ExxonMobil Chemical Company
13501 Katy Freeway
Houston, Texas 77079-1398

W.A. Jennings
Global Oxo Marketing Manager
Intermediates

March 29, 2011

ExxonMobil Chemical

Office of the Secretary
U.S. Consumer Product Safety Commission
4330 East West Highway
Bethesda, MD 20814

cpsc-os@cpsc.gov

RE: CHAP on Phthalates

Dear Sir:

ExxonMobil Chemical Company (ExxonMobil) is herein submitting information to assist the Chronic Hazard Advisory Panel (CHAP) on Phthalates with its deliberations, in particular, with its consideration of the cumulative effect of phthalates used in children's toys and childcare products. Our comments focus on diisononyl phthalate (DINP) and diisodecyl phthalate (DIDP), which are produced by ExxonMobil, and we are providing in-depth toxicological information on those phthalates as well as comments on cumulative risk assessment methodology.

At the outset, we note that the purpose of the CHAP is to provide a report to the CPSC, which the Commission can then use to inform its determination of whether to continue the interim prohibition of DINP and DIDP in children's products and whether to regulate use of any children's products containing phthalates. Thus, while there is a wide range of factors the CHAP is to consider, its deliberations and report will be of most use to the Commission if focused on the effects of phthalates and phthalate substitutes as they are used in toys and childcare articles.

We further note that Congress's charge to the CHAP is to consider the effect of cumulative exposures to phthalates. This is not a mandate to undertake a quantitative cumulative risk assessment; rather, the CHAP has discretion to consider the cumulative effect in any manner supported by the best available science, including a qualitative approach. Further, Congress did not direct the CHAP to include chemicals besides phthalates in its consideration of cumulative effects.

Discussion at the CHAP meetings has focused on the possibility of doing a cumulative risk assessment based on the hypothesized "rat phthalate syndrome." As discussed in the attached technical document, ExxonMobil is concerned that use of this term in and of itself is problematic, because the term is imprecise, potentially misleading, and not sufficiently supported to be used in hazard assessment as a substitute for evaluation of more specific endpoints. ExxonMobil questions whether there is sufficient scientific foundation for using this "syndrome" as the basis for a cumulative risk assessment of any set of chemicals. In any event, the existing data are clear that DINP and DIDP should not be included in any cumulative assessment based on "rat phthalate

syndrome" (or on endocrine disruption in general). This conclusion is strongly supported by the robust databases for DINP and DIDP, as discussed in the attached technical document. ExxonMobil urges that the CHAP carefully consider the information in that document.

Biomonitoring data demonstrate that aggregate exposures to DINP and DIDP are well below conservatively-derived Acceptable Daily Intake values. As demonstrated by the CPSC's studies on migration of DINP from PVC and on mouthing times, children's exposures from mouthing PVC objects containing DINP would be very low, even under a worst-case scenario. Due to the physical chemical properties of DIDP, exposures to PVC objects plasticized with DIDP would be yet lower. Therefore, these substances do not pose a health concern from use in children's products.

DINP and DIDP have unusually robust toxicology databases. They also have something that very few other industrial chemicals have – both robust biomonitoring data and the means to convert that biomonitoring data to estimated exposures using methodology that has been widely accepted. Thus, we are able to do for DINP and DIDP what often cannot be done in risk assessment – compare actual population exposure data to the ADI's derived from the robust toxicological data, giving us high confidence that human exposures to these two compounds are well below health benchmarks. Many alternative plasticizers do not have the robust toxicology databases nor the biomonitoring data which is available for DINP and DIDP.

For this reason, it is important that the CHAP's assessment be grounded in clearly supported science. It is also important that each phthalate be evaluated individually, and that DINP and DIDP not be presumed to pose concerns demonstrated for any other phthalates simply because they share the name "phthalate." The extensive data demonstrate that high molecular weight phthalates such as DINP and DIDP have distinct toxicological profiles. The CHAP must consider these differences in order to provide sound guidance to the CPSC.

If you have any questions or wish further information, please contact Angela Rollins at 281-870-6439.

Sincerely,

Attachment

cc: Commissioner Robert (Bob) Adler
Michael Babich, CPSC Chemical Hazards Program Coordinator
Mary Ann Danello, Directorate for Health Sciences Associate Executive Director
Cheryl Falvey, General Counsel
Commissioner Thomas Moore
Commissioner Nancy Nord
Commissioner Anne Northup
Chairman Inez Tenenbaum

COMMENTS TO

THE CONSUMER PRODUCT SAFETY COMMISSION

CHRONIC HAZARD ADVISORY PANEL ON PHTHALATES

by

EXXONMOBIL CHEMICAL COMPANY

March 29, 2011

Technical Contributors:

Ammie N. Bachman, Ph.D.
Kevin M. Kransler, Ph.D.

INTRODUCTION

THE ROLE OF DINP AND DIDP IN THE CHAP ASSESSMENT AND THE SIGNIFICANCE OF THEIR DATABASES

These comments are submitted by ExxonMobil Chemical Company and focus on DINP and DIDP – two phthalates that are subject to an interim ban on use in any children's toy or childcare article that can be placed in the mouth. The information in the attached technical document supports four key points.

1. A primary objective of the CHAP is to provide scientific support for the CPSC's determination whether to maintain the interim prohibition on uses of DINP and DIDP in toys and childcare articles. Toward that end, the CHAP should conduct a separate evaluation of each of these compounds, determine what effects relevant to humans are seen in laboratory studies at what doses, and compare adverse effect levels to likely exposures from toys and other childcare products. Considerable work product is already available from prior assessments by the CPSC and other agencies. Cumulative risk assessments pertaining to DINP and DIDP, if any are conducted, should focus on effects observed in studies of these compounds, and should include other phthalates only to the extent they cause the same effects. A cumulative risk assessment that focuses on effects seen in studies of phthalates that are already permanently banned from toys and other childcare articles will be of questionable utility to any decisions the CPSC must make to meet its obligations under the Consumer Product Safety Improvement Act (CPSIA).

2. Extensive biomonitoring data gathered by the CDC show that children's, pregnant women's and general population exposures to DINP and DIDP from all sources are very low, and far below their most conservative acceptable daily intake (ADI) values that have been calculated by the CPSC for each compound. Reliable methods have been developed for using the biomonitoring data to estimate exposures, with no adjustment needed (e.g. for fasting) (Aylward *et al.*, 2010). These exposures can then be compared to the calculated ADI. The most conservative ADIs derived by CPSC are based on liver and kidney effects that are of doubtful relevance to humans, but exposures from biomonitoring are well below even these very conservative ADIs. Further, the CPSC has previously calculated potential exposures to DINP from mouthing soft plastic toys and found that reasonably anticipated exposures are two orders of magnitude below the most conservative ADI, leading to the conclusion that exposures to DINP from mouthing soft plastic toys and from other children's products are *not* likely to present a health hazard to children (Babich *et al.,* 2004). DIDP, which did not have significant use in toys and other children's products that might be mouthed, was not assessed, but the same conclusion would be expected, given DIDP's low toxicity profile and physical and chemical properties.

3. Extensive developmental and reproductive toxicity data are available for DINP and DIDP, and for each the findings are very different from what has been reported in studies of other phthalates. Neither compound has been shown to cause cryptorchidism, hypospadias, or gross reproductive tract malformations. There is no strong evidence that either compound affects sperm. Additionally, neither affected fertility in definitive multi-generation studies. AGD and nipple retention were examined and found to be unaffected in the DIDP two-generation reproduction

study. Some equivocal, transient, high dose findings have been reported in some DINP studies with respect to AGD, nipple retention, and fetal testosterone levels, but the biological and toxicological significance of these findings is questionable in light of other studies that have not found similar effects, and the absence of other evidence of adverse effects on male reproductive tract development and reproductive performance. Two large DINP studies are being conducted at the Hamner Institutes and were designed specifically to address these and other male reproductive tract development and performance endpoints; reports should be available for consideration by the CHAP before its final report is due, and will add significantly to the available weight of the evidence. However, this much is clear now: The currently available data for DIDP and DINP present very different toxicity profiles compared to other phthalates that have been associated with the so-called "rat phthalate syndrome." The available data do not provide a sound scientific basis for including either compound in a cumulative risk assessment based on the vague and imprecise "rat phthalate syndrome."

4. <u>At the July CHAP meeting, recognition was given to the critical importance of problem formulation at the outset of any cumulative risk assessment exercise and the need specifically to incorporate that step into the CHAP deliberations</u>. This is indeed very important here for two reasons. First, problem formulation can help determine whether cumulative risk assessment (quantitative or qualitative) is needed in this case for any combination of phthalates. Second, problem formulation can help in determining how any cumulative risk assessment that might be deemed necessary should be conducted. As already noted, the CPSC's most immediate responsibility under the CPSIA is to determine if the interim ban on DINP, DIDP (and DnOP) should be continued, and thus the utility of a cumulative risk assessment focusing on phthalates that are subject to a permanent ban is unclear at best. Moreover, screening-level cumulative risk assessments that have been conducted thus far do not support the need for more refined cumulative risk assessments of any phthalates (Benson, 2009; Kortencamp and Faust, 2010). In each case, the Hazard Index was well below 1. Further, DINP was an insignificant contributor in each case (DIDP was not included). Thus, these screening exercises do not demonstrate concern for any combination of phthalates, and certainly not for DINP or DIDP, and do not demonstrate a need for an actual cumulative risk assessment. If the CHAP nevertheless persists in considering approaches to conducting a cumulative risk assessment that involves DINP and/or DIDP, it should focus on endpoints observed in studies of those compounds. The CHAP should not include either compound in a cumulative risk assessment based on toxicity endpoints that have not been demonstrated for DINP and DIDP or that have not been shown to result in adverse outcomes.

The CHAP's Charge to Consider Cumulative Effects of Phthalates

Section 108(a) of the Consumer Product Safety Improvement Act of 2008 (CPSIA) banned three phthalates[1] – DBP, BBP and DEHP – from any children's toy or childcare article in commerce in the United States.[2]

CPSIA §108(b) places an interim restriction on three other phthalates[3] – DnOP, DINP and DIDP – in any child care article or children's toy that can be placed in the mouth.[4]

Congress delegated to the Consumer Product Safety Commission (CPSC) the task of deciding whether this interim prohibition should be made permanent through rulemaking. CPSIA §108(b)(3). To inform the Commission's decision, Congress directed that the CPSC appoint a Chronic Hazard Advisory Panel (CHAP) "to study the effects on children's health of all phthalates and phthalate alternatives as used in children's toys and child care articles". CPSIA §108(b)(2)(A).

Congress spelled out the elements of the charge to the CHAP, stating that it was "to complete an examination of the full range of phthalates that are used in products for children" and lists factors to be considered as part of that examination. CPSIA §108(b)(2)(B). Among these, the CHAP is to:

- "consider the potential health effects of each of these phthalates both in isolation and in combination with other phthalates";

and

- "consider the cumulative effect of total exposure to phthalates, both from children's products and from other sources, such as personal care products".

CPSIA §108(b)(2)(B)(ii) & (iv).

Of note, Congress *did not* require the CHAP to conduct a quantitative cumulative risk assessment. The CPSIA does not define the word "consider", but the first dictionary definition of that word is "to think about carefully".[5] This provides a wide berth for the nature of the CHAP's examination. At one end, after consideration, the CHAP could conclude that the science is not yet at a point to enable an assessment of the nature and extent of the cumulative effect of exposure to phthalates. At the other end, the CHAP could attempt an intensive, detailed, quantitative cumulative risk assessment. There are numerous possibilities in-between, including a qualitative determination that one or more phthalates are unlikely to contribute to a cumulative effect with other phthalates. Another possibility is that the CHAP could determine exposures to phthalates are low and, even

[1] Di-Butyl Phthalate (DBP), Butyl Benzyl Phthalate (BBP), Di-2EthylHexyl Phthalate (DEHP)
[2] The three prohibited phthalates may be present at trace levels – up to 0.1%. A children's toy is a toy designed or intended for a child 12 years or under; a child care article is a product designed or intended to facilitate sleep or feeding of children age 3 and under or to help children 3 and under with sucking or teething. CPSIA §108(e)(1)(B)-(C).
[3] Di-n-octyl Phthalate (DnOP), Di-IsoNonyl Phthalate (DINP), Di-IsoDecyl Phthalate (DIDP).
[4] The phthalates may be present at levels up to 0.1%. Note that DnOP is not a commercial product; for that reason, we do not further refer to it in this document.
[5] Online Merriam-Webster Dictionary, at http://www.merriam-webster.com/dictionary/consider.

with some degree of cumulative effect, are unlikely pose a risk to human health in children's toys and childcare articles. This could be accomplished through use of conservative screening methodologies as opposed to a full cumulative risk assessment.

Another point to note regarding the charge to the CHAP is that the two items directing consideration of cumulative effects – quoted above – are limited to phthalates only. The CHAP is charged to generally "consider possible similar health effects of phthalate alternatives used in children's toys and child care articles", CPSIA §108(b)(2)(B)(viii), but it is not charged to include any chemicals that are not phthalates in its evaluation of cumulative effects.

In its discussions to date, the CHAP has expressed interest in performing a cumulative risk assessment based on the hypothesized "rat phthalate syndrome" (or "androgen insufficiency syndrome"). This document discusses the science demonstrating that neither DINP nor DIDP causes "rat phthalate syndrome" and therefore these phthalates should not be included in a cumulative risk assessment based on that outcome. Further, data for aggregate exposures to DINP and DIDP shows that those exposures are far below conservative acceptable daily intake values.

Because a primary purpose of the CHAP is to inform the Commission's determination of whether to continue the interim prohibition of DIDP and DINP, it may be suboptimal to start by considering an effect common to several phthalates (especially phthalates banned from children's products). The best approach might be to first determine what effects, of relevance to humans, DINP and/or DIDP causes in laboratory studies, and then ask whether other phthalates exhibit those same effects. If so, the CHAP could consider the cumulative effect of such phthalates for that endpoint.

It is important for the CHAP to understand that the inclusion of DINP and DIDP in the CPSIA was not an indication of a Congressional determination that these phthalates pose a risk to human health when used in children's products. They were included because of an amendment introduced by Senator Feinstein of California, based on legislation enacted in California in 2007.[6]. The California legislation was adopted to mirror legislation enacted in the European Union in 2005.[7] That legislation reflected a political decision to include DINP and DIDP on a precautionary basis;[8] even though comprehensive EU risk assessments concluded there was no concern from existing uses of DINP (including toys) and DIDP.[9] In each set of legislation, the restrictions on DINP and DIDP have been less than those for DBP, BBP and DEHP, reflecting their low toxicity profiles.[10] In the CPSIA, Congress chose to include DINP and DIDP on an interim basis, following the California and EU legislation. However, it also provided for the convening of the CHAP to

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[6] *See, e.g.*, remarks of Senator Diane Feinstein, 154 Cong. Rec. S1511 (daily ed. March 4, 2008); remarks of Representative Joe Barton, 154 Cong. Rec. H7582 (daily ed. July 30, 2008).

[7] *See, e.g.*, F. Ma, 2007 Legislative Summaries, http://democrats.assembly.ca.gov/members/a12/Legislation/2007/default.aspx.

[8] Directive 2005/84/EC of the European Parliament and of the Council, whereas clause 12, 2005 O.J. (L344) 40-43 (regarding phthalates in toys and childcare articles), http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2005:344:0040:0043:en:PDF.

[9] Summaries of the risk assessments are available at http://ecb.jrc.ec.europa.eu/documents/Existing-Chemicals/RISK_ASSESSMENT/SUMMARY/dinpsum046.pdf and http://ecb.jrc.ec.europa.eu/documents/Existing-Chemicals/RISK_ASSESSMENT/SUMMARY/didpsum041.pdf.

[10] *See, e.g.*, Directive 2005/84/EC, whereas clause 12, *supra* note 8.

investigate, in part, whether DINP and DIDP in fact would pose a risk if used in children's products.

For the reasons below, ExxonMobil believes the science strongly shows that DINP and DIDP pose very little risk to human health, whether considered individually or in conjunction with other phthalates. As these phthalates are far better studied than any alternatives, it makes good public health sense to discontinue the interim prohibition on their use.

Human Exposures to DINP and DIDP Are Low

Both DINP and DIDP have been included in the biomonitoring work of the Centers for Disease Control and Prevention (CDC), as well as work of other investigators. The largest body of data consists of urinary concentrations in samples collected under the US National Health and Nutrition Examination Survey (NHANES) (CDC, 2009; 2011). Metabolic data is also available that enable conversion of these urinary concentrations to actual exposures. These data are very valuable in assessing the potential for health effects from DINP and DIDP exposures.

In addition, the NHANES data provides exposure data for other phthalates. This enables answering the initial question for deciding whether a cumulative risk assessment is warranted: whether there are co-exposures to chemicals. For multiple phthalates, there is sufficient evidence of widespread human exposure due to their use in a wide range of consumer products (CDC 2009; 2011).

The following summarizes the biomonitoring data for DINP and DIDP, focusing on populations of interest for the CHAP's evaluation.

The Physical and Chemical Properties of DINP and DIDP Limit their Exposures

The primary use of DINP and DIDP is as plasticizers for polyvinyl chloride (PVC), for products such as floor tile, wire and cable insulation, and other applications where there is a need for a flexible plastic that is tough and durable. Phthalates as plasticizers are intrinsically bound within the PVC polymer matrix and only severe conditions (e.g. solvent extraction) will lead to significant migration from the PVC. Migration and dispersion of high molecular weight phthalates such as DINP and DIDP is further limited by their extremely low water solubilities and vapor pressures. In practice, migration occurs only at a very low rate; hence phthalates such as DINP and DIDP have a low potential for exposure. With significant advances in analytical techniques for the analysis of urinary concentrations of phthalate metabolites, and mathematical techniques for estimating intake, uncertainties about exposures to phthalates have been greatly reduced. However, it is critical to consider that the measurement of an environmental chemical in a person's blood or urine does not by itself mean that the chemical causes or is associated with disease. At face value, risk cannot be inferred from metabolite concentrations measured in biologic media, such as urine. The concentrations must be transformed with mathematical calculations to exposure estimates so that appropriate comparisons with points of departure, derived from laboratory animal studies can be made. Only then can the interpretation of exposure data be put into the context of risk.

As discussed below, exposures estimates for DINP and DIDP in women of reproductive age, pregnant women and children are low and well below the lowest acceptable daily intakes (ADI)

calculated by the CPSC: 120 µg/kg/day for DINP[11] and 150 µg/kg/day for DIDP[12]. These ADIs are based on liver effects: CPSC ADIs based on reproductive/developmental effects are higher.

Converting Urinary Metabolite Data to Exposure Estimates
Two calculation methods can be used for estimating DINP and DIDP exposure; given urinary concentrations of metabolites. In one calculation, daily intake is calculated as a function of creatinine corrected urinary metabolite, Equation 1 (David, 2000; Kohn et al., 2000):

$$\text{Equation 1: } DI = [UC \times CE / (F_{UE} \times 1000)] \times [MWd/MWm]$$

DI is daily intake (µg/kg/day), UC is the creatinine corrected urinary metabolite concentration (µg/kg), CE is the creatinine excretion rate (mg/kg/day) for adults (Tietz, 2006) and children (Remer *et al.*, 2002) and is used to account for differences in urine dilution (Preau et al., 2010), F_{UE} is the fractional urinary excretion rate of the metabolite (unitless) (Anderson et al., 2011; Koch and Angerer, 2007).[13] MWd and MWm are the molecular weights of DINP and the metabolite, respectively (David, 2000; Kohn et al., 2000).

A second equation for calculating exposure estimates is used when spot urine samples are not creatinine corrected, Equation 2 (Wittassek *et al.*, 2007).

[11] An ADI of 120 µg/kg/day was calculated for DINP based on the effect of spongiosis hepatis observed in the chronic toxicity studies. Spongiosis hepatis is of doubtful relevance to humans, and therefore, the ADI based on this endpoint is overly conservative. Karbe and Kerlin (2002), and MacSween *et al*. (2003) provide evidence that spongiosis hepatis is a spontaneous is a degenerative change seen in aging rats without a counterpart in human hepatic pathology. Careful review of rodents over the last twenty or more years by the National Toxicology Program has led to only a rare incidence of neoplasms arising from stellate cells in mice (13 cases from more than 90,000 mice), but these lesions differ morphologically from spongiosis hepatis. There was no evidence of a lesion resembling spongiosis hepatis in a review of 163 human livers (Su *et al*., 1997). Indeed, in the chapter on liver neoplasia from a definitive text on human liver disease, Pathology of the Liver edited by R.N.M. MacSween et al (2003)., the authors state: "To the best of our knowledge no human counterpart of the spongiotic pericytoma [spongiosis hepatis] has ever been described." Reports of lesions with similar characteristics in humans or non-human primates are also not found in the literature. This lesion or lesions with similar appearances are not described in any of a number of standard texts on neoplasia or systemic pathology in domestic animals, and there are no reports of this lesion in dogs. The only other species in which this lesion has been reported is the teleost fish (Couch, 1991). Given the large number of laboratory dogs and primates that have been exposed to a broad variety of chemicals over a considerable number of years, the absence of descriptions of this lesion would support the view that spongiosis hepatis observation in male rats and teleost fish are not relevant to human hazard assessment.

[12] An ADI of 150 µg/kg/day was calculated for DIDP based on a 13-week dietary exposure study in Beagle dogs. However, for risk characterization purposes this study it not appropriate due to its severe limitations. As described in the study report, gross necropsy examinations did not reveal any consistent compound-related alterations, only minor microscopic changes were noted and there was a lack of significant dose-response in severity and number of animals affected for these effects. More significantly, this study was not conducted to a standardized protocol, not conducted according to Good Laboratory Practice (GLP), had only 3 animals per sex per dose, and the results were not subjected to statistical analysis. As assessed using the Klimisch criteria for study reliability, this study scores a 3 – Not Reliable (Klimisch *et al*., 1997).

[13] The fractional urinary excretion rates (F_{UE}) of several DINP metabolites have been determined in volunteer studies (Anderson *et al.*, 2011; Koch and Angerer, 2007). No F_{UE} values are currently available for DIDP. However, they are anticipated to be similar to those for DINP, and for purposes of the calculations presented here, values for DINP are used.

Equation 2: $DI = [UC_{pm} \times UV_{24} / (F_{UE} \times BW)] \times [MWp]$

DI is daily intake (µg/kg/day), UC_{pm} is the urinary metabolite concentration (µmol/l), UV_{24} is the 24-hour urine volume (l/day), F_{UE} is the fractional urinary excretion rate of the metabolite (unitless) (Anderson et al., 2011; Koch and Angerer, 2007), BW is body weight (kg) and MWp is the molecular weight of the parent compound.

Exposure Estimates in Women of Reproductive Age and Pregnant Women
In the United States, the NHANES database contains data on urinary concentrations of various phthalate metabolites, including those of DINP and DIDP, in a cross-sectional sampling cohort representative of the US population (Calafat *et al.*, 2011; Centers for Disease Control and Prevention, 2009, 2011). When the data are parsed by sex and age, three different stages for female reproductive potential can be identified: pre-reproductive age (6-11), reproductive age (12-40) and post-reproductive age (40+). Utilizing equation 1, exposure estimates can be determined for these groups, Tables 1 and 2[14].

For women of reproductive age (12-40), regardless of ethnicity, DINP exposure estimates average 1 µg/kg/day (95% 7.8 µg/kg/day). DIDP exposure estimates are slightly lower; mean 0.5 µg/kg/day (95% 2.5 µg/kg/day). These values are similar to those calculated for the total population (males/females aged 6-60) (e.g. 1-2 µg/kg/day). Additionally, there is no difference in exposure estimates for the cohorts of younger (6-11) and older (40 +) women, although exposures to DIDP in females aged 6-11 were slightly higher[15].

Table 1 - DINP Exposure estimates in the 2005/2006 NHANES dataset (µg/kg-bw/day)

2005/2006 NHANES Dataset	**Non-Hispanic White**		**Hispanic**		**Non-Hispanic Black**		**Total**	
Age (Reproductive Stage)	**Mean**	**95%**	**Mean**	**95%**	**Mean**	**95%**	**Mean**	**95%**
6-11 (pre-reproductive age)	1.82	7.47	0.18	7.30	1.55	8.03	1.77	7.64
12-40 (reproductive age)	1.02	6.78	1.16	10.33	0.89	6.25	1.03	7.86
40+ (post-reproductive age)	1.27	6.98	1.32	10.40	0.86	4.50	1.20	7.28
Total US population (M/F, aged 6-60) (2005/2006)	1.53	9.09	1.44	9.68	1.18	7.09	1.46	9.32

[14] The data used for the tables are for the 2005-06 survey period (CDC, 2009). The CDC recently released the data tables with concentrations measured in samples from 2007/2008 survey (CDC, 2011). Within the margin of error, the DINP and DIDP urinary metabolite concentrations for 2007/2008 are comparable to those for 2005/2006. Updated exposure estimates will be provided in a future submission.
[15] On a relative basis, i.e., that ratio of the estimates for the two groups, the difference could be interpreted as large. On an absolute basis, however, given the margins of error, they are essentially equivalent. For example, the 95% confidence interval for the geometric mean is approximately 1-2 µg/kg/day, so that a value of 1.03 is essentially equivalent to a value of 1.77.

Table 2 - DIDP Exposure estimates in the 2005/2006 NHANES dataset (µg/kg-bw/day)

2005/2006 NHANES Dataset	Non-Hispanic White		Hispanic		Non-Hispanic Black		Total	
Age (Reproductive Stage)	**Mean**	**95%**	**Mean**	**95%**	**Mean**	**95%**	**Mean**	**95%**
6-11 (pre-reproductive age)	1.30	6.27	1.02	5.07	0.01	7.04	1.27	5.99
12-40 (reproductive age)	0.61	2.95	0.52	1.70	0.47	2.13	0.56	2.56
40+ (post-reproductive age)	0.73	3.07	0.76	4.05	0.51	2.84	0.71	3.03
Total US population (M/F, aged 6-60) (2005/2006)	0.71	3.66	0.71	3.66	0.71	3.66	0.75	3.72

Several studies are available that have examined DINP metabolite urinary concentrations in pregnant women. Exposure estimates calculated from these data also average approximately 1 µg/kg/day, similar to the estimates women of reproductive age, Table 3. A single study reported DIDP urinary metabolite concentrations in pregnant women, Table 4. Collectively, pregnant women have DINP and DIDP exposure estimates that are no different than the general population.

Table 3 - DINP Exposure estimates in pregnant women [16]

			DINP (µg/kg-bw/day)	
Reference	**Sampling year**	**n (age)**	**Mean**	**95%**
(Ye *et al.*, 2008)	2002-2006	99 Pregnant Women (18-41)	1.18	13.48
(Ye *et al.*, 2009)	2004-2006	11 Pregnant Women (15-53)	1.75	n.r.
(Suzuki *et al.*, 2009)	2005/2006	50 Pregnant Women	0.06	4.38
(Berman *et al.*, 2009)	2006	19 Pregnant Women (24-41)	0.74	n.r.
(Lin *et al.*, 2011)	2001/2002	100 Pregnant women (25-35)	0.05	0.20

Table 4 - DIDP Exposure estimates in pregnant women

			DIDP (µg/kg-bw/day)	
Reference	**Sampling year**	**n (age)**	**Mean**	**95%**
(Berman *et al.*, 2009)	2006	19 Pregnant Women (24-41)	0.41	n.r.

Exposure Estimates in Children (Aged 2 – 18)

Compared to data for the general population, there are fewer biomonitoring data available for estimating exposures of infants and young children. However, several studies have examined DINP and DIDP urinary metabolites in children between 2 and 18 years of age, Tables 5 and 6. Similar to adults, children's exposure estimates for DINP and DIDP from all sources are low and well below the ADIs calculated by CPSC.

[16] n.r. = not reported

Table 5 - DINP Exposure estimates in children

			DINP (µg/kg-bw/day)	
Reference	**Sampling year**	**n (age)**	**Mean**	**95%**
(Becker *et al.*, 2009)	2003-2006	137 (3-5)	3.95	18.14
	2003-2006	145 (6-8)	3.62	16.15
	2003-2006	149 (9-11)	3.38	18.47
	2003-2006	168 (12-14)	2.63	11.18
(Boas *et al.*, 2010)	2006/2007	342 Female children (4-9)	2.13	3.03
	2006/2007	503 Male children (4-9)	2.25	3.41
(Calafat *et al.*, 2011)	2005/2006	356 (6-11)	2.35	8.16
	2005/2006	702 (12-19)	1.58	9.15
(Lin *et al.*, 2011)	2003/2004	30 children (2-3)	1.92	2.00
	2006/2007	59 children (5-6)	0.95	3
(Koch *et al.*, 2011)	2007	108 children (5-6)	2.4	9.5

Table 6 - DIDP Exposure estimates in children

			DIDP (µg/kg-bw/day)	
Reference	**Sampling year**	**n (age)**	**Mean**	**95%**
(Koch *et al.*, 2011)	2007	108 children (5-6)	0.3	1.20

Understanding Exposures from Toys

Urinary biomonitoring data represent the aggregate of all sources of exposure. Since the CHAP is charged with determining risks posed to children from toys and childcare articles that can be placed in the mouth, understanding DINP and DIDP exposure from toys is necessary.

In 2002, the CPSC completed a detailed assessment of potential children's exposure to DINP from mouthing of plastic toys (United States Consumer Products Safety Commission, 2002). A comprehensive observational study of child mouthing activity (United States Consumer Products Safety Commission, 2002, Tabs F and G) and a state-of-the-art study of migration of DINP from toys (United States Consumer Products Safety Commission, 2002, Tabs I and J) were performed; these enabled significantly refined exposure estimates for children from the mouthing of toys. As documented, "The staff concluded that oral exposure to DINP from mouthing soft plastic toys, teethers and rattles is not likely to present a health hazard to children. Since children mouth other children's products less than they do toys, teethers and rattles, and since dermal exposure is expected to be minimal, staff does not believe that other children's products are likely to present a health hazard to children"; subsequently published as (Babich *et al.*, 2004; United States Consumer Products Safety Commission, 2002).

The migration extraction study utilized 41 plastic children's products, containing 133 articles which could be mouthed by small children. Of the 85 articles that contained soft plastic, 36 were found to contain DINP (42% of the articles). Using the "head-over-heels" method, migration rates ranged from 1.0 to 11.1 µg/10 cm^2/min, with a mean of 4.1 µg/10 cm^2. This value was then calibrated to previously reported data for an *in vivo* "chew-and-spit" DINP migration rate of a standard 10 cm^2 disk so that the need for *in vivo* data for each product containing DINP was eliminated.

The observational study represents one of the largest and most comprehensive mouthing studies to date. The survey included 169 children aged 3-36 months. Trained observers watched each child for 12 twenty minute periods over 2 days. Items mouthed were placed into one of 13 categories, with soft plastic toys a specific category. This study found that the largest single non-pacifier category was anatomy (fingers, hands, skin). Soft plastic toys represented only a small part of mouthing time. Further, the results of the observational study indicated a mean mouthing time for soft plastic toys of 1.3 minutes/day for the 3-12 month age group and 1.9 minutes/day for the 12-24 month age group (the age group with the highest mouthing time) (Table 7).

Based on the migration data and the comprehensive observation mouthing study, the estimated oral exposure to DINP from the mouthing of soft plastic toys is 0.07, 0.08, and 0.03 µg/kg/day for children aged 3 to <12, 12 to <24 and 24 to <36 months, respectively (Table 7). Therefore, CPSC concluded "that oral exposure to DINP from mouthing soft plastic toys, teethers and rattles is not likely to present a health hazard to children" (Babich *et al.*, 2004).

Table 7 - DINP Exposure estimates from the mouthing of toys

Age in Months	Basic Case – Soft Plastic Toys, 42% with DINP - Mean (95th percentile) (µg/kg-bw/day)	Hypothetical Case – Soft Plastic Toys, 100% with DINP - Mean (95th percentile) (µg/kg-bw/day)	Mouthing Time in minutes/day – Mean (95th percentile)
3 to < 12	0.07 (0.44)	0.17 (0.94)	1.3 (7.1) N=54
12 to < 24	0.08 (0.53)	0.22 (1.11)	1.9 (8.8) N=66
24 to < 36	0.03 (0.12)	0.07 (0.27)	0.8 (3.3) N=49

Current Methodologies for Estimating Exposures are Accurate

At recent CHAP meetings, there has been discussion of whether the equations used to estimate exposures from biomonitoring data yield accurate results. Based on data for various media, researchers have concluded that exposures to DINP and DIDP likely come primarily from dietary sources (Clark *et al.*, 2011). Because of this exposure pattern, there can be significant variability in metabolite urinary concentrations throughout the day (Hildenbrand *et al.*, 2009; Preau *et al.*, 2010; Wittassek *et al.*, 2010). Since there is rapid elimination of DINP and DIDP metabolites, and the dietary pathway appears to be the predominate exposure route, it was hypothesized that concentrations of metabolites in urine a few hours following last food consumption might be higher than concentrations in individuals who report food consumption at a time greater than the metabolite half-live (10-12 hours for DINP and DIDP).

In the 2005/2006 NHANES dataset, participants with morning appointments older than the age of 12 were requested to fast beginning at 11:00 pm the previous night. Participants in the afternoon and evening sessions were not directed to fast, and reported fasting times reflect typical times since the previous meal. In a submission to the CHAP for the December 2-3, 2010 meeting, Dr. Aylward reported that approximately 50% of participants reported a fasting time of 6 hours or less; 25% reported a fasting time of more than 12 hours (Aylward *et al.*, 2011).[17] Thus, fasting times are either relatively short (representing time between meals) or fairly long (reflecting an overnight fast). Using DEHP as a case-study, Dr. Aylward demonstrated that urinary DEHP metabolite levels were related to fasting times in the NHANES 2005-2006 data set in a complex fashion, with urinary concentrations increasing with increasing fasting time up to approximately 8 hours, and

[17] http://www.cpsc.gov/about/cpsia/chap/urinaryDEHP.pdf

decreasing with longer fasting times. This metabolite pattern does not support application of a simplistic "correction" factor related to fasting time in order to account for the rapid elimination of these compounds in interpretation of urinary biomonitoring data. Further, Dr. Aylward showed that the distributions of summed metabolite levels were not significantly different between individuals reporting short and long fasting times (<= 8 vs. >8 hours). Levels of MEHP, which is more transient than the other DEHP metabolites, do demonstrate a statistically significant difference by category of fasting time. However, MEHP represents a very minor fraction of the total excretion of DEHP metabolites. Therefore, reliance on the summed metabolites, including those with longer urinary excretion half-lives, provides a more stable indicator of exposure level. Discussion by CHAP members at the December 2-3, 2010 meeting considered the possibility of arbitrarily adding a 2 or 3 fold adjustment factor to account for fasting. There is no reason to use such an arbitrary adjustment factor because quality data and analysis are available to accurately determine exposure with and without fasting. When data is available, adjustment factors are not scientifically justified.

Conclusion on Exposures

The CHAP is charged with "examining the likely levels of children's, pregnant women's, and others' exposure to phthalates based on a reasonable estimation of normal and foreseeable use and abuse of such products." Based on the currently available data, for every segment of the population, exposures estimates for DINP converge on a mean of 1-2 µg/kg/day; estimates for DIDP are slightly less. When these exposures are compared to the most conservative ADIs calculated by the CPSC (120 µg/kg/day for DINP and 150 µg/kg/day for DIDP), it is clear that DINP and DIDP are likely not posing risk to human health, including that of adults, children, and developing fetuses. Additionally, current methodologies to estimate exposures are sufficient and do not required the arbitrary inclusion of uncertainty factors to account for fasting. Specific to toys and child care articles, the conclusion of the 2002 CHAP is still accurate, "…oral exposure to DINP from mouthing soft plastic toys, teethers and rattles is not likely to present a health hazard to children. Since children mouth other children's products less than they do toys, teethers and rattles and since dermal exposure is expected to be minimal, staff does not believe that other children's products are likely to present a health hazard to children" (Babich *et al.*, 2004).

Do Exposures Contribute to Common Adverse Outcomes?

The CHAP is charged with considering the potential health effects of the full range of phthalates. Based on CHAP discussions, it appears the hypothesized "rat phthalate syndrome" may be chosen as the "common endpoint" to focus their review and to conduct a cumulative risk assessment

The term "rat phthalate syndrome" was coined to encompass a group of adverse health effects observed in male rats from exposures during the critical window of male reproductive tract development (Gray and Foster, 2003). However, the basis for classifying this group of effects as a "syndrome" specifically attributable to phthalates as a class is weak and imprecise. There are significant differences in toxicity between the low molecular weight phthalates and the high molecular weight phthalates, such as DINP and DIDP. When these differences in toxicity are appropriately taken into consideration, it is clear that the inclusion of DINP and DIDP in a cumulative risk assessment based on the "rat phthalate syndrome" is not warranted, since neither substance induces the adverse outcomes of maldevelopment of the male reproductive tract that are observed with low molecular weight phthalates.

Phthalate Differentiation - Definition of Low Molecular Weight (LMW) Phthalates and High Molecular Weight (HMW) Phthalates

For phthalate plasticizers, there are two main families differentiated according to their structure and molecular weight: LMW and HMW. Substantial toxicology data exist for most phthalates which demonstrates that a subset of phthalates is toxicologically differentiated by their effects on reproductive and/or developmental parameters. Specifically, significant adverse reproductive/developmental effects are associated with LMW phthalates and not with HMW phthalates.

LMW phthalates are those with alkyl side chains whose alcohol carbon backbones range from C3 – C6. Members of this group include DEHP, DBP, BBP, Di-IsoButyl Phthalate (DIBP), Di-Pentyl Phthalate (DPP) and Di-IsoHeptyl Phthalate (DIHP). These LMW phthalates are classified as reproductive and developmental toxins (Category 1B under the UN Globally Harmonized System and the European Union (EU) Classification, Labeling and Packaging Regulation) due to significant adverse health effects observed in rodent studies.

HMW phthalates are those with alkyl side chains whose alcohol carbon backbones are C7 or greater. Members of this group include DINP, DIDP and Di-(2-PropylHeptyl) Phthalate (DPHP). Based on comprehensive data and evaluations these substances are not classified in the EU or under the Globally Harmonized System as reproductive and developmental toxins as they do not produce adverse reproductive or developmental effects in laboratory animal studies.[18]

Because of these toxicological differences among phthalates, it is important that each phthalate's hazard profile be fully evaluated separately.

"Rat Phthalate Syndrome" – A Hypothesis for LMW Phthalate-Induced Male Reproductive Tract Effects

The suite of effects induced by LMW phthalates, which has led to a conclusion that they are endocrine disruptors, has collectively been described by some researchers as the "rat phthalate syndrome". The suite of adverse effects, as defined by Gray and Foster (2003) includes: decreased anogenital distance, nipple retention, infertility, decreased sperm count, cryptorchidism, hypospadias, and other reproductive tract malformations such as testicular, epididymal, and gubernacular cord agenesis. The validity of this hypothesized syndrome for use in a phthalate cumulative risk assessment is questionable.[19] A control incidence of this syndrome has never been established and the threshold for inclusion based on incidence and severity of each effect has never been defined, though it has been suggested that one effect or merely a proposed sentinel event is enough to warrant inclusion. In addition, a number of non-phthalate compounds induce one or more of the included effects which belies the specificity of this description to "phthalates" only.

[18] The very low molecular weight (VLMW) phthalates Di-Methyl Phthalate (DMP – carbon side chains of one carbon) and Di-Ethyl Phthalate (DEP – carbon side chains of two carbons) are used in cosmetics and toiletries are not classified for reproductive effects, unlike phthalates with C3-C6 backbones.

[19] For this reason we put quote marks around the term "rat phthalate syndrome". Another term that has been proposed is androgen insufficiency syndrome, which has the merit of not being overbroad with respect to phthalates and underbroad with respect to other chemicals. However, as discussed below, each effect is not necessarily related to androgen levels.

Furthermore, while these effects are observed with LMW phthalates, a weight of the evidence review of all available data indicates that DINP and DIDP do not induce the effects characteristic of the "rat phthalate syndrome". As evidence, LMW phthalates (DiBP, DBP, BBP, and DEHP) which clearly induce the effects characterized as the "rat phthalate syndrome": hypospadias, cryptorchidism, decreased anogenital distance, nipple retention, changes in androgen sensitive tissue weight and infertility, are classified in the EU as reproductive and developmental toxins (European Chemicals Bureau, 2004, 2007, 2008). As demonstrated below, this is not true for DINP or DIDP which are not classified in the EU (European Chemicals Bureau, 2003a, b). Thus, it is inappropriate to name this group of effects as a syndrome attributable to phthalates as a class.

Moreover, the mode(s) of action leading to the observed effects included in the hypothesized "rat phthalate syndrome" is not known. In addition, a molecular target(s) of the phthalates has not been identified and likely differs based on the phthalate (i.e. pharmacodynamic differences). Nonetheless, a reduction of fetal testosterone and/or a reduction in insulin-like 3 peptide hormone biosynthesis (insl3) during the critical window of male reproductive tract development have been hypothesized to be critical contributors or common key events predictive of the "rat phthalate syndrome"; each is discussed in more detail below. However, a number of non-overlapping disrupted pathways may result in the varied and complex responses. This complexity highlights the need to carefully examine the specific toxicity, adverse health effects, and associated events for each individual phthalate.

Given the differences noted between the LMW and HMW phthalates, it is plausible that multiple modes of action may be at play; observation of a single precursor event (e.g. reduced testosterone) may not be predictive of the suite of effects described above, as exemplified by the data available for DINP. Moreover, there is no data available to suggest that DIDP triggers any of the proposed sentinel events or any of the downstream effects of "rat phthalate syndrome". Thus, there is no justification for classifying DIDP as a substance that induces the "rat phthalate syndrome".

Role of insl3 in "Rat Phthalate Syndrome"
Insulin-like hormone 3 (insl3) is a peptide hormone produced by the Leydig cells of the testes which has been shown to be associated with gubernacular defects and cryptorchidism when reduced (Adham *et al.*, 2000; Nef and Parada, 1999; Zimmermann *et al.*, 1999). Specifically, insl3 induces the gubernacular cord to differentiate and mature, thus facilitating the first phase of testes descent from the kidney area to the inguinal region during fetal life (Zimmermann *et al.*, 1999). Mice without a functional insl3 gene display cryptorchid testes and normal androgen levels. Androgen also plays a role in testis descent by acting to regress the cranial suspensory ligament during the first phase of testis descent. In the untreated (control) female rodent fetus, the gubernacular cord involutes in the absence of insl3 and the cranial suspensory ligament develops in the absence of testosterone to maintain the position of the ovaries near the kidneys (Howdeshell *et al.*, 2008a).

Two studies have examined the effect of DINP on insl3 mRNA levels. In one study, an increase in insl3 mRNA was observed 2 days following the last dose (i.e. GD 19.5) of DINP. However, the authors suggested that the increase may have been due to a "rebound effect" from the low testosterone production at the time dosing was initiated (i.e. GD 13.5) (Adamsson *et al.*, 2009). Results of a second study were presented in a poster recently at the 2011 Society of Toxicology

meeting; preliminary data suggested DINP did not affect insl3 mRNA levels (Lambright *et al.*, 2011). Therefore, consistent with data from the definitive 2-generation study and developmental toxicity studies where cryptorchidism was not observed (see below), DINP likely does not affect insl3. While not yet examined, DIDP is also not likely to affect insl3 levels since cryptorchidism is not observed in the definitive 2-generation studies and developmental toxicity studies (Hushka *et al.*, 2001).

Role of Fetal Testosterone in "Rat Phthalate Syndrome"
In order to assess the role of altered fetal testosterone as a critical contributor or common key event predictive of the "rat phthalate syndrome", current knowledge of the role of testosterone in the developing male fetus needs to be understood. Steroidogenesis in the fetal rodent and human testis has been reviewed in detail (Scott *et al.*, 2009), and key events are described here.

Altered testosterone levels in the rat fetus may be due to growth and differentiation factors (paracrine factors)
Beginning at gestational day (GD) 14.5 to 15.5, testicular testosterone production is initiated in the rat (Habert and Picon, 1984; Warren *et al.*, 1972). The mechanism for initiation is somewhat unclear as luteinizing hormone (LH) secretion, a primary stimulatory hormone, does not start until embryonic day 17.5 (Aubert *et al.*, 1985). This suggests that testosterone production is largely regulated either autonomously or by paracrine factors during embryonic days 15.5 – 17.5 (Scott *et al.*, 2009). This time period has been termed the "masculinization programming window" and is thought to be the critical window for androgen influence necessary for morphological differentiation of the male genitalia (e.g. epididymis, vas deferens, seminal vesicles, prostate, penis, scrotum and perineum) (Scott *et al.*, 2009). Following this programming window and a peak in fetal testosterone on approximately embryonic day 18 (Livera *et al.*, 2006), LH levels begin to rise and influence gonadotropic function. Based on these events and given the most common dosing regimen (i.e. single or repeated dose during GD 7 - GD 21) in short term *in vivo* rat studies, altered testosterone levels may be a result of disrupted paracrine factor action and or influence (Scott *et al.*, 2009). This hypothesis has been largely untested.

Humans differ from rats in aspects of testicular steroidogenesis
Fundamental control of steroidogenesis in the fetal rat is not identical to that of a human fetus. This point is important since it is frequently claimed that the pathway (sexual differentiation) that phthalates disrupt in the fetal male rat is highly conserved in all mammals and is known to be critical for human reproductive development. Indeed, commonalities exist between humans and rodents during the period of sexual differentiation (i.e. the time when a fetus can be morphologically distinguished as being male) and to some extent masculinization. However, a clear difference is noted in the stimulatory mechanisms for testicular steroidogenesis during the critical period when masculinization of the reproductive tract is being programmed. As described for the rat, the 2 day time period (GD 15.5-17.5) during which testosterone is produced and masculinization occurs is largely LH-independent (Scott *et al.*, 2009). Human fetal testosterone production begins around gestational week 8 and is mainly controlled by chorionic gonadotropin (hCG), a hormone not produced by rodents. By gestation week 12, hCG begins to decline and LH levels are seen to rise, although hCG is two to six times more potent than LH on a weight basis and may continue to strongly stimulate steroidogenesis through week 20 (Dufau *et al.*, 1972; Lee and

Ryan, 1973). Unlike rodents, paracrine factors likely have a secondary or supporting role in human testosterone secretion and are not seen to initiate production.

Basic differences in the steroidogenic cascade are also noted. The principle form of circulating cholesterol differs between rats and humans. HDL is the primary source taken up by the SRB-1/HDL receptor on the Leydig cell in rats and LDL is the primary source taken up by the LDL receptor on the Leydig cell in humans. In addition, the preferred steroid biosynthetic pathway converting cholesterol to testosterone differs; the Δ4 pathway (i.e. progesterone and its intermediate 17α-hydroxyprogesterone) predominates in rats while the Δ5 pathway (i.e. pregnenolone and its intermediates, 17α-hydroxypregnenolone and DHEA) is the predominant mechanism of testosterone synthesis in humans. These differences must be considered when characterizing the relevance of reported rodent effects and their extrapolation to human hazard characterization and risk assessment.

Existing data do not support relevance to humans of reduced fetal testosterone in rats
Species differences in response to phthalates have become more apparent in the recent literature. *In utero* exposure of mice and rats to DBP results in multinucleated germ cell formation and an increase in seminiferous tubule diameter, yet rats only exhibit suppression of fetal Leydig cell steroidogenesis (Gaido et al., 2007). This difference could be a species specific effect of DBP exposure on fetal Leydig cell SREBP2 activity; however the underlying mechanism is unknown (Johnson et al., 2011).

Limited data have been reported from studies in which effects of phthalates have been tested on human fetal testes. Lambrot et al., 2008 investigated the effect of MEHP on human fetal testes recovered during the first trimester (7-12 weeks) of gestation. MEHP had no effect on basal or LH-stimulated testosterone and did not affect proliferation and apoptosis of Sertoli cells. Reduced mRNA expression of anti-Müllerian hormone was reported and a reduced number of germ cells (via increased apoptosis) were also seen. Similarly, Hallmark *et al*. (2007) reported no effect on human fetal testis explants cultured with 10^{-3}M MBP for up to 48hrs. This included measurement of intratesticular testosterone levels and cytochrome P450 side chain cleavage enzyme expression as well as Leydig cell aggregation. However, the authors questioned the utility and validity of the *in vitro* system. Human fetal testes have also been xenotransplanted within the renal subcapsular space of a nude rat host followed by three days exposure to DBP (Heger *et al.*, 2010, 2011). Results, presented in abstract form, indicate DBP did not affect steroidogenic gene expression. An increase in multinucleated gonocytes (MNGs) per total number of germ cells was reported although the significance of this effect is not known. Therefore, limited data using human tissue has not indicated any effect by phthalates on the Leydig cell or suppression of testosterone. This highlights the need for further research but also calls into question the relevance of testosterone reduction in rats by phthalates for human health risk assessment.

DINP and DIDP Do Not Induce "Rat Phthalate Syndrome"
The following section reviews the available data from studies which have specifically investigated DINP and DIDP, including data on a suggested critical contributor, testosterone reduction, and each of the effects proposed to be within the hypothesized "rat phthalate syndrome". Infertility, the most severe outcome of disruption of male reproductive tract development, is also discussed.

We also note that, because of the hypothesized role of fetal testosterone in "rat phthalate syndrome", coupled with designation of low molecular weight phthalates as "endocrine disruptors", there is a tendency to assume all phthalates are endocrine disruptors and therefore capable of inducing "rat phthalate syndrome". Attachment A discusses the data pertinent to an analysis of whether a chemical is an endocrine disruptor and the corresponding data for DINP and DIDP. The weight of the evidence demonstrates that neither of these high molecular weight phthalates is an endocrine disruptor.

In addition to the data discussed below for DINP, robust developmental studies of DINP, consisting of a gavage study using 144 pregnant rats and a dietary study using 100 pregnant rats, are being conducted by the Hamner Institutes. These studies were designed to provide strong statistical power for analyzing, collectively, the kinetics and fetal testes effects of DINP and post-natal effects including nipple retention and AGD as well as any malformations of the male reproductive tract including hypospadias, cryptorchidism, and epididymal malformations, both gross and histological and the endpoints attributed to the hypothesized "rat phthalate syndrome." The in-life portions of the studies are completed, final analysis is nearing completion, and a report is being prepared. ExxonMobil anticipates that the results from the study will be available to the CHAP in time for incorporation into its report. We ask that the CHAP carefully consider the study results at that time, as these data will be important to the overall weight of the evidence and conclusions for DINP.

DINP Induces a Transient Decrease in Fetal Testosterone Levels in High Dose Gavage Studies
Several short term *in vivo* studies have been conducted (as discussed in full in Attachment A) in rats that specifically evaluated the potential for DINP-induced effects on plasma/testicular testosterone production or content (Adamsson *et al.*, 2009; Boberg *et al.*, 2011; Borch *et al.*, 2004; Gray *et al.*, 2000; Lee *et al.*, 2006a; Lee *et al.*, 2006b). For comparison, the results of those studies are summarized (Table 8). Of those, two studies, one examined only a single dose of DINP, and in the other, effects were observed only in one dose group in the middle of the dose range (Boberg *et al.*, 2011; Borch *et al.*, 2004). The four remaining studies reported no effects for various testosterone measurements at multiple time points following exposure (Adamsson *et al.*, 2009; Boberg *et al.*, 2011; Gray *et al.*, 2000; Lee *et al.*, 2006a; Lee *et al.*, 2006b).

While two studies have reported an effect on fetal testosterone levels at GD 21, limitations of the studies should be taken into consideration. Both studies that saw an effect used high doses of DINP (e.g. 750 mg/kg/day). In addition, a clear dose-response was not demonstrated (Boberg *et al.*, 2011). At time points post-GD 21, no effects on fetal testosterone levels were observed, indicating the effects observed at the early time point are transient.

As described above, there is an ongoing gavage study with DINP at the Hamner Institutes; it includes an evaluation of fetal testicular testosterone at GD 19. As these data will be important to the overall weight of the evidence and conclusions for DINP, the CHAP should carefully consider this robust study when it becomes available.

Table 8 - Studies that examined DINP effects on plasma/testicular testosterone production or content

					Testosterone Concentrations			
	Route/Strain	Dose (mg/kg)	Exposure Duration	Testosterone Measurement	Blood Serum	Blood Plasma	Intratesticular content	Testicular production
(Gray *et al.*, 2000)	G/SD	750	GD 14 – PND 3	PND 90	**No effect**	n.d.	n.d.	n.d.
(Borch *et al.*, 2004)	G/W	750	GD 7 – GD 21	GD 21	n.d.	**No effect**	**(+) approximately 60% reduction**	**(+) approximately 60% reduction**
(Lee *et al.*, 2006a)	D/W	5 50 500 1100	GD 15 – PND 21	PND 140	**No effect**	n.d.	n.d.	n.d.
(Lee *et al.*, 2006b)	D/W	5 50 500 1100	GD 15 – PND 21	PND 7	**No effect**	n.d.	n.d.	n.d.
(Adamsson *et al*., 2009)	G/SD	250 750	GD 13.5 – GD 17.5	GD 19.5	n.d.	n.d.	**No effect**	n.d.
(Boberg *et al*., 2011)	G/W	300 600 750 900	GD 7 – PND 17	GD 21	n.d.	**No effect**	**(+)approximately 40% reduction (600 mg/kg only)**	**No effect**
(Boberg *et al*., 2011)	G/W	300 600 750 900	GD 7 – PND 17	PND 90	n.d.	n.d.	**No effect**	n.d.

G: Gavage, D: Diet, SD: Sprague-Dawley, W: Wistar, n.d.: no data

There is No Direct Measure of Fetal Testosterone; However, Existing DIDP Data Demonstrate a Lack of Concern for Determining Effects on Fetal Testosterone

Data are not available concerning fetal testicular testosterone levels following administration of DIDP during the critical window of susceptibility; however, the lack of any evidence for adverse male reproductive tract development or associated endpoints such as nipple retention and AGD suggests that, even if testosterone levels were affected, the significance of that event would be questionable and clearly would not impact fertility or development of the male reproductive tract. As fertility has not been affected at doses up to ~750 mg/kg/day, determining the effect of DIDP on testosterone levels at doses at or above the limit dose (i.e. 1000 mg/kg/day or greater) would not be informative.

DINP and DIDP Do Not Induce Permanent Changes in Anogenital Distance

Anogenital distance is a sexually dimorphic trait in laboratory rodents and humans; rodent males exhibit a distance 2 – 2.5 fold greater than females. Androgens are responsible for normal AGD elongation in neonatal males (Clemens *et al.*, 1978; Hotchkiss *et al.*, 2007; Imperato-McGinley *et al.*, 1985). In laboratory animals, agents that are androgen receptor antagonists will induce a decrease in AGD in males.[20]

Anogenital distance was reported to be unaltered in two studies in which: a single dose of 750 mg/kg/day DINP was administered by gavage (Gray *et al.*, 2000), doses up to ~2500 mg/kg/day were administered via the diet (Masutomi *et al.*, 2003).

Boberg *et al.* (2011) reported a small (6%) but statistically significant decrease in anogenital distance in males exposed to DINP at 900 mg/kg/day on post natal day 13. However, the authors

[20] As described in Attachment A, DINP is not an androgen receptor antagonist (Takeuchi *et al.*, 2005).

reported there was no difference between treated animals and controls on post natal day 90, suggesting the effect was transitory.

Lee *et al.* (2006b) reported a significant decrease in anogenital distance at all doses tested (0, 40, 400, 4000, or 20000 pm in the diet on GD 15 through PND 21) on post natal day 1. However, these results are suspect because of the very small difference between the control (2.5) and the treated (< 0.1 below 2.5) normalized values for all dose groups. This finding was reported as being statistically significant in each dose group, yet with a unit number potentially as low as 16 animals, the statistical findings seem suspect and draw into question whether this is a reporting error, especially since potent anti-androgens that were also studied in this report exhibited no effect and this measurement. As pointed out by Foster and McIntyre (2002), "a 2 to 3% change in anogenital distance although measurable is unlikely to be biologically of importance and in isolation would not necessarily be considered adverse".

As described above, there are ongoing dietary and gavage studies with DINP at the Hamner Institutes; those studies include evaluation of AGD at GD 19, PND 2, 14, and 49. As these data will be important to the overall weight of the evidence and conclusions for DINP, the CHAP should carefully consider this comprehensive robust study when it becomes available.

Anogenital distance was specifically examined as part of the two-generation reproductive toxicity study protocol used for DIDP (Hushka *et al.*, 2001). DIDP (0.02, 0.06, 0.2 or 0.4% diet) did not affect AGD in the F_1 or F_2 pups when examined on post natal day 0.

DINP and DIDP Do Not Induce Permanent Nipple Retention

Nipple retention in males is thought to be a sensitive endpoint downstream of a reduction in fetal testosterone and has been assessed in several studies. As discussed earlier, further studies are warranted to determine if fetal reductions in testosterone are necessary and sufficient to produce this effect. The development of the rodent nipple is sexually dimorphic (Kratochwil, 1971; Kratochwil and Schwartz, 1976). Although mammary gland development begins similarly in both male and female rodent fetuses, offspring female rats and mice have nipples but males do not. In the developing rodent fetus, di-hydroxy testosterone produced locally from fetal testosterone causes regression of the nipple anlagen (Imperato-McGinley *et al.*, 1986; Kratochwil, 1977, 1986). This process can be disrupted, and these offspring subsequently display nipples. However, further studies are warranted to determine if fetal reductions in testosterone are necessary and sufficient to produce this effect.

As reported in Gray *et al.* (2000), data for DINP indicated that at 13 days of age, infant males with areolas were observed at an incidence of 22% compared with controls (0%). At approximately 5 months of age, 2/52 male pups displayed permanent nipples where the number of nipples equaled 1 and 6 for each of the two males. This effect was considered to be a malformation and was reported collectively with 2 other malformations as statistically significant, although the endpoint on its own was not statistically significant. The range of historical control values is important for understanding the low incidence effects. In this study the control incidence for areola retention was reported to be zero, but in a subsequent study from the same lab using the same rat strain, control values are reported as 14% (Ostby *et al.*, 2001a) which confounds interpretation of the results of the earlier study.

Boberg *et al.* (2011) reported a significant increase in nipples in males exposed to DINP at 750 and 900 mg/kg/day (average of 3 nipples in each dose group) as compared to controls (average of 2 nipples) on post natal day 13. However, there was no difference in the number of nipples in males between control and treated animals on post natal day 90. Since nipple retention was not observed on post natal day 90, the utility of this endpoint for hazard assessment is questionable.

The biological and/or toxicological significance of nipple retention observed in early postnatal male rats is questionable. Studies examining the effects of in utero exposure to finasteride, a 5α – reductase inhibitor, demonstrated that finasteride exposure induced nipple/areola retention in perinatal male rats, but the effects were temporary (Clark *et al.*, 1990), similar to the finding of Boberg *et al.* (2011) and Carruthers and Foster (2005). Furthermore, unlike rats, human males do not lose their nipples, significantly challenging the relevance of this endpoint for use in human hazard assessment or by extension to cumulative risk assessment.

As referenced above, there is an ongoing dietary study with DINP at the Hamner Institutes; it includes evaluation of nipple retention at PND 14 and PND 49. As these data will be important to the overall weight of the evidence and conclusions for DINP, the CHAP should carefully consider this robust study when it becomes available.

Nipple retention was specifically examined as part of the two-generation reproductive toxicity study protocol used for DIDP (Hushka *et al.*, 2001). DIDP (0.02, 0.06, 0.2 or 0.4% diet) did not induce male nipple retention in the F_1 or F_2 pups when examined on post natal day 12-13.

DINP and DIDP Do Not Induce Cryptorchidism, Hypospadias or General Reproductive Tract Malformations

Gross male reproductive tract malformations, such as cryptorchidism or hypospadias, have not been reported in any studies for DINP or DIDP; including, the definitive two-generation reproductive toxicity studies (Hushka *et al.*, 2001; Waterman *et al.*, 2000), and a number of other *in vivo* studies previously mentioned (Adamsson *et al.*, 2009; Boberg *et al.*, 2011; Borch *et al.*, 2004; Gray *et al.*, 2000; Hellwig *et al.*, 1997; Kwack *et al.*, 2009; Lee and Koo, 2007; Lee *et al.*, 2006a; Lee *et al.*, 2006b; Masutomi *et al.*, 2004; Masutomi *et al.*, 2003; Waterman *et al.*, 1999).

Reported in Gray *et al.* (2000), four of 52 adult males (from three litters) exposed perinatally to DINP exhibited a malformation: one displayed a fluid-filled testis, a second displayed paired testicular and epididymal atrophy, the third displayed bilateral testicular atrophy and the fourth displayed unilateral epididymal agenesis with hypospermatogenesis and scrotal fluid-filled testis devoid of spermatids. The low incidence of reported effects was without any dose response, using a small number of rats, and effects are of unclear significance. The collective incidence of effects in DINP treated animals was 7.7% (compared to 82% with DEHP treated animals). No endpoint on its own was significantly different from control values; rather, different effects were pooled to produce the 7.7% incidence. This type of data manipulation is not routinely performed in toxicological safety evaluations, nor is it considered good statistical practice. Based on the above points (historical control data and pooling of data to achieve significance), the significance of the reported findings is questionable.

Likewise, DINP does not induce general reproductive tract malformations manifested as decreased weights in androgen sensitive tissues: levator ani/bulbocavernosus muscles (LABC), seminal vesicles, ventral prostrate, glans penis, bulbouretral gland, and epididymis (Adamsson *et al.*, 2009; Boberg *et al.*, 2011; Gray *et al.*, 2000). These findings are not unexpected since, as discussed above, DINP only induces transient effects on fetal testosterone.

Some effects in androgen sensitive tissue weight were reported by Lee and Koo (2007) in which a study similar in design to the Hershberger assay was utilized. However, both DINP and DIDP did not induce consistent changes in these androgen sensitive tissues. A significant decrease in seminal vesicle weight was observed in all DINP dose groups while a significant decrease in LABC weight was only observed in the high dose group. Seminal vesicle weight and ventral prostrate weight were significant decreased in the DIDP high dose group. Regardless of control group, the weights of the sex accessory tissues from the administered groups showed no consistent or dose-related significant differences from the testosterone-only animals. In both of these cases, the data do not meet the Organisation for Economic Co-operation and Development (OECD) or Environmental Protection Agency (EPA) criteria for being classified as having a positive results since not all tissues were effected and no dose-response was observed.

As referenced above, there is an ongoing dietary study with DINP at the Hamner Institutes; it includes evaluation of phallus malformation, preputial separation, a full suite of reproductive organ weights at PND 49 and a comprehensive review of testes and epididymal histopathology at PND 2 and PND 49. As these data will be important to the overall weight of the evidence and conclusions for DINP, the CHAP should carefully consider this robust study when it becomes available.

There Is No Strong Evidence DINP or DIDP Adversely Affects Sperm
Two studies have examined sperm counts in male rats exposed to DINP (Boberg *et al.*, 2011; Kwack *et al.*, 2009). Boberg *et al.* (2011) reported that on post natal day 90, a small but significant ($p = 0.048$) *increase* in sperm count was observed in male offspring from dams that were exposed to 900 mg/kg/day DINP between gestation day 7 and post natal day 17; however, based on an increase in sperm counts measured as sperm per gram cauda epididymis and a slight decrease in epididymis weight, the authors concluded that "these data may indicate that DINP does not affect testicular sperm production". Conversely, Kwack *et al.* (2009) reported a reduction in sperm count (~25%) in adult males exposed to 500 mg/kg/day DINP for 4-weeks beginning at 28 days of age. Kwack *et al.* (2009) also noted no effect on sperm quality or motility.

Kwack *et al.* (2009) also examined sperm parameters in animals exposed to DIDP. No effect on sperm count was observed. The authors reported statistically significant decreases in sperm motion/quality parameters such as: straight-line velocity, curvilinear velocity, straightness, and linearity.

The reductions observed in Kwack *et al.* (2009) are of questionable relevance since higher doses of DINP and DIDP were used in the definitive two-generation reproductive toxicity studies where no effects on fertility were reported in males that would have been exposed to each substance for a longer period of time, including both the P and F_1 generations. Fertility is dependent not only on having adequate sperm count, but also on having normal sperm quality. When sperm quality is good, (i.e. normal motility as demonstrated for DINP in Kwack *et al.* (2009), then a significant

reduction in sperm count is required to affect fertility (Parker, 2006). Furthermore, Kwack *et al.* (2009) did not assess reproductive performance in these animals, critical to the interpretation of their findings.

DINP and DIDP Do Not Affect the Onset of Puberty or Male Mating Behavior
DINP exposure during gestation had no effect on the age of prepupital separation in male rats (Gray *et al.*, 2000; Masutomi *et al.*, 2003). Furthermore, as reported by Lee *et al.* (2006a; 2006b), the frequency of copulatory behaviors in post natal week 20 animals was unaffected by DINP at doses of 400 or 4000 ppm (number of mountings, number of intromissions, number of ejaculations, and post ejaculation interval). These observations support the findings of the definitive two-generation reproductive and developmental toxicity study in which there are no adverse effects reported for male fertility parameters (Waterman *et al.*, 2000).

As referenced above, there is an ongoing dietary study with DINP at the Hamner Institutes; it includes evaluation of preputial separation at PND 49. As these data will be important to the overall weight of the evidence and conclusions for DINP, the CHAP should carefully consider this robust study when it becomes available.

Similar to DINP, DIDP did not affect the age of preputial separation in F_1 or F_2 animals examined in the comprehensive two-generation reproductive toxicity test (Hushka *et al.*, 2001).

DINP and DIDP Do Not Impair Fertility
Both DINP and DIDP have not been shown to alter male fertility in laboratory animals in the definitive two-generation reproductive and developmental toxicity study (Hushka *et al.*, 2001; Waterman *et al.*, 2000).[21] Impaired fertility would be considered the decisive concern and ultimate result of the collective effects described for the male reproductive tract and termed "rat phthalate syndrome". As previously described, there were no effects on male fertility parameters or reproductive performance in either the parental (P) or first filial (F_1) generation. These studies demonstrate that adult males (P) exposed to DINP or DIDP prior to mating are successfully able to reproduce. More importantly, the reproductive capacity of the F_1 generation males, which were exposed to both chemicals throughout their lifetime, is unaltered. Therefore, it is clear that DINP and DIDP do not impair fertility[22].

Conclusion: DINP and DIDP Do Not Induce "Rat Phthalate Syndrome"
There has been speculation or an assumption that the combination of phenomena associated with exposure to low molecular weight phthalates in laboratory rodents, "rat phthalate syndrome," can

[21] Conducted according to EPA Health Effects Test Guideline OPPTS 870.3800 and in accordance with the principles of Good Laboratory Practices.

[22] For its monographs of seven phthalates, the National Toxicology Program Center for Evaluation of Risks to Human Reproduction (NTP-CERHR) created a scale ranging from clear, some, or limited evidence of adverse effects to insufficient evidence for a conclusion to limited, some or clear evidence of no adverse effects. The conclusion with respect to reproductive toxicity was "limited evidence of no adverse effects" for DINP and "some evidence of no adverse effects" for DIDP. In contrast, the conclusion for BBP for male reproductive toxicity was "some evidence of adverse effects" and for DBP and DEHP the conclusion for reproductive toxicity was "clear evidence of adverse effects." The NTP-CERHR evaluations can be accessed at http://cerhr.niehs.nih.gov/evals/.

be extended to include high molecular weight phthalates and is relevant to humans. Proposed key events critical to the induction of the hypothesized "rat phthalate syndrome" include a decrease in fetal testosterone and insl3 (Gray and Foster, 2003; National Research Council, 2008). It is important to again emphasize that the mechanisms underlying these effects remained ill-defined.

A decrease in fetal testosterone levels has been observed in two studies with DINP (Boberg *et al.*, 2011; Borch *et al.*, 2004); however, it appears to be a transient effect (Boberg *et al.*, 2011). Furthermore, there is a strong disconnection between this observed hormone change and the lack of predicted adverse phenotypes. The most sensitive phenotypic endpoints for the identification of "rat phthalate syndrome" are decreased anogenital distance and nipple retention (Carruthers and Foster, 2005; Gray *et al.*, 2009; National Research Council, 2008; Wilson *et al.*, 2007). While Boberg *et al.* (2011) reported a significant decrease in anogenital distance in males gestationally exposed to DINP (900 mg/kg/day) on post natal day 13 (approximately 6%), there was no difference between treated animals and controls on post natal day 90; the effect was transitory. Additionally, there was no effect on nipple retention at either time point. No effects on AGD or nipple retention were observed in the definitive two-generation reproductive toxicity test on DIDP (Hushka *et al.*, 2001).

Additionally, both DINP and DIDP have been shown not to induce hypospadias, cryptorchidism, or alter the androgen sensitive tissues. Furthermore, in the definitive two-generation reproductive toxicity tests, DINP and DIDP had no effect on fertility or developmental parameters.

Overwhelmingly, the data clearly indicate that both DINP and DIDP do not induce the adverse effects hypothesized to be part of "rat phthalate syndrome". Therefore, the applicability of the "syndrome" for hazard assessment is not supported for either substance. Limited research suggests that DINP induces a reduction in fetal testosterone synthesis. However, use of decreased testosterone as the sentinel event predictive of adverse effects is problematic as DINP does not induce the effects consistent with the hallmarks of the "rat phthalate syndrome." In addition, species specific differences in sensitivity to phthalate induced disruption in testosterone are clear. Recent and developing evidence indicates that humans are more similar to mice in that both seem to be refractory to phthalate induced testosterone reductions. Therefore, the relevance of this endpoint for human hazard or cumulative risk assessment is highly questionable.

Should a Cumulative Risk Assessment be Conducted and Will It Increase Accuracy Concerning Risk?

Cumulative risk typically refers to the accumulation of risk from multiple chemical and/or non-chemical stressors that may interact to produce an additive, synergistic, or antagonistic effect. This concept is different from aggregate risk assessment which refers to the sum of the risks resulting from exposures to the same chemical via multiple sources and multiple routes. Chemical mixtures risk assessment is encompassed within cumulative risk: two or more chemicals are involved which may cause the same or different effects to a target population (e.g., different organophosphates with the same mode of action, tailpipe exhaust with multiple chemicals having similar and different effects, etc.). Cumulative risk may also be defined broadly to refer to accumulation of risk from multiple unrelated sources (e.g., combined chemical and non-chemical risks).

Cumulative risk assessment (CRA) requires extensive scientific knowledge and currently has significant uncertainty. Expert testimony at the February 4, 2010 hearing of the Senate Environment and Public Works Committee's Subcommittee on Superfund, Toxics, and Environmental Health on "Current Science on Public Exposures to Toxic Chemicals" supported this view.[23] For example, the National Institute of Environmental Health Sciences (NIEHS) Director, Linda Birnbaum, recognized that the science of cumulative risk from multiple chemical exposures is only beginning to develop and that major research in this area is needed.[24]

The CHAP has discussed the National Academy of Sciences (2008) recommendation that phthalates and other chemicals that affect male reproductive development in animals, including compounds that affect testosterone production, be considered in a CRA. These approaches would be all encompassing, highly complex assessments without precedent. While this type of assessment would provide a complete understanding of how all chemical and non-chemical stressors contribute to an individual's risk, the data development and methodological validations would be vast, complex and time intensive. As discussed below, considerable planning would be required to conduct such an assessment which far exceeds the CPSIA §108(b)(2)(B) charge to the CHAP which stated the CHAP is to "consider the potential health effects of each of these phthalates both in isolation and in combination with other phthalates" and "consider the cumulative effect of total exposure to phthalates, both from children's products and from other sources, such as personal care products."

EM believes conservative assumptions built into individual chemical risk assessments (the current regulatory approach) in most cases will account for potential risks from chemicals in combination at low exposure. In addition, given the state of the science in cumulative risk assessment, there exists no methodology at present to incorporate comprehensive cumulative risk, including chemical and non-chemical stressors, as a routine component of chemical analysis. The scientific community has proposed several different approaches to cumulative risk, including those of World Health Organisation's (WHO) International Programme on Chemical Safety, US National Research Council, and EPA; however, a number of key issues are still being debated. Assessing when a cumulative risk assessment is necessary, defining which chemicals should be included and extrapolating to relevant exposures are all critical gaps in the current knowledge for assessing cumulative risk.

Critical Consideration to Problem Formulation Has Not Been Conducted

The first step when considering any risk assessment should be problem formulation in which the development of the objectives and scope help characterize the problem. The U.S. EPA Framework for Cumulative Risk Assessment (2002) recommends that planning and scoping the risk assessment should begin with a dialogue among all stakeholders. As cumulative risk assessments may be very complex, involving several chemicals and other stressors and/or responses, a detailed

[23] Written statements and archived webcast are at http://epw.senate.gov/public/index.cfm?FuseAction=Hearings.Hearing&Hearing_ID=8a722315-802a-23ad-4e9a-b8477139e63f

[24] Testimony given to US Senate Committee on Environment and Public Works Subcommittee on Superfund, Toxics and Environmental Health hearing on Current Science on Public Exposures to Toxic Chemicals February 4, 2010 (minute 56.10 of archived flash video at http://epw.senate.gov/public/index.cfm?FuseAction=Hearings.Choose&Hearing_id=8a722315-802a-23ad-4e9a-b8477139e63f).

plan is required at the onset of the assessment to identify all of the issues that need to be addressed. The problem formulation needs to consider the goals of the risk management, the purpose of the assessment, the scope and depth of the analysis, the analytical approach, and the resources available for the assessment. This problem formulation is usually an iterative process during which data gaps are identified and addressed and key knowledge is refined to a level that allows the risk assessment to proceed with the required degree of certainty. The process of problem formulation has largely been missing from recent discussions concerning the necessity and impact of any cumulative assessment of phthalates.

In the case of the CPSIA phthalates, problem formulation has largely been bypassed; however, this becomes a key consideration for the CHAP. In fact, during the July CHAP meeting, the statement was made "… generally that [problem formulation] is terribly important and often neglected and the process starts with a state of problem formulation and defining the context, I think that we're about to do that and we're very near to that point. That step is so important. That helps us to decide what kind of information we actually need. We need to define the context and the problem." This point should not be forgotten, as problem formulation helps focus goals and define the scope and depth of the analysis. We note again that, ultimately, information obtained from the CHAP's evaluation needs to help inform the CPSC's determination of whether to continue the interim ban on DINP, DIDP and DnOP in *children's toys and childcare articles that can be placed in the mouth*. Therefore, the CHAP's efforts should be focused on estimates of cumulative effects for children, versus other subpopulations, and on exposures from children's products.

Although the scope and underlying rationale for the immediate need to conduct a quantitative cumulative risk assessment of phthalates is not clear, the CHAP has discussed the following as perhaps a loose justification. First, evidence of concurrent exposure to phthalates, in general, is supported by biomonitoring data (Centers for Disease Control and Prevention, 2009; 2011). Second, some phthalates have been described as inducing a spectrum of effects described as the "rat phthalate syndrome" (see above discussion). Observation of any one of, or just a proposed critical contributor to, this collection has been suggested as enough evidence to describe an agent as inducing the "rat phthalate syndrome;" even though each effect, singly, may arise through the disruption of multiple pathways. Third, a number of studies report an increased incidence or response of phthalate-induced toxicity when administered together as opposed to administered singly (Howdeshell *et al.*, 2007; Howdeshell *et al.*, 2008a; Howdeshell *et al.*, 2008b; Jarfelt *et al.*, 2005; Martino-Andrade *et al.*, 2008; Rider *et al.*, 2008; Rider *et al.*, 2009). These studies are largely conducted with doses at or slightly below the observable response range and conclude that the combined effects are consistent with dose addition. Importantly, no data have been generated to support interaction at doses orders of magnitude lower than the rat NOAEL, or exposures close to that estimated for humans. A discussion on the validity of dose addition for human-relevant exposures is included below. The usefulness of the CHAP's report to CPSC would be enhanced by carefully considering the uncertainties, assumptions, and gaps associated with conducting a cumulative risk assessment, if one is undertaken, and fully characterizing each in the context of any conclusions reached.

Transparent Criteria for Establishing a Chemical Group Have Not Been Proposed
The National Academy of Sciences report (2008) recommended that phthalates and other chemicals that induce the general "androgen-insufficiency syndrome" in animals be considered in

a CRA. Given the previous discussion concerning the clear toxicological differences between LMW and HMW phthalates, and the inability to fully support the identification of a relevant predictive marker for human risk; broadly grouping "phthalates" for the purpose of estimating human risk is of questionable practice. As indicated above, a number of non-overlapping disrupted pathways may result in the varied and complex responses observed among different phthalates and other chemicals known to affect the male reproductive tract. This complexity highlights the need to carefully examine the specific toxicity, adverse health effects, and associated events for each individual chemical. By basing a cumulative risk assessment on broad criteria such as "adverse health outcomes", "the same phenomenological effect" or even having the same family name, the assessment not only becomes more complex but less accurate for estimating human risk.

As discussed above, the selection of DBP, BBP, DEHP, DnOP, DINP and DIDP for inclusion in the CPSIA was based on historical political processes, not on a scientific judgment. In addition, the CPSIA charge to the CHAP does not require a quantitative cumulative risk assessment. We believe that neither DINP nor DIDP qualifies for inclusion in a cumulative risk assessment based on male reproductive tract effects or hypothesized sentinel effects of the "syndrome". However, even where screening assessments have included DINP on that basis, they show that the contribution of DINP to risk is negligible. The following discusses these studies.

Conservative Screening Approaches Demonstrate DINP Poses Low Risk[25]

If problem formulation identifies a need to consider conducting a cumulative risk assessment, then proposed cumulative risk methodologies can be used as practical screening tools to confirm or disprove the concern identified during problem formulation. As described, the HI approach can provided useful information pertaining to the level of concern for phthalates as a mixture and help identify which phthalates in the mixture likely drive the concern. The published literature (Benson, 2009; Kortenkamp and Faust, 2010) suggests that the level of concern for adverse effects as a result of current exposures to a mixture of phthalates is very small and does not approach the point at which additional analysis is needed, bearing in mind that these screens are designed to represent an overly conservative estimate and a worst case scenario.

The Hazard Index (HI) approach was developed by the EPA in the early 1980's and has primarily been used as a screening method which enabled EPA to consistently compare risks and alternative remedial strategies, but not to accurately describe or characterize risk. Using this approach, an HI is calculated for a mixture, irrespective of each chemical's target organs, by taking the sum of the hazard quotients for the individual compounds present in that mixture. A hazard quotient (HQ) is the ratio of the estimated exposure to the acceptable level of exposure (e.g., reference dose RfD). The HI approach has the advantage of being a defined, transparent methodology where extensive data are not needed and uncertainty is well incorporated. No appreciable concern is assumed if the sum of the hazard quotients does not exceed a value of 1. If the HI exceeds the value of 1, an adjusted HI (HI_A) can be calculated by combining hazard quotients only for chemicals with similar target organ toxicity. The approach overcompensates for uncertainty and hence is highly inaccurate when it is used to characterize risk as it produces excessively high estimates of toxicity and risk.

[25] Likely, DIDP would exhibit hazard indices similar to or less than DINP due to its low exposures and low potential for toxicity.

Three significant layers of conservatism inherent to the HI approach are as follows.

1. Dose-addition (DA) – Dose addition is based on the idea that all components in a mixture behave as if they are simple dilutions of one another. DA implies that every toxicant in the mixture contributes, in proportion to its toxic unit, to the overall mixture toxicity. This oversimplification introduces a high degree of conservatism and uncertainty.

2. No Observed Effect Level (NOAEL)/Lowest Observed Effect Level (LOAEL) to describe dose-response data – Point estimates, such as NOAELs and LOAELs, neither represent effect concentrations nor effect levels. Both are empirically based on experimental design and are not an accurate representation of the intrinsic hazard value of a chemical. Since point estimates do not represent equi-effective doses, the use of them in a CRA introduces an additional layer of conservatism and uncertainty into the HI approach.

3. Modified Points of Departure (MPOD) – Adjustment/uncertainty factors used in the calculation of the MPOD are quantitative judgments of qualitative deficiencies in the database and are typically based on default values. The use of these uncertainty factors results in the conservative estimate of an MPOD, and by extension, a conservative HI value.

Two initial phthalate cumulative risk screens have employed a hazard index approach where the critical "effect" included multiple developmental endpoints, as summarized in Table 2 (Benson, 2009; Kortenkamp and Faust, 2010). Benson (2009) employed a hazard index (HI)/relative potency factor (RFP) approach for six phthalates (DBP, DiBP, BBP, DEHP, DPP, and DINP). A reference dose for each of the phthalate esters was derived, based purportedly on adverse male reproductive and/or developmental effects, and a potency factor relative to DEHP was assigned. Using exposure data from (Wittassek and Angerer, 2008) and (Kohn *et al.*, 2000) for EU and US populations, respectively, hazard quotients were calculated and then summed to determine the HI. This screening exercise indicated that humans are likely not suffering adverse developmental effects from current environmental exposures to the six phthalates as a mixture.

Kortenkamp and Faust (2010) also utilized the HI approach for the examination of phthalates (DBP, DiBP, BBP, DEHP and DINP) and other chemicals (vinclozolin, prochloraz, procymidone, linuron, fenitrothion, p,p'-DDE). For each of these chemicals, a reference dose was defined based on a point of departure based on adverse male reproductive and/or developmental effects. Using exposure data from US and EU populations, hazard quotients and the hazard index was calculated. Like the findings of Benson (2009), Kortenkamp and Faust (2010) demonstrated that the contribution of phthalates to the overall risk was small and suggested that concern for adverse reproductive effects from current environmental exposures to humans from the six phthalates as a mixture is low. In addition this study indicates that reduction in phthalate exposure would not significantly reduce the risk of the total mixture as they are of low toxicity and low exposure.

In both of these examples, the hazard index approach helped to highlight which phthalates in the mixture are most likely to drive the toxicity of the mixture. Consistent with each individual chemical's ability to induce developmental and reproductive effects in rodents, the hazard quotients for DEHP and DBP were much larger than for DINP which indicates that DINP does not

significantly contribute to the overall "phthalate" mixture toxicity due to its low toxicity for the chosen endpoint and low exposure. Therefore, as a screening exercise the HI serves as a first step to evaluate a mixture of chemicals in a highly conservative manner; however, it does not accurately determine a risk metric for humans nor does it serve as an adequate assessment with which to base regulatory restrictions.

The HI is dependent on the availability of comparable high quality hazard and exposure data for each component of the mixture. DIDP was not included in the published HI screens, for good reason. In each approach, data available on "rat phthalate syndrome" effects or a decrease in testosterone was used as the basis for deriving a modified point of departure. There is no evidence to suggest DIDP induces any of the effects chosen including nipple retention, decreased AGD, or decreased testosterone. Therefore, inclusion of DIDP based on any male reproductive tract effect, or any "rat phthalate syndrome" effect, or a decrease in testosterone is unjustifiable.

Table 9 - Phthalate Cumulative Risk Screens Indicate a Hazard Index < 1

(Benson, 2009)		(Kortenkamp and Faust, 2010)	
	Hazard Quotient		Hazard Quotient
DBP	0.02	DBP	0.06
DiBP	0.001	DiBP	0.008
BBP	0.004	BBP	0.012
DEHP	0.01	DEHP	0.12
DINP*	0.002	DINP*	0.001
Hazard Index	0.037	Hazard Index	0.201

* The point of departure chosen in these studies for DINP was a reduction in testosterone observed at a single high dose (750 mg/kg) (Borch *et al.*, 2004). Use of decreased testosterone as the sentinel event predictive of adverse male reproductive tract effects or as a surrogate effect for the "rat phthalate syndrome" is not supported by the studies on DINP which show no evidence of adverse health effects in association with the reversible reduction in testosterone (observed at high oral gavage doses). In addition, species specific differences reported in the literature question the relevance of this endpoint for human hazard or cumulative risk assessment.

Current Assumptions and Data Gaps Require Scrutiny
Discussions concerning how a cumulative risk assessment could be conducted have led to some proposals and assumptions which need to be scrutinized.

Assumption 1: A cumulative risk assessment could be conducted on a group of phthalates as indicated in the CPSIA based on evidence of their ability to similarly disrupt male sexual differentiation in reproductive toxicity models in rats (i.e. exhibited effects characteristic of the androgen insufficiency syndrome).

As discussed in the introduction above, the six phthalates named in the CPSIA were not originally grouped based on any toxicological similarities. In contrast, the six were included as a result of a historical political process that had included DINP and DIDP despite comprehensive risk assessments indicating no unacceptable risk from their current uses.

At approximately the same time as the passage of the CPSIA, the NAS 2008 recommended that "Accordingly, the cumulative risk assessment of phthalates should consider any chemical that leads to disturbance of androgen action and is thus capable of inducing any of the effects on the

development of the male reproductive system that are characteristic of phthalate exposure." The statement implies that disturbance of androgen action indicates capacity for inducing hypospadias, cryptorchidism, reproductive tract malformations, a decrease in Leydig cell function, a decrease in AGD and or a decrease in fertility.

The weight of the evidence suggests for DINP that although testosterone is transiently reduced late in gestation, effects on the development of the male reproductive system are not observed at doses below the commonly accepted limit dose of 1000-2000 mg/kg/day. Therefore, a number of non-overlapping disrupted pathways may result in the varied and complex responses induced by the LMW phthalates. This complexity highlights the need to carefully examine the specific toxicity, adverse health effects, and potentially associated events for each individual phthalate. Given the differences noted between the LMW and HMW phthalates, it is plausible that multiple modes of action may be at play; observation of a single precursor event (e.g. reduced testosterone) may not be predictive of the capacity for "inducing any of the effects on the development of the male reproductive system that are characteristic of phthalate exposure."

Assumption 2: Combination effects of phthalates with other anti-androgens can be approximated by using dose addition.

Mixtures assessments have been conducted primarily with LMW phthalates to determine if effects on the developing male rat reproductive tract are additive in nature, specifically if they display dose addition at doses well above estimated human exposures.[26] A single study has been conducted *in vivo* which tested the interaction effect of DINP and DEHP on testicular testosterone production (Borch *et al.*, 2004). Thirty-two dams were dosed with either 300 mg DEHP/kg bodyweight per day, 750 mg DINP/kg bodyweight per day, or a combination of these doses. Male fetuses were examined on gestation day 21, and blood and testes were collected for hormone analysis. The authors reported that a factorial statistical analysis revealed no statistically significant interaction between the effects of DEHP and DINP. In contrast, the assumption of dose-addition appears to be supported by the mixtures studies with LMW phthalates, again testing doses at or near the observable effect region (Ghisari and Bonefeld-Jorgensen, 2009; Howdeshell *et al.*, 2007; Howdeshell *et al.*, 2008a; Howdeshell *et al.*, 2008b; Jarfelt *et al.*, 2005; Martino-Andrade *et al.*, 2008; Rider *et al.*, 2008; Rider *et al.*, 2009).

The assumption of dose addition as the basis for conducting a cumulative risk assessment for humans is highly conservative (i.e. dose-addition is assumed at levels below a threshold of response) and not well supported in the published literature. As stated by Borgert *et al*. (2004), dose addition may be a conservative assumption [for some effects] of chemicals when they are present at concentrations at or above their NOAELs, but that independence becomes more predictive when the concentrations of the component chemicals are well below their individual NOAELs. It is important to point out that the reason low dose mixtures may be less than additive is that the mode of action could be different below the NOAEL.

[26] For mixtures of components that are determined to act through a common mode of action, the likelihood of toxicity associated with a mixture is determine by adding the doses of the components, where the concept of threshold is applied to the dose of the complete mixture, rather than to the doses of the individual components. The assumption for dose addition is that components are essentially toxicological "clones" of one another such that the relative proportions of each in a mixture are treated as dilutions of one another.

Borgert *et al*. (2004) also indicates that it is premature to assume dose addition for chemicals that appear to be mechanistically similar and to assume response addition models only for chemicals that appear to be mechanistically dissimilar. Because these simple models were developed for binary mixtures, their applicability to more complex mixtures is uncertain. Dose addition should be correlated with specific mechanistic features for particular toxic effects before the approach is generalized.

Gap: Additional Justification Is Needed for Aspects of Exposure Estimates Being Considered by the CHAP

Inherent in the extrapolation of cross-sectional sample data to the population are uncertainty and variability. Uncertainty represents a lack of knowledge about factors affecting exposure whereas variability arises from true heterogeneity across people, places or time (United States Environmental Protection Agency, 1997). More simply stated, uncertainty can lead to inaccurate or biased estimates whereas variability can affect the precision of the estimates and the degree to which they can be generalized. Therefore, it is of paramount importance that when addressing phthalate exposure estimates, the CHAP provides clear scientific justification for its choices, particularly when distinguishing and correcting variability and uncertainty. Rigorous quantitative analysis is of little value for use in the decision-making process if results are not clearly presented.

There have been discussions at the CHAP meetings on which population exposure estimates should be used in a cumulative risk assessment; mean or 95th%. Some support for use of the 95th% estimate for each phthalate has been expressed. Consideration must be given on whether or not this is a realistic scenario or if it substantially overestimates exposure. For example, it is unlikely that an individual in the population would be in the upper echelon of exposure estimates for all phthalates simultaneously. Therefore, the use of these high exposure estimates could significantly overestimate cumulative exposures. It is realistic to use the mean exposure estimates for purposes of the cumulative risk assessment.

A preliminary illustration of a cumulative risk screening for the phthalates in the NHANES database was presented at the December 2010 CHAP meeting, where a hazard index was calculated per individual. All individuals within a subpopulation (e.g. children ages 6-11) were plotted so that the number of individuals whose HI exceeded 1 could be identified. Drawbacks of the methodology as presented include: 1) estimations of exposure for DINP assumed exposure at the limit of detection for all "below the limit of detection" values, resulting in overestimation; and 2) the assessment was limited to those phthalates included in the NHANES database. Assessment of the statistical validity of the method is not possible on the basis of the information presented at the meeting, as the underlying calculations were not clearly conveyed. If the CHAP pursues this methodology, we request that a thorough explanation of the methodology be publicly provided so that any issues can be raised for the CHAP's consideration earlier rather than later in the process.

Conclusions

The two hazard index screens further support the previously discussed observation that all phthalates are not toxicologically equivalent. Even when an inappropriate endpoint is used[27], DINP has been shown to be a minimal contributor to any cumulative assessment on phthalates due to its low toxicity and very low exposure whereas LMW phthalates are seen to drive the risk associated with phthalate-induced effects on the male reproductive tract.

The published screening assessments described above based the points of departure on various effects on the male reproductive tract, or effects presumed to presage male reproductive tract effects, which is an overly conservative approach. The point of departure used in both published assessments for DINP was a reduction in testosterone. Detailed analysis of the full manifestation of the "rat phthalate syndrome" indicates a multifactorial basis; therefore, a mere reliance on decreased testosterone synthesis as a predictive marker is likely simplistic and inaccurate for the purposes of estimating human risk. In addition, species specific differences in sensitivity to phthalate-induced disruption in testosterone are clear. Humans are more similar to mice in that both seem to be refractory to the androgen modulation. Therefore, the relevance of this endpoint for human hazard or cumulative risk assessment is questionable and should not be used to include DINP in a cumulative risk assessment based on male reproductive effects. DIDP has not and should not be included in a cumulative risk assessment based on any "rat phthalate syndrome" effects or a decrease in testosterone as there is no evidence to suggest DIDP induces any of these effects. Therefore, inclusion of DIDP based on any male reproductive tract effect (i.e. any "rat phthalate syndrome" effect) or a decrease in testosterone is unjustifiable.

Areas of limited evidence need to be highlighted and incorporated into any conclusions regarding cumulative risk. Cumulative risk assessment based on adverse health outcomes is a new area for risk assessors and screening methodologies help to characterize "worst case scenarios". However, these methodologies incorporate a number of untested assumptions including dose addition at human relevant doses, steady state exposure levels, and the absence of additional interactions which either increase the effects (synergy) or diminish the effects (antagonism) of a single chemical. In addition, factors such as the ability to adapt and compensate for as well as repair damage are largely ignored in current cumulative assessments. Without consideration of these data gaps, the characterization of risk is largely inaccurate and does not serve to inform rationale and scientific decisions regarding regulation of products.

[27] The point of departure chosen in these studies for DINP was a reduction in testosterone observed at a single high dose (750 mg/kg) (Borch *et al.*, 2004). Use of decreased testosterone as the sentinel event predictive of adverse male reproductive tract effects or as a surrogate effect for the "rat phthalate syndrome" is not supported by the studies on DINP which show no evidence of adverse health effects in association with the reversible reduction in testosterone (observed at high oral gavage doses). In addition, species specific differences reported in the literature question the relevance of this endpoint for human hazard or cumulative risk assessment.

Attachment A: DINP is not an Endocrine Disruptor

Because of the hypothesized role of fetal testosterone in "rat phthalate syndrome", coupled with designation of low molecular weight phthalates as "endocrine disruptors", there is a tendency to assume all phthalates are endocrine disruptors and therefore capable of inducing "rat phthalate syndrome". This attachment discusses the data pertinent to an analysis of whether a chemical is an endocrine disruptor and the corresponding data for DINP and DIDP. The weight of the evidence demonstrates that neither of these high molecular weight phthalates is an endocrine disruptor.

Definition of an Endocrine Disruptor
Endocrine disruption is not a toxicological end point per se, but a functional change that leads to an adverse health effect. One of the earliest consensus definitions was developed during a multi-stakeholder conference in Weybridge, England during 1996. This led to further conferences and considerations within respected scientific programs such as the World Health Organization's International Programme on Chemical Safety (IPCS), which also developed a consensus definition.

The definitions are:

- Weybridge definition (1996) [28]: "An endocrine disruptor is an exogenous substance that causes adverse health effects in an intact organism, or its progeny, secondary to changes in endocrine function."

- IPCS definition (2002) [29]: "An endocrine disruptor is an exogenous substance or mixture that alters function(s) of the endocrine system and consequently causes adverse health effects in an intact organism, or its progeny, or (sub) populations."

Each of these definitions contains two critical components. First, and intuitively, is the recognition that an endocrine disruptor modulates the endocrine system. Second, and critical to the definition, is that this modulation leads to an adverse health effect. Each day the population undertakes a variety of activities that modulate the endocrine system, for example, exercising or use of contraceptives, and is exposed to chemicals that modulate the endocrine system, such as fructose. While these activities and chemicals modulate the endocrine system, they are not and should not be considered endocrine disruptors; they do not lead to adverse health effects.

The same tenet must be upheld for xenobiotics; not every substance that causes endocrine modulation within the range of homeostatic or (adaptive) physiological responses should be considered an endocrine disruptor. Within the range of physiological responses, substances may also act or be suspected to act on the endocrine system via endocrine mechanisms for which no relationship to an adverse health effect can be established. These substances should be discriminated from those that result in changes in the endocrine system leading to clear adverse

[28] European Commission, European workshop on the impact of endocrine disruptors on human health and wildlife. Weybridge, UK, Report No. EUR 17549, Environment and Climate Research Programme, DG XXI. Brussels, Belgium. 1996.
[29] International Programme on Chemical Safety - World Health Organization, Global Assessment of the State-of-the-Science of Endocrine Disruptors. 2002.

health effects, consistent with the above definitions. Only substances that cause adverse effects on functionality or composition of tissues, organs, or organ systems via endocrine modes of action should be categorized as having 'endocrine disruptor properties' and characterized as endocrine disruptors. Using the previously established IPCS definition of endocrine disruption, the OECD developed a conceptual framework for identifying endocrine disruptors. This is discussed below.

OECD Conceptual Framework for Identifying Endocrine Disruptors
The OECD conceptual framework for identifying endocrine disruptors was developed to provide a framework for the identification, testing and assessment of potential endocrine disruptors.[30] It is intended to apply to both new and existing substances as well as different chemical sectors such as pharmaceuticals, industrial chemicals and pesticides.

The conceptual framework agreed by the EDTA[31] in 2002 is not a testing scheme, but rather a tool box in which various tests can contribute information for the detection of the hazards of endocrine disruption. Organized into five levels each corresponding to a different level of biological complexity, study data are identified and organized. The end result is a weight of evidence assessment in the context of the ICPS definition of an endocrine disruptor; i.e. a substance that modulates the endocrine system in a manner that leads to adverse health effects.

DINP and DIDP are not Endocrine Disruptors
There are sufficient data to conclude that both DINP and DIDP do not modulate the endocrine system in a manner that leads to adverse health effects. Therefore, DINP and DIDP are not endocrine disrupting substances when evaluated according to the OECD Conceptual Framework and using the commonly recognized definitions of an endocrine disruptor. The following summarizes the data for DINP and DIDP, following the OECD flow from in vitro to short-term in vivo studies to definitive one- and two-generation studies.

In vitro Study Reports on DINP and DIDP
Available *in vitro* studies with DINP and DIDP have examined binding to both the androgen and estrogen receptors as well as the ability to modulate active iodide uptake in the thyroid. No significant responses were observed with either DINP or DIDP in any of the *in vitro* assays. *In vitro* data needs to be evaluated carefully as to whether they provide meaningful data; in some cases the test substance of interest was not always examined. Both DINP and DIDP are rapidly metabolized to the monoester which is likely the toxic entity. As discussed, extensive *in vivo* data support the conclusion that DINP and DIDP do not interact with the androgen or estrogen receptor and have no effect on the thyroid function.

In vitro Studies - DINP
Harris *et al.* (1997) screened a series of phthalate esters, including DINP, for estrogenic activity using a recombinant yeast screen. The recombinant yeast screen utilized the human estrogen receptor integrated into the main yeast genome and expressed in a form capable of binding to estrogen response elements, controlling the expression of the reporter gene lac-Z. DINP was tested at concentrations ranging from 10^{-3} M to $5x10^{-7}$ M and produced inconsistent results. DINP

[30] http://www.oecd.org/document/58/0,3343,en_2649_34377_2348794_1_1_1_1,00.html
[31] Sixth meeting of the task force on endocrine disrupters testing and assessment

was also tested for the ability to stimulate proliferation of human breast cancer cells (MCF-7 and ZR-75 cells). DINP had no effect in the MCF-7 assay. In the ZR-75 cells, DINP induced proliferation to a significantly greater extent than the control. Under *in vivo* conditions, DINP is rapidly metabolized to MINP (Koch and Angerer, 2007; McKee *et al*., 2002); therefore testing the diester *in vitro* is not representative of the *in vivo* response.

Zacharewski *et al.* (1998) examined the estrogenic activities of eight phthalates, including DINP, in both *in vitro* and *in vivo* test systems. DINP did not induce displacement of [^{3}H] estradiol in rat uterine cytosol. Additionally, in a estrogen sensitive luciferase reporter gene assay in MCF-7 and HeLa cells, DINP did not induce any measureable response at any of the doses tested (up to 10^{-5} M). Finally, no significant growth of *S. cervisiae* strain PL3 transformed with human estrogen receptor cDNA in selective medium with DINP. The authors also examined the effects of the eight phthalates, including DINP, in two *in vivo* assays; an uterotrophic assay and vaginal cell cornification assay. Mature ovariectomized rats were dosed with 1, 20, 200, or 2000 mg/kg/day DINP for 4 consecutive days with assessment occurring on day 5. In two separate experiments, DINP caused a minimal yet significant decrease in uterine weight in the 2000 mg/kg/day dose group. No effects were observed in vaginal cell cornification assay for DINP. Based on these results, a NOAEL of 200 mg/kg/day can be inferred for decreases in uterine weight. As stated previously, under *in vivo* conditions DINP is metabolized to MINP (Koch and Angerer, 2007; McKee *et al.*, 2002).

Akahori *et al.* (2005) used combined quantitative structure-activity relationship (QSAR) models from discriminant and multi-linear regression analysis to predict the binding potency to human estrogen receptor alpha (ERα) and compared these results to an *in vitro* human ERα binding assay. In the *in vitro* assay, DINP exhibited minimal human ERα binding; reported as the relative binding affinity (logRBA = -3.49). When examined in the computer models, weak binding was predicted for DINP.

Breous *et al.* (2005) investigated possible effects of DINP on the transcriptional activity of sodium/iodide symporter (NIS) which mediates the active transport of iodine in the thyroid. PC C13 cells were transfected with the human NIS reporter in a luciferase expression vector along with a vector containing the NIS promoter and upstream enhancer and cultured with test material for 48 hours. No effect was observed with DINP on the transcriptional activity of NIS.

Takeuchi *et al.* (2005) characterized the activities of the human ERα, human ERß and human androgen receptor (AR) using a reporter gene assay in Chinese Hamster Ovary (CHO) cells. CHO cells were transfected with plasmids containing human ERα, ERß, or AR along with a luciferase reporter plasmid. As compared to the controls estradiol and dihydroxy-testosterone, DINP did not show any estrogenic/anti-estrogenic or androgenic/anti-androgenic activity at the tested concentrations (up to 10^{-5} M).

Wenzel *et al.* (2005) investigated the potential of six phthalates to modulate basal iodide uptake mediated by the sodium/iodide symporter (NIS) in a rat thyroid cell line, FRTL-5. Results indicated that DINP showed no cytotoxicity at levels $<10^{-2}$M but at high concentrations (10^{-4}M), enhanced basal iodide uptake by these cells.

Mlynarcikova *et al.* (2007) investigated the effects of DINP on progesterone and estradiol production in primary cultures of porcine ovarian granulosa cells. Cells were incubated in the presence or absence of 3 phenols, 3 phthalates (up to 10^{-4} M) or human recombinant Follicle Stimulating Hormone (hFSH, 1g/ml). Steroid levels were measured by radioimmunoassay (RIA). DINP did not induce any significant effect on basal or hFSH stimulated progesterone production. Further, DINP did not induce any significant effect on basal estradiol production. A decrease in hFSH stimulated estradiol production was associated with DINP at all dose levels.

Akahori *et al.* (2008) examined a series of chemicals in a human ERα binding assay and compared the results to observations from an *in vivo* uterotrophic assay performed according to the OECD guideline 440 and in compliance with good laboratory practices (GLP). DINP exhibited minimal human ERα binding in the *in vitro* assay; reported as the relative binding affinity (logRBA = -3.49).

Kruger *et al*. (2008) tested DINP in two chemically activated luciferase gene expression (CALUX) bioassays, one in recombinant mouse Hepa1.12cR cells to assess the AhR function and the other in CHO cells to assess AR function. DINP had no effect on AhR or AR activity in the tested dose range (10^{-10} – 10^{-2} M).

Ghisari and Bonefeld-Jorgensen (2009) investigated the potential *in vitro* thyroid hormone-like and estrogenic activities of a range of widely used plasticizers, including DINP. Thyroid hormone disrupting potential was determined by the effect on the TH-dependent rat pituitary GH3 cell proliferation (T-screen). Estrogenicity potential was assessed using MVLN cells, stably transfected with an estrogen receptor (ER) luciferase reporter vector. DINP did not induce a significant effect on GH3 cell proliferation, indicating that it did not mediate thyroid receptor activity. Further, DINP did not induce any effects in the ER assay at doses up to $5x10^{-5}$ M.

In vitro Studies - DIDP

Harris *et al.* (1997): A series of phthalate esters, including DIDP, were screened for estrogenic activity using a recombinant yeast screen. The recombinant yeast screen, a gene for a human estrogen receptor was integrated into the main yeast genome and was expressed in a form capable of binding to estrogen response elements, controlling the expression of the reporter gene lac-Z (when receptor is activated, lac-Z is expressed). DIDP was tested at concentrations ranging from 10^{-3} M to $5x10^{-7}$ M. DIDP showed no effects in any of these screens performed. It should be noted that these *in vitro* assays have investigated one mechanism of action only, the ability of phthalates to act as estrogen agonists. More importantly, it should also be noted that these were tests of phthalate diesters.. Under *in vivo* conditions, DIDP is rapidly metabolized to its monoester MIDP (General Motors Research Laboratory 1983); therefore testing the diester *in vitro* is not representative of the *in vivo* response.

Zacharewski *et al.* (1998) examined the estrogenic activities of eight phthalates, including DIDP, in both *in vitro* and *in vivo* test systems. DIDP did not induce displacement of [^{3}H] estradiol in rat uterine cytosol. Additionally, in an estrogen sensitive luciferase reporter gene assay in MCF-7 and HeLa cells, DIDP did not induce any measureable response at any of the doses tested (up to 10^{-5} M). Finally, there was no significant growth of *S. cervisiae* strain PL3 transformed with human estrogen receptor cDNA in selective medium with DIDP. The authors also examined the effects of the eight phthalates, including DIDP, in two *in vivo* assays; an uterotrophic assay and a vaginal cell

cornification assay. Mature ovariectomized rats were dosed with 1, 20, 200, or 2000 mg/kg/day DIDP for 4 consecutive days with assessment occurring on day 5. In two separate experiments, DIDP caused a minimal yet significant decrease in uterine weight in the 1 mg/kg/day dose group only. No effects were observed in vaginal cell cornification assay for DIDP. Under *in vivo* conditions the diesters are metabolized to monoesters which are not estrogen receptor agonists (Koch and Angerer, 2007; McKee *et al.*, 2002).

Breous *et al*. (2005) investigated possible effects of DIDP on the transcriptional activity of sodium/iodide symporter (NIS) which mediates the active transport of iodine in the thyroid. PC C13 cells were transfected with the human NIS reporter in a luciferase expression vector along with a vector containing the NIS promoter and upstream enhancer and cultured with test material for 48 hours. A slight effect was observed with DIDP on the transcriptional activity of NIS but the biological relevance of this weak effect is not clear due to the artificial nature of reporter assays.

Takeuchi *et al.* (2005) characterized the activities of the human ERα, human ERß and human androgen receptor (AR) using a reporter gene assay in Chinese Hamster Ovary (CHO) cells. CHO cells were transfected with plasmids containing human ERα, ERß, or AR along with a luciferase reporter plasmid. As compared to the controls estradiol and dihydroxy-testosterone, DIDP did not show any estrogenic/anti-estrogenic or androgenic/anti-androgenic activity at the tested concentrations (up to 10^{-5} M).

Wenzel *et al*. (2005) investigated the potential of six phthalates to modulate basal iodide uptake mediated by the sodium/iodide symporter (NIS) in a rat thyroid cell line, FRTL-5. Results indicated that DIDP showed no cytotoxicity at levels $<10^{-2}$M. At high concentrations (10^{-4}M), DIDP enhanced basal iodide uptake by these cells. The biological relevance of these effects is unclear due to the artificial nature of the cell based assay.

Mlynarcikova *et al.* (2007) investigated the effects of DIDP on progesterone and estradiol production in primary cultures of porcine ovarian granulosa cells. Cells were incubated in the presence or absence of 3 phenols, 3 phthalates (up to 10^{-4} M) or human recombinant Follicle Stimulating Hormone (hFSH, 1g/ml). Steroid levels were measured by radioimmunoassay (RIA). DIDP did not induce any significant effect on basal or hFSH stimulated progesterone production. Further, DIDP did not induce any significant effect on basal estradiol production.

Akahori *et al*. (2008) investigated the relationship between the *in vitro* ER binding and *in vivo* uterotrophic assays. The authors compared the results from these assays for 65 chemicals spanning a variety of chemicals classes. The DIDP binding affinity (log RBA) value of -3.46 was one of the lowest reported and far below the cut-off level (-2.63) that could induce estrogenic/ anti-estrogenic activities in the uterotrophic assay, indicating that DIDP does not have estrogenic/ anti-estrogenic properties.

Kruger *et al*. (2008) tested DIDP in two chemically activated luciferase gene expression (CALUX) bioassays, one in recombinant mouse Hepa1.12cR cells to assess the AhR function and the other in CHO cells to assess AR function. DIDP had a slight effect on AhR in the highest dose group, but no effect or AR activity in the tested dose range (10^{-10} – 10^{-2} M).

Ghisari and Bonefeld-Jorgensen (2009) investigated *in vitro* the potential for thyroid hormone-like and estrogenic activities of a range of widely used plasticizers. The TH disrupting potential was determined by the effect on the TH-dependent rat pituitary GH3 cell proliferation (T-screen). The estrogenic activities of the compounds were assessed in MVLN cells, stably transfected with an estrogen receptor (ER) luciferase reporter vector. Results were variable with DIDP being reported as causing a small increase in GH3 proliferation at one concentration only. DIDP did not have an effect on ER transactivation.

In vivo Study Reports on DINP and DIDP
In vivo data examining the effects of DINP and DIDP on the male reproductive tract is robust. DINP and DIDP show no significant adverse effects in the Uterotrophic Assay and no consistent significant adverse effects in the Hershberger Assay. In non-validated research studies for anti-androgenic effects, DINP showed none, minor or inconsistent effects at high doses, with no or limited evidence of a dose response. While one animal study shows no effects on fetal testicular testosterone, one study shows variable effects with no dose response, and one study shows reduced fetal testicular testosterone at a single high dose. This effect on testosterone appears to be occurring at high doses only and without adverse health effects being seen in animals

In vivo Studies - DINP
In vivo data examining the effects of DINP on the male reproductive tract is robust. A number of short term *in vivo* studies have been performed although protocols have varied as have endpoints of interest making comparisons and conclusions on consistency difficult. DINP shows no significant adverse effects in the Uterotrophic Assay, and no consistent significant adverse effects in the Hershberger Assay. In non-validated research studies for anti-androgenic effects, DINP showed none, minor or inconsistent effects at high doses, with no or limited evidence of a dose response. While one animal study shows no effects on fetal testicular testosterone, one study shows variable effects with no dose response, and one study shows reduced fetal testicular testosterone at a single high dose. This effect on testosterone appears to be occurring at high doses only and without adverse health effects being seen in animals.

In general, the short-term exposure studies are informative and have identified particular endpoints of interest including testosterone synthesis, nipple retention, AGD, and epididymal malformations, but do not invalidate the conclusions from the comprehensive 2-generation reproductive toxicity study. It has been proposed by Carruthers et al (2005) that there is a critical window of susceptibility for the developing male fetal reproductive system for LMW phthalates in rodent studies (gestation day 16 – 19). This critical window is fully assessed in the 2-generation reproductive studies. The 2-generation study design assesses the effects of continuous exposure in the F1 and F2 generations. Both a 1-generation and a comprehensive 2-generation reproductive toxicity study have been performed where rats were exposed to DINP in the feed at various doses. No significant differences in male mating, male fertility, female fertility, female fecundity, or female gestational indices were noted. Mean days of gestation were unaffected by treatment as well as the mean sex ratio of the treated offspring when compared with controls. While the parameters of anogenital distance and nipple/areola retention were not specifically part of the test protocol (not included in the test guidelines in effect at the time of the study), these study data indicate that DINP does not adversely affect male reproductive development or fertility at doses up to approximately 1000 mg/kg/day (the highest dose tested).

In Vivo Studies – Short Term Exposure -- DINP
Hellwig *et al.* (1997) administered DINP by gavage at 0, 40, 200, and 1000 mg/kg/day to 8-10 sperm-positive Wistar females/group on gestation day 6 through day 15. The dams were sacrificed on day 20 and implantation sites were examined. Fetuses were weighed and examined for external malformations; half of the fetuses were examined for skeletal malformations and the other half for visceral malformations. Maternal toxicity at the high dose consisted of reduced food consumption and increased relative liver and kidney weights. There were no treatment-related effects on the number of live fetuses/dam or fetal weight. The only fetal effects were evident at the highest dose by a statistically significant increase in percent fetuses per litter with variations. These variations consisted of rudimentary cervical and/or accessory 14^{th} ribs. A modest increase in dilated renal pelves in the high-dose group was also noted. There were no maternal or fetal effects at 40 or 200 mg/kg/day. A maternal and fetal NOAEL of 200 mg/kg/day was determined.

Waterman *et al.* (1999) using Crl:CDBR mated female rats, DINP was administered by gavage at doses of 0, 40, 200, 500 or 1000 mg/kg/day on gestation day 6 through day 15. Overt signs of maternal toxicity were not apparent at any dose level. Transient signs of maternal toxicity at 1000 mg/kg/d, as indicated by slight reductions in body weight gain and food consumption were observed; however, normal weight and food consumption patterns were observed during the late gestation period, after exposure ceased, possibly indicating a recovery effect. A significant increase in fetuses with skeletal lumbar rudimentary ribs and with visceral (dilated renal pelves) variations at 1,000 mg/kg/d on a per litter basis was observed. Therefore, the maternal and fetal NOAELs were determined to be 500 mg/kg/day.

Gray *et al*. (2000) treated pregnant rats with DINP via gavage at a dose of 750 mg/kg/day from gestation day 14 thru post natal day 3. There were no treatment related effects on fetal body weight or anogenital distance (AGD) on post natal day 2. As infants, males in the DEHP, BBP and DINP groups were reported as displaying areolas (87, 70, 22% respectively and reported as statistically significant). There were no treatment related effects reported in androgen sensitive tissue weights: testes, LABC, seminal vesicles, ventral prostrate, glans penis, and epididymis. Four of 52 adult males (from three litters) exposed perinatally to DINP exhibited malformation: one displayed a fluid-filled testis, a second displayed paired testicular and epididymal atrophy, the third displayed bilateral testicular atrophy and the fourth displayed unilateral epididymal agenesis with hypospermatogenesis and scrotal fluid-filled testis devoid of spermatids. The incidence of effects in DINP treated animals was 7.7%, compared to 82% with DEHP treated animals. No other single endpoint (nipple retention, epididymal agenesis, fluid filled testes, and testes weight) on its own was significantly different from control values. In addition different effects were pooled to produce the 7.7% incidence. Only by pooling of these different effects was statistical significance demonstrated. This type of data manipulation is not routinely performed in toxicological safety evaluations, nor is it considered good statistical practice. There were no effects on testosterone levels. Two of 52 animals displayed permanent nipples at 3 months of age (number of nipples = 1 and 6 for each of the two males). The range of historical control values is important for understanding the low incidence of observed effects. In this study the control incidence for areola/nipple retention was reported to be zero, but in a subsequent study, control values are reported as 14% (Ostby *et al.*, 2001b). Based on the above points the significance of the reported findings is questionable.

Ostby *et al*. (2001a) (an abstract that was not peer-reviewed) treated pregnant rats with DINP via gavage at a dose of 1000 or 1500 mg/kg/day from gestation day 14 thru post natal day. At post natal day 2, males exposed to 1500 mg/kg/day DINP displayed reduced anogenital distance. On post natal day 13, there was an increase in the percentages of males with areolas; the control group exhibited a 14% incidence rate for this endpoint.

Masutomi *et al.* (2003) exposed pregnant rats to DINP at doses of 400, 4000, or 20,000 ppm (approximately 30-66, 307-657, and 1100-2657 mg/kg/day) via the diet between gestation day 15 and post natal day 10. Maternal body weight, accompanied by decreased food consumption, was reduced in the 20,000 ppm dose group on gestation day 20 and post natal day 10. Fetal body weight gain was significantly decreased in the 20,000 ppm dose group males on post natal days 10 and 21. No effect was observed on AGD at post natal day 2. Post natal day 27 absolute and relative testis weight was decreased in the 20,000 ppm dose group. DINP treatment had no effect on the onset of puberty in male or female animals. No treatment related effects were noted in androgen-sensitive tissue weight; testis or prostrate. Some histopathological changes in males were noted in the 20,000 ppm dose group; degeneration of meiotic spermatocytes, vacuolar degeneration of Sertoli cells and scattered debris in epididymal ducts.

Masutomi *et al.* (2004) exposed pregnant rats to DINP at doses of 400, 4000, or 20,000 ppm (approximately 30-66, 307-657, and 1100-2657 mg/kg/day) via the diet between gestation day 15 and post natal day 10. At both post natal week 3 and 11, DINP had no effect on pituitary cells positive for luteinizing hormone, follicle stimulating hormone, or prolactin in male and female animals. Additionally, there was no effect on pituitary weight in either sex at this time point.

Borch *et al*. (2004) Maternal exposure to DINP at 750 mg/kg on gestation days 7-21 induced reduced ex vivo testicular testosterone production and *in vivo* testosterone levels in testes and plasma of male fetuses at ED 21. However, the utility of this study for hazard identification and risk assessment is limited by several factors. First, the study utilized only one very high dose of DINP. Second, there were no adverse phenotypic effects reported in the study, therefore it is unclear if the observed decrease in testosterone content is in-fact a toxicologically significant response. Finally, the authors measured the testosterone levels on gestation day 21, a time point after the developmental surge of testosterone that occurs during gestation day 16-18 in the rat. After gestation day 18, plasma testosterone levels are naturally declining in the fetal rat, thus, conclusions regarding reductions in testosterone synthesis are unreliable when assayed at this time point.

Lee *et al*. (2006a; 2006b) Pregnant rats were fed DINP (0, 40, 400, 4000 ppm) from gestation day 15 through weaning (PND21). On post natal day 1, males displayed significantly decreased body weight, anogenital distance and bodyweight normalized anogenital distance. On post natal day 7, serum estradiol was significantly decreased in the 40 ppm females. No effects were observed on serum testosterone levels in male and females or estradiol levels in males. Relative mRNA levels of granulin precursor gene were significantly decreased in 40, 400, and 20000 ppm the female hypothalamus on PND7. Relative mRNA levels of p130 were significantly increased in all male dose groups on PND7. On post natal week 21, weanling male rats in the 40 ppm exposure group displayed a significant decreased in mating behavior mounts, ejaculations, and intromissions. No

other effects in mating behavior were observed in the other male dose groups. DINP did not have any effect on serum luteinizing hormone, serum follicle stimulating hormone or serum testosterone at post natal week 20 in both male and female rats. Female rats exhibited a significant dose-dependent decrease in lordosis during post natal week 20[32].

Lee and Koo (2007) reported a study designed similar to the Hershberger bioassay screen to test the anti-androgenic properties of a series of chemicals. DINP was administered at concentrations of 20, 100 and 500 mg/kg by oral gavage to castrated rats for 10 days. No effects were observed on animal bodyweight, liver weight, kidney weight or adrenal weight. DINP did not induce consistent changes in the androgen sensitive tissues. A significant decrease in seminal vesicle weight was observed in all dose groups while a significant decrease in levator ani/bulbocavernosus muscles (LABC) weight was only observed in the high dose group.

Kwack *et al.* (2009) reported a reduction in sperm count (~25%) in adult males exposed to 500 mg/kg/day DINP for 4-weeks beginning at 28 days of age. The reduction observed in Kwack *et al.* (2009) is of questionable relevance since higher doses of DINP were used in the definitive two-generation reproductive toxicity study where no effects on fertility were reported in males that would have been exposed to DINP for a longer period of time, including both the P and F_1 generations. Fertility is dependent not only on having adequate sperm count, but also on having normal sperm quality. When sperm quality is good (i.e. normal motility as demonstrated in Kwack *et al.* (2009)), then a significant reduction in sperm count is required to affect fertility (Parker, 2006). Furthermore, Kwack *et al.* (2009) did not assess reproductive performance in these animals, critical to the interpretation of their findings.

Adamsson *et al*. (2009) maternal exposure to DINP at 250 or 750 mg/kg on embryonic days (EDs) 13.5–17.5 did not down-regulate the activity of steroidogenesis in ED 19.5 male rat fetus. Protein expression levels of testicular and adrenal StAR, P450scc, 3β-HSD and androgen receptor (AR) did not show any changes. Further no morphological change in the testis was noted. Therefore, no effect on testosterone synthesis, or expression of the genes and proteins associated with testosterone synthesis were observed in this study.

Boberg *et al.* (2011) reported a study in which pregnant rats were exposed to DINP at concentrations of 0, 300, 600, 750 and 900 mg/kg/day between gestation day 7 to post natal day 17. On gestation day 21, at doses ≥ 600 mg/kg/day, DINP produced a significant increase in multinucleated gonocytes in male pups. No effects on plasma testosterone levels, plasma luteinizing hormone levels or testicular testosterone production was observed in male pups on gestation day 21. A significant decrease in testicular testosterone content was only observed in the 600 mg/kg/day dose group. As assessed on post natal day 13, male perinates in the 900 mg/kg/day dose group displayed significantly decreased bodyweights, anogenital distance and anogenital distance normalized to the cubed root of body weight. Perinatal males in the 750 and 900

[32]Interpretation of these studies is uncertain for several reasons: 1) perinatal data was analyzed on a per individual basis versus the more accepted methods that account for the correlations of outcomes among pups from the same litter (Ryan, 1992; Milliken and Johnson, 1994); 2) the numbers of examined pregnant dams and litters are not reported, confounding assessment of the statistical analyses; 3) litters were culled to 8 pups at PND 5, a controversial practice in developmental toxicology (Palmer and Ulbrich, 1997), which may have affected the results, and 4) body weights and food consumption were not reported, making calculation of the dose uncertain, particularly for a developmental study in which body weight and food consumption vary through pregnancy.

mg/kg/day dose group displayed a significant increase in the number of retained nipples. No effects were observed in female perinates at this time point. As assessed on post natal day 22, DINP did not produce any effects on androgen sensitive tissue weights: right testis, left testis, left epididymis, prostrate, LABC, bulbouretral gland or seminal vesicle. Additionally, at this time point, no effects on anogenital distance were observed. On post natal day 90, there were no effects on the histology of the male reproductive organs: seminal vesicles, prostate and testis (including no instances of mononuclear gonocytes). Also, there were no effects on testis testosterone. Sperm parameters were also examined on post natal day 90. Percent of motile sperm, an assessment of sperm quality, was significantly decreased in the 600, 750 and 900 mg/kg/day dose groups. No effects were observed on an additional parameter of sperm quality, velocity. A significant increase in sperm count was observed in the 900 mg/kg/day dose group. The authors concluded that "these data may indicate that DINP does not affect testicular sperm production". In a series of behavioral studies, no effects were noted in motor activity levels in young and adult offspring. Additionally, radial arm maze performances were unaffected in both male and female rats exposed to DINP. Based on these results, the NOAEL for gestation day 21 and post natal day 90 animals was 300 mg/kg/day.

In Vivo Studies – Definitive Two-Generation Reproduction Toxicity Study -- DINP
Waterman *et al.* (2000) describes both a one generation and a two generation reproductive toxicity study.

In the one generation study, groups of 30 male or female Crl:CDBR, VAF Plus rats were administered DINP in the feed at doses of 0, 0.5, 1.0, or 1.5% w/w for 10 weeks prior to mating. The females were exposed throughout mating, gestation, and lactation until post natal day (PND) 21. The males were killed immediately after the mating period. Parental effects included a statistically significant lower mean body weight, as well as suppression in body weight gain, primarily observed in the mid and high-dose groups. The greatest decrease from controls was observed during the postpartum period. Similarly statistically significant lower mean food consumption was observed primarily in the mid and high-dose groups. Statistically significant increases in the mean and absolute and/or mean relative liver and kidney weights of both male and female animals at all dose levels tested were observed. Males in the high dose group exhibited a statistically significant increase in the mean absolute and relative right testis weight, left testis and right epididymis weights and the mean relative left epididymis and seminal vesical weights. High dose females showed a significant decrease in the mean absolute and relative right ovarian and mean absolute left ovarian weights.

No significant differences in male mating, male fertility, female fertility, female fecundity, or female gestational indices were noted. Mean days of gestation were unaffected by treatment as well as the mean sex ratio of the treated offspring when compared with controls. Offspring effects were noted for a number of parameters. The mean live birth index, day 4 survival index, day 14 survival index and lactation index of the high-dose offspring were statistically significantly decreased. Dose related decreases in mean offspring body weight were observed during the postnatal period (PND 0-21). There were statistically significant lower mean body weights in the high-dose males and females, mid dose females at all weighing intervals and in mean offspring body weight of the mid dose males on PND 0, 1, 7, 14 and 21. Statistically significant lower mean body weights in the low-dose males on PND 0, 1, 14, and 21 and low-dose females at all weighing

intervals was also observed. Based on increases in liver and kidney weights from 0.5%, no NOAEL could be determined for parental systemic toxicity. No effect was observed on fertility parameters indicating a reproductive NOAEL of 1000 mg/kg/day; however, a decrease of live birth and survival indices occurred at 1.5% which led to a NOAEL of 1% (622 mg/kg/day for parental males during pre-mating).

A two generation study was designed based on the results of the one generation range finding study. Crl:CDBR VAF Plus rats (30/group) were fed DINP in the diet at 0.2, 0.4, or 0.8% (w/w) for 10 weeks prior to mating, and through gestation and lactation. There were no treatment-related deaths and no clinical signs which were judged to be directly related to treatment with DINP in P1 and P2 animals. During gestation, significantly lower mean food consumption in the P2 high-dose females compared with controls was noted without an associated decrease of the body weight change during gestation days 0-21. During the postpartum period, parental toxicity was limited to a lower mean body weight in the high dose P1 females on post partum days 14 and 21 which corresponded to significant body weight gain suppression during the overall postpartum interval and was associated with decreased mean food consumption. Lower mean body weights were observed in the P2 high-dose females with an associated decrease of mean food consumption but without an associated decrease of the body weight gain. Statistically significant increases in the mean absolute and mean relative liver weights in P1 and P2 in both sexes at 0.4% and 0.8% were observed. Microscopic hepatic changes were noted from 0.2% in P1 and P2 animals. High-dose males exhibited a statistically significant increase of relative right and left epididymal weights in P2 animals with a concurrent increase (not statistically significant) of absolute epididymis weight. *There were no statistically significant differences in male mating, male fertility, female fertility, female fecundity or female gestational indices in P1 generation. A slight decrease, not statistically significant, of male mating, male fertility, female fertility, and female fecundity indices was observed in P2 generation*. Mean days of gestation of the P1/P2 treated and control animals were equivalent. No treatment-related clinical findings and no biologically significant differences in the F1 or F2 offspring survival indices were observed between the treated and control offspring or gross post-mortem findings. There were statistically significant, dose-related, lower mean offspring bodyweights in all treatment groups compared with controls during the F1 or F2 generations. However, when the litter size was taken into account, effects were significant only in high-dose males on PND 0, in males and females of the mid and high-dose levels on PND 7 and 14 and in all treated animals on PND 21. In addition, the weights of all F1 and F2 treated offspring were within the historical control range of the laboratory with the exception of the F2 high-dose males and females on PND 0 and the F2 high-dose males on PND 1 (considering litter size). These findings were considered by the laboratory to be a result of maternal stress and/or direct effects of DINP via exposure through lactation. Studies with other phthalates concluded that these decreases were apparently due to decreased food consumption by the dams and changes in the quality or quantity of milk. Thus the laboratory concluded that the lower body weights in the pups might have resulted from decreased milk consumption.

Based on the microscopic liver changes observed from 0.2%, the NOAEL for parental systemic toxicity is considered to be lower than 0.2% (114 to 395 mg/kg bw/day depending on the period considered). No NOAEL can be derived from this study, but a LOAEL for offspring is 0.2%, emphasizing a trend observed similarly in males and females, based on the dose dependent reduced mean body weights of the treated offspring. The LOAEL is approximate since pups switched diet

from milk to solid food between PND 14 and 21, but may be estimated to be 159 mg/kg/d, the lowest dose of the maternal estimated range (159 - 395 mg/kg/d) during post-partum. No statistically significant differences were observed in reproductive indices indicating a reproductive NOAEL of 0.8% (1000 mg/kg/day). *Together, these robust one- and two-generation study data indicate that DINP does not affect male reproductive development or fertility at doses up to approximately 1000 mg/kg/day (the highest dose tested).*

In vivo Studies - DIDP
Lee and Koo (2007) reported a study designed similar to the Hershberger bioassay screen to test the anti-androgenic properties of a series of chemicals. DIDP was administered at concentrations of 20, 100 and 500 mg/kg by oral gavage to castrated rats for 10 days. No effects were observed on animal bodyweight, liver weight, kidney weight or adrenal weight. DIDP did not induce consistent changes in the androgen sensitive tissues. A significant decrease in seminal vesicle weight and ventral prostrate weight was observed only in the 500 mg/kg dose group. No other effects were reported.

Waterman *et al.* (1999) performed a guideline developmental toxicity study with DIDP conducted at doses of 100, 500, and 1000 mg/kg between gestation days 6-15. Evidence of slight and transient signs of maternal toxicity at 1,000 mg/kg/d (significant reversible decrease of body weight gain and food consumption) was observed; suggesting a conservative NOAEL of 500 mg/kg/d for maternal toxicity. The only statistically significant changes observed in the fetus were skeletal variations (supernumerary cervical and rudimentary lumbar ribs) on a per litter basis in the high dose group. Rudimentary ribs are a common finding in rat fetuses and should not be regarded as associated with malformations since they are likely related to transient maternal stress (Hood and Miller, 2006). It should be noted that supernumerary ribs were located in the cervical region which is less common, but the biological significance of cervical supernumerary ribs is uncertain (Hood and Miller, 2006). A NOAEL of 500 mg/kg/d may be assumed for skeletal variations.

Hellwig *et al.* (1997) administered DIDP by gavage at 0, 40, 200, and 1000 mg/kg/day to 8-10 sperm-positive Wistar females/group on gestation day (GD) 6 through day 15. On GD 20, dams were terminated and uteri removed and examined. All live fetuses were weighed, sexed, and examined externally for morphologic abnormalities. Maternal toxicity at the high dose consisted of reduced food consumption and increased relative liver and kidney weights. There were no treatment-related effects on the number of live fetuses/dam or fetal weight. The only fetal effects were evident at the highest dose by a statistically significant increase in percent fetuses per litter with variations. These variations consisted of rudimentary cervical and/or accessory 14th ribs. A modest increase in dilated renal pelves in the high-dose group was also noted. There were no maternal or fetal effects at 40 or 200 mg/kg/day. The maternal and fetal NOAELs were 200 mg/kg/day. There were no changes observed in fetal morphology or maternal response indicative of endocrine mediated toxicity.

Hushka *et al.* (2001) reported a two-generation study in which four groups of Crl:CDBR, VAF Plus rats (30 rats/sex/group) were administered daily in the diet DIDP (assumed 100% pure) at doses of 0-0.2-0.4 and 0.8%. In addition to the 30 rats/sex/groups, satellite groups of 20 female rats each were treated with the control diet and the high-dose diet during the P_1 generation. Offspring from these animals were utilized in cross-fostering and switched diet experiments to determine if

removal of exposure to DIDP would permit recovery from the expected body weight effects. In the cross fostering study, F_1 generation pups from 10 satellite group 1 (control) litters and 10 satellite group 4 (0.8%) litters were switched on PND 0. In the switched diet satellite study, all the surviving pups of the F_1 generation not selected for the P_2 generation were allowed to become adults. On PND 21, all pups from group 4 were fed control diet and the pups from group 1 were fed group 4 diet. These animals received switched diets for the duration of the P_2 premating period.

Parental toxicity was limited to increased liver and kidney weights in males and females and a statistically significant reduction of body weight gain and/or decreased food consumption in P_1 and P_2 high-dose females during the postpartum period. There were no changes in reproductive organ weight in P_1 or P_2. In P_1 and P_2, there were no statistically significant differences in homogenization resistant spermatid counts, total cauda sperm counts or progressive sperm motility between the treated and control males. In P_1 there were no statistically significant differences in male mating, male fertility, female fertility or female fecundity indices between treated and control animals. *There were no statistically significant treatment-related changes in reproductive organ weights as well as in reproductive indices.* For parental systemic toxicity, based on minor liver changes from the lowest dose, no NOAEL can be determined and a LOAEL of 0.2% (103 to 361 mg/kg bw/d given that received doses are widely dependent on the period considered) can be assumed. No overt signs of reproductive toxicity were reported; therefore the NOAEL for parental reproduction toxicity was 0.8%, the highest dose tested.

There were dose-related decreases in the live birth and Day 4 survival indices (number of live pups at day 4x100/number of live pups at day 0) during the F_1 generation. In the F_2 offspring, reduced survival was again on day 1 and 4 in several groups. It should be noted that in the follow-up two-generation reproduction toxicity study in rat conducted at doses of 0, 0.02, 0.06, 0.2, 0.4% DIDP in diet, the decrease in pup survival was confirmed: a decrease in survival indices (day 1 and day 4) was observed at 0.2% and higher and no effect at the lower doses of 0.02% and 0.06%. In F_1 body weights of the high dose male and female offspring were reduced on PDN 0 (4-6% lower than control); reduced body weight gain continued during the postnatal period (up to 23%) lower than controls, but recovered following weaning (0-7% on day 0 of P_2). In F_2, body weights of the high-dose male and female offspring were reduced on PDN 0 (6-9% lower than control); reduced body weight gain continued during the postnatal period (up to 22% lower than controls). There were no statistically significant differences for preputial separation between treated and control males measured in F_1 offspring. In the females, the mid (33.5 days) and high (34.2 days) dose groups exhibited a statistically significant later maturation for vaginal patency (opening) compared with controls (32.2 days). In F_2, four (out of 123) high-dose male offspring were noted with undescended testes at 21 days. *For offspring survival a decrease in survival indices (day 1 and day 4) from the lowest dose in F_2 generation leads to a LOAEL of 0.2%. For decrease of offspring body weight in F_1 and F_2 generations observed following maternal exposure to 0.8%, a NOAEL of 0.4% (253 to 761 mg/kg bw/d given that received doses are widely dependent on the period considered) can be assigned for developmental effects.*

Satellite studies
In the cross fostering satellite study, offspring born to high-dose dams and cross-fostered to control dams on PND 0 exhibited body weights which were not different from main study control offspring throughout the postnatal phase. Conversely, the mean body weights of the offspring

cross-fostered to the high-dose dams were statistically significant lower (up to 19%) than the main study control offspring of both sexes on PND 14 and 21. This indicates that DIDP may be transferred through the milk but at a low level, evidenced by a low decrease of body weight; a statistical level of significance was obtained when lactation exposure effects and direct toxicity via feed (solid food is absorbed by pups from PND 14) were combined.

In the switched diet phase, weaning from high-dose animals was given control diet, while weaning from control animals was given high-dose diet. The high-dose offspring of both sexes switched to control diet displayed signs of recovery in body weight immediately after weaning and displayed normal growth patterns. However a trend toward lower body weight similar to the main study high-dose males was observed after day 42.

Hushka *et al.* (2001) also reported a follow-up two-generation reproductive toxicity study in which five groups of Crl:CDBR, VAF Plus rats (30 rats/sex/group) were administered daily in the diet DIDP (assumed 100% pure) at doses of 0, 0.02, 0.06, 0.2 and 0.4%.

In the P_1 and P_2 generations, there were statistically significant increases in the mean absolute and relative liver and kidney weights. The majority of P_1 and P_2 animals throughout the groups were free of observable abnormalities at postmortem examination. *There were no statistically significant differences in male mating, male fertility, female fertility, female fecundity, or female gestational indices between treated and control animals in the P_1 or P_2 generation.* For parental systemic toxicity, based on liver and kidney changes in the P_2 males a NOAEL (0.06%) can be determined (33 to 76 mg/kg bw/d, given that received doses are widely dependent on the period considered). Up to the highest dose tested no overt signs of reproductive toxicity were reported and no effect was observed on fertility parameters.

There were no biologically significant differences in F_1 survivorship between the treated and control offspring and all survival indices were within the historical control range. In the F_2 generation, there was a dose-related decrease in the Day 1 and Day 4 survival indices, with statistically significant decreases being observed in the 0.2% dose group (4% and 10%, respectively) and 0.4% dose group (6% and 13%, respectively) compared with controls. There were no treatment-related clinical signs observed in the F_1 or F_2 offspring of any group and the majority of offspring in all groups were free of observable abnormalities from PND 0-21 and during the post weaning periods. *There were no statistically significant differences in F_1 or F_2 offspring mean PND 0 AGD between treated and control animals of either sex. Nipple retention was similar between treated and control offspring of both sexes: the majority of females in all groups had six nipples retained on PND 13/14, while all males in all groups had zero.* In the F_1 animals, there were no statistically significant differences in age or weight at preputial separation between treated and control male offspring. There were no statistically significant differences in age or weight at vaginal patency between treated and control female offspring. For offspring toxicity, a decrease in survival indices (day 1 and day 4) in F_2 generation leads to a NOAEL of 0.06% (33 mg/kg bw/d, lowest estimated dose for 0.06% DIDP in diet). No effect was observed on development landmarks assessed at any dose tested.

Conclusion

In general, the short-term exposure studies are informative and have identified particular endpoints of interest including testosterone synthesis, nipple retention, AGD, and epididymal malformations, but do not invalidate the conclusions from the comprehensive 2-generation reproductive toxicity studies. It has been proposed by Carruthers et al (2005) that there is a critical window of susceptibility for the developing male fetal reproductive system for LMW phthalates in rodent studies (gestation day 16 – 19). This critical window is fully assessed in the 2-generation reproductive studies (Hushka *et al.*, 2001; Waterman *et al.*, 2000). The 2-generation study design assesses the effects of continuous exposure in the F_1 and F_2 generations. A 1-generation and a comprehensive 2-generation reproductive toxicity study have been performed for each of DINP and DIDP. No significant differences in male mating, male fertility, female fertility, female fecundity, or female gestational indices were noted. Mean days of gestation were unaffected by treatment as well as the mean sex ratio of the treated offspring when compared with controls. The parameters of anogenital distance and nipple/areola retention were not specifically part of the test protocol used at the time of the DINP study (not included in the test guidelines in effect at the time of the study); however, they were examined as part of the DIDP experimental protocol. DIDP did not induce nipple retention, affect AGD, induce hypospadias or cryptorchidism or induce gross male reproductive tract malformations (Hushka *et al.*, 2001).

Based on the comprehensive one-generation and two-generation reproductive studies and the developmental toxicity studies conducted on DINP and DIDP, it can be concluded that neither DINP nor DIDP are endocrine disruptors. The adverse health effects mediated via an endocrine mechanism (e.g. cryptorchidism, hypospadias, and significant testicular pathology) which are seen with LMW phthalates in laboratory animals are <u>not</u> seen with DINP or DIDP.

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As referenced in this submission, robust developmental studies consisting of a gavage study using 144 pregnant rats and a dietary study using 100 pregnant rats are being conducted by the Hamner Institutes. These studies were designed to provide strong statistical power for analyzing, collectively, the kinetics and fetal testes effects of DINP and the endpoints attributed to the hypothesized "rat phthalate syndrome." DIDP was not included in this study since its comprehensive two-generation reproductive toxicity study studied endpoints attributed to the hypothesized "rat phthalate syndrome," in which no effects were reported for those endpoints (e.g. nipple retention, AGD, hypospadias, cryptorchidism or gross male reproductive tract malformations (Hushka *et al.*, 2001). The in-life portions of the studies are completed, final analysis is nearing completion, and a report is being prepared. ExxonMobil anticipates that the results from the study will be available to the CHAP in time for incorporation into its report. We ask that the CHAP carefully consider the study results at that time, as these data will be important to the overall weight of the evidence and conclusions for DINP.

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