, and F2 rat (OECD TG 416)	1	55 (study report in 2001)	- estrogenic effect, NOAEL 2,000 mg/kg/d (uterotrophic assay)	62
Carboxylates	DINCH	- NOAEL 1,000 mg/kg/d for GE in F0 rat + NOAEL 100 mg/kg/d for GE in F1 rat - NOAEL 1,000 mg/kg/d for DE in F2 rat (OECD TG 416)	No score	55 (study report in 2003),63	+ anti-androgenic effect, LOAEL 1,000 mg/kg/d (decrease of anogenital distance in male)	65
		- NOAEL 1,000 mg/kg/d for ME in F0 rat - NOAEL 1,000 mg/kg/d for DE in F1 rat (OECD TG 415)	No score	17,62	- anti-androgenic effect	63
Citrates	ATBC	+ NOAEL 100 mg/kg/d, LOAEL 300 mg/kg/d in F0 male rat + NOAEL 300 mg/kg/d, LOAEL 1,000 mg/kg/d in F0 female rat (No guideline)	No score	17,57,62	- estrogenic effect (ER luciferase reporter assay) - androgenic effect (AR luciferase reporter assay) + steroidogenesis (↑ CYP3A4 gene expression, SXR agonist)	21
		+ NOAEL 100 mg/kg/d, LOAEL 300 mg/kg/d for ME in F0 rat + NOAEL 100 mg/kg/d, LOAEL 300 mg/kg/d for DE in F1 rat (OECD TG 416)	No score	17,62	- estrogenic effect (ER binding assay, ovariectomized uterine weight) - androgenic effect (AR binding assay)	66
	BTHC	?			?	

Table 4. Continued

Category	Alternatives	Reproductive toxicity [a]			Endocrine disruption	
		Endpoint	Klimisch score	Ref.	Endpoint	Ref.
Organophosphates	DEHPA	+ NOAEL 150 mg/kg/d, LOAEL 750 mg/kg/d in F0 male rat - NOAEL 750 mg/kg/d in F0 female rat (28 d repeated dose)	1	55 (study report in 2013)	?	
	TEHPA	?			+ steroidogenesis (GR transactivation assay)	67
	TCP	?			+ estrogenic effect (ER transactivation assay) - anti-estrogenic effect (ER transactivation assay) + anti-androgenic effect (AR transactivation assay) + steroidogenesis (PXR, GR transactivation assay)	67
					+ steroidogenesis (H295R cell assay)	22
Sebacates	DBS	?			- estrogenic effect (ER binding assay, ovariectomized uterine weight) - androgenic effect (AR binding assay)	66
	DOS	?			?	
Terephthalates	DEHT	- NOAEL 500-700 mg/kg/d (1.0% diet, male) and 800-1000 mg/kg/d (1.0% diet, female) for reproductive toxicity in F0 rat + NOAEL 150-200 mg/kg/d (0.3% diet, male) and 250-300 mg/kg/d (0.3% diet, female) for DE in F1 rat (OECD TG 416)	1	17,55 (study report in 2001),62	- estrogenic effect, NOAEL 2,000 mg/kg/d (Uterotrophic assay)	62,68
					- anti-androgenic effect, NOAEL 750 mg/kg/d	62,69
Trimellitates	TETM	+ NOEL 100 mg/kg/d for reduced number of spermatids in F0 male rat - NOAEL 1,000 mg/kg/d in F0 female rat (OECD TG 421)	2	17,55 (study report in 1998)	+ estrogenic effect (ERα and ERβ luciferase assay)	71
		- NOEL 500 mg/kg/d for reproductive toxicity in F0 male rat - NOEL 500 mg/kg/d for DE in F1 rat (OECD TG 422)	2	55 (study report in 2001)	- estrogenic effect (ERα binding assay)	72
Vegetable oil derivatives	COMGHA	- NOAEL 25,000 ppm (1,159 mg/kg/d in male, 2,200 mg/kg/d in female) for ME in F0 rat - NOAEL 25,000 ppm (1,320 mg/kg/d in male, 2,262 mg/kg/d in female) for DE in F1 rat (OECD TG 416)	1	59	- anti-androgenic effects	59

Table 4. Continued

Category	Alternatives	Reproductive toxicity [a]			Endocrine disruption	
		Endpoint	Klimisch score	Ref.	Endpoint	Ref.
	ESBO	- NOEL 1,000 mg/kg/d for PE in F0 rat - NOEL 1,000 mg/kg/d for fetotoxicity in F1 rat (OECD TG 415)	2	55 (study report in 1993),59	- estrogenic effect (ERα binding assay)	72
					- estrogenic effect (ER binding assay, ovariectomized uterine weight) - androgenic effect (AR binding assay)	66
Others	ASE	+ NOAEL 600 ppm (68 mg/kg/d), LOAEL 3,000 ppm for reduction in body weight gain in F0 rat + NOAEL 600 ppm (68 mg/kg/d), LOAEL 3,000 ppm for DE in F1 rat (OECD TG 415)	1	55 (study report in 2002a),59	?	
Others	GTA	- NOAEL 1,000 mg/kg/d for reproductive toxicity in F0 rat - NOAEL 1,000 mg/kg/d for DE in F1 rat (OECD TG 422)	1	55 (study report in 1998),62	?	
	TXIB	+ NOAEL 276 mg/kg/d (4.5 mg/g in diet), LOAEL 15 mg/g in diet for ME in F0 male rat + NOAEL 359 mg/kg/d (4.5 mg/g diet), LOAEL 15 mg/g for ME in F0 female rat (OECD TG 421)	1	55 (study report in 2001),62	?	
		- NOEL 750 mg/kg/d for ME in F0 rat (OECD TG 422)	2	55 (study report in 1993),62		

[a] Test guideline was indicated in parenthesis. In relation to reproductive toxicity endpoints, studies with Klimisch score 1 and 2 were considered. The Klimisch score 1 and 2 indicate that the data were of good quality, usually test data from studies conducted according to internationally recognized test guidelines (or similar to) and good laboratory practice.
Abbreviation: AR: androgen receptor, ER: estrogen receptor, FE: fertility, GE: general effect, GR: glucocorticoid receptor, LOAEL: lowest observed adverse effect level, ME: maternal effect, NOEL: no observed effect level (greatest concentration or amount of a substance, found by experiment or observation, that causes no effects of target organisms distinguishable from those observed in normal organisms of the same species under the same defined conditions of exposure), NOAEL: no observed adverse effect level (greatest concentration or amount of a substance, found by experiment or observation, which causes no detectable adverse effects of target organism under defined conditions of exposure), PE: parental effect, PXR: pregnane X receptor, +: effect confirmed, -: no evidence for adverse effect, ?: data gap.

TG 421, and its NOAEL was considered to be 1,000 mg/kg/d for F0 reproduction and 300 mg/kg/d for F1 development.[55-56] In a one-generation reproductive toxicity study (OECD TG 415), the highest dose (1,080 mg/kg/d) of DEHA led to reductions in maternal body weight gain and mean pup weight in an F1 rat.[55,57,59] DEHA induced an increase in follicle atresia in ovaries at 1,000 mg/kg/d in a repeated-dose 28 day oral toxicity study.[55,58] Since data on reproductive toxicity are lacking for DINA and DIDA, further research is critical.

Although the molecular structures of DEHP and DEHA are analogous, a number of studies have confirmed that the endocrine effects of DEHA are not similar to that of DEHP.[18,31,51,60] For example,

no anti-androgenic activity in male rats [18,51,60] and no estrogenic activity in human breast MVLN cells[31] were observed. However, DEHA did affect steroidogenesis in male rats.[61] Further studies are needed to investigate the potential endocrine disruptions of DBA, DINA, and DIDA.

2) Benzoates

DEGDB has been found to show toxicity in the F1 generation.[55,62] In a two-generation test according to OECD TG 416, the lowest NOAEL in F1 rats was 3,300 ppm (165 mg/kg/d) for DEGDB.[55,62] In terms of data specifically examining endocrine activity, DEGDB and DPGDB did not exhibit estrogenic activity up to 2,000 mg/kg/d.[62] Further investigation of other possible endocrine disrupting effects (e.g., anti-androgenic activity) are needed.

3) Carboxylates

The toxicity of Hexamoll® DINCH® from BASF was generally lower than that of DEHP. However, the Klimisch score was not confirmed in the REACH registration dossier. In several studies, no reproductive toxicities were observed; the key NOAELs were 100-1,000 mg/kg bw/d.[17,55,62-63] BASF states that DINCH® does not cause reproductive toxicity.[64] However, this chemical has been assigned a moderate hazard rating for endocrine activity based on the decreased anogenital distance in male offspring and on the effects observed in thyroid glands.[65] For example, a significant decrease in anogenital distance was reported in male rats (1,000 mg/kg bw/d).[65] In a two-generational reproduction toxicity test (OECD TG 416), treatment with DINCH® induced slight hypertrophy/hyperplasia of the thyroid follicular epithelia in F1 females, while fertility or reproductive performance were not affected.[65] Thus, further studies are needed to fully explore possible endocrine disrupting effects.

4) Citrates

Current data indicate that ATBC has reproductive toxicity resulting in decreased body weight and changes in organ weights, even though a Klimisch score was not confirmed.[17,57,62] In a two-generation reproductive toxicity study (OECD TG 416), the lowest NOAEL for both parental animals and offspring was found to be 100 mg/kg/d ATBC.[17,62] ATBC was the strongest activator of steroid xenobiotic receptor among the plasticizers evaluated. However, this chemical did not stimulate other nuclear receptors, including human estrogen receptor (ER) α, ERβ, and androgen receptor (AR) as measured using luciferase reporter assays.[21,66] Based on the collected information, further investigation is needed for BTHC in terms of reproductive toxicity and endocrine activity.

5) Organophosphates

Among the selected organophosphates, a NOAEL for DEHPA (150 mg/kg/d in male rat and 750 mg/kg/d in female rat) was reported in the REACH registration dossier for reproductive toxicity.[55] However, no data is available on the reproductive toxicity of TEHPA and TCP. In terms of endocrine disruption, TEHPA showed anti-glucocorticoid receptor activity.[67] TCP induced ERα and ERβ-mediated transcriptional activity, and inhibited androgenic activity.[67] Exposure to TCP significantly increased E2 and T hormone production in H295R cells at 0.1 mg/L.[22] Potential effects on other endocrine systems need to be investigated in future studies.

6) Sebacates

For DBS, no estrogenic effect in the ER binding assay and no androgenic effect in the AR binding assay were reported.[66] However, more extensive data set for the reproductive toxicity of the two sebacates DBS and DOS are needed for future studies.

7) Terephthalates

DEHT, a phthalate ester stoichiometrically

eqivaluent to DEHP, has been shown to have potential reproductive and developmental toxicity. For example, the NOAEL for reproductive toxicity was 1.0% in the diet (500-700 mg/kg/d for males and 800-1,000 mg/kg/d for females), and was 0.3% in the diet for offspring toxicity (150-200 mg/kg/d for males and 250-300 mg/kg/d for females) in a two-generation reproductive toxicity test.[17,55,62] The ability of DEHT to induce anti-androgenic effects in male offspring was assessed by giving pregnant rats 750 mg/kg/d DEHT; no changes were observed.[62,69] Results of a uterotrophic assay in which immature females were given up to 2,000 mg/kg/d DEHT indicate that this compound does not possess estrogenic activity.[62,68] It was noted, however, that limited data were available to assess potential thyroid effects.

8) Trimellitates

Reproductive toxicities were studied in rats at three doses (100, 300, and 1,000 mg/kg/d) of TETM.[17,55] Examination of testes found decreased spermatocytes and spermatids in males at the two highest dose levels. No effects of TETM were detected on body weight and reproductive organ weights in maternal rats and no changes in viability or body weight were detected in the offspring. On the basis of this study, a NOEL for males was 100 mg/kg/d and a NOAEL for females was 1,000 mg/kg/d.[17,55] TETM exhibited estrogenic activity in both the ERα and ERβ luciferase assay,[70] but the other study showed no affinity for ERα.[71] Further research on reproductive toxicity and endocrine disruption are needed before TETM can be routinely used as a PVC plasticizer.

9) Vegetable oil derivatives

For COMGHA, no effects on reproduction were observed: a NOAEL was found to be 25,000 ppm in rats.[59] For ESBO, the maternal NOEL was set at 1,000 mg/kg/d.[55,59] Several studies have confirmed that COMGHA and ESBO do not have endocrine effects: no anti-androgenic effects of COMGHA[59] and no estrogenic and androgenic effects of ESBO[66,71] were observed.

10) Others

For ASE, a NOAEL of 600 ppm (68 mg/kg/d) for reproductive toxicity (OECD TG 415) was observed.[55,59] In rats exposed to 40, 200, and 1,000 mg/kg/d of GTA, no significant adverse effects on reproductive parameters were observed.[55,62] In a combined repeat dose and reproductive/developmental toxicity test, a NOEL of 750 mg/kg/d was established for TXIB.[55,62] In another combined study, a NOAEL for reproductive toxicity was 276 mg/kg/d in males and 359 mg/kg/d in females.[55,62] However, critical data on endocrine disruption for ASE, GTA, and TXIB were unavailable.

V. Conclusion

The present study summarized the estrogenic and androgenic potentials of 15 phthalate diesters and 21 alternatives, and identified knowledge gaps. Several phthalate diesters were found to possess estrogenic and anti-androgenic activity, and produced adverse effects on the male reproductive system. DEHP was the most potent compound among phthalate diesters; alternatives had a lower toxic potential than DEHP. Among the selected alternatives, nine compounds (DBA, DINA, DIDA, BTHC, DEHPA, DOS, ASE, GTA, and TXIB) have not yet been fully investigated for estrogenic potential. Therefore, further studies are needed to fully explore these properties.

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