Australian Government
Department of Health and Ageing
NICNAS

Phthalates Hazard Compendium

A summary of physicochemical and human health hazard data for 25 phthalate chemicals

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Draft

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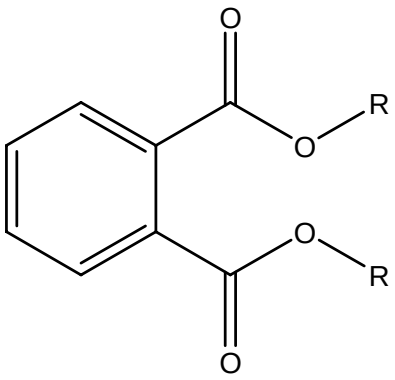
1. INTRODUCTION

This hazard compendium contains information on the physicochemical properties and human health hazards associated with 25 phthalate chemicals that are listed on the Australian Inventory of Chemical Substances and are currently or likely to be used industrially in Australia. Given continued concerns over health effects associated with phthalates, the aim of this compendium is to summarise the hazard profiles of each of these individual phthalates based on information from scientific reviews and recent literature updates and to include, where possible, comment on toxicological and molecular structural similarities and differences. Moreover, the compendium highlights the datasets available for individual phthalates and for those for which few data are available, extrapolates, where possible, likely toxicological effects based on structurally similar phthalates. Identification and structural features of the 25 phthalates are outlined in Table 1.

Background

Phthalates are chemicals used in a variety of industrial processes and consumer products. Structurally, phthalates are characterised by a diester structure consisting of a 1,2-benzenedicarboxylic acid head group linked to two ester side chains (see Figure 1). Generally, the term phthalate esters is used to identify the *ortho* form of 1,2-benzenedicarboxylic acid where the carboxylic acid functional groups and ester side chains arise from adjacent carbon atoms of the benzene ring. The terms isophthalates and terephthalates identify the *meta* and *para* structural configurations respectively.

Figure 1. Typical molecular structure of phthalate esters



The most common phthalates possess ester side chains ranging from C_1 to approximately C_{13}. Side chains may be linear eg. dibutyl phthalate, branched eg. diisobutyl phthalate or a combination of linear, branched and ringed structures eg. butyl benzyl phthalate (BBP). Commonly, both side chains are structurally identical (as depicted in Figure 1), but for some phthalates eg. BBP they differ. The structural characteristics of the ester side chains affect the physicochemical and toxicological properties of the phthalate.

Phthalates are the most common group of chemicals used as plasticisers (plastic softeners) worldwide. They are also used commonly as surfactants. Accordingly, as a chemical group, they are ubiquitous. The physicochemical properties that impart

usefulness as plasticisers also permit migration and leaching of phthalates from polymer substrates. This potential for leaching from products manufactured from plastics combined with a well recognised toxicity profile for some phthalates has led to concern over potential health impacts particularly from use in consumer products where widespread public exposure to phthalates is possible.

NICNAS Phthalate Assessments

In Australia, concern over health effects of phthalates led to phthalates being nominated to the NICNAS Candidate List from which chemicals requiring assessment are chosen on a priority basis. The Australian Inventory of Chemical Substances contains over 100 different phthalates. As a result of regulatory reviews, literature searches and a call for information from industry in 2004, 25 phthalate chemicals were identified as currently or potentially in industrial use in Australia. These 25 phthalates are listed in Table 1 and have been the subject of individual hazard assessments by NICNAS. Data from these hazard assessments are summarised in this hazard compendium. The individual hazard assessments are published separately, in parallel with this compendium.

For all 25 phthalate chemicals, the compendium contains summarised information, where available, on use, physicochemical properties, acute toxicity, irritation and sensitisation potential and more detailed evaluations of genotoxicity, repeat dose toxicity, carcinogenicity and reproductive and developmental toxicity. Data gaps are highlighted and extrapolations have been made where appropriate.

Historically, studies of the health effects of phthalates have identified reproductive and developmental toxicity as endpoints of particular concern. Information was sought from industry on general use, but also to determine those phthalates in use in applications associated potentially with repeated or long term exposure of the public and especially exposure of the young. Details of the use patterns of the 25 phthalate chemicals are available in this compendium. Subsequently, 9 of these 25 phthalates were identified in actual or potential use in children's toys, childcare articles and/or cosmetics. Therefore, in addition to hazard assessments, human health risk assessments are being conducted by NICNAS on these 9 phthalates for these consumer applications. These 9 phthalates were declared as Priority Existing Chemicals (PECs) for risk assessments on 7 March 2006. The declaration notice is available on the NICNAS website at http://www.nicnas.gov.au/Industry/Existing_Chemicals/PEC_Declarations.asp.

These PEC risk assessments are in progress and the hazard assessments of these 9 phthalates will form part of the risk assessments. The individual phthalates undergoing these human health risk assessment are identified in Table 1.

In the individual hazard assessments, frequent use was made of information obtained from international, peer-reviewed sources. For quotations of data sources, this compendium adopts the nomenclature of the hazard assessments in using an asterix to note references quoted, but not directly obtained, from reliable secondary sources such as regulatory reviews.

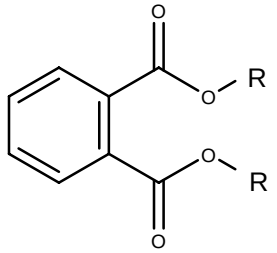
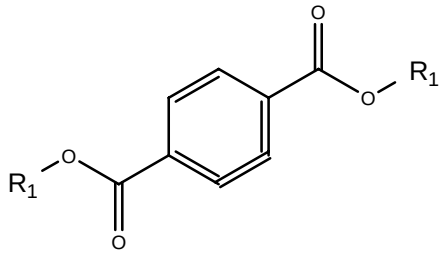
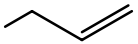
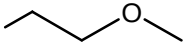
Categorisation and Data Extrapolation

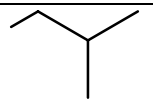
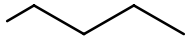
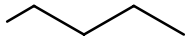
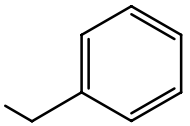
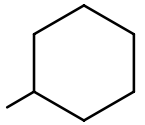
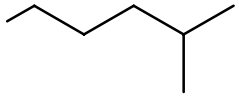
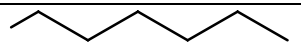
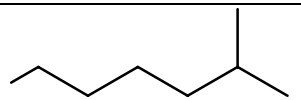
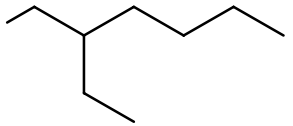
In order to determine appropriate test plans or to read-across hazard data for phthalates for which data are incomplete, regulatory reviews of groups of phthalates examining a range of health endpoints have been conducted (Phthalate Esters Panel HPV Testing Group, 2001; OECD, 2004). In these reviews, groupings of phthalates were examined on the basis of incremental structural differences allowing categorisation and identification of physicochemical and toxicological trends. Although this compendium has compiled data on a range of phthalates, the aim of this compendium and the method by which individual phthalates were included for assessment differ from these previous reviews. In this compendium, the 25 individual phthalate chemicals were included not on the basis of structural similarities to facilitate read-across for missing data, but on the basis of their current use in Australia with a view to summarising the hazard profile and highlighting the extent of data available for each chemical. Nevertheless, where possible, hazard endpoints are extrapolated for poorly characterised phthalates from information for similar phthalates within these 25 and, in some cases, with reference to information from well characterised phthalates not included for detailed review here.

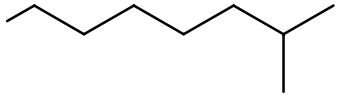
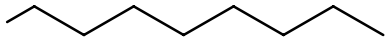
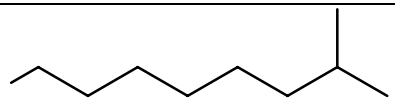
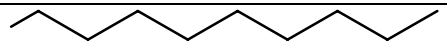
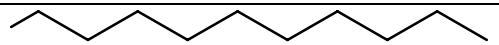
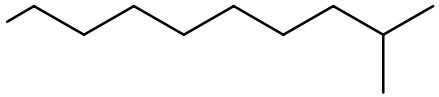
The Phthalate Esters Panel HPV Testing Group (2001) derived 3 categories of phthalates based on use, physicochemical and toxicological properties. Low molecular weight phthalates were defined as those produced from alcohols with straight-chain carbon backbones of ≤ C3. High molecular weight phthalates were defined as those produced from alcohols with straight-chain carbon backbones of ≥ C7 or ring structure. An identical description of high molecular weight phthalates as ≥ C7 was used by OECD (2004) for their review of the high molecular weight phthalates category. Transitional phthalates were defined as those produced from alcohols with straight-chain carbon backbones of C4-6. Phthalates of this backbone length have been associated previously with reproductive and developmental toxicity (Foster et al., 1980; Oishi and Hiraga, 1980a,b; Lamb et al., 1987; Heindel et al., 1989).

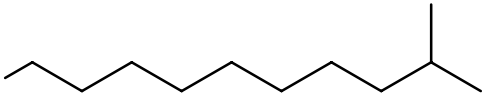
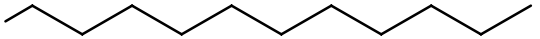
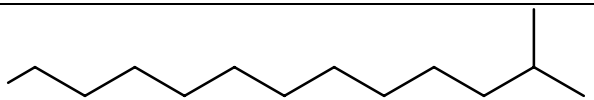
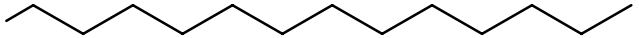
The structural properties that distinguish members of these phthalate categories is the presence of branched or linear side chains with a predominant fraction with backbones (ie. the linear portion of the side chains) falling within the appropriate carbon number ranges. For the purposes of discussion in this compendium, reference to low, transitional and high molecular weight phthalates refers to those with backbone lengths of ≤ C3, C4-6 and ≥ C7 respectively, as defined within the above reviews.

Table 1. Identification of 25 phthalates reviewed for this compendium

Backbone C Length	Chemical Name	Common Name	CAS no.	Molecular Formula	MW	Structure
C1	1,2-Benzenedicarboxylic acid, dimethyl ester	Dimethyl phthalate (DMP) *	131-11-3	C10H10O4	194.19	 R = CH_3
C1 para	1,4-Benzenedicarboxylic acid, dimethyl ester	Dimethyl terephthalate (DMT)	120-61-6	C10H10O4	194.20	 R_1 = CH_3
C2	1,2-Benzenedicarboxylic acid, diethyl ester	Diethyl phthalate (DEP) *	84-66-2	C12H14O4	222.30	R = 
C3 (double bond)	1,2-Benzenedicarboxylic acid, di-2-propenyl ester	Diallyl phthalate (DAP)	131-17-9	C14H14O4	246.27	R = 
C3	1,2-Benzenedicarboxylic acid, bis(2-	Bis(2-methoxyethyl) phthalate (DMEP) *	117-82-8	C14H18O6	282.30	R = 

	methoxyethyl) ester					
C3	1,2-Benzenedicarboxylic acid, bis(2-methylpropyl) ester	Diisobutyl phthalate (DIBP)	84-69-5	C16H22O4	278.35	R = 
C4	1,2-Benzenedicarboxylic acid, dibutyl ester	Dibutyl phthalate (DBP) *	84-74-2	C16H22O4	278.34	R = 
C4, C5	1,2-Benzenedicarboxylic acid, butyl phenylmethyl ester	Butylbenzyl phthalate (BBP) *	85-68-7	C19H20O4	312.35	R =  R_1 = 
C4 (ring)	1,2-Benzenedicarboxylic acid, dicyclohexyl ester	Dicyclohexyl phthalate (DCHP)	84-61-7	C20H26O4	330.46	R = 
C5	1,2-Benzenedicarboxylic acid, dihexyl ester, branched and linear	Diisohexyl phthalate (DIHP)	68515-50-4	C20H30O4	334.00	R = 
C6	1,2-Benzenedicarboxylic acid, dihexyl ester	Di-n-hexyl phthalate (DnHP)	84-75-3	C20H30O4	334.40	R = 
C6-rich	1,2-Benzenedicarboxylic acid, di-C6-8-branched alkyl esters, C7-rich	Diisoheptyl phthalate (DiHepP)	71888-89-6	C22H34O4	363.00	R = 
C6	1,2-Benzenedicarboxylic acid, bis(2-ethylhexyl) ester	Diethylhexyl phthalate (DEHP) *	117-81-7	C24H38O4	390.56	R = 

C7	1,2-Benzenedicarboxylic acid, diisooctyl ester	Diisooctyl phthalate (DIOP)	27554-26-3	C24H38O4	390.62	R = 
C8	1,2-Benzenedicarboxylic acid, dioctyl ester	Di-n-octyl phthalate (DnOP) *	117-84-0	C24H38O4	390.60	R = 
C7-9	1,2-Benzenedicarboxylic acid, di-C7-9-branched and linear alkyl esters	Di-C7-9 alkyl phthalate (Di-C7-9 PE)	68515-41-3	C24H38O4	390.60	R = C_7H_{15} to C_9H_{19}
C8, C9	1,2-Benzenedicarboxylic acid, diisononyl ester 1,2-Benzenedicarboxylic acid, di-C8-10-branched alkyl esters, C9-rich	Diisononyl phthalate (DINP) *	28553-12-0, 68515-48-0	C26H42O4	420.60	R = 
C9	1,2-Benzenedicarboxylic acid, dinonyl ester	Dinonyl phthalate (DNP)	84-76-4	C26H42O4	418.60	R = 
C6-10	1,2-Benzenedicarboxylic acid, di-C6-10-alkyl esters	Di-C6-10 alkyl phthalate (610P or Di-C6-10 PE)	68515-51-5	C27H44O4	434.40	R =  (based on C10 length)
C9-11	1,2-Benzenedicarboxylic acid, di-C9-11-branched and linear alkyl esters	Di-C9-11 alkyl phthalate (Di-C9-11 PE)	68515-43-5	C28H46O4	446.70	R = C_9H_{19} to $C_{11}H_{23}$
C9, C10	1,2-Benzenedicarboxylic acid, diisodecyl ester 1,2-	Diisodecyl phthalate (DIDP) *	26761-40-0, 68515-49-1	C28H46O4	447.00	R = 

	Benzenedicarboxylic acid, di-C9-11-branched alkyl esters, C10-rich					
C10	1,2-Benzenedicarboxylic acid, diundecyl ester, branched and linear	Diisoundecyl phthalate (DIUP)	85507-79-5	C30H50O4	474.70	R =  (branched only shown)
C11	1,2-Benzenedicarboxylic acid, diundecyl ester	Diundecyl phthalate (DUP)	3648-20-2	C30H50O4	474.70	R = 
C12-rich	1,2-Benzenedicarboxylic acid, di-C11-14-branched alkyl esters, C13 rich	Diisotridecyl phthalate (DITDP)	68515-47-9	C34H58O4	530.80	R =  (based on C13 length)
C13	1,2-Benzenedicarboxylic acid, ditridecyl ester	Ditridecyl phthalate (DTDP)	119-06-2	C34H58O4	530.80	R = 

***** Phthalates currently subject to human health risk assessments for use in children's toys, childcare articles and cosmetics

2. PHYSICOCHEMICAL PROPERTIES

A comprehensive dataset on physicochemical properties was available for the majority of the 25 phthalates in this compendium. Data were incomplete for 4 phthalates and only for one phthalate, DIHP, were no data available.

Physicochemical data are summarised in Table 2. Unless stated, values are for standard pressure and temperature conditions of 101.325 kPa and 25°C.

The 25 phthalates in this review are generally clear to yellow, oily liquids at ambient temperatures with high boiling points (ranging from about 157ºC to 501ºC). Nearly all have melting points below -25ºC. The exceptions are DMP (5.5ºC), DMT (141ºC), DCHP (63-65ºC), DIUP and DUP (-9ºC).

Physicochemical properties alter with increasing molecular weight and backbone length. The phthalates exhibit an eight-fold increase in octanol-water partition coefficient and an order of magnitude of ten for decrease in vapour pressure as backbone length increases from 1 to 13 carbons. Water solubility is also inversely related to molecular weight and backbone length. Lower molecular weight phthalates exhibit slight to moderate water solubility with the highest being 4.3 g/L for DMP, whereas higher molecular weight phthalates are insoluble.

Information on the use patterns of phthalates indicate generally that the lower molecular weight phthalates are used as surfactants whilst the higher molecular weight phthalates are used as plasticisers. Phthalates are readily soluble in most organic solvents and miscible with alcohol, ether and most oils (Phthalate Esters Panel HPV Testing Group, 2001).

Table 2. Summary of physicochemical properties

Backbone C Length	Phthalate	Melting Point (ºC)	Boiling Point (ºC)	Density (kg/m^3)	Vapour Pressure (kPa)	Water Solubility (g/L)	Partition Coefficient (log Kow)
C1	DMP	5.5	284	1190	<1.33 x 10^{-3} (20ºC)	4.3 (20ºC)	1.47 - 2.12
C1 para	DMT	141	No data	No data	<1.33 x 10^{-3}	1.9 x 10^{-2}	2.25
C2	DEP	No data	298	1120	2.19 x 10^{-4}	1	2.47 – 2.51
C3 (double bond)	DAP	-70	157 (0.67 kPa)	1120	2.13 × 10^{-5}	1.48 x 10^{-1} (20ºC)	3.23 (20 ºC)
C3	DMEP	-40	340	1170 (15°C)	<1.30 x 10^{-2} (20ºC)	9 x 10^{-1} (20°C)	2.9
C3	DIBP	-37	320	1038	1 x 10^{-5} (20°C)	1 x 10^{-3}	4.11
C4	DBP	-69	340	1045 (20˚C)	9.7 x 10^{-6}	1 x 10^{-2} (20˚C)	4.57
C4, C5	BBP	<-35	370 (1.01 kPa)	1114-1122	8.0 x 10^{-8}	2.8 x 10^{-3}	4.84
C4 (ring)		66	222 – 228 (0.5 kPa)	1383 (20ºC)	13.3 x 10^{-3} (150ºC)	4 x 10^{-3} (24ºC)	3-4 (temp not specified)
C5	DIHP	No data	No data	No data	No data	No data	No data
C6	DnHP	-27.4	350	1011	6.67 x 10^{-7}	5 x 10^{-5}	6.30
C6-rich	DiHepP	-45	398	994 (20°C)	9.33 x 10^{-8}	1.7 x 10^{-5} (22°C)	6.87
C6	DEHP	-55	230	980-985	3.4 x 10^{-6} (20°C)	3 x 10^{-6}	7.50
C7	DIOP	-45	230 (0.53 kPa)	986 (20°C)	1.33 (200°C)	<1 x 10^{-1} (20°C)	No data
C8	DnOP	-25	390	978	1.33 x 10^{-8}	5 x 10^{-6}	8.06
C7-9	Di-C7-9 PE	-48 – -45	398 – 454	965	(6.81 – 93.30) x 10^{-9}	(6.10 – 170) x 10^{-7}	6.9 – 8.6
C8, C9	DINP	-50	>400	975 (20°C)	6 x 10^{-8} (20°C)	6 x 10^{-5} (20°C)	8.8

C9	DNP	No data	413	972 (20ºC)	1.33×10^{-2} (205ºC)	Insoluble	>2.12
C6-10	Di-C6-10 PE	-50	250 (0.5 kPa)	974 – 979 (20°C)	$<1 \times 10^{-4}$ (20°C)	$<2 \times 10^{-4}$ (20°C)	>3.5 (20°C)
C9-11	Di-C9-11 PE	-48 – -9	454 – 501	960	$(4.97 - 68.10) \times 10^{-10}$	$(<1.70 - 6.10) \times 10^{-7}$	8.6 – 10.3
C9, C10	DIDP	-45	>400	970 (20°C)	5.1×10^{-8}	2×10^{-7} (20°C)	8.8
C10	DIUP	-9°	501	964	4.97×10^{-10}	4.41×10^{-9}	10.3
C11	DUP	-9°	501	954	4.97×10^{-10}	4.41×10^{-9}	10.3
C12-rich	DITDP	-37	501	950	3.63×10^{-11}	7×10^{-8}	12.1
C13	DTDP	-37	501	950	3.63×10^{-11}	7×10^{-11}	12.1

3. PHTHALATE USES

In 2004 and 2006, detailed information was sought from industry regarding the industrial uses of phthalates in Australia. From general industry information, regulatory reviews and literature searches, a number of individual phthalate chemicals were identified as likely to be in current industrial use and for these, specific information was sought on use patterns. Table 3 contains summarised descriptions of the industrial use patterns for the 25 individual phthalates.

For several phthalates (DnHP, Di-C6-10 PE, Di-C9-11 PE), information on use in Australia was not available, despite information on overseas uses. Nevertheless, these were included for hazard assessment on the basis of their frequent and common use overseas and therefore the possibility that such uses may occur in the future in Australia.

In reviews of phthalates, lower molecular weight phthalates are often associated with uses as surfactants, whilst the higher molecular weight phthalates are associated with uses as plasticisers. The lower molecular weight phthalates were identified in Australia in applications as plasticisers for a variety of industrial substrates including electrical wiring insulation, automotive parts, surface protection coatings and adhesives. They were also noted in use as surfactants in fragrance bases for household cleaning and personal care products.

The Australian use patterns indicate that generally, the higher molecular weight phthalates are used as plasticisers in applications similar to those of the lower molecular weight phthalates. However, they are not noted as being used as surfactants. The transitional phthalates such as DBP, DEHP are identified in specific applications as being used as both plasticisers and surfactants.

Several phthalates (9) were identified being in use or with the potential for use in children's toys, childcare articles or cosmetics. These are the subject of separate human health risk assessments for these applications and are highlighted in Table 3.

Table 3. Summary of phthalate uses in Australia

Backbone C Length	Phthalate	Uses
C1	DMP*	For minerals separation; mining and construction coatings; plasticiser in PVC, rubber, automotive parts, children's toys; fabrication of fibreglass, paints, nitrocellulose, cellulose acetates; fragrance bases for household cleaning and cosmetic products.
C1 para	DMT	For analytical research.
C2	DEP*	In epoxy resins, household cleaning, cosmetics, personal care and pharmaceutical products, perfumes and children's toys. Also used as an alcohol denaturant.
C3 (double bond)	DAP	As primary plasticiser for insulation materials, flexible fibreglass topcoat laminates, adhesives and injection moulding materials.
C3	DMEP*	Plasticiser for PVC consumer products including play and exercise balls.
C3	DIBP	Plasticiser for PVC and rubber; industrial adhesives and catalyst systems for polypropylene and fibreglass manufacture.
C4	DBP*	In automotive adhesives; mining and construction coatings; explosives, rocket propellants, textiles and leather treatments. Plasticiser in nitrocellulose lacquers, elastomers, rubber, epoxy resins, children's toys, exercise balls, and screen printing inks; fragrance bases for household, personal care and cosmetic products.
C4, C5	BBP*	In automotive adhesives, coatings, sealants and paints. A speciality plasticiser for nitrocellulose lacquers and acrylic coatings, screen printing inks, polyurethane forklift wheels and in PVC consumer products including gumboots, children's toys, play and exercise balls. Also used in military specified topcoats for metal substrates.
C4 (ring)	DCHP	In adhesive manufacture and screen printing inks.
C5	DIHP	In auto transmission lubricants.
C6	DnHP	No 2004/2006 information is available.
C6-rich	DiHepP	A specialist PVC plasticiser; also used in screen printing inks.
C6	DEHP*	In coatings, adhesives and resins for flooring, waterproofing, PVC labels, surface repair resin moulds, epoxy and polyurethane products, rubber components in brake assemblies and hot melt adhesives for automotive assembly and repair. Also used for cable sheathing/insulation. Used in fragrance bases for perfumery and cosmetic products.
C7	DIOP	In rubber compounds for automotive hoses and parts.
C8	DnOP*	Plasticiser in automotive and industrial hose, plastic processing, and for the manufacture of PVC conveyer belts, insulation materials, polyurethane surface coatings, floor finishers, and adhesives. Also in imported children's toys, play and exercise balls.
C7-9	Di-C7-9 PE	Plasticiser for automotive refinishing paints.
C8, C9	DINP*	In manufacture of cable insulation, adhesives, laminations, PVC automotive products, sheets, films, adhesive tapes, surfactants, vinyl

		flooring and interface-backing for products such as carpets. Also used in screen printing inks and children's toys, play and exercise balls.
C9	DNP	For analytical research.
C6-10	Di-C6-10 PE	No 2004/2006 information is available.
C9-11	Di-C9-11 PE	No 2004/2006 information is available.
C9, C10	DIDP*	For automotive parts, automotive and domestic interior vinyl, hoses, gaskets, insulation/protection coatings, adhesives, packaging materials, industrial flooring, paints, surfactants, plastics, flame-retardants, PVC films, children's toys and exercise balls.
C10	DIUP	In automotive sealants/adhesives and flame retardant compounds.
C11	DUP	In photographic paper dispersion coating, printing inks and flame-retardant polyurethane resins for construction.
C12-rich	DITDP	In air compressor lubricants.
C13	DTDP	Imported as finished encapsulating and blocking compounds for telephone cable maintenance.

* Phthalates identified in use or with the potential for use in children's toys, childcare articles and cosmetics and subject to human health risk assessments for these applications

4. TOXICOKINETICS

There are at least some toxicokinetic data available for many of the 25 phthalates, with the majority of testing conducted via the oral and dermal route. No data are available for DIHP, DnHP, DiHepP, DNP, Di-C6-10 PE, Di-C9-11 PE, DIUP, DUP, DITDP and DTDP.

Limited data on absorption are available. Data, mostly from rats and with one human study on DEHP, suggest phthalates are readily absorbed via the oral route. A clear trend was noted in dermal absorption with data collected from five phthalates, DEP, BBP, DEHP, DINP and DIDP. Studies indicated a decrease in dermal absorption with increasing side chain length. The only information available on inhalation is on DIDP, which was readily absorbed from the lung.

There is minimal or no evidence of accumulation in rodent tissues.

Studies on several phthalates indicate that they are rapidly metabolised and excreted in the urine and faeces. They undergo phase I biotransformation, that is, primary metabolism into their hydrolytic monoesters by hydrolysis of one of their ester bonds. Further enzymatic oxidation of the alkyl chain occurs in some of the phthalates, resulting in more hydrophilic oxidative metabolites. Monoesters and the oxidative metabolites of phthalates may continue to undergo phase II biotransformation to produce glucuronide conjugates with increased water solubility.

5. ACUTE TOXICITY, IRRITATION AND SENSITISATION

Data for acute toxicity and sensitisation endpoints are summarised in Table 4.

Acute Toxicity

The 25 phthalates have been studied extensively for acute toxicity, with all but one phthalate tested for acute oral and more than half for dermal toxicity. Inhalation toxicity testing data were available for 9 phthalates. Available test data indicate that overall the reviewed phthalates have a low order of acute toxicity via oral, dermal and inhalation routes. DAP showed the highest acute oral toxicity (LD50 of 656-891 mg/kg bw). This low molecular weight phthalate contains short ester backbones (C3) each incorporating a single carbon double bond.

Irritation & Sensitisation

Nearly all phthalates have been tested for skin and eye irritation. In contrast, very few have been tested for respiratory irritation. Almost all testing revealed an absence of effects for skin and eye irritation with only minimal irritant effects recorded for a few. Overall, the reviewed phthalates are not associated with skin and eye irritation.

The majority of phthalates were tested for skin sensitisation with all but one showing negative results. DAP tested positive for sensitisation *in vivo* in a local lymph node assay. Overall, these phthalates can be regarded as chemicals with low skin sensitisation potential.

Table 4. Summary of acute toxicity data

		Acute Toxicity			**Irritation**			**Skin Sensitisation**
Backbone C Length	**Phthalate**	**Oral LD50 (mg/kg bw)**	**Dermal LD50 (mg/kg bw)**	**Inhalation LC50 (mg/L)**	**Skin**	**Eye**	**Respiratory**	
C1	DMP	Rat: 2860 – 10000	Rat: 38000		ME	ME		negative
C1 para	DMT	Rat: 4390 – >6590	Guinea pig: >5000	Rat: >6	ME	ME		negative
C2	DEP	Rat: >5600 – 31000	Rat: >11000	Rat: 7.50	ME	ME		negative
C3 (double bond)	DAP	Rat: 656 – 891	Rabbit: 3300	Rat, 1h: 5.20 – 10.31	negative	negative		positive (LLNA)
C3	DMEP	Rat: 3200-6400	Guinea pig: >1171	Rat, 6h: >770-1595 ppm	ME	ME		negative
C3	DIBP	Rat: 16000 – 60320			ME	negative		negative
C4	DBP	Rat: 6300 – 8000	Rabbit: >20000	Rat, 4h: ≥15.68	ME	ME	ME	negative
C4, C5	BBP	Rat: 2330 – 20400	Rat: 6700		negative	ME		negative
C4 (ring)	DCHP	Rat: >3200	Rabbit: >300	Rat: >3.20	ME	ME		insufficient data
C5	DIHP				negative			negative
C6	DnHP	Rat: 29600	Rabbit: >20 (mL/kg bw)		ME			
C6-rich	DiHepP	Rat: >10000	Rabbit: >3160		ME	ME		negative
C6	DEHP	Rat: 30600 – >40000	Rabbit: 24750	Rat: >10.62	ME	ME	insufficient data	negative
C7	DIOP	Rat:	Rabbit:		ME	negative		

		>22000	>3160					
C8	DnOP	Rat: 53700	Guinea pig: 75 (mL/kg bw)		ME	ME	insufficient data	negative
C7-9	Di-C7-9 PE	Rat: >15000 – >20000			negative	negative		negative
C8, C9	DINP	Rat: >10000 (CAS 68515-48-0); >40000 (CAS 28553-12-0)	Rabbit: 3160 (CAS 68515-48-0)	Rat: >4.4	ME	ME		negative
C9	DNP	Rat: >2000			Insufficient data	Insufficient data		
C6-10	Di-C6-10 PE	Rat: >2000			ME	ME		
C9-11	Di-C9-11 PE	Rat: >6200 – 19700			negative	negative		negative
C9, C10	DIDP	Rat: >29100	Rabbit: >3160	Rat: >12.54	ME	ME		negative
C10	DIUP	Rat: >15800						negative
C11	DUP	Rat: >15800		Rat, 6h: >1.8	negative	ME		negative
C12-rich	DITDP	Rat: >10000	Rabbit: > 3160		ME	ME		negative
C13	DTDP	Rat: >2000						

ME – minimal effects
Blank cells – no data
LLNA – local lymph node assay

6. GENETIC TOXICITY

The amount of genotoxicity data available for individual phthalates varies widely, ranging from no data, to only *in vitro* data, to a comprehensive set of *in vitro* and *in vivo* data. A summary of genotoxicity data for the 25 phthalates is outlined in Table 5.

The standard testing strategy for genotoxicity is two *in vitro* tests (a bacterial reverse mutation assay and an in vitro cytogenetic test with mammalian cell culture). If the results of the two *in vitro tests* are negative, further *in vivo* testing is not required (European Union, 1996; NICNAS, 2006). Based on this testing strategy, 12 phthalates are considered to have adequate data for drawing conclusions regarding their genotoxicity. The remaining 13 phthalates have either insufficient or no data.

For those 12 phthalates where genotoxicity data are sufficient, the following 11 are considered non-genotoxic: DMT, DMP, DBP, BBP, DiHepP, DEHP, DnOP, DINP, DIDP, DUP and DTDP. The low molecular weight phthalate DAP also had several studies available, but these included positive results *in vitro* mammalian mutation and clastogenicity studies, an equivocal result in an *in vivo* chromosome aberrations assay, and negative results from *in vitro* bacterial mutation assays and *in vivo* mouse micronucleus and sex-linked recessive lethal assays. Whilst mutagenic *in vitro* and generally negative evidence *in vivo*, it is not possible to conclude the genotoxic potential of this phthalate.

For those phthalates where data are not available or inadequate, genotoxic potential was assessed on a weight of evidence basis. Consideration was given to high molecular weight phthalates DIOP, Di-C6-10 PE, Di-C7-9 PE, DNP, Di-C9-11 PE, DITDP and DIUP, for which there was no or limited data. These seven phthalates are considered unlikely to be genotoxic based on the negative mutagenicity data for the category as a whole, including data on the 7 phthalates reviewed here (where data was available) and other high molecular weight phthalates reviewed by Phthalate Esters Panel HPV Testing Group (2001) and OECD (2004). The outcome of this read-across approach to characterise the genotoxicity potential for high molecular weight phthalates is in accordance with the general understanding that chemicals with bulky substituents and high molecular weight are likely to be of lower genotoxic potential than their smaller counterparts because they are less effective in interacting with DNA.

The two phthalates with alkyl carbon backbones of C5-6, DnHP and DIHP, are structurally similar analogues, one with a linear side chain (DnHP) and the other branched (DIHP). Both phthalates have only one *in vitro* study available. DnHP was negative in bacterial mutagenicity tests whereas DIHP was negative in a mouse micronucleus assay. When assessed together, and noting the generally negative genotoxicity profile of phthalates of a similar molecular weight, DnHP and DIHP are considered unlikely to be genotoxic.

For other smaller phthalates, DEP, DMEP and DIBP, that exhibited genotoxic activities in one or more *in vitro* genotoxicity or *in vivo* dominant lethal assays, their genotoxic potential cannot be determined in the absence of definitive *in vivo* genotoxicity data. The genotoxic potential of DCHP, a dicyclohexyl phthalate is also

undetermined due to insufficient available data, with only negative bacterial mutation assays available for assessment.

Overall, apart from DAP, DEP, DMEP, DIBP and DCHP for which their genotoxic potential cannot be determined, the reviewed phthalates are considered to be chemicals with low genotoxic potential.

Table 5. Summary of genotoxicity data

Backbone C Length	**Phthalate**	**Key Study Summary** ***In vitro***	**Key Study Summary** ***In vivo***	**Overall Conclusion**
C1	DMP	Negative in majority bacterial mutation assays (±S9) Positive in sister chromatid exchange assay (+S9) Negative in chromosomal aberrations assays	Negative in sister chromatid exchange assay Negative in dominant lethal assay	Non-genotoxic
C1 para	DMT	Negative in bacterial mutation assays Negative in sister chromatid exchange Negative in micronuclei assay Negative in UDS assays	Negative in micronucleus assay Negative in sex-linked recessive lethal assay	Non-genotoxic
C2	DEP	Negative in majority bacterial mutation assays (±S9) Negative in chromosomal aberrations assays Positive in sister chromatid exchange assay (+S9) Association between human urinary MEP levels and increased DNA damage (using in vitro comet assay) in sperm (sample size = 141)	No data	Insufficient data
C3 (double bond)	DAP	Negative in majority bacterial mutation assays Negative in bacterial microscreen assay Positive in chromosome aberrations and sister chromatid exchange (at the top dose) (+S9) Positive in micronuclei assays (+S9) Positive with dose-related increases in mouse lymphoma assay (±S9)	Equivocal in chromosome aberration assays (Positive at the top dose in one group but negative in another in the same study) Negative in mouse micronucleus tests Negative in sex-linked recessive lethal assays	Mutagenic *in vitro*, with generally negative evidence *in vivo*.
C3	DMEP	No data	Positive in dominant lethal assay (increased resorptions and reduced implantation at top dose)	Insufficient data
C3	DIBP	Negative in bacterial mutation assays Positive in comet (DNA damage) assay using human mucosa	No data	Insufficient data

		(sample size = 70)		
C4	DBP	Negative in majority bacterial and yeast mutation assays Negative in bacterial DNA repair test Negative in chromosomal aberrations and sister chromatid exchange assays Negative in mouse lymphoma assay	Negative in sex-linked gene mutation assay Negative in chromosomal aberration assays	Non-genotoxic
C4, C5	BBP	Negative in bacterial mutation assays Negative in chromosomal aberrations assay Negative in mouse lymphoma assays	Negative in sex-linked recessive lethal Negative in dominant lethal mutation assays Negative in micronuclei assay	Non-genotoxic
C4 (ring)	DCHP	Negative in bacterial mutation assays	No data	Insufficient data
C5	DIHP	Negative in mouse micronuclei assay	No data	Unlikely to be genotoxic
C6	DnHP	Negative in bacterial mutation assays	No data	Unlikely to be genotoxic
C6-rich	DiHepP	Negative in bacterial mutation assays Negative in chromosomal aberrations assay	No data	Non-genotoxic
C6	DEHP	Negative in bacterial and fungi mutation assays Negative in Primary DNA damage, sister chromatid exchange and chromosomal aberrations assays Negative in mammalian mutation assays	Negative in chromosome aberrations and DNA damage assays	Non-genotoxic
C7	DIOP	Negative in majority bacterial mutation assays	No data	Unlikely to be genotoxic
C8	DnOP	Negative in bacterial mutation assays Negative in direct DNA damage assay	No data	Non-genotoxic
C7-9	Di-C7-9 PE	No data	No data	Unlikely to be genotoxic
C8, C9	DINP	Negative in bacterial mutation assays Negative in chromosomal aberrations assay	Negative in cytogenetic assay	Non-genotoxic

		Negative in mouse lymphoma assay Negative in unscheduled DNA synthesis assay		
C9	DNP	No data	No data	Unlikely to be genotoxic
C6-10	610P or Di-C6-10PE	Equivocal in mouse lymphoma assay ± S9 (non-dose related increases)	No data	Unlikely to be genotoxic
C9-11	Di-C9-11 PE	Negative in bacterial mutation assays	No data	Unlikely to be genotoxic
C9, C10	DIDP	Negative in bacterial mutation assays Negative in mouse lymphoma assays	Negative in mouse micronuclei assay	Non-genotoxic
C10	DIUP	No data	No data	Unlikely to be genotoxic
C11	DUP	Negative in bacterial mutation assays Negative in mouse lymphoma assay	No data	Non-genotoxic
C12-rich	DITDP	Negative in bacterial mutation assays	No data	Unlikely to be genotoxic
C13	DTDP	Negative in bacterial mutation assays Negative in chromosomal aberrations assays	No data	Non-genotoxic

Insufficient data - less than 2 *in vitro* tests (a bacterial reverse mutation assay and an in vitro cytogenetic test with mammalian cell culture) available.
Unlikely to be genotoxic - based on data from similar phthalates.

7. REPEAT DOSE TOXICITY AND CARCINOGENICITY

Repeat Dose Toxicity

Most of the 25 phthalates have been evaluated for repeat dose toxicity with the majority of testing conducted via the oral route (Table 6). However, the extent of data and the time periods for testing vary considerably. Across the phthalates, data ranged from extensive eg. DEHP, DBP, DINP to little or no data available eg. DIHP, DIOP, DNP, DIUP and DITDP.

Adverse effects related to repeat dose toxicity were observed for a variety of organs/tissues such as liver, kidney, testes, thymus, pituitary, epididymus, intestines, lungs, stomach, spleen, pancreas and bladder. For the majority of phthalates, toxicity was noted at doses at or above 100 mg/kg bw/d. Di-6-10 alkyl phthalate and DTDP showed the smallest lowest observed adverse effect level (LOAEL) values at 45 and 50 mg/kg bw/d respectively. The most common target organs were the liver and kidney with effects seen in these tissues at the lowest doses. However, the severity of effects in these target organs and the doses at which effects were seen varied for different phthalates.

Particular phthalates were associated with specific effects in organs other than liver and kidney. For example, for the terephthalate DMT, the primary target organ was the bladder with effects related to urinary hyperplasia from the formation of urinary calculi.

Liver Effects

Across all phthalates, liver changes were the most commonly identified effects from repeated exposure. Increases in absolute and/or relative liver weight was a common finding. Overt hepatocellular hypertrophy, hyperplasia, vacuolation and necrosis were reported for some phthalates at high doses. Proliferation of peroxisomes, as evidenced by enzyme or lipid changes or quantitation of peroxisome numbers, was a common but not universal observation. Despite the common understanding of hepatic peroxisome proliferation being a pleiotropic response involving both increases in numbers and sizes of both hepatocytes and peroxisomes, certain phthalates eg. DAP, DCHP showed evidence of hepatocellular hypertrophy and/or hyperplasia without accompanying evidence indicating the proliferation of peroxisomes.

For certain well studied phthalates eg. DEHP, DIDP, DINP, several lines of evidence such as liver weight and enzyme changes, serum lipid changes and/or histopathologic observations clearly indicate peroxisome proliferation as a toxicological effect. These data confirm early comparative studies of the peroxisome proliferating effects of phthalates showing that amongst a range of phthalates tested, these were the most potent (Barber et al., 1987).

Relevance of Liver Effects for Humans

For the 25 phthalates assessed, limited information was available on repeated exposure in humans. For some phthalates, health effects information from surveys of occupational exposures was available, but interpretation of such studies was inevitably complicated by confounding effects of exposure to multiple phthalates or

other non-phthalate chemicals in the workplace and/or poor determination of exposure levels at which effects were noted.

Information on repeat dose effects in primates was available but only for two phthalates, DEHP and DINP. For these phthalates, significant changes were observed in mice and rats for liver organ weights, histopathology and liver enzymes but these effects were not replicated in monkeys. In chronic tests of DEHP in marmoset monkeys and DINP in both marmoset or cynomolgus monkeys, only minor decreases in body weight and body weight gains were noted at high doses. NOAELs of 2500 mg DEHP/kg bw/d and 500 mg DINP/kg bw/d respectively were reported.

Historically, liver enlargement (hepatomegaly), hepatocyte hypertrophy and hyperplasia and proliferation of peroxisomes are recognised effects in rodents from exposure to compounds regarded as peroxisome proliferators. In rodents, peroxisome proliferation has been shown to be mediated through the peroxisome proliferator-activated nuclear receptor alpha (PPARα) (Ward et al., 1998; Klaunig et al., 2003). Using PPARα-null mice, Lapinskas et al. (2005) recently showed that expression of PPARα is necessary for both DEHP and DBP induced liver enlargement and induction of fatty acid metabolising enzymes confirming earlier knockout mouse studies of this receptor (Ward et al., 1998).

The comparative unresponsiveness of the primate liver to peroxisome proliferators is explained on the basis of decreased tissue levels of PPARα, genotypic variations rendering the primate liver receptor less active compared to rodents and species differences in phthalate hydrolysis and production of active phthalate metabolites (Tugwood et al., 1996; Palmer et al., 1998; Woodyatt et al., 1999). On this basis, it is considered that phthalate-induced hepatomegaly in rodents, when related to peroxisome proliferative effects, is unlikely to be a toxicological effect that is relevant for humans.

Kidney Effects

As well as liver effects, kidney effects were reported for a number of phthalates. Kidney changes were the most prominent effect of DMP exposure in rats at high doses. Increases in kidney weight were a common finding often in the absence of direct evidence of renal cellular hypertrophy, hyperplasia or renal peroxisome proliferation. Occasional changes in urinanalysis parameters in the absence of histopathologic changes suggested compromised renal tubular function.

Renal necrotic changes were reported for DAP, DEHP, DINP, DIDP and DTDP. For DINP and DIDP, nephropathy in male rats was attributed to a species-specific accumulation of alpha 2μ-globulin. Retrospective histological studies of kidneys in male rats exposed to DINP noted accumulation of alpha 2μ-globulin in areas of cellular proliferation accompanying renal tubular nephropathy. Alpha 2μ-globulin nephropathy is regarded as a species-specific effect in male rats with no relevance to humans (Caldwell, 1999a).

Similar to liver effects, kidney effects observed in rodents from repeated phthalate exposure were not replicated in primates. For DINP and DEHP, whereas changes in kidney organ weights, urine chemistry and histopathology (including chronic progressive neuropathy) were observed in mice and several species of rats, these

effects were not replicated in studies in marmoset and cynomolgus monkeys. With DEHP in marmoset monkeys and DINP in both marmoset and cynomolgus monkeys, minor decreases in body weight and body weight gains but no specific organ changes were noted at high doses (NOAEL of 2500 mg DEHP/kg bw/d and 500 mg DINP/kg bw/d respectively).

Categorisation and Data Extrapolation

Looking across the repeat dose toxicity data for the 25 phthalates, correlations between backbone length and severity of repeat dose effects or doses at which repeat dose effects were seen were not apparent. Liver and kidney were the most common target organs regardless of backbone length. In general, effects did not occur at doses below 100 mg/kg bw/d.

Insufficient data were available to determine repeat dose toxicity effects for DIHP, DIOP, DNP, DIUP and DITDP. Other than DIHP with a backbone carbon length of C5, all of these could be regarded structurally as high molecular weight phthalates on the basis of backbone lengths of ≥ C7.

Considering single metrics of toxicity such as LOAELs, values ≥ 1000 mg/kg bw/d were reported for higher molecular weight phthalates (eg. DnHP and DUP) as well as low molecular weight phthalates (eg. DEP, DMP). Moreover, another two high molecular weight phthalates, Di-6-10 alkyl phthalate and DTDP were associated with the lowest LOAEL values (45 and 50 mg/kg bw/d respectively). Therefore, for those phthalates currently with insufficient information, the severity of effects expected from repeat doses is difficult to predict. However, liver and kidney effects from repeat doses would be expected, particularly at high doses.

Carcinogenicity

Few phthalates have been tested adequately for carcinogenicity (Table 6). Of those that have been tested in long-term *in vivo* studies (commonly in rodents), the majority show carcinogenic effects with several common tumour types reported. For the most well studied phthalate DEHP, tumour induction was reported in several rodent species. In contrast, for the well studied phthalates DCHP, DMT and DEP subject to dietary studies in rats ranging from 18 months to 2 years, no treatment related tumours were reported.

For those phthalates with adequate data, the doses at which carcinogenic effects were observed varied but generally were similar or higher than doses associated with repeat dose toxic effects in the same species.

In carcinogenicity studies of different phthalates, tumours were reported in a variety of tissues including the urinary tract, forestomach, haematopoietic system, pancreas, testes, kidney and liver. Mononuclear cell leukaemia in rats and hepatocellular adenomas or carcinomas in rats and mice were the most common tumour types. Also, in all studies reporting hepatocellular neoplasms, peroxisome proliferative effects as noted by changes in enzyme activities or serum lipid levels or histopathological observations were reported.

Overall, phthalates that have been adequately tested for genotoxicity showed a negative genotoxic profile. In addition, peroxisome proliferators are generally

considered to be non-genotoxic (Ashby et al., 1994). This suggests that phthalates showing positive results from carcinogenicity studies are unlikely to be genotoxic carcinogens and that a threshold is likely to exist.

There is scientific consensus linking liver tumours in rodents with peroxisome proliferation mediated by the nuclear receptor, proliferator-activated nuclear receptor alpha (PPARα). Peroxisome proliferation is a recognised effect of toxicity in rodents for some phthalates, mediated through PPARα (Ward et al., 1998; Klaunig et al., 2003). Using PPARα-null mice, Lapinskas et al. (2005) recently showed that expression of PPARα is necessary for both DEHP and DBP induced liver effects (hepatomegaly and induction of fatty acid metabolising enzymes) confirming earlier knockout mouse studies (Ward et al., 1998). Moreover, an association between PPARα and liver cancer is demonstrated through knock-out mice studies which showed the inability of the potent peroxisome proliferator and hepatocarcinogen Wy-14,643 to induce either increased cellular proliferation or hepatocarcinogenicity in PPARα-deficient animals (Lee et al., 1995; Peters et al., 1997).

For those phthalates tested for carcinogenicity in primates, neoplasms seen in rodents were not replicated in this species. Klaunig et al. (2003) analysed the relationship between animal bioassays of carcinogenicity mediated through PPARα and their relevance for human carcinogenicity. Data show that there are significant species differences in reactivity to peroxisome proliferators with respect to hepatomegaly, peroxisome proliferation and tumour formation between rodents and primates. This is explained in part by the differences in the expression of PPARα in human versus rodent hepatocytes, species differences in transcriptional accessory proteins and differences in oxidative stress defences (reviewed by O'Brien et al., 2005). Overall, therefore, the mechanisms by which peroxisome proliferators induce liver tumours in rodents are regarded as not relevant for humans.

Mononuclear Cell Leukaemia

Increased incidences of mononuclear cell leukaemia (MCL) were reported in carcinogenicity studies for several phthalates – DAP, BBP, DEHP and DINP. All increased incidences of MCL were reported in F344 rats. MCL was not reported in other species. MCL is regarded as a common neoplasm in aged F344 rats and is rare in other rat strains. This neoplasm has not been found in other mammalian species and has no histologically comparable tumour type in humans (Caldwell, 1999b). Therefore, this tumour type observed in rats is regarded as unlikely to be relevant to humans.

Leydig Cell Tumours

Testicular interstitial (Leydig) cell tumours are reported in animals following repeat exposure to some phthalates. Leydig cells are testicular interstitial cells whose primary function is the production of testosterone. A dose-related increase in the incidence of Leydig cell tumours (as well as hepatic tumours) in Sprague Dawley rats was reported for DEHP (Berger, 1995; Voss et al., 2005). Spontaneous Leydig cell tumours are not common in this rat strain, in contrast to F344 rats (Prentice and Meikle, 1995). A review by Cook et al. (1999) evaluating the relevance of this tumour type to humans noted that the hypothalamo-pituitary-testis axis in rats and humans are similar and so most classes of substances that induce Leydig cell tumours in rats by disruption of this axis may have similar effects in humans. In a recent human biopsy

study, Leydig cell "micronodules" were commonly found in testicular biopsies from men with impaired spermatogenesis and reproductive hormone imbalances, suggesting that alterations in Leydig cells might be of developmental origin (Holm et al., 2003). However, it is suggested that human Leydig cells may be less sensitive than rodents in proliferative responses (Cook et al., 1999).

Categorisation and Data Extrapolation

Looking across the carcinogenicity data for the 25 phthalates, correlations between backbone length and prevalence of carcinogenic effects, or the doses at which these effects were seen, were not apparent. More than half of the 25 phthalates reviewed had not been subject to adequate testing for carcinogenicity and so attempts to correlate carcinogenic potential with structural differences and then to extrapolate on this basis to phthalates with inadequate data is not possible. For these, neoplasms in animals might be expected from long term exposure where significant proliferative effects are observed. Also, the modes of action by which some arise eg. liver tumours, MCL may not be relevant to humans. However, Leydig cell tumours seen in animals may be relevant to humans.

Table 6. Summary of repeat dose toxicity and carcinogenicity

Backbone C Length	Phthalate	Repeat Dose Toxicity	Carcinogenicity
C1	DMP	Rat: LOAEL = 2000 mg/kg bw/d: ↓ body weight gain. NOAEL = 1000 mg/kg bw/d. High doses: nephritis, ↑ liver weight; ↓ liver cholesterol and total lipids; liver damage.	No *in vivo* carcinogenicity studies are available. Negative in *in vitro* cell transformation assay. Negative in *in vivo* Swiss CD-1 mouse (m) initiation/promotion study.
C1 para	DMT	Rat: LOAEL = 636 mg/kg bw/d: ↓ body weight gain. NOAEL = 313 mg/kg bw/d. High doses: bladder calculi and urothelial hyperplasia. Diffuse hepatocellular swelling (one study).	Wistar rat, B6C3F1mouse, 2 year dietary study: no treatment-related carcinogenicity (300 mg/kg bw/d).
C2	DEP	Rat: LOAEL = 3200-3700 mg/kg bw/d; (m-f): ↑ in relative liver, kidney, stomach and small intestine weight (no histopathological abnormalities). NOAEL = 750-770 mg/kg bw/d (m-f).	F344/N rat 2 year dermal study: no treatment-related carcinogenicity; ↓ fibroadenomas of the mammary glands (f). B6C3F1 mouse 2 year dermal study: ↑ hepatocellular ademomas or carcinomas combined (dose-related in males only from 280 mg/kg bw/d). Negative in Swiss CD-1 mouse (m) initiation/promotion study.
C3 (double bond)	DAP	Rat: LOAEL = 100mg/kg bw/d; hepatocellular hypertrophy and renal tubular necrosis (f) NOAEL = 50 mg/kg bw/d (f) High doses: Acute necrotizing colitis, lung discolouration, hepatic cellular hyperplasia, periportal necrosis and fibrosis.	F344 rat 2 year dietary study: LOAEL = 100 mg/kg bw/d: ↑ mononuclear cell leukaemia (MCL) (f). No NOAEL established. B6C3F1 mouse 2 year dietary study: ↑ forestomach papillomas (equivocal causality).
C3	DMEP	Rat: LOAEL = 100 mg/kg bw: ↓ haemoglobin and haematocrit values. No NOAEL assigned. High doses: ↓ thymus and testes weights; testicular atrophy.	No data are available.

C3	DIBP	Rat: LOAEL = 5%: ↓ body and testes weights (m) and ↑ liver weight (m+f). NOAEL = 1% (in diet).	No data are available.
C4	DBP	Rat: LOAEL = 752 mg/kg bw/d: ↑ liver and kidney weight; ↓ hepatocellular lipid deposition; ↑ palmitoyl-CoA oxidase activity and ↓ T3 levels. NOAEL = 152 mg/kg bw/d. Rat (inhalation): NOAEC = 509 mg/m^3: systemic effects. LOAEC = 1.18 mg/m^3, local effects in upper respiratory system. High doses: liver, kidney, testes effects. PP noted.	No *in vivo* carcinogenicity studies are available. Negative in *in vitro* cell transformation assays.
C4, C5	BBP	Rat: LOAEL = 381 mg/kg bw/d (m): ↑ kidney weight, pancreatic islet enlargement with cell vacuolization and peri-islet congestion, liver discolouration and cellular necrosis. NOAEL = 151 mg/kg bw/d (m) Rat (inhalation): LOAEC = 789 mg/m^3: ↑ liver and kidney weights NOAEC = 218 mg/m^3. High doses: testes, epididymis, prostate, liver, kidney, spleen and pancreas effects. PP noted.	F344 rat 2 year dietary study: ↑ MCL; ↑ pancreatic tumours (not with diet restriction). LOAEL = 720 mg/kg bw/d. F344/N rat 2 year dietary study: ↑ pancreatic acinar cell adenoma and adenoma and carcinoma (combined). LOAEL = 500 mg/kg bw/d. B6C3F1 mouse 2 year dietary study: no carcinogenic effects. Positive in 1 of 3 in vitro cell transformation assays.
C4 (ring)	DCHP	Rat LOAEL (7 days) = 500 mg/kg bw/d: ↑ liver weight No NOAEL assigned. High doses: liver and testes effects. No PP noted.	Insufficient data
C5	DIHP	No data are available.	No data are available.
C6	DnHP	Rat: LOAEL = 1824 mg/kg bw/d; hepatocellular necrosis, fat accumulation, loss of glycogen; ↑ liver enzymes. No NOAEL was established.	No data are available.
C6-rich	DiHepP	Rat (2-gen. repro study): LOAEL = 222-750 mg/kg bw/d; ↑ liver, kidney and pituitary weights; centrilobular	F344 rat, B6C3F1 mouse 4 week dietary study: ↑ hepatic DNA synthesis and peroxisomal beta-oxidation; no effect on gap junctional intercellular

		hepatocellular hypertrophy and vacuolation, dilated renal pelves/hydronephrosis and hypertrophy of the pituitary pars distalis. NOAEL = 50-168 mg/kg bw/d. PP noted.	communication.
C6	DEHP	<u>Liver</u> Rat: LOAEL = 146.6 mg/kg bw/d: ↑ liver weight and PP. NOAEL = 28.9 mg/kg bw/d. <u>Kidney</u> Rat: LOAEL = 146.6 mg/kg bw/d:↑ kidney weight. NOAEL = 28.9 mg/kg bw/d. <u>Testes</u> Rat: LOAEL = 37.6 mg/kg bw/d: mild to moderate Sertoli cell vacuolation. NOAEL= 3.7 mg/kg bw/d. High doses: hepatomegaly; PP; testicular tubular atrophy; renal papilla mineralisation, tubule cell pigmentation and chronic progressive nephropathy.	F344 rat 2 year dietary study: LOAEL = 146.6 mg/kg bw/d: ↑ hepatocellular adenomas and carcinomas and ↑ MCL. NOAEL = 28.9 mg/kg bw/d. Sprague-Dawley rat (m) lifetime (~ 3 year) dietary study: ↑ hepatocellular adenomas and carcinomas; ↑ benign Leydig cell tumors. B6C3F1 mouse 2 year dietary study: LOAEL = 292 mg/kg bw/d : ↑ hepatocellular adenomas and carcinomas. NOAEL = 98 mg/kg bw/d. Syrian golden hamster 23 month inhalation study: no sig. ↑ in tumour incidence.
C7	DIOP	Insufficient data.	No *in vivo* carcinogenicity studies are available. Negative in an *in vitro* cell transformation assay.
C8	DnOP	Rat: LOAEL = 350 mg/kg bw/d: ultrastructural changes in liver and thyroid. NOAEL = 37 mg/kg bw/d. High doses: ↑ liver weight, vacuolization and necrosis; ↓ centrilobular glycogen. PP not generally noted.	Rat (species not identified) 15 month dietary study: "numerous" liver nodules – no additional information. Sprague-Dawley rats (m): 26 week dietary study: ↑ gamma-glutamyltransferase-positive foci in liver.
C7-9	Di-C7-9 PE	Rat: LOAEL = 120 mg/kg bw/d:↓ haemoglobin levels and red blood cell counts and ↑ urinary cell excretion. NOAEL = 60 mg/kg bw/d. High doses: Testicular tubular atrophy; hepatic centrilobular degeneration, hepatocyte swelling, fatty vacuolation. PP	No data are available.

		noted.	
C8, C9	DINP	Rat: LOAEL = 358-442 (m-f) mg/kg bw/d; ↑ liver weight, liver histopathological changes, serum enzyme changes. NOAEL = 88-108 mg/kg bw/d (m-f). High doses: Liver enlargement and necrosis, renal tubular necrosis (alpha 2 μ -globulin related?). PP noted.	F344 rat 2 year dietary study: LOAEL = 358 mg/kg bw/d (m): ↑ MCL, hepatocellular neoplasia, renal tubule cell carcinomas. NOAEL = 88 mg/kg bw/d (m) B6C3F1 mouse 2 year dietary study: LOAEL = 335 mg/kg bw/d (f): ↑ hepatocellular adenomas and carcinomas. NOAEL = 112 mg/kg bw/d (f) Positive in 1 of 7 *in vitro* cell tranformation assays.
C9	DNP	Insufficient data.	No data are available.
C6-10	Di-C6-10 PE	Rat: LOAEL (2-gen. repro study) = 45 mg/kg bw/d: ↑ liver and kidney weights (F1 f). No NOAEL was established. High doses: pale livers with lobulation and discolouration, slight cell necrosis (m). PP noted.	No *in vivo* carcinogenicity studies are available. Negative in an *in vitro* cell transformation assay.
C9-11	Di-C9-11 PE	Rat: LOAEL (2-gen. repro study) = 1000 mg/kg bw/d: ↑ liver weight in young rats, liver histopathological changes and ↓ body weight in mature rats, ↑ in palmitoyl CoA oxidase activity. NOAEL = 500 mg/kg bw/d.	No data are available.
C9, C10	DIDP	Rats: LOAEL = 120 mg/kg bw/d (f): ↑ liver weight. NOAEL = 60 mg/kg bw/d. High doses: ↑ liver and kidney weight; nephropathy (m) (alpha 2 μ -globulin related?) PP noted.	No *in vivo* carcinogenicity studies are available. Positive in 1 of 2 *in vitro* cell transformation assays.
C10	DIUP	No data are available	No *in vivo* carcinogenicity studies are available.

			Negative in an *in vitro* cell transformation assay.
C11	DUP	Rat: LOAEL = 1145 mg/kg bw/d: ↑ liver weight. NOAEL = 282 mg/kg bw/d. High doses: ↑ liver and kidney weights. PP noted.	No data are available
C12-rich	DITDP	No data are available.	No data are available.
C13	DTDP	Rats: LOAEL = 50 mg/kg bw/d, ↑ liver weight, hypertrophy of centrilobular hepatocytes, ↓ body weight gain. NOAEL = 10 mg/kg bw/d. High doses: liver and kidney effects. PP not noted.	No data are available

f – female; m - male; PP – peroxisome proliferation

8. REPRODUCTIVE AND DEVELOPMENTAL TOXICITY

Reproductive Toxicity

Historically, health impacts associated with phthalates have been linked most strongly to reproductive and developmental toxicity. Initial animal studies of phthalates revealed reproductive effects in the adult male (eg. decreased testes weight and reduce sperm production in rats) at relatively high doses (> 250 mg/kg bw/d). However, more recently, effects on the male reproductive system have been shown at lower doses following gestational exposure to some phthalates.

Traditional hazard assessments consider reproductive toxicity separate from developmental toxicity. Reproductive toxicity is tested by exposing sexually mature adults to a chemical and examining the effects on reproductive capacity. Developmental toxicity is studied by exposing pregnant dams and looking for effects on the foetuses. However, neither of these tests generally detect effects that only appear postnatally. Thus, chemicals that affect the developing reproductive system following prenatal exposure may also affect sexual maturation or induce functional reproductive disorders that are only apparent at maturity. In this review of reproductive and developmental endpoints, effects of phthalates on the adult will be reviewed separately from effects induced following prenatal exposure.

Reproductive effects induced in adulthood

Animal studies

Having reviewed toxicity data for 25 phthalates, it is apparent that although not all phthalates have been studied to the same extent, some phthalates induce a similar pattern of effects in adult test animals (Table 7). The most sensitive reproductive endpoint in animals is effects on the male such as decreased fertility and testes weight as well as effects on male accessory organs. At the histological level, seminiferous tubule atrophy and sloughing of cells into the tubule lumen have been frequently reported. For example, mild vacuolations in the Sertoli cells were observed in male rats exposed to 500 ppm DEHP whereas at higher doses (5000 ppm) mild to moderate seminiferous tubule atrophy and decreased testes weight were noted (Poon et al., 1997).

Male reproductive toxicity is mainly associated with phthalates where the backbone is 4-6 carbon atoms in length and not with phthalates with shorter or longer chains. BBP, DBP, DIBP, DnHP, DiHepP, and DEHP all demonstrated effects on male reproductive organs most notably, decreased testes weight. This observation of reproductive effects associated with C4-6 backbone lengths has been noted by others in comparative studies eg. Foster et al. (1980), Oishi and Hiraga, (1980a,b), Lamb et al. (1987), Heindel et al. (1989) and in reviews such as by Phthalate Esters Panel HPV Testing Group (2001). In this latter review, phthalates with backbones consisting of 4-6 carbons are categorised as transitional phthalates, in contrast with low (≤ C3) and high (≥ C7) molecular weight phthalates. In comparative studies, a number of different phthalates of varying lengths were tested for effects on fertility and/or testes weight. Potency, as measured by the lowest dose affecting the number of fertile pairs, increased as the length of the side chain increased from 3 to 6 (Heindel et al., 1989; Lamb et al., 1987). Phthalates with backbones of 8 carbons (DnOP) or 2 carbons

(DEP) had no effect on the most sensitive reproductive endpoints. In other studies, rats were gavaged for 4-7 days with a range of phthalates (Foster et al., 1980; Oishi & Hiraga, 1980). Relative testis weights (and testicular zinc content) were reduced for the phthalates with 4-6 carbon backbones (DBP, DIBP, DPentP, DHexP, DEHP), but not for those with backbones of 1-3 (DMP, DEP, DnPropP) or ≥ 7 (DnHepP, DnOP).

However, it is important to note that a backbone length of C4-6 is not a clearcut determinant of testicular toxicity. For example, in a comparative toxicity study of C2-4 isomers of monobutyl phthalate (MBP), isomers with backbone lengths of C3 (-*iso*-butyl and –*sec*-butyl phthalate) and C4 (-*n*-butyl phthalate) all induced testicular atrophy and altered zinc metabolism in rats, but –*tert*-butyl phthalate (C2) did not (Foster et al., 1981). In addition to these comparative studies, in a two-generation reproductive study of the low molecular weight phthalate DEP, there was no effect on male reproductive organ weights up to doses of 1016-1375 mg/kg bw/d (male-female) in Sprague Dawley rats (Fujii et al., 2005). However, there was an increased frequency of abnormal and tailless sperm in the F1 generation that did not affect fertility.

Within the transitional phthalate group (C4-6), there appears to be species differences with respect to sensitivity to testicular toxicity (Gray et al., 1982). With DBP, rats and guinea pigs were most responsive, mice were relatively resistant and hamsters were insensitive. Similar results were reported for DEHP. Urinary metabolic profiles of rats and hamsters suggested that differences in susceptibility to testicular toxicity from DBP may be due to differences in metabolism (Foster et al., 1982a). In a non-rodent study, DEHP was also shown to induce testicular effects in the ferret (Lake et al., 1976). In contrast, studies in primates report an insensitivity to DEHP-induced testicular toxicity.

Studies have generally indicated that high molecular weight phthalates are unlikely to be male reproductive toxins. None of the high molecular weight phthalates reviewed (Di-C7-9, DnOP, Di-C9-11, DIDP, DTDP) affected fertility or other aspects of the male reproductive system. Only for DINP were effects on the testes observed. However, these were equivocal with increased testes weight observed in repeat dose toxicity studies and in the F0 generation of a two-generation study in rats but decreased testes weight observed in the F1 generation and in repeat dose studies in mice. DINP is a complex mixture of branched isomers and one author ascribed the effects seen with DINP in a developmental toxicity study as likely to be due to the presence of low amounts of lower molecular weight phthalates (Gray et al., 2000). Overall, it can be concluded that the high molecular weight phthalates are unlikely to affect fertility. Therefore, although there are no data for DNP, DUP, DIUP and DITDP, it is expected that these high molecular weight phthalates would not induce reprotoxic effects in adults.

For the low molecular weight phthalates, DMP, DMT and DIBP, male reproductive effects were observed only at high doses. DMT is structurally distinct from other phthalates reviewed in possessing a *para* configuration of ester side chains, in contrast to the *ortho* configuration of the other phthalates. With DEP with a C2 backbone, effects on testosterone levels and sperm were observed at 197 mg/kg bw/d. However, DEP did not affect testes weight, a common effect of transitional phthalates.

The phthalates DAP, DMEP and DCHP do not possess simple straight or branched carbon backbones so it is not possible to extrapolate effects based on the above observations. DMEP was associated with decreased testes weight in a number of limited studies. DAP had no effect on testes weight in a combined reproductive/developmental toxicity study at doses that induced maternal lethality. DCHP induced testicular atrophy in rats gavaged with 4.2 g/kg bw/d but not lower doses (Grasso, 1978).

Human studies

A number of studies have attempted to correlate phthalate monoesters in urine with semen quality (Duty et al., 2003; 2004; Jonsson et al., 2005). Effects on semen parameters have been reported to be associated with some phthalates. However, methodological problems in these studies such as the use of a subfertile population, single spot urine sampling and/or the presence of other phthalates make it difficult to conclude that effects on semen quality are related only to exposure to a particular phthalate. In an *in vitro* study, human sperm suspensions were incubated with a range of phthalates for up to 18 hours (Fredricsson et al., 1993). Sperm motility was most affected by DEHP and DBP but at least one other motion variable was affected by each of the phthalates tested including DMP and DEP, not thought to be reproductive toxicants.

Pan et al (2006) studied gonadotropin and gonadal hormone levels of 74 male workers exposed to high levels of DBP and DEHP. Urinary levels of the metabolites MBP and monoethylhexyl phthalate (MEHP) (normalized to creatinine) were significantly higher in exposed workers compared with controls. Free testosterone was significantly lower in exposed workers and was negatively correlated with MBP and MEHP.

Mode of action

The mode of action of phthalates on the male reproductive system has been most extensively studied in rats using the transitional phthalates DEHP, DBP and BBP. Given the similarity of effects induced by the transitional phthalates, it is reasonable to assume that the mode of action would be similar between phthalates of this group. Results from both *in vivo* and *in vitro* studies have indicated that the Sertoli cell is the main target for DEHP-induced testicular toxicity and that the monoester metabolite is the active testicular toxicant (Gray and Beamand, 1984; Gray and Gangolli, 1986; Sjoberg et al, 1986; Chapin et al., 1988). However, effects on Leydig cells have also been reported (Jones et al. 1993), albeit at phthalate monoester dose levels orders of magnitude higher than those affecting Sertoli cell function (Foster, 2005). Leydig cells may be the initial testicular target in foetal animals (reviewed in Foster, 2005; 2006; David, 2006).

The function of the Sertoli cell in adult life is to support spermatogenesis. The number of Sertoli cells determines the number of germ cells that can be supported, sperm number and testes size (reviewed in Sharpe et al., 2003). The final number of Sertoli cells in the adult is determined during the time of Sertoli cell proliferation. This occurs during foetal or neonatal life but more importantly, in the peripubertal period. In the rat, both stages occur by day 30 (mainly during weaning) while in the human the periods occur before 12-18 months then later, at 10-13 years. At puberty, Sertoli cells become functionally mature and are non-proliferative. Tight junctions form

between cells effectively sealing off the developing germ cells from direct access to the blood stream (around post-natal day (PND) 10-20).

Studies show that the earliest changes after treatment with dipentyl phthalate (DpentP) or DEHP were vacuolation of the Sertoli cell followed by degenerative changes in ultrastructure of Sertoli cells, spermatocytes, and spermatids (Gangoli, 1982; Foster et al, 1982b; Creasy et al., 1983). Thus, the germ cells changes may be secondary to effects on the Sertoli cells (Creasy et al., 1987; Richburg & Boekelheide, 1986*). This leads to decreases in seminiferous tubule diameter and ultimately testes weight.

Some phthalates (eg. DEHP and DBP) show an age dependency for induction of testicular toxicity with neonatal animals more sensitive than pubertal animals which are in turn more sensitive than adult animals for a given dose. This coincides with Sertoli cell maturation and the development of the blood-testis barrier. Younger animals responded to a much lower dose or produced a more serious lesion with comparable doses on a mg/kg bw/d basis.

One study with DEHP also demonstrated that the number of Sertoli cell nuclei per tubule were reduced in neonatal rats with 2-3 week old rats showing loss of spermatocytes but not of Sertoli cells (Dostal et al., 1988). In 6 and 12-week old rats, spermatids and spermatocytes were lost. These results demonstrated that Sertoli cells were most sensitive during their proliferative stage.

Leydig cells secrete testosterone and in postnatal and adult rats are also affected by some phthalates. However this effect may be secondary to tubular damage. DEHP, BBP and DBP induced decreased serum testosterone levels in adult rats and mice but other results were inconsistent. DEHP administered to mice significantly reduced the concentration of testosterone and zinc in the testis, but no testicular atrophy was observed (Oishi and Hiraga 1980a). However, oral administration of DEHP to 5-week-old rats increased the concentration of testosterone in the testis and reduced that in serum (Oishi and Hiraga 1980b). Testosterone in testes was also significantly reduced in rats following oral exposure to DEP and DMP but increased in rats fed diets containing DBP or DIBP (Oishi & Hiraga, 1980a). However serum testosterone was also decreased for BBP and DBP (Agarwal et al., 1985; Piersma et al., 1999). Decreased testosterone levels may explain the decreased weights of accessory sex organs (seminal vesicle and prostate) in male rats.

There is limited evidence to support the induction of a postnatal compensatory mechanism as short-term exposure to DEHP led to decreases in testosterone levels while longer exposures led to increases in testosterone with accompanying Leydig cell proliferation (Akingbemi et al., 2001; 2004). Although others note delays in male puberty through DEHP-induced inhibition of testis testosterone production and lower serum testosterone levels in rodents (Gray et al., 2006), a compensatory mechanism from long-term exposure has not been widely described.

A number of mechanisms have been proposed to explain the effects on the testes, including loss of zinc from critical cellular targets and inhibition of FSH responsiveness and participation of the Fas signalling system in the initiation of germ cell apoptosis (Foster, 2005). There are experimental data to support these hypotheses but the mechanism of action has not yet been fully elucidated.

Developmental Effects

There are at least some developmental toxicity data for the majority (19/25) of the assessed phthalates (summarised in Table 7). The data are derived from 2 generation studies and prenatal developmental toxicity studies which terminate at term or later in the offspring's life. It is clear that different phthalates induce different effects (Table 7). Observed developmental effects can be grouped into male reproductive, skeletal variations and lactational effects. Some phthalates induce all three types of effects. For these phthalates, reproductive effects are more sensitive endpoints than skeletal or lactation effects which are induced at higher doses. In contrast, some phthalates induce no developmental effects.

Developmental effects of phthalates have been recently extensively reviewed by Corton & Lapinskas (2005), Foster (2005; 2006) and David (2006). Several authors, including Gray et al., (2000) and Phthalate Esters Panel HPV Testing Group (2001), have suggested grouping phthalates on the basis of developmental effects.

All of the different developmental effects noted above have been reported following exposure to transitional phthalates. Prenatal exposure to transitional phthalates induces an array of malformations, variations and developmental effects. These include decreased pup weight at birth and through weaning, skeletal variations particularly increased frequency in lumbar ribs, malformations of the male reproductive system notably hypospadias and feminisation of male sexual differentiation as typified by decreased anogenital distance (AGD), delayed preputial separation and retained thoracic nipples. Reproductive effects in the developing male pup appear to be the most sensitive developmental endpoint. For example, oral exposure to BBP induced a significant increase in the frequency of supernumerary lumbar ribs at doses at and above 1000 mg/kg be/d whereas AGD was significantly reduced from 750 mg/kg bw/d. Other effects included decreased testes weight.

In the cross-phthalate studies described earlier (Lamb et al., 1987; Heindel et al., 1989), 7 phthalates (DEP, di-n-propyl phthalate (DnPropP), DBP, DPentP, DnHP, DEHP, DnOP) were tested using a continuous breeding protocol and cross-over trial in CD-1 mice at three different doses in feed. None of the dams treated at the highest doses with DEHP, DnHP, DPentP or DPropP produced a viable litter in the cross-over study with untreated males. DPentP or DPropP have not been reviewed by NICNAS but are C4-5 phthalates. DBP markedly reduced fecundity. There was no effect on litter size, pup viability or weight for DEP or DnOP.

A further cross-phthalate study examined the effects of different phthalates on sexual differentiation (Gray et al., 2000). In this study, rats were gavaged with BBP, DEHP, DINP, DEP or DMP at a single dose level from gestation day (GD) 14 to PND 3. There was no effect on litter size but decreased AGD and retained nipples as well as male reproductive tract malformations were induced by DEHP, BBP and DINP but not with low molecular weight phthalates DEP or DMP. In a more restrictive study, gavage with DBP, DEHP, BBP or DPentP but not DEP or DMP during GD 12-19 induced decreased AGD distance (Liu et al., 2005). These cross-phthalate studies indicate that effects on male reproductive development are largely restricted to the transitional phthalates of 4-6 carbon backbones.

More recent transgenerational studies on transitional phthalates have been able to detect more subtle effects of the developing male reproductive system, such as effects on AGD, time of preputial separation and retention of thoracic nipples in the male. These effects were induced at lower doses than other reproductive effects. As a whole, these phthalates at similar doses can also induce minor maternal effects such as increased liver or kidney weights in the absence of effects on food intake or maternal weight gain.

The biological significance of these developmental effects needs to be considered. Carney et al (2004) examined the effect of feed restriction (FR) from GD 7, through gestation and lactation until weaning on CD rats for a number of developmental toxicity end points. Food was restricted by 10, 30 or 50% of the control group. They found that pup weight and AGD was decreased on PND 4 in both males and females of the 50% feed restricted group only but the ratios of AGD to body weight were nearly identical across groups. This suggests that the changes in absolute AGD were merely due to a smaller size of the pups. This finding points to the critical importance of normalizing AGD to a measure of body size in order to avoid ‘‘false positives’’ due to reduced pup size. In this feed restriction study, normalization to absolute body weight on PND 4 appeared to sufficiently correct for changes in body size, even when differences in pup body weight were large.

Based on the individual hazard assessments for BBP, DBP, DEHP, DCHP, and DiHepP and reviews by others of DPenP and DnHepP, the transitional phthalates have all been shown to induce decreased AGD in prenatally exposed male pups only. Many of the studies included normalisation for pup body weight or used body weight as a covariate. If the endpoint was an indirect function of reduced pup weight (which was a feature of some phthalates at high doses), then it might be expected that AGD might also be reduced in female pups as occurred in the feed restriction study (Carney et al., 2004). This was not the case. Also, this endpoint, decreased AGD relative to body weight, commonly remained statistically significant in these studies. Moreover, long-term follow-up studies showed that DBP-induced changes in AGD were permanent, indicating an adverse developmental response and further underlining AGD as a biologically significant endpoint for these phthalates.

Puberty onset is influenced by sex steroid hormones, body weight, body composition, and other general factors. The feed restriction study described earlier (Carney et al., 2004) demonstrated that puberty onset was greatly dependent on body weight. Feed restriction in which body weights at puberty onset (preputial separation or vaginal opening) were reduced slightly (<3% in females; <11% in males) resulted in a slight delay (1.2 days) of mean age of puberty onset. However, when body weight at puberty onset was decreased by 15% (females) or 23% (males), mean age of puberty onset was delayed by approximately 6 days (males and females). Studies by other workers (Stoker et al., 2000; Marty et al., 2003) suggest that body weight reductions of approximately 10–15% at the time of puberty onset can result in several days delay in mean age of puberty onset. None of the transitional phthalates that induced preputial separation (DBP, BBP, DiHepP, DEHP) induced body weight reductions sufficient enough to question the validity of the observed delay in timing of preputial separation.

Malformations of the male reproductive system have been reported following gestational exposure to the transitional phthalates, DBP, BBP, DEHP and to a lesser

extent, DINP. Malformations include hypospadias, agenesis of accessory organs and cryptorchidism (Gray et al., 2000; Mylchreest et al., 2000; Barlow et al., 2004; Tyl et al., 2004). Malformations were generally induced at higher doses compared to other effects. For example, for DBP, the LOAEL for nipple retention was 100 mg/kg bw/d but malformations were only observed at 500 mg/kg bw/d (Mylchreest et al., 2000; Barlow et al., 2004;). Similar observations have been made for BBP (Tyl et al., 2004) and DEHP (Andrade et al., 2006) although in the latter case, decreased AGD (BBP) and delayed preputial separation (DEHP) were the more sensitive endpoints of male sexual differentiation.

The testes were also targeted when exposure to different phthalates occurred during gestation and early postnatal life. Within the transitional phthalates, testicular atrophy and/or decreased testis weight was induced in two-generation and gestational studies. There is some evidence that exposure that begins during prenatal life requires lower doses to induce testicular effects. For example, in a two-generation study with BBP, testes weight was reduced in F1 but not F0 at 750 mg/kg bw/d (Nagao et al., 2000; Tyl et al., 2004; Aso et al., 2005). Similar effects were also noted for DiHepP (McKee et al., 2006), DBP (Wolf et al., 1999) and DEHP (Wolfe & Layton, 2003).

For a number of phthalates, an increased frequency of skeletal variations, particularly short supernumerary lumbar ribs, was noted following gestational exposure at higher doses. For example, there was an increased frequency of supernumerary ribs in rats following prenatal exposure to 1000 mg BBP /kg bw/d (NOAEL was 250 mg/kg bw/d, Uriu-Adams et al., 2001) but decreased AGD was induced at 100 mg/kg bw/d (Aso et al., 2005). Increased frequency of skeletal variations was the most sensitive endpoint for one low molecular weight phthalate (DEP) and four high molecular weight phthalates (DIDP, DINP, Di-C7-9 PE, Di-C9-11 PE).

The presence of short supernumerary ribs (cervical and lumbar) is one of the more common anomalies seen in developmental studies in laboratory rats, mice and rabbits. Both variations are more common in mice, with lumbar ribs more common than cervical ribs. The incidence of supernumerary lumbar ribs in rat strains range from less than 1% to 28% among control animals but most report an incidence of less than 10% (reviewed by Chernoff and Rogers, 2004). In mice, the range is 3-32% but can range up to 62%. Lumbar ribs have been induced by a variety of chemical agents with and without accompanying maternal toxicity. Cervical ribs are more rarely induced by chemicals.

Other factors such as maternal stress in mice, but not rats, can lead to increased frequency of lumbar ribs perhaps reflecting the greater susceptibility to the anomaly in mice (Chernoff and Rogers, 2004). Maternal feed restriction from GD 6-17 in Sprague Dawley rats resulting in a 63% decrease in dam weight did not increase the frequency of supernumerary ribs (Fleeman et al., 2005). A number of studies where a high incidence of short lumbar ribs was chemically induced in rats and mice have shown a postnatal disappearance by PND 40 (Wickramaratne, 1988*; Chernoff et al., 1991*; Rogers et al., 1991*; Foulon et al., 2000*). Chernoff and Rogers (2004) hypothesised that the short lumbar rib had become part of the lateral vertebral processes. A similar phenomenon, with respect to cervical ribs, is known to occur in humans (Bagnall et al., 1984*; McNally et al., 1990*; Schumacher et al., 1992*). The

critical period of supernumerary rib formation is GD 7-8 in the mouse and GD 8-9 in the rat.

The high molecular weight phthalates appear generally not to induce developmental effects. There were no effects on sexual differentiation and/or AGD following prenatal exposure to the high molecular weight phthalates, Di-C7-9 PE, Di-C9-11 PE or DIDP. However, an increased frequency of skeletal variations, particularly short supernumerary lumbar ribs, was induced following gestational exposure to DIDP, DINP, Di-C7-9 PE, Di-C9-11 PE. For DIDP, Di-C7-9 PE and Di-C9-11 PE, there was increased frequency of skeletal variations at and above 1000 mg/kg bw/d following gestational oral exposure on GD 1-19 (Fulcher et al., 2001; Waterman et al., 1999; Hellwig et al., 1997). Because the incidence of supernumerary ribs observed in these studies was outside the historical control range of their respective laboratories, they were interpreted as indicative of slight developmental effects. However, as mentioned above, these effects are common anomalies seen in developmental studies. DINP is a high molecular weight phthalate and a complex mixture that weakly induced effects in male pups including testicular atrophy, retained thoracic nipples and male reproductive system malformations (Gray et al., 2000). The authors of this study surmised that the effects were due to the presence of low amounts of lower molecular weight phthalates.

Of the low molecular weight phthalates, DMP and DEP appear not to induce developmental effects. Prenatal exposure to DEP or DMP at high doses (~ 3200 mg/kg bw/d) had no effect on embryo/foetal development in a number of studies (Plasterer et al., 1985; Hardin et al., 1987; Field et al., 1993; Gray et al., 2000). There was no effect on sexual differentiation and/or AGD following prenatal exposure to DEP or DMP (Gray et al., 2000). However, in one well-described two-generation reproduction toxicity study, rats were fed diets up to 1016-1375 mg/kg bw/d DEP (Fujii et al., 2005). The main effect, reduced pup weight at weaning and minor developmental delay in the high dose group, could be attributed to lactational rather than gestational effects. However, dam weight was unaffected during this period; in fact dam body weight gain was significantly increased during lactation in the high dose group. There was an increased frequency of skeletal variations, primarily rudimentary extra lumbar ribs but no clear dose response and it is unknown whether the frequency fell within the historical control range.

In contrast to DMP and DEP, studies with the low molecular weight phthalate DIBP revealed some developmental effects. At less than maternally toxic doses in rats, DIBP decreased foetal weight and increased the incidence of undescended testes (Saillenfait et al., 2006). Also, in male foetuses at term, DIBP decreased testicular testosterone production *ex vivo* and testosterone levels in testes and plasma, decreased AGD and induced pathological changes in the testes including clustering of small Leydig cells and vacuolisation of Sertoli cells (Borch et al., 2005). These data concur with testicular effects observed with C3 isomers of methylisobutyl phthalate (MIBP) (Foster et al., 1981) and indicate that low molecular weight phthalates approaching the C4 carbon backbone content of transitional phthalates are likely also to display developmental effects.

The low molecular weight phthalates DAP and DMEP do not possess side chains that are simple linear or branched carbon structures. Therefore, it is not possible to infer

for these phthalates a similar toxicity profile to DMP and DEP. There are no developmental studies following oral or inhalation administration of DMEP. The metabolites of DMEP, 2-ME (2-methoxyethanol) and methoxyacetic acid (MAA) have been evaluated. 2-ME (also referred to as ethylene glycol monomethyl ether) is an important industrial solvent (Lanigan et al., 1999). Both induce malformations, principally skeletal, in developmental studies. Skeletal effects were not fully described but included mainly limb malformations. Increased frequency of supernumerary ribs was not described. These metabolites are not structurally similar to any of the straight-chain phthalate metabolites and therefore may not have a common site of action. DAP had no effect on offspring viability, growth and development from conception to early lactation in a combined reproductive/developmental toxicity study up to 150 mg/kg bw/d, although effects on new-borns and live new-borns were not evaluated in the 3 of 10 dams that died or were sacrificed at this dose. Neither DMEP nor DAP has been tested in transgenerational reproductive toxicity tests.

The remaining low molecular weight phthalate DMT possesses a *para* configuration of ester side chains, in contrast to the *ortho* configuration of all other phthalates reviewed. Although a low molecular weight phthalate on the basis of backbone carbon length, structural differences limit comparisons to the other low molecular weight phthalates reviewed. Limited studies indicate mild developmental effects with reduced pup weights observed at weaning in a 1-generation reproduction toxicity study (Krasavage et al., 1973).

Overall, the transitional phthalates induce a recognisable pattern of malformations including decreased AGD, delayed preputial separation and retained thoracic nipples in male pups. At higher doses, hypospadias and cryptorchidism are induced as well as increased frequency of supernumerary ribs. The finding of similar responses to prenatal exposure to the transitional phthalates suggests a common mode of action. In contrast, the high molecular weight phthalates have no significant effect on male sexual differentiation and, for these, skeletal variations were the most sensitive endpoint. However, the finding of supernumerary ribs is considered a minor and potentially reversible effect in the absence of other signs of developmental toxicity. For low molecular weight phthalates, the findings are less consistent, with developmental effects associated with some but not others. No developmental effects are observed with the lowest molecular weight phthalates with simple linear sidechains (DMP, DEP).

Therefore, overall, the reproductive/developmental toxicity profile for poorly tested phthalates is likely to be generally predictable on the basis of these backbone length categorizations ie. ≤ C3; C4-6; ≥ C7. That said, current data for one low molecular weight phthalate (DIBP) indicate that backbone lengths (C3) approaching those of the transitional phthalates (C4-6) may also show some developmental effects similar to the transitional phthalates.

Mode of action

It has been hypothesised that transitional phthalates produce antiandrogen effects by inhibiting foetal testosterone production which in turn lowers testicular and whole body testosterone to female levels (David, 2006; Foster, 2006). The transitional phthalates appear to induce antiandrogen effects but differ from known androgen

receptor antagonists such as flutamide, vinlozolin and procymidone in the type of effects induced. These chemicals induce hypospadias but have less effect on testosterone-dependent tissues such as the testis, whereas DEHP and DBP have both been shown to reduce foetal testosterone *in vivo*. Neither phthalate nor their monoester metabolites acted as androgen receptor ligands *in vitro*. Thus, while the phthalate diesters and monoesters appear to have antiandrogenic properties, they do not appear to interact with the rat or human androgen receptor (Mylchreest et al., 1999; Parks et al., 2000; Foster et al., 2001; McKee et al., 2004; Takeuchi et al., 2005).

The critical period for reproductive development in the rat is GD 12-20. Sertoli cells secrete anti-mullerian hormone (AMH) that promotes the regression of ducts that would later form the female reproductive system. Leydig cells secrete two hormones, testosterone and insulin-like factor 3 (insl3). Testosterone promotes the development of androgen-dependent tissues such as the vas deferens, epididymis and seminal vesicles and may also play a role in Sertoli cell proliferation. Insl3 is important in testicular descent. At other sites within the developing foetus, testosterone is converted to the more potent 5a-dihydrotestosterone (DHT). DHT induces the development of the prostate and external genitalia and is responsible for the growth of the perineum to produce the larger normal male AGD and induces apoptosis in male nipple anlagen. Interference with Leydig cells would thus explain the main effects of transitional phthalates on the developing male foetus.

There is some experimental evidence to support the hypothesis that transitional phthalates produce antiandrogen effects by inhibiting foetal testosterone production and insl3. Gestational exposure to BBP, DBP or DEHP induced a decrease in expression of insl3 in rat foetal testes (Wilson et al., 2004) perhaps explaining the increased incidence of cryptorchidism. Serum testosterone was reduced following gestational treatment with BBP (Nagao et al., 2000), DEHP (Parks et al., 2000) or DBP (Mylchreest et al., 2002; Lehmann et al., 2004). In histological evaluations of testes, Leydig cells were hyperplastic or found in clusters following prenatal exposure to BBP (Aso et al., 2005), DBP (Lee et al., 2004; Mylchreest et al., 2002), DEHP (Shirota et al., 2005) or DIBP (Borch et al., 2006). Sertoli cell proliferation was decreased following prenatal exposure to DEHP (Li et al., 2000). The seminiferous tubules of animals exposed to DBP and DEHP were also abnormal with multinucleated gonocytes appearing among the germ cells (Parks et al., 2000; Mylchreest et al., 2002; Barlow & Foster, 2003; Andrade et al., 2006; Borch et al., 2006; Grande et al., 2006). This was followed by decreased numbers of spermatogonia and spermatocytes (Barlow & Foster, 2003). The effect on the Sertoli cells may be a direct or an indirect consequence of decreased testosterone levels. Thus, the cellular target of the transitional phthalates may be both Leydig and Sertoli cells. Inhibition of the cellular function of the Leydig cell may perturb testosterone and insl3 synthesis resulting in disturbances in the normal development of the male reproductive tract whilst interference with Sertoli cells may result in failure to proliferate with subsequently depleted germ cells.

Human data

A number of studies have attempted to correlate maternal phthalate monoester concentrations in urine or breast milk with indicators of abnormal male development (Main et al., 2006; Swan et al., 2005). No association was found between phthalate

monoester levels in maternal breast milk and cryptorchidism but correlations were found between monoethyl phthalate (MEP), MBP and monomethyl phthalate (MMP) (but not MEHP or MiBP) levels in breast milk and reproductive hormone levels in 3 month old infants (Main et al., 2006). Urinary concentrations of MEP, MBP and MBzP were significantly inversely related to anogenital index (AGI) (Swan et al., 2005). There was no correlation between AGI and MEHP, MiBP and MMP. This study has been criticised by the Cosmetic and Fragrance Associations of America and Europe (McEwen and Renner 2006). These authors suggested that AGD is more likely to be proportional to height rather than weight (used to calculate AGI) and that maternal phthalate urinary concentrations were not normalized for urine volume. The reliability of the measurement of AGD has not been verified in humans. The one study that did assess the reliability of AGD measures found that the correlation of AGD with body weight was 0.48 in males and that body length may be a better predictor of AGD than weight (Salazar-Martinez et al., 2004). Therefore, currently, the human data are insufficient to determine a causal association between phthalate exposure (on an individual or group basis) and developmental effects.

Table 7. Summary of reproductive and developmental effects

Backbone C length	Phthalate	LOAEL (mg/kg bw/d) & endpoint	
		Fertility[1]	**Development**
C1	DMP	> 2000	> 3570
C1 para	DMT	> 636	313: ↓ pup weight at weaning
C2	DEP	Rat: 197-267 (m-f): ↓ serum testosterone levels, ↑ frequency of abnormal and tailless sperm	*Two generation study* Rat: Maternal: 1016-1375 (m-f): ↑ rel liver wt (F_0, F_1), ↑ rel kidney wt (F_1), Developmental: 1016-1375 (m-f): ↓ pup weight at PND 21 (F_1 males, F_2) and PND 4-21 (F_1 females), delayed age of onset of vaginal opening (F_1). *Developmental study* Rat: Maternal: 1910(m-f): ↓ body weight Developmental: 3200(m-f): ↑ skeletal variations (supernumerary ribs)
C3 (double bond)	DAP	Rat: 150	Insufficient data
C3	DMEP	1000: ↓ testes weight, testes atrophy	291 (intraperitoneal) ↑ skeletal & visceral variations
C3	DIBP	2000: ↓ testes weight, histology	*Developmental study* Maternal: 500 Developmental: 500: ↓ pup weight and ↑ trans-abdominal testes migration

C4	DBP	509-794 (m-f): ↓ epididymis weight (F_0)	*Two generation study* 256-385 (m-f) ↓ F_1 sperm counts ↑ F_1 testes atrophy *Gestation study* 100: seminiferous tubule atrophy, retained nipples
C4, C5	BBP	400: atrophy of testis, seminal vesicle and epididymis	*Two generation study* 100: ↓ body weight and AGD
C4 (ring)	DCHP	Rat: 80-107 (m-f): ↓ sperm head counts and focal seminiferous tubule atrophy in F_1 males.	Rat: 80-107 (m-f) : ↓ AGD & retained nipples in F_2 males.
C5-6	DIHP	No data	No data
C6	DnHP	380: ↓ number of litters/pair	380: ↑ pup mortality
C6-rich	DiHepP	419-1360 (m-f): ↓ reproductive organ weight	*Developmental study*: 750: ↑ resorptions and malformations *Two generation study*: 222-750 (m-f): ↓ AGD in F_2
C6	DEHP	140: ↓ fertility	14: small testes, seminiferous tubule atrophy
C7	DIOP	No data	No data
C8	DnOP	>7500	>7500
C7-9	Di-C7-9 PE	> 1000	1000: ↑ skeletal variations (lumbar ribs)

C8, C9	DINP	Rat: 742 (m): ↓ testes weight	*Two generation study*: Rat: 118-359 (m-f): ↓ pup weight at weaning *Developmental study*: Rat: 750: ↑ areolas/nipples in males; ↑ male reproductive malformations 1000: ↑ skeletal variations
C9	DNP	No data	No data
C6-10	Di-C6-10 PE	450 (F_0): ↓ seminal vesicle weight	450: ↓ litter survival, ↓ pup weight
C9-11	Di-C9-11 PE	> 1000	500: ↑ skeletal variations (lumbar ribs)
C9, C10	DIDP	NE	*Two generation study:* 134-352: ↓ pup survival in F_2 *Developmental study:* 500: ↑ skeletal variations (cervical and lumbar ribs)
C10	DIUP	No data	No data
C11	DUP	No data	No data
C12-rich	DITDP	No data	No data
C13	DTDP	> 250	> 250

[1] LOAEL derived from exposure to adult male. F1 ie including prenatal exposure included for comparison.
f – female; m – male; NE – not established

9. SUMMARY AND CONCLUSIONS

This compendium contains summarised physicochemical and human health hazard information for 25 phthalate chemicals. Analysis of human health hazards included all standard health endpoints – acute toxicity, irritation and sensitisation potential, genotoxicity, repeat dose toxicity, carcinogenicity and reproductive and developmental toxicity. The toxicological data for all endpoints are summarised in Table 7.

Historically, concerns over the use of phthalates, from a human health viewpoint, have been associated particularly with the potential for reproductive/developmental effects. Underlining these concerns is the issue that the structural and physicochemical properties of certain phthalates that impart usefulness as plasticisers also permit migration and leaching, with the resultant potential for human exposure, particularly from soft plastics. The potential for exposure, combined with a recognised toxicity profile for some particular phthalates, has raised concerns over potential health risks from phthalates, especially when used in consumer applications.

As evident from the chemical features depicted in Table 1, phthalates have a common diester structure consisting of a benzene/dicarboxylic acid "head" with two attached ester side chains. However, the phthalates differ structurally in the position, length and composition of ester side chains. Almost all of the phthalates included for review here were of the *ortho* configuration, with carboxylic acid groups and attached side chains arising from adjacent carbon atoms of the benzene ring. These are commonly known as phthalate esters. A single phthalate of the *para* configuration (dimethyl terephthalate, DMT) was also reviewed.

Given the commonalities in "head" structure, molecular weight differences for phthalates arise from differences in the structure and composition of ester side chains, with molecular weights increasing with increasing side chain length. References in this compendium to low (≤ C3), transitional (C4-6) and high (≥ C7) molecular weight phthalates refers to the carbon length of the side chain backbones and is in accordance with phthalate categorisations from previous reviews (Phthalate Esters Panel HPV Testing Group, 2001; OECD, 2004).

Overall Results and Data Extrapolation

Reflecting both human health and environmental concerns, information searches for this compendium revealed that many phthalates have been subject to regulatory scrutiny and tested extensively for human health and environmental effects. The hazard profiles of these 25 individual phthalates in this compendium, and as summarised in Table 7, are based on information from publicly available reviews combined with the results of recent literature searches up to September, 2006.

A review of physicochemical properties for the 25 phthalates shows that water solubility decreases and partition coefficients increase with increasing molecular weight. This is in line with information on the use of phthalates revealing that generally the lower molecular weight phthalates are used as surfactants whilst the higher molecular weight phthalates are used as plasticisers.

Although *in vivo* animal data or *in vitro* assay data were available for many phthalates, the amount of information for health effects from human studies was limited. For some phthalates, health effects information from surveys of occupational exposures was available, but interpretation of such studies was always complicated by confounding effects of exposure to multiple phthalates or other non-phthalate chemicals in the workplace and/or poor determination of exposure levels at which effects were noted. Literature reviews reveal clearly that the health effects of phthalates, especially with respect to reproductive and developmental effects in humans, is the subject of continued interest and study.

As the human health data are limited, the hazard profiles for the 25 phthalates are based predominantly on animal data. Rodent studies showed that phthalates are readily absorbed via the oral route. A clear trend was noted in dermal absorption data collected for five phthalates, DEP, BBP, DEHP, DINP and DIDP showing decreases in dermal absorption with increasing side chain length. The only information available on inhalation absorption of phthalates showed that DIDP was readily absorbed from the lung.

Phthalates are rapidly metabolised and excreted via the urine and faeces. Minimal tissue accumulation of phthalates was evident in rodent studies. Following absorption, phthalates undergo metabolism into monoesters by hydrolysis of one ester bond. Data for some phthalates shows further enzymatic oxidation of the alkyl chain resulting in numerous hydrophilic oxidative metabolites. Monoesters and oxidative metabolites may continue to undergo biotransformation to produce glucuronide conjugates prior to excretion.

All but one phthalate had been tested for acute oral toxicity with most also tested for dermal toxicity. Data for inhalation toxicity were available only for 9 phthalates. All phthalates showed low acute toxicity via these test routes.

Almost all phthalates had been tested for skin/eye irritation and skin sensitisation. Almost none had been tested for respiratory irritation. No effects or only minimal irritant effects were reported. The majority of phthalates were tested for skin sensitisation with all but one showing negative results. The low molecular weight phthalate DAP tested positive for sensitisation *in vitro.* No *in vivo* data were available. This phthalate is distinct structurally from other phthalates reviewed here in that it features short ester backbones of a low molecular weight phthalate but each containing a single double carbon bond. Overall, the phthalates can be regarded as possessing low irritant and skin sensitisation potential.

The amount and type of data available from genotoxicity testing varied widely. Other than for 5 lower molecular weight phthalates for which data were equivocal or lacking and therefore conclusions could not be drawn, the majority of phthalates were regarded as possessing low genotoxic potential.

Repeat dose testing mostly via the oral route was available for the majority of phthalates, although the extent of data and the time periods for testing varied considerably. For the majority of phthalates, repeat dose toxicity was noted at high doses (≥ 100 mg/kg bw/d). The most common target organs were the liver and kidney with effects commonly seen in these tissues at the lowest doses. Liver effects ranged

from increases in organ weights through to hypertrophy, hyperplasia, vacuolation and necrosis. Kidney effects were reported for a number of phthalates. These ranged from organ weight increases through to necrosis. Toxic effects were not always accompanied by direct evidence of peroxisome proliferation. In contrast to other phthalates, DMT, a terephthalate, induced effects in the urinary tract related to hyperplasia from the formation of calculi.

A limited number of phthalates were subject to testing in primates. The repeat dose effects of DEHP and DINP in the liver and kidney in rodents were not replicated in limited studies in marmoset or cynomolgus monkeys. On the basis of questions over the relevance to humans of the peroxisome proliferative effects seen in rodents, supported by observations of the lack of effects in primates, the potential for repeat dose effects in these organs in humans from exposure to these phthalates is questioned.

Most of the 25 reviewed phthalates have not been tested adequately for carcinogenicity. Of those that had been tested in long-term *in vivo* studies (commonly in rodents), the majority showed carcinogenic effects with several common tumour types reported. Mononuclear cell leukaemia in F344 rats and hepatocellular adenomas or carcinomas in rats and mice were the most common tumour types. Also, in all studies reporting hepatocellular neoplasms, evidence of peroxisome proliferative effects was reported. On the basis of this evidence, these tumour types are not considered relevant to humans. In addition, mononuclear cell leukaemia has no direct comparable tumour type in humans and so prevalence of this neoplasm is not regarded as a relevant for human health.

Leydig cell tumours were reported in rodents following DEHP exposure. In a human biopsy study, Leydig cell "micronodules" were commonly found in testicular biopsies from men with impaired spermatogenesis and reproductive hormone imbalances, suggesting that alterations in Leydig cells might be of developmental origin (Holm et al., 2003). However, although it is suggested that human Leydig cells may be less sensitive than rodents in proliferative responses (Cook et al., 1999), Leydig cell tumours induced by phthalates may be relevant to humans.

For those phthalates tested for carcinogenicity in primates, neoplasms seen in rodents were not replicated in this species. Phthalates which had been adequately tested for genotoxicity showed a negative genotoxic profile. Therefore, phthalates that show carcinogenic effects are likely to act by a non-mutagenic mechanism and demonstrate a threshold for these effects.

Reproductive and developmental toxicity data were available for the majority of phthalates. Common reproductive effects consisting of decreased fertility, decreased testes weight and alterations in accessory organs in males were reported. Seminiferous tubule atrophy was frequently observed. These effects appeared to be associated predominantly with the transitional phthalates of carbon backbone lengths of C4-6.

Studies generally indicated that high molecular weight phthalates (≥C7) were not male reproductive toxins. Except for DINP, none of the high molecular weight phthalates reviewed affected fertility or other aspects of the male reproductive system. Only for DINP were effects on the testes observed. However, these were equivocal.

Similarly, studies generally indicated that low molecular weight phthalates (≤C3) were not male reproductive toxins, with effects observed for DMP, DMT and DIBP only at high doses. For DEP, effects on testosterone levels and sperm were observed at modest doses (197 mg/kg bw/d) but no decreases in testes weight were noted as seen with transitional phthalates.

Toxicity data from two-generation studies showed the most sensitive developmental endpoints to be male reproductive effects and skeletal variations. Lactational effects were also seen with some transitional phthalates. For transitional phthalates, prenatal exposure induced an array of malformations, variations and developmental effects. Decreased AGD in males was a common finding for the transitional phthalates.

The high molecular weight phthalates appeared generally not to induce developmental effects. Increased frequencies of skeletal variations, common variations seen in developmental studies, were observed following gestational exposure to some high molecular weight phthalates at high doses.

Of the low molecular weight phthalates, DMP and DEP appeared not to induce developmental effects. A number of studies revealed no effects on embryo/foetal development with prenatal exposure to these phthalates at high doses. A single 2-generation reproduction toxicity study of DEP showed reduced pup weight at weaning and minor developmental delays at high doses. In contrast, separate studies with the low molecular weigh phthalate DIBP and the metabolite MIBP revealed decreased foetal weights and increased incidence of undescended testes, decreased testosterone levels in testes and plasma, decreased AGD and pathological changes in the testes. These effects suggest that low molecular weight phthalates approaching the C4 carbon backbone content of transitional phthalates are likely also to display developmental effects. DAP, DMEP and DMT possess unique molecular configurations compared to the other low molecular weight phthalates which, along with minimal available developmental data limited comparisons of developmental effects.

Overall, a comparison of toxicity information for the 25 phthalates reviewed showed that phthalates as a chemical group cannot be regarded as all possessing a similar toxicological profile. Generalisations may be drawn with respect to certain health endpoints. For example, acute toxicity, skin and eye irritation and sensitisation potential were low for all phthalates tested. However, specific patterns of toxicity were associated with groups of phthalates delineated by the ester backbone length. The widely held view of particular reproductive and developmental toxicity associated with the transitional phthalates ie. those with backbones of C4-6, is supported by reviews of these 25 phthalates.

In addition, phthalates with structures deviating from the common single bonded branched or linear carbon side chains showed toxicity profiles that often differed from phthalates of similar molecular weight. For example, DAP with short double bond-containing ester side chains displayed high acute oral toxicity relative to almost all other phthalates and showed positive results in individual tests within endpoints such as skin sensitisation, genotoxicity, repeat dose toxicity and reproductive/developmental toxicity. These positive results indicate a toxicological reactivity that contrasts with the overall low toxicity profile of other lower molecular weight phthalates reviewed. Therefore, ester backbone length alone without regard for

additional structural features should not be relied on as predictive of potential health impacts for the current reviewed phthalates or for alternative phthalate chemistries.

Table 7. Summary of hazard information

Backbone C Length	Phthalate	Oral LD50 (mg/kg bw)	Dermal LD50 (mg/kg bw)	Inhalation LC50 (mg/L)	Skin Irritation	Eye Irritation	Resp. Irritation	Skin Sensitisation	Genotoxicity	Repeat Dose Toxicity: LOAEL/NOAEL (mg/kg bw/d) and effects	Carcinogenicity: doses (mg/kg bw/d) and tumour type	Fertility: lowest LOAEL (mg/kg bw/d) and effects	Development: lowest LOAEL (mg/kg bw/d) and effects
C1	DMP	Rat: 2860 – 10000	Rat: 38000		ME	ME		negative	Non-genotoxic	Rat: LOAEL = 2000; NOAEL = 1000; Liver & kidney.	Negative *in vitro;* Mouse: negative *in vivo* init/prom. study	> 2000	> 3570
C1 para	DMT	Rat: 4390 – >6590	Guinea pig: >5000	Rat: >6	ME	ME		negative	Non-genotoxic	Rat: LOAEL = 636; NOAEL = 313; Urinary tract.	Rat, mouse: negative	> 636	313: ↓ Pup weight at weaning.
C2	DEP	Rat: >5600 – 31000	Rat: >11000	Rat: 7.50	ME	ME		negative	Insufficient data	Rat: LOAEL = 3200-3700 (m-f); NOAEL = 750-770; Liver, kidney, stomach, small intestine.	Rat: negative; Mouse: ↑ ademomas/carcinomas combined (dose-related males only from 280); Mouse: negative *in vivo* init/prom. study	197-267: ↓ serum testosterone; ↑ freq. abnormal and tailless sperm (F_0)	Maternal: 1016-1375: Liver, kidney. Developmental: 1016-1375: ↓ Pup weight; delayed age of onset of vaginal opening.
C3 (double bond)	DAP	Rat: 656 – 891	Rabbit: 3300	Rat, 1h: 5.20 – 10.31	negative	negative		positive	Mutagenic *in vitro*; equivocal and negative evidence *in vivo*.	Rat: LOAEL = 100 (f); NOAEL = 50 (f); Liver, kidney, lung.	Rat: LOAEL = 100; No NOAEL assigned; MCL. Mouse: Forestomach papillomas.	150	Insufficient data
C3	DMEP	Rat: 3200-6400	Guinea pig: >1171	Rat, 6h: >770-1595 ppm	ME	ME		negative	Insufficient data	Rat: LOAEL = 100; No NOAEL assigned; Blood, thymus, testes		1000: ↓ Testes weight and atrophy	291 (IP): ↑ Skeletal & visceral variations.
C3	DIBP	Rat: 16000-60,320			ME	negative		negative	Insufficient data	Rat: LOAEL = 5%; NOAEL = 1% (in diet); Liver, testes.		2000: ↓ Testes weight; histology.	Maternal and Developmental: 500: ↓ Pup weight and ↑ trans-abdominal

													testes migration.
C4	DBP	Rat: 6300 – 8000	Rabbit: >20000	Rat, 4h: ≥15.68	ME	ME	ME	negative	Non-genotoxic	Rat: LOAEL = 752; NOAEL = 152; Liver, kidney Rat (inhalation): NOAEC = 509 mg/m^3; LOAEC = 1.18 mg/m^3; Liver, kidney, testes.	Negative *in vitro*.	509: ↓ Epididymus weight (F_0).	100: Seminiferous tubule atrophy, retained nipples
C4, C5	BBP	Rat: 2330 – 20400	Rat: 6700		negative	ME		negative	Non-genotoxic	Rat: LOAEL = 381; NOAEL = 151; Liver, kidney, pancreas. Rat (inhalation): LOAEC = 789 mg/m^3; NOAEC = 218 mg/m^3; Testes, liver, kidney, spleen, pancreas	Rat: LOAEL = 500 – 720: ↑ MCL; ↑ pancreatic tumours; ↑ pancreatic acinar cell adenoma and adenoma/carcinoma (combined). Mouse: negative Positive *in vitro* (1 of 3 studies)	400: atrophy of testis, seminal vesicle and epididymis	100: ↓ body weight and AGD.
C4 (ring)	DCHP	Rat: >3200	Rabbit: >300	Rat: >3.20	ME	ME		Insufficient data	Insufficient data	Rat: LOAEL = 500; No NOAEL assigned; Liver, testes.	Insufficient data	80-107 (m-f): ↓ sperm head counts and focal seminiferous tubule atrophy	80-107 (m-f): ↓ AGD & retained nipples.
C5	DIHP				negative			negative	Unlikely to be genotoxic				
C6	DnHP	Rat: 29600	Rabbit: >20 mL/kg bw		ME				Unlikely to be genotoxic	Rat: LOAEL = 1824; No NOAEL assigned; Liver		380: ↓ Number of litters/pair.	380: ↑ Pup mortality.
C6-rich	DiHepP	Rat: >10000	Rabbit: >3160		ME	ME		negative	Non-genotoxic	Rat: LOAEL = 222-750; NOAEL = 50-168; Liver, kidney, pituitary.	Rat, mouse: ↑ hepatic DNA synthesis and peroxisomal beta-oxidation.	419-1360: ↓ reproductive organ weight.	222-750: ↓ AGD in F_2
C6	DEHP	Rat: 30600 – >40000	Rabbit: 24750	Rat: >10.62	ME	ME	Insufficient data	negative	Non-genotoxic	Rat: LOAEL = 146.6; NOAEL = 28.9; Liver, kidney. LOAEL = 37.6;	F344 rat: LOAEL = 146.6; NOAEL = 28.9; Adenomas, carcinomas, MCL.	140: ↓ Fertility.	14: Small testes, seminiferous tubule atrophy.

										NOAEL= 3.7; Testes.	Sprague-Dawley rat: Adenomas, carcinomas; benign Leydig cell tumours. Mouse: LOAEL = 292; NOAEL = 98; Adenomas and carcinomas. Syrian golden hamster: Inhalation: negative.		
C7	DIOP	Rat: >22000	Rabbit: >3160		ME	negative			Unlikely to be genotoxic	Rat, dog: Insufficient data	Negative *in vitro*.		
C8	DnOP	Rat: 53700 mg/kg bw	Guinea pig: 75		ME	ME		negative	Non-genotoxic	Rat: LOAEL = 350; NOAEL = 37; Liver, thyroid	Rat: Insufficient data.	NE	NE
C7-9	Di-C7-9 PE	Rat: >15000 – >20000			negative	negative		negative	Unlikely to be genotoxic	Rat: LOAEL = 120 NOAEL = 60 Liver, testes, blood		> 1000	1000: ↑ Skeletal variations (lumbar ribs).
C8, C9	DINP	Rat: >10000 (CAS 68515-48-0); >40000 (CAS 28553-12-0)	Rabbit: 3160 (CAS 68515-48-0)	Rat: >4.4	ME	ME		negative	Non-genotoxic	Rat: LOAEL = 358-442 (m-f); NOAEL = 88-108 (m-f); Liver, kidney.	Rat: LOAEL = 358 (m); NOAEL = 88 (m); ↑ MCL, hepatocellular neoplasia, renal tubule cell carcinomas. Mouse: LOAEL = 335 (f); NOAEL = 112 (f); ↑ Hepatocellular adenomas and carcinomas. Positive *in vitro* (1 of 7 studies).	742 (m): ↓ Testes weight.	118-359 (m-f): ↓ Pup weight at weaning.
C9	DNP	Rat: ≥2000			Insufficient data	Insufficient data			Unlikely to be genotoxic	Mouse: Insufficient data.			

C6-10	Di-C6-10 PE	Rat: >2000			ME	ME			Unlikely to be genotoxic	Rat: LOAEL = 45; No NOAEL assigned; Liver, kidney.	Negative *in vitro*.	450 (F_0): ↓ Seminal vesicle weight	450: ↓ Litter survival, ↓ pup weight
C9-11	Di-C9-11 PE	Rat: >6200 – 19700			negative	negative		negative	Unlikely to be genotoxic	Rat: LOAEL = 1000; NOAEL = 500; Liver.		> 1000	500: ↑ Skeletal variations (lumbar ribs).
C9, C10	DIDP	Rat: >29100	Rabbit: >3160	Rat: >12.54	ME	ME		negative	Non-genotoxic	Rat: LOAEL = 120; NOAEL = 60; Liver, kidney.	Positive *in vitro* (1 of 2 studies).	NE	134-352: ↓ Pup survival in F_2
C10	DIUP	Rat: >15800						negative	Unlikely to be genotoxic		Negative *in vitro*.		
C11	DUP	Rat: >15800		Rat, 6h: >1.8	negative	ME		negative	Non-genotoxic	Rat: LOAEL = 1145; NOAEL = 282; Liver, kidney.			
C12-rich	DITDP	Rat: >10000	Rabbit: >3160		ME	ME		negative	Unlikely to be genotoxic				
C13	DTDP	Rat: >2000							Non-genotoxic	Rat: LOAEL = 50; NOAEL = 10; Liver, kidney.		> 250	> 250

AGD – anogenital distance
MCL – mononuclear cell leukaemia
ME – minimal effects
NE – not established
Blank cell – no data available

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