

Review

# NTP center for the evaluation of risks to human reproduction reports on phthalates: addressing the data gaps

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## Abstract

Between 1998 and 2000 an Expert Panel convened by the National Toxicology Program's Center for the Evaluation of Risks to Human Reproduction (NTP-CERHR) reviewed information related to the developmental and reproductive toxicity of seven phthalate esters; DBP, BBP, DnHP, DEHP, DnOP, DINP, and DIDP. Information on exposures was also considered. The objectives were to determine whether any of these phthalates posed potential human reproductive risks, and if so, to define the circumstances. The Expert Panel also identified some areas of uncertainty. These assessments, ultimately published in 2002, concluded that reproductive risks were minimal to negligible in most cases although some specific uses were considered potentially more problematic. Since the evaluations were completed, numerous studies dealing with both hazard characterization and underlying mechanism have been carried out. Additionally, exposures of the general population have been much better characterized through the use of urinary measurements developed by the Centers for Disease Control (CDC).

This additional information makes several important points. First, calculations based on the urinary metabolite measurements indicate that exposures within the general population are at levels similar to or lower than the estimates used by the NTP-CERHR. The demonstration that exposures were not underestimated by the CERHR has removed a substantial portion of the uncertainty. Second, new hazard characterization studies on several phthalates have established NOAELs similar to or higher than those used by the Expert Panel. Thus, these data demonstrate that, to the extent that the rodent data are useful for human health risk assessment, the no effect levels and dose–response relationships are now more precisely defined. In some cases, the no effect levels may be substantially higher than those estimated by the Expert Panel. Third, studies of underlying mechanism and/or hazard characterization studies in other species suggest that primates may be less sensitive than rodents to the reproductive effects of certain phthalates. Finally, the two specific situations that the CERHR identified as potentially problematic, the exposure of young children to DINP through the use of toys or to DEHP from medical devices, have been assessed by the responsible regulatory authorities. The Consumer Product Safety Commission concluded that exposure to DINP from toys was well below effect levels in animals, and, therefore, there was no risk. The Food and Drug Administration estimates of exposures from medical devices indicated that for some limited, intensive medical procedures, DEHP exposures could be similar to or greater than the NOAELs selected by the NTP-CERHR. However, the FDA also acknowledged that more recent information indicates that the NOAELs identified in rodent studies may be substantially higher than values previously proposed by the NTP-CERHR. In summary, much of the uncertainty identified by the CERHR has now been addressed, and the overall conclusions that levels of concern are minimal to negligible in most situations are much better established. The overall objective of this report is to summarize this new research and comment on its relevance to the NTP-CERHR assessments.

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## 1. Background

In 1999, the Center for the Evaluation of Risks to Human Reproduction (CERHR) of the National Toxicology Program convened an Expert Panel to review developmental and re-

productive toxicology data for seven phthalate esters. The goals were to:

- (1) interpret for and provide to the general public information about the strength of the scientific evidence that a given exposure or exposure circumstance poses a risk to reproduction and the health and welfare of children;
- (2) provide regulatory agencies with objective and scientifically rigorous assessments of reproductive/developmental health effects associated with exposure

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to specific chemicals, including descriptions of any uncertainties associated with the assessment of risks; and

(3) identify important research and testing needs [1,2].

The Expert Panel completed its reviews of di-*n*-butyl phthalate (DBP), butyl benzyl phthalate (BBP), di-*n*-hexyl phthalate (DnHP), di-*n*-octyl phthalate (DnOP), di-2(ethylhexyl) phthalate (DEHP), di-isononyl phthalate (DINP), and di-isodecyl phthalate (DIDP) in October, 2000, although the reports were not published in the peer reviewed literature until the end of 2002 [3–9]. The monographs were not assessments of risk in a classical sense, that being reserved for regulatory agencies. Rather, the Expert Panel expressed “degrees of concern”, based on a semi-quantitative relationship between the No Adverse Effect Levels (NOAELs) in animal (primarily rat) studies, and expected levels of human exposure. For most of the phthalates and most situations, concerns were characterized as negligible or minimal, by which the Expert Panel meant that estimated human exposures were at least three orders of magnitude below the NOAELs from animal studies. However, for some specific uses higher degrees of concern were expressed.

In its identification of research and testing needs, the Expert Panel identified a number of issues, some of which were general, relating to most if not all of the phthalates reviewed, whereas others were related to specific phthalate esters. General issues included better assessments of phthalate exposures, greater understanding of the relevance to humans of the results of rodent studies, and fuller definition of species differences in pharmacokinetics and metabolism. Issues related to specific phthalates included the need for additional hazard assessment studies, work-place exposure data, information on exposures relating to specific substance uses, and resolution of assumptions related to “route-to-route” extrapolations. Since the completion of the Expert Panel review, a number of studies addressing these various data gaps have been completed, and new information relevant both to the conclusions reached by the Expert Panel and the certainty with which these conclusions were expressed is now available. The objectives of this report are to review these recent developments and discuss their relevance to the various phthalate assessments.

Three principal recommendations related to the phthalates reviewed by the Expert Panel were to:

- quantify more precisely the exposures to the general population and to specific subgroups when appropriate,
- conduct hazard characterization studies in rodents, principally two-generation reproductive toxicity studies on principal phthalates, and
- assess the relevance to humans of the rodent data, particularly the effects of phthalates on male reproductive tract development.

The more recent information relating to each of these issues and recommendations will be discussed below.

## 1.1. General issues for phthalate esters

### 1.1.1. Exposure

The potential for phthalate exposure within the general population has been under study for many years. In the past, assessments involved identification of phthalate levels in various media and estimation of exposure as a consequence of contact with these media using both deterministic and probabilistic models. Estimates of this type were used by the Expert Panel to define human exposure for purposes of determining the levels of concern for the various phthalates reviewed. Recently, Clark et al. [10,11] compiled the information on phthalate levels in various media and estimated exposures for several of the major phthalates using probabilistic statistical techniques. Clark’s estimates are similar to those used by the Expert Panel. However, procedures developed by the U.S. Centers for Disease Control and Prevention (CDC) now allow phthalate exposures to be assessed directly in human populations via non-invasive techniques. It has been known for many years that phthalate esters are rapidly converted to their corresponding monoesters and then to the ultimate metabolites that are excreted in the urine (e.g. [12,13]). The procedures developed by the CDC now permit metabolite levels in human urine to be quantified [14]. A pilot study was conducted in which levels of metabolites of seven phthalates, including DBP, BBP, DnOP, DEHP and DINP, were measured in the urine of 289 people [15]. (Two other monoesters, metabolites of di-ethyl phthalate and dicyclohexyl phthalate were also quantified, but as these specific phthalates were not reviewed by the NTP-CERHR, the data are not directly relevant to this review.) The study group, referred to as the reference sample, was not representative of the US population—the age distribution was 20–60 years, it contained 56% women, and it was weighted towards minority groups [16]. Of particular interest to Blount et al. was that the urinary levels of the monoester metabolites of DEHP and DINP, two of the most widely used phthalates, were substantially lower than metabolite levels of lower molecular weight species [15].

These urinary metabolite levels were then used to calculate ambient exposures [17,18]. As shown in Table 1, the calculations yielded exposure estimates generally similar to or lower than those used by the Expert Panel in its assessments. Subsequent studies involving a group of more than 2500 individuals, considered to be representative of the US population [19,20], provided results similar to those of the reference group [15] although urinary levels for the monoesters of BBP and DBP were lower than previously reported values (Table 2).

Although these initial studies provided information on the population at large, the CDC data did not address exposures of young children as the study population contained only individuals 6 years of age and older. This was potentially a significant data gap as young children may have disproportionate exposures due to differences in diets, exposure patterns, and physiological parameters. Further,

Table 1

Estimated exposures ( $\mu\text{g}/\text{kg}$  per day) to the general population based on extrapolated intake from urinary metabolites in 289 individuals as compared to estimates used by the CERHR Expert Panel

Phthalate	CERHR estimate	David [17]		Kohn et al. [18]	
		Mean	95th percentile	Median	95th percentile
DBP ( $\mu\text{g}/\text{kg}$ per day)	2–10 (“significant uncertainty” expressed)	1.6	6.9	1.5	7.2
BBP ( $\mu\text{g}/\text{kg}$ per day)	2 (“low to moderate confidence” expressed)	0.73	3.3	0.88	4.0
DnHP ( $\mu\text{g}/\text{kg}$ per day)	$\leq 3$ –30 (estimate based on DEHP data)	No data	No data	No data	No data
DEHP ( $\mu\text{g}/\text{kg}$ per day)	3–30	0.60	3.0	0.71	3.6
DnOP ( $\mu\text{g}/\text{kg}$ per day)	$\leq 3$ –30 (estimate based on DEHP data)	Not estimated <sup>a</sup>	Not estimated	0.01	1.0
DINP ( $\mu\text{g}/\text{kg}$ per day)	$\leq 3$ –30 (estimate based on DEHP data)	0.21	1.1	Not estimated <sup>a</sup>	1.7
DIDP ( $\mu\text{g}/\text{kg}$ per day)	$\leq 3$ –30 (estimate based on DEHP data)	No data	No data	No data	No data

The methods for calculating exposures are given in the respective papers.

<sup>a</sup> The mean urinary metabolite levels of DnOP and DINP were below the limits of detection at the 50th percentile level. David [17] and Kohn et al. [18] treated these data differently for calculation purposes.

Table 2

Geometric mean exposure estimates (expressed as  $\mu\text{g}/\text{kg}$  per day) for various phthalates in the US population

Phthalate ester	Reference population (289 individuals) <sup>a</sup>	Representative US population (2541 individuals) <sup>b</sup>
BBP ( $\mu\text{g}/\text{kg}$ per day)	0.73 (3.34)	0.43 (2.08)
DBP ( $\mu\text{g}/\text{kg}$ per day)	1.56 (6.87)	0.86 (3.86)
DEHP ( $\mu\text{g}/\text{kg}$ per day)	0.60 (3.05)	0.61 (3.51)
DnOP	<LOD <sup>c</sup>	<LOD
DINP ( $\mu\text{g}/\text{kg}$ per day)	0.21 (1.08)	<LOD (0.73)

<sup>a</sup> Calculated phthalate intake based on the geometric mean values for urinary metabolites [17]. Data are from a 289 person reference population and corrected for creatinine [15]. The 95th percentile values are given in parentheses.

<sup>b</sup> Calculated phthalate intake based on the geometric mean values for urinary metabolites using the method of David [17]. Data are from a population of 2541 individuals, considered to be representative of the US population [20]. The 95th percentile values are given in parentheses.

<sup>c</sup> LOD is the level of detection. For mono-octyl phthalate, the monoester of DnOP, the LOD was 0.9 ng/ml and for mono-isononyl phthalate, the monoester of DINP, the LOD was 0.8 ng/ml [15].

young children were considered by the CERHR Expert Panel to be a potentially susceptible population, based on evidence that some phthalates affect male reproductive tract development in juvenile rodents. Subsequently, a pilot study involving 19 children, averaging approximately 1-year-old,

was conducted [21]. Metabolites of DBP, BBP and DEHP were found in urine, but metabolites of DnOP and DINP were below detection levels. Within this group of children, calculated exposures to the detected phthalates were higher than the adult levels but generally below the estimates used by the NTP-CERHR (Table 3). Thus, the data indicated that young children might be somewhat more highly exposed than adults but do not appear to be grossly different from the general population. Young children have lower body weights than adults and have relatively higher rates of ingestion. Thus, it is possible that children's exposures could also be higher when expressed on a body weight basis. However, the number of individuals examined was small (19); it is unlikely that a representative group was sampled; and the standard deviations associated with the measurements were larger than the means. Further, the DBP data were skewed by a single high value. The authors acknowledged the large variation, and suggested that multiple samples might be required to fully assess the exposures of these individuals. This seems to be the most reasonable way of reducing the uncertainty in these specific measurements. At any event, the exposure levels at the 95th percentile are well below levels of concern established by the EPA [22].

The study of the reference population also suggested that exposures to some of the lower molecular weight phthalates, particularly DBP, were higher in young women specifically

Table 3

Calculated intake of phthalates by infants (~1 year of age) and children (aged 6–11)<sup>a</sup>

Phthalate	Representative US population (2541 individuals 2003) <sup>b</sup>	Infants, approximately 1-year-old (19 individuals) <sup>c</sup>	Children aged 6–11 (328 individuals) <sup>d</sup>
BBP ( $\mu\text{g}/\text{kg}$ per day)	0.43	1.64	0.80
DBP ( $\mu\text{g}/\text{kg}$ per day)	0.86	2.65	0.91
DEHP ( $\mu\text{g}/\text{kg}$ per day)	0.61	2.57	0.57
DnOP	<LOD	<LOD	<LOD
DINP	<LOD	<LOD	<LOD

<sup>a</sup> Calculated phthalate intake based on the geometric mean values for urinary metabolites using the method of David [17].

<sup>b</sup> Data are from a population of 2541 individuals, considered to be representative of the US population [20].

<sup>c</sup> Data are from a group of 19 infants, averaging approximately 1 year of age [21].

<sup>d</sup> Data are from a subset of the 2541 individuals [20] and encompass a group of 328 children aged 6–11.

than in the general population [18], possibly because of their use in personal care products [23]. More specifically, of the 10 individuals with the highest monobutyl phthalate levels (i.e. >300 µg/g creatinine), 9 were women. The highest of these had a urinary metabolite level of 2763 µg/g creatinine, which corresponds to an estimated exposure of 113 µg/kg per day [18].

The value of 113 µg/kg per day has been carried forward into assessments by several authors who have questioned whether a sufficient margin of exposure exists to assure safety [24,25]. However, inasmuch as those data came from a single individual in a pilot study, it is important to determine the extent to which they reflect the US population. In general the urinary metabolite levels measured in subsequent studies of representative populations [19,20] were lower than those in the reference group [26]. The CDC also conducted a demographic analysis and separated the data by gender. This analysis provided some evidence that women had higher levels of urinary monobutyl phthalate than men with overall differences of approximately a factor of 2 [20]. The 95th percentile level in all women (131 µg/g creatinine) corresponded to an external exposure of 5.2 µg/kg per day, within the range of 2–10 µg/kg per day which the Expert Panel considered to be of minimal to negligible concern [4] and also well below the EPA reference dose of 100 µg/kg per day [22]. In another study which quantified monobutyl phthalate metabolite levels in the urine of a group of 35–39-year-old African-American women, Hoppin et al. [27] found mean concentrations of 52.7 µg/g creatinine with a maximum of 157.3. These values corresponded to external exposures of 1.7 and 6.2 µg/kg per day, respectively (Table 4). As a final point, Brock et al. [28] reported that preliminary evidence indicated that phthalate metabolite excretion

by pregnant women was at levels similar to or lower than the reference population. In summary, it is possible that, as a group, women exhibit a slightly greater exposure to DBP than men. However, the more recent studies have not replicated the extreme urinary metabolite levels found in the pilot study. The most recent report from CDC [26] reported that urinary metabolite levels for low molecular weight phthalates are all lower than initially reported with median levels of monobutyl phthalate specifically being approximately half the values reported by Blount et al. [15] from the pilot study. If there are individuals as highly exposed as suggested by the pilot study, they must be rare. Perhaps the pilot study contained some individuals with unusual exposures; alternatively, there may have been something unusual about some of the samples. Both of these possibilities merit additional consideration. Further studies to assess sources of exposure are ongoing (e.g. [23]) and may provide additional information on this point. Additionally, whether or not pregnant women (as opposed to the “women of child-bearing age” identified in the reference study [15]) are more highly exposed than the general population remains unclear but should be a priority for further work, since male reproductive development in humans occurs primarily during the gestational period [29].

The CDC studies provide very detailed information on the exposure to phthalates in the general population. Of particular note is that the data indicated that phthalate exposures were similar to or lower than estimates of calculated intakes previously derived from phthalate levels in various media. This confirms that there are no unidentified sources of phthalates that contribute significantly to exposures of the general population. Additionally, the urinary metabolite data indicate that phthalate exposures within the population at large are similar to or lower than estimates used by the CERHR

Table 4  
Calculated intake of phthalates by women (given in µg/kg per day)

Phthalate	All individuals in the representative US population <sup>a</sup>	Women in the reference population <sup>b</sup>	Women in the representative US population <sup>c</sup>	African-American women <sup>d</sup>
BBP	Geometric mean = 0.43 µg/kg per day	Median = 1.2 µg/kg per day; 95th percentile = 4.5 µg/kg per day	Median = 0.56 µg/kg per day; 95th percentile = 2.91 µg/kg per day	Geometric mean = 0.79 µg/kg per day; maximum = 4.35 µg/kg per day
DBP	Geometric mean = 0.86 µg/kg per day	Median = 1.7 µg/kg per day; 95th percentile = 32 µg/kg per day	Median = 1.12 µg/kg per day; 95th percentile = 5.15 µg/kg per day	Median = 1.71 µg/kg per day; maximum = 6.18 µg/kg per day
DEHP	Geometric mean = 0.61 µg/kg per day	Median = 0.71 µg/kg per day; 95th percentile = 3.8 µg/kg per day	Median = 0.67 µg/kg per day; 95th percentile = 3.27 µg/kg per day	Median = 1.28 µg/kg per day; maximum = 15.49 µg/kg per day
DnOP	Median ≤ LOD	Median ≤ LOD; 95th percentile = 0.65 µg/kg per day	Median ≤ LOD; 95th percentile = 0.62 µg/kg per day	Median = 0.06 µg/kg per day; maximum = 15.59 µg/kg per day
DINP	Median ≤ LOD	Median ≤ LOD; 95th percentile = 3.7 µg/kg per day	Median ≤ LOD; 95th percentile = 0.68 µg/kg per day	Median = 0.73 µg/kg per day; maximum = 26.85 µg/kg per day

<sup>a</sup>Calculated phthalate intake based on the geometric mean values for urinary metabolites using the method of David [17]. Data are from a population of 2541 individuals, considered to be representative of the US population [20].

<sup>b</sup>Data from Kohn et al. [18]. Reported as women of childbearing age. Approximately 289 individuals.

<sup>c</sup>Data from CDC [20]. The calculations utilized the method of David [17].

<sup>d</sup>Data are from a population of 46 African-American women [27]. The calculations utilized the method of David [17].

Expert Panel in its assessments. Thus, these more precise exposure estimates provide additional confidence that the overall conclusions by the Expert Panel of minimal to negligible concerns for the general population are reasonable and well supported. The CDC plans to continue to measure the levels of urinary metabolites for the foreseeable future, providing information that may be useful to assess exposure trends. It may also be possible to glean additional insights from more detailed demographic analysis of the data [16,20].

### 1.1.2. Pharmacokinetics and metabolism

There are species differences in pharmacokinetics and metabolism relating at least to absorption and specific target organ doses. Rodents efficiently convert orally administered phthalates to the corresponding monoesters, the forms in which they are rapidly absorbed (e.g. [12,13]). Across a wide range of doses, at least 50% of orally administered DEHP is absorbed by rats [30,31]. In contrast, absorption by primates of high molecular weight phthalates is more limited. Based on a study in which marmosets were given 2500 mg/kg per day, it was estimated that the maximum internal dose which could be achieved was similar to levels in rodents given 100–200 mg/kg per day DEHP [31]. Similar data were obtained in studies in cynomolgus monkeys [30]. In a more recent study, at doses ranging from 30 to 500 mg/kg per day, absorption by rats was greater than that in marmosets with differences being approximately two- to three-fold based on peak blood levels and approximately seven-fold based on the area under the curve [32]. Further studies showed that primates excrete phthalates in the bile to a much greater extent than do rodents, and, therefore, much of what is absorbed in primates may not be distributed to the target organs identified from rodent studies [32,33]. Consequently, at equivalent external exposure levels, target organ (i.e. testicular) doses in rodents may be significantly higher than in primates.

Differences in absorption between rodents and primates at high exposure levels have been documented for many years [30,31], but recent data also suggest that there are differences at levels approximating ambient exposures. Based on urinary excretion data, initial volunteer studies suggested that humans absorbed relatively lower amounts than rodents when given doses in the range of 10–30 mg DEHP [34]. In more recent studies, volunteers were given phthalates at levels approximating ambient exposures. The data indicated that humans absorbed 65–80% of monobutyl phthalate (given as DBP) and monobenzyl phthalate (from BBP, in humans monobenzyl phthalate is the preferred metabolite of BBP with only 6% being converted to MBP) but only 12–14% of the corresponding monoesters of either DEHP or DnOP [35]. Thus, at least for high molecular weight phthalates (i.e. >C8), the amount of monoester absorbed by humans is significantly lower than that absorbed by rodents, even at phthalate exposures in the  $\mu\text{g}/\text{kg}$  range. Consequently, at least for high molecular weight phthalates, humans experi-

ence lower internal doses and lower target organ doses than rodents at equivalent external exposure levels. Further, the relatively large internal doses of high molecular weight phthalates associated with some of the rodent effects may not be achievable in humans under any circumstances.

### 1.1.3. Relevance of rodent data to humans

The last of the general issues identified by the CERHR Expert Panel was the relevance of rodent findings to humans. This is particularly important in light of reports that some phthalates had profound effects on the development of the reproductive tract in male offspring when given to dams at the end of the gestational period (e.g. [36–39]). Others have extended these observations (e.g. [40–42]), showing that the late gestational period is a sensitive window for rodents. There have been questions about the relevance to humans of results of phthalate studies in rodents for many years. Nevertheless, the Expert Panel took the position that, in the absence of definitive information to the contrary, the rodent data would be assumed to be relevant to humans and appropriate for use in risk evaluation.

The Expert Panel was aware that phthalates induced testicular atrophy in rodents (e.g. [43–45]) but not in primates [46–48], suggesting the possibility of species-related differences. However, there was some uncertainty as to whether the primates had been exposed prior to achieving sexual maturity. Consequently, the Expert Panel considered the evidence for species-specificity to be inconclusive and listed studies of the effects of phthalate treatment during the juvenile phase on male sexual development in non-human primates as a critical data need.

To respond to that need, a study of the effects of repeated DEHP treatment on the development of the male reproductive tract in the marmoset monkey (*Callithrix jacchus*) was undertaken [49]. This species was chosen, in part, because it has been shown to be a good model for human sexual development [50]. Treatment was initiated when the marmosets were approximately 100 days of age (weaning), the earliest time at which treatments by gavage were feasible in these animals. The animals were given 100, 500 or 2500 mg/kg per day on a daily basis for 65 weeks, until approximately 18 months of age. This exposure period covered the juvenile period as marmosets reach sexual maturity at approximately 400–450 days [51]. At the end of the treatment period, the animals were sacrificed and examined. Of those animals completing the treatment period, six males per group were sacrificed for gross examination, and three males per group were perfused with 0.1 M *S*-collidine, 2% paraformaldehyde, 3% glutaraldehyde. The examinations included gross and histologic evaluation of principal organs. The testes and accessory organs were subjected to light and electron microscopic examination, and measurements of hormone levels and sperm counts were carried out. As shown in Figs. 1 and 2, DEHP treatment had no significant effects on liver weights or testicular weights in the marmosets whereas

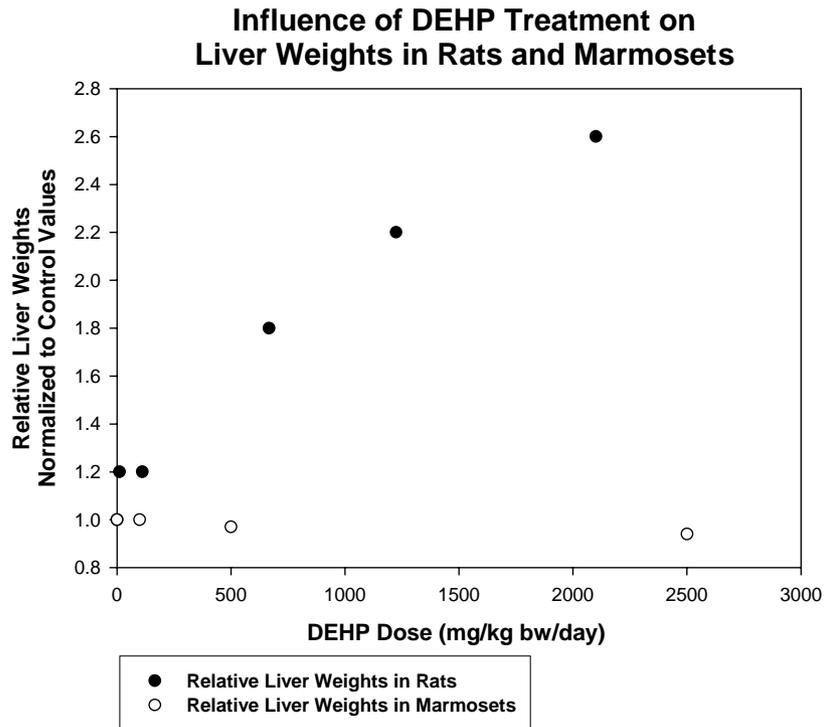


Fig. 1. The liver weight data for rats (○) were taken from 21 days studies in juvenile male rats [52]. The liver weight data for marmosets (●) were taken from a 65-week juvenile marmoset study [49]. The data were normalized as percent of the respective control values.

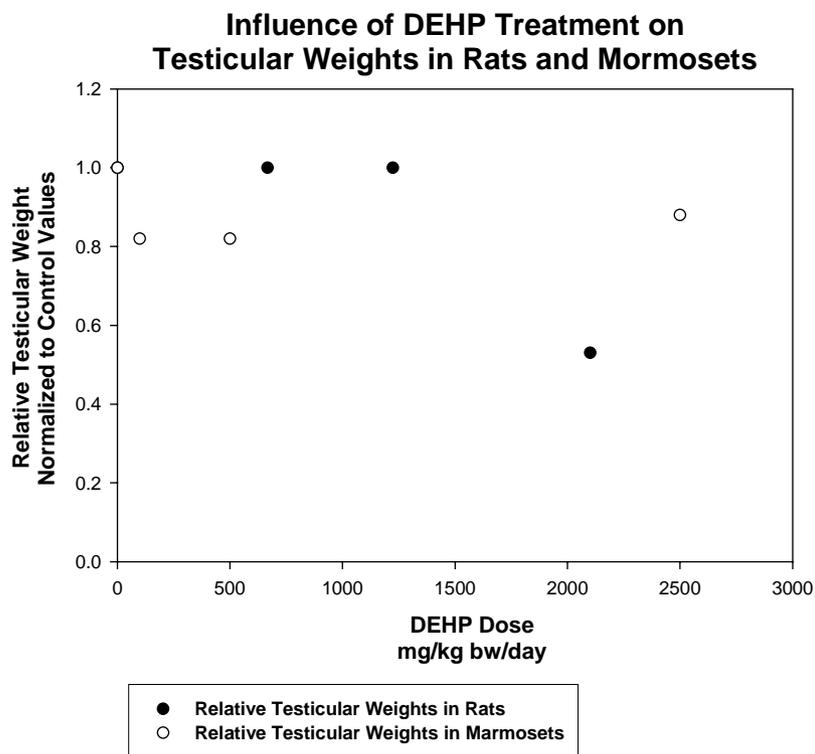


Fig. 2. The testes weight data for rats (○) were taken from 21 days studies in juvenile male rats [52]. The testes weight data for marmosets (●) were taken from a 65-week juvenile marmoset study [49]. The data were normalized as percent of the respective control values.

weights of these organs were significantly affected in rodents at equivalent doses. Weights of the other accessory male reproductive organs in marmosets were similarly unaffected by treatment. The microscopic evaluations did not reveal any testicular lesions, and there were no differences in sperm counts [49]. Thus, this study demonstrated that daily administration of DEHP during the juvenile period did not affect male reproductive tract development in the marmoset.

#### 1.1.4. Mechanisms of phthalate-mediated effects on male reproductive development in rodents

Although not specifically identified as a data need, the mechanism(s) underlying the male reproductive development is obviously also very important. Substantial progress has been made in understanding the mode of action, although there is still much to learn. One report suggested that some phthalates might interact with androgen receptors [53]. However, further studies indicated that the effects of phthalates are not receptor-mediated (e.g. [54–59]). To further test whether phthalates were capable of producing androgen receptor-mediated effects, all of the commercially important phthalates and their corresponding monoesters were tested for agonist and antagonist effects on the androgen receptor. These tests were performed in the yeast human androgen receptor assay [60] and the HepG2 AR Reporter Gene Assay [61]. As shown in Table 5, negative results were produced in all tests at levels up to  $10^{-5}$  M, the highest concentration tested. These data provide further evidence that those phthalates that affect male reproductive development in rodents do so by processes that do not involve receptor interactions.

Evidence is emerging that the testicular effects of some phthalates may be a consequence of reduced testosterone biosynthesis [57,63–65] and that the effects may differ depending on the point of male reproductive development at which exposure occurs. Exposure during the late gestational and early lactational periods, the time at which testicular development occurs in rats, results in structural malformations in the male reproductive tract whereas exposure during the period of sexual maturation produces testicular atrophy. The consequences also differ as exposure during development may lead to permanent changes whereas the effects of later exposures seem reversible [66].

Of particular importance is the issue of possible species differences, more specifically, would one expect humans to respond in the same way as rodents, and, if so, would they be more or less sensitive? As indicated above, there are pharmacokinetic differences providing evidence that, at equivalent external exposures levels, humans have significantly lower internal doses than rodents. There are also differences between rodents and humans related to timing of sexual development. In rodents, the principal developmental events occur at the end of the gestational cycle whereas in humans much of male sexual development takes place during the first trimester (e.g. [29]). Additionally, there may be species differences in reversibility of effect. Sharpe and co-workers [50,67] has reported that an experimentally-induced reduction in Sertoli cell number in the neonatal period is permanent in rats but reversible in primates.

Additionally, pharmacodynamic differences between humans and rodents may also be important. Based on a study that compared wild-type versus PPAR $\alpha$ -null mice, Ward et al. [68] concluded that the “results provide evidence that PPAR $\alpha$ -dependent processes played a role in the testicular effects but that PPAR $\alpha$ -independent processes were also involved”. (This point is discussed in more detail in the section on DEHP.) Available data suggest at least four processes that could influence testosterone levels including: (a) cholesterol mobilization; (b) cholesterol uptake by Leydig cells; (c) androgen biosynthesis; and (4) androgen metabolism. PPAR $\alpha$  activation apparently plays a role in several but perhaps not all of these steps. For example, phthalates and other peroxisomal proliferating agents may inhibit cholesterol mobilization as a consequence of their hypolipidemic effects and may also reduce cholesterol uptake [64]. There are other aspects of cholesterol uptake and androgen biosynthesis that may be inhibited by some phthalates [63–65] by processes that may be unrelated to PPAR $\alpha$  induction. However, PPAR $\alpha$  activation also appears to stimulate aromatase activity in rodent liver, and this may affect the balance between testosterone and  $\beta$ -estradiol [69]. The extent to which PPAR $\alpha$  induction is involved in the production of testicular tract malformations in rodents is very pertinent to the overall assessment of human risk and certainly merits further study.

Table 5  
Interaction of selected phthalate diesters and monoesters with the human androgen receptor<sup>a</sup>

Phthalate	Yeast AR reporter assay	HepG2 AR reporter assay	Monoester (phthalate)	Yeast AR reporter assay	HepG2 AR reporter assay
Diethyl phthalate	Negative	Negative	Monoethyl-	Negative	Negative
Butyl benzyl phthalate	Negative	Negative	Monobenzyl-	Negative	Negative
Di-isohexyl phthalate	Negative	Negative	Monoisohexyl-	Negative	Negative
Di-isooheptyl phthalate	Negative	Negative	Monoisooheptyl-	Negative	Negative
Di- <i>n</i> -octyl phthalate	Negative	Negative	Mono- <i>n</i> -octyl-	Negative	Negative
Di-isononyl phthalate	Negative	Negative	Monoisononyl-	Negative	Negative
Di-isodecyl phthalate	Negative	Negative	Monoisodecyl-	Negative	Negative

<sup>a</sup> As indicated in the text, the phthalates and monoesters were tested in both the yeast and the HepG2 androgen receptor assays. The substances were tested over a range of concentrations with  $10^{-5}$  M being the highest. This procedure was used to assure consistency with a previous study of estrogen receptor binding [62].

### 1.1.5. Assessment of “levels of concern”

As noted above, the Expert Panel expressed its conclusions in terms of “levels of concern”, with minimal or negligible concern corresponding to a margin of exposure (between estimated exposure and the animal NOAEL) of 1000 or more. The use of margins of exposure as a means of defining concern (or risk) also merits comment. It is important to remember that the use of margins of exposure is a regulatory convention developed to provide ample margins of safety when information on human sensitivity relative to animal sensitivity is lacking. The typical default assumptions are that the average human may be as much as an order of magnitude more sensitive than a rodent (intra-species factor), and the most sensitive human may be as much as an order of magnitude more sensitive than the average (interspecies factor). Additional “safety” or “uncertainty” factors, usually factors of 3 or 10, are sometimes added to account for other uncertainties, typically the absence of developmental and/or reproductive toxicity studies. For the major phthalates assessed by the Expert Panel, the animal data are extensive and human exposures are quite well defined. Pharmacokinetic and pharmacodynamic evidence (some discussed under phthalate-specific issues) from studies with humans and non-human primates indicate that rodents are likely to be more sensitive to phthalate-induced effects than humans, so the use of a full interspecies factor of 10 is actually quite conservative. This information adds additional confidence to the degrees of concern expressed by the Expert Panel in most situations. Given that general human exposures are well below the no effect levels in rodents, potential human risk for developmental and reproductive toxicity from phthalates at environmental exposure levels is highly unlikely.

## 2. Substance-specific issues

### 2.1. BBP

The Expert Panel identified two specific needs, a database sufficient to characterize hazards and a better definition of exposure. More specifically, the Expert Panel stated that “[T]here is not an adequate database to determine NOAELs/LOAELs for male or female reproductive effects from perinatal exposure” and recommended multi-generation reproductive toxicity studies with “endocrine-sensitive” endpoints [3]. As described in more detail below, two reproductive toxicity studies in rats have been conducted. The Expert Panel relied principally on exposure estimates from the International Program on Chemical Safety (IPCS) of approximately 2  $\mu\text{g}/\text{kg}$  bw per day. There were also data on BBP levels in food from the UK Ministry of Agriculture, Fisheries and Food (MAFF) that the Expert Panel converted to estimates of 0.11–0.29  $\mu\text{g}/\text{kg}$  per day. The Expert Panel expressed some uncertainty in these numbers in part because they were based on measurements on BBP in food and did not consider exposure from other

sources and because there was considerable variation in the estimates. As shown in Table 2, mean exposures to BBP in the US population are less than 1  $\mu\text{g}/\text{kg}$  per day, with 95th percentile values in the range of 2–3  $\mu\text{g}/\text{kg}$  per day. Thus, the exposure estimate from IPCS, on which the Expert Panel ultimately relied, seems quite reasonable. The analysis from Clark et al. [11] indicated that for most segments of the population, food constitutes >90% of the dose. Thus, with respect to exposure assessment, the identified needs have now been satisfied.

#### 2.1.1. Hazard characterization studies of BBP

In one of the two recently completed studies [70], BBP was given to SD rats in daily oral gavage doses of 20, 100, and 500 mg/kg per day. There were no significant effects on mating index (number copulated/number cohabiting), fertility index (number pregnant/number copulated), gestation length, or delivery index (number delivered/number pregnant) at any treatment level. Body weights of high-dose FO parental males were significantly reduced, but there were no significant changes in weights of any of the reproductive organs and no apparent histological differences. There were also no effects on sperm parameters. Levels of testosterone and T<sub>4</sub> were reduced whereas FSH and prolactin levels were elevated in the high-dose group. In the F<sub>1</sub> generation there were no statistically significant effects on number of fetuses born, live births, sex ratio or viability during the lactational period. PND 0 weights were significantly reduced in the mid- and high-dose groups, and offspring weight gains were significantly reduced in the high-dose group.

There was a small (but statistically significant) reduction in anogenital distance (AGD) in high-dose group males. Among offspring sacrificed at the end of weaning, there were body weight reductions in both sexes from the high-dose group, significant reductions in testicular and ovarian weights, and an increase in uterine weight. FSH and TSH levels were reduced in high-dose group F<sub>1</sub> males (TSH was also reduced in mid-dose F<sub>1</sub> males), but there were no apparent effects on testosterone, T<sub>4</sub>, or prolactin levels. Histopathological evaluation revealed testicular abnormalities in high-dose group males but no apparent effects in females. There was a small but statistically significant increase in the age at preputial separation among high-dose group males, but no effects among females on age at vaginal opening or estrous cyclicity. Mating parameters were unaffected. Terminal sacrifice revealed reduced body weights in males from the 100 and 500 mg/kg per day groups, as well as reductions in gross testis and epididymis weights, but the organ weight differences were not statistically significant when expressed as fraction of body weight. There were no significant body or organ weight changes in females. There were some changes in hormone parameters in the males but no effects on sperm parameters and no changes in hormone levels in females. The pathological investigation revealed some testicular abnormalities in the 500 mg/kg per day group males but no effects in males from lower groups and no effects on

females. There were no effects on F<sub>2</sub> offspring. In summary, daily doses of up to 500 mg/kg had no apparent effects on classical reproductive parameters. There were some body weight and testicular effects in the high-dose males, but no effects in females. There were very minimal effects at the 100 mg/kg per day level including elevated kidney weights but without histopathological changes, inconsistent changes in hormonal levels, and a reduction in F<sub>1</sub> PND 0 weight that may have been confounded by litter size effects and was not replicated in the second generation. The authors considered 20 mg/kg per day to be the overall NOAEL with 100 mg/kg per day as the LOAEL.

A second two-generation reproductive toxicity study of BBP in Sprague–Dawley rats also used dietary administration [71]. The study design incorporated all of the requirements of U.S. EPA OPPTS testing guidelines for reproductive toxicity assessment as well as the specific enhancements listed below. In addition, the study was performed and reported in compliance with U.S. EPA Good Laboratory Practice standards. Study features which went beyond the OPPTS guideline requirements included:

- (1) measurement of AGD and body weight for all live F<sub>1</sub> and F<sub>2</sub> offspring at birth on PND 0;
- (2) standardization of F<sub>1</sub> and F<sub>2</sub> litters to 10 pups (with as even a sex ratio as possible) on PND 4 to minimize the potential confounding effects of litter size on offspring survival and growth during lactation. All culled pups on PND 4 were subjected to an external and visceral examination, and special attention was paid to the male reproductive organs;
- (3) examination of all F<sub>1</sub> and F<sub>2</sub> male preweanling pups on PND 11–13 for the presence of retained nipples and/or areolae;
- (4) expansion of the necropsy at weaning on PND 21, in addition to the required necropsy of three pups/sex/litter, all pups sacrificed at that time were necropsied, with special attention paid to the male reproductive organs;
- (5) sperm analysis including epididymal sperm number, motility, and morphology; enumeration of testicular homogenization-resistant spermatid heads for calculation of daily sperm production (DSP); and efficiency of DSP in all F<sub>0</sub> and F<sub>1</sub> adult males at scheduled necropsy;
- (6) additional histopathological examination of F<sub>0</sub> and F<sub>1</sub> adult males in all groups which exhibited gross lesions or did not sire live litters (also F<sub>0</sub> and F<sub>1</sub> females if they did not produce live litters), and/or if there was evidence of potential treatment-related histopathologic findings in any organs at the high dose.

Thirty animals per sex per dose level received 0, 750, 3750 or 11,250 ppm BBP in their feed for two generations, one litter per generation. The target dietary doses equated to approximately 0, 50 or 250 mg/kg per day in the control, low- and mid-dose groups, respectively. The high-dose of 11,250 ppm was equivalent to a daily intake of about 750 mg/kg BBP, a dose reported by Gray et al. [40] to cause

very high incidences of male reproductive system malformations in rats from gavage exposure to the dam on GD 14 through PND 3. Signs of systemic toxicity were observed in high-dose parental animals. F<sub>1</sub> but not F<sub>0</sub> high-dose males exhibited reduced body weight gain throughout the entire pre-breed and mating periods. High-dose F<sub>0</sub> and F<sub>1</sub> females exhibited reduced body weights throughout the study. F<sub>0</sub> and F<sub>1</sub> males and females exhibited increased absolute and relative liver weights and increased relative liver weights in F<sub>1</sub> males at 11,250 ppm. This was also seen in F<sub>1</sub> males at 3750 ppm. The increased liver weight was probably due to hepatic peroxisome proliferation since the phthalates, including BBP [72,73], are known inducers of proliferation of peroxisomes in the rodent liver. The observation of histopathologic lesions in the liver supported but did not confirm induction of liver peroxisomes.

There were no effects on reproductive status or functions in F<sub>0</sub> males or females at any dietary dose. In the F<sub>1</sub> generation, mating and fertility indices were reduced in the high-dose group. Among F<sub>1</sub> males, reduced absolute (but not relative) weights of testes, epididymides, and seminal vesicles/coagulating gland, and reduced absolute and relative prostate weights were observed in the high-dose group. Also reduced in the high-dose group were epididymal sperm concentration, motility, and progressive motility. Increased gross and histopathologic findings were reported for the testis and epididymis of the high-dose group. F<sub>1</sub> females from the high-dose group exhibited reduced uterine implantation sites, and reductions in total and live pups per litter on PND 0 (with no increase in dead pups per litter). There was also evidence of increased absolute and relative uterine weights, but with no histopathologic lesions in female reproductive organs. High-dose animals exhibited reduced ovarian weights but these occurred in the absence of any effects on ovarian primordial follicle counts at this dietary dose. Body weights per litter (sexes combined) of F<sub>1</sub> and F<sub>2</sub> offspring during lactation exhibited significant reductions. At necropsy, both F<sub>1</sub> male and female weanlings at 11,250 ppm exhibited reduced terminal body weights, reduced absolute (but not relative) thymus weights, reduced absolute and relative spleen weights, and reduced absolute and increased relative brain weights. F<sub>1</sub> male weanlings also exhibited reduced absolute and relative testes weights at 11,250 ppm and decreased absolute epididymal weights, with relative epididymal weights unaffected. F<sub>1</sub> weanling females exhibited reduced absolute ovarian and uterine weights, with relative weights of both organs unaffected. Male F<sub>1</sub> and F<sub>2</sub> pups from the high-dose group exhibited reduced AGD at birth and delayed acquisition of puberty (in F<sub>1</sub> males and females), retention of nipples and areolae and male reproductive system malformations. F<sub>1</sub> males from the mid-dose group were observed to have shortened AGD at PND 0.

High-dose F<sub>2</sub> males and females at weaning exhibited reduced terminal body weights, reduced absolute (but not relative) thymus weights, reduced absolute and relative spleen weights, and increased relative (with no effect on absolute)

brain weights. The F<sub>2</sub> males also exhibited reduced absolute and relative testes weights but no effects on absolute or relative epididymal weights, and an increased incidence of gross findings in the male reproductive organs, all at 11,250 ppm only. AGD was significantly reduced in mid and high-dose male offspring, and areolae retention was significantly increased in high-dose males. F<sub>2</sub> female weanlings exhibited reduced absolute (with no effect on relative) ovarian weights at 11,250 ppm and increased absolute uterine weight (with no effect on relative uterine weight) at 3750 ppm, with no effects on uterine weight at 11,250 ppm. There were no treatment-related gross findings in the female weanlings.

The no observable adverse effect level (NOAEL) for reproductive effects was 3750 ppm (~250 mg/kg per day). The NOAEL for developmental toxicity was 3750 ppm (~250 mg/kg per day), and the no observable effect level (NOEL) was 750 ppm (~50 mg/kg per day), based on the reduced AGD in F<sub>1</sub> and F<sub>2</sub> males at birth at 3750 ppm. There were no effects on reproductive development, structures, or functions at the 750 ppm (50 mg/kg per day) level.

As mentioned above, Gray et al. [40] reported that BBP given by oral administration to pregnant Sprague–Dawley rats at 750 mg/kg per day from gestational day 14 to postnatal day 3 produced a number of developmental effects including a significant reduction in mean birth weight and a significant increase in males exhibiting incomplete preputial separation. There were significant reductions in weights of testes and accessory organs and weights of levator ani plus bulbocavernosus (LABC) muscles. There was a significant increase in nipples per male, and a significant reduction in AGD. There were also several animals with testicular malformations of various kinds. These data provided evidence that, at high doses, BBP can produce testicular effects in rats. The effects seem similar to those of DBP (discussed in the next section), but that is not surprising as, in rats, BBP is metabolized primarily to monobutyl phthalate [74]. In contrast, in humans, BBP is predominantly metabolized to the monobenzyl metabolite [35]. Nevertheless, the Gray data have more utility in defining mechanism than in assessing risk as only a single treatment level, much higher than those used in other studies, was evaluated, and it provided no information on dose–response relationships.

In summary, the data now available address the questions raised by the Expert Panel and provide sufficient information to better define the degree of concern and substantially increase the level of confidence in the overall assessment. There were no apparent effects on female rats in either of the multi-generation studies, making the NOAEL for reproductive effects in female rats >750 mg/kg per day. NOAEL's of 20 mg/kg per day for male reproductive effects have been independently reported by NTP [75] and Nagao et al. [70]. However, the 1997 NTP report that BBP decreased caudal epididymal spermatozoa concentration in a 10-week feeding study could not be replicated by NTP in a 26-week feeding study conducted in the same strain of rat (F344/N) at higher dose levels than those used in the 10-week study.

The NOAEL of 20 mg/kg per day proposed by Nagao derive mainly from the observation of reduced F<sub>1</sub> offspring PND 0 body weight at 100 mg/kg per day—a finding that was not observed in the F<sub>2</sub> generation of that study or replicated by Tyl et al. [71]. Additionally, there were more offspring in the 100 mg/kg per day group, and this may have also contributed to the body weight differences. The most comprehensive study of BBP reproductive toxicity is the study of Tyl et al. [71] which established an F<sub>0</sub> and F<sub>1</sub> parental systemic and F<sub>1</sub> reproductive no observable adverse effect level (NOAEL) of 3750 ppm (~250 mg/kg per day). The offspring toxicity NOAEL derived from that study was 3750 ppm (~250 mg/kg per day), and the offspring toxicity no observable effect level (NOEL) was 750 ppm (~50 mg/kg per day), based on the reduced AGD in F<sub>1</sub> and F<sub>2</sub> males at birth at 3750 ppm, with no effects on reproductive development, structures, or functions at that dietary dose. From these studies, the reproductive NOAEL for BBP should be no lower than 50 mg/kg per day. Piersma et al. [76], used benchmark dose techniques to estimate 95 mg/kg per day as the dose associated with a 1% increase in abnormal testis location, the most sensitive indicator of the development of the male reproductive tract. As mean exposures to BBP in the general population are less than 1 µg/kg per day, the margin of exposure is ≥50,000. The Expert Panel had determined a “negligible concern” for male reproductive effects from adult exposure, but they were unable to ascribe a level of concern for the postnatal consequences of BBP exposure. However, now that the data addressing concerns expressed by the Expert Panel have been provided, it would seem reasonable, based on the wide margins of exposure, to now conclude “negligible concern” for postnatal consequences as well.

## 2.2. DBP

According to the Expert Panel, reproductive toxicity and male reproductive development were adequately assessed. Areas for further work included assessing the potential effects of DBP on female rats and non-rodent species and defining the window of sensitivity for effects on male reproductive tracts in rats [4]. There was also a recommendation to extend the current PBPK model to include parameters for pregnant women and fetuses. As described below, several in utero and multi-generation studies have now been conducted which address most if not all of the concerns expressed by the Expert Panel. Additionally, DBP exposure within the general population has been much more precisely defined; however, there may still be some questions relating to the extent of and sources for exposure of young women (as described above). There are efforts ongoing to extend the current PBPK models to pregnant women and fetuses, but to date this work has not advanced to the publication stage. As for studies of the effects of DBP in non-rodent species, the strategy followed was to test DEHP first, as described elsewhere in this report. As noted by the Expert Panel,

the results of such studies on DEHP are likely applicable to DBP.

With respect to female rats, the Expert Panel noted that “Adult female functional reproductive toxicity (decreases in fertility) has been noted in rats; however, the data do not permit confident characterization of dose effects below 250 mg/kg bw per day [4]”. Further investigation of the dose-related effects of DBP on female rats was recommended. A recent two-generation reproduction study in Sprague–Dawley rats assessed the effects of dietary administration of DBP [77]. Test material was administered in the diet at levels of 1, 4, 10, 100, 1000 and 10,000 ppm (0.1, 0.2, 1.7, 6, 60 and 600 mg/kg per day). There were small but statistically significant effects on AGD, preputial separation, and testicular descent in males from the 10,000 ppm group. There were also decreases in testosterone and 5- $\alpha$ -androstane-3- $\alpha$ , 17 $\beta$ -diol levels in the 10,000 ppm male fetuses. However, there were no effects in females in any dose group, and no effects on males receiving less than 10,000 ppm. Thus, the overall NOAELs, defined by this study were Male:60 Female:600 mg/kg bw per day. In a parallel study rats (Sprague–Dawley and Wistar) were given 600 mg/kg per day by oral gavage. The effects observed appeared to be more profound than those associated with dietary administration, suggesting that dose administration rate has an important influence on the magnitude of the effects observed [77] and on the NOAEL used to compare to human exposures (which are predominantly dietary). There are similar data from Ema et al. [78] who evaluated anti-androgenic effects in male offspring exposed in utero to dietary levels of 100, 330, or 660 mg DBP/kg per day. The NOEL identified by Ema et al. was 330 mg/kg per day, a value that is substantially higher than the 50 mg/kg per day used by the Expert Panel from the Mylchreest et al. [39] oral gavage study. Finally, the more recent data from Patel et al. [77] did not substantiate earlier observations [79] which were cited by the Expert Panel. The CERHR position on reproductive toxicity (no NOAEL, LOAEL = Male:52 Female:80 mg/kg bw per day) is based on data which has not been replicated. The data obtained from Patel is more robust and should be used in preference to previous value (NOAEL = Male:60 Female:600).

With respect to the “window of sensitivity” question, the Expert Panel stated that the “known current window in rats, 12–20 days, is still quite wide from a rodent ontogenesis perspective”. Several recent studies [74,80,81] provide evidence that, in the rat, the critical period for male reproductive development is more likely gestational days 15–17 or 18. However, in a larger sense, this question may be somewhat academic. In rats male sexual development starts late in gestation and continues after birth until the animals reach sexual maturity, with the period of greatest sensitivity being the last few gestational days. In contrast, male sexual development in humans occurs earlier in the gestational period, and then becomes largely quiescent until puberty when sexual maturation occurs (e.g. [29]). Thus, the establishment

of the “window of sensitivity” in rats may be useful in the development of an experimental model, but may not be directly relevant to human health risk assessment as the time course of male reproductive tract development in humans and rodents is quite different.

With respect to an assessment of the effects of DBP on development in non-rodent species, the reader is referred to the summary of studies of the effects of DEHP on male reproductive development and the discussion of the strategy to test DEHP as a model compound in evaluating species differences.

In summary, the data now available address the questions raised by the Expert Panel and provide sufficient information to better define the degree of concern and increase the level of confidence in the overall assessment. There were no apparent effects on female rats in either of the multi-generation studies. Thus, the concern over the potential for reproductive effects in female rats based on a LOAEL of 80 mg/kg per day [79] should be reconsidered as more recent data indicate that the NOAEL in females may in fact be greater than 600 mg/kg per day [77]. Although there have been several new investigations of effects in male rats, none has suggested a NOAEL <50 mg/kg per day, the value used by the Expert Panel in its assessment. As the mean exposures to DBP in the general population are below 1  $\mu$ g/kg per day, the margin of exposure is  $\sim$ 50,000. The Expert Panel expressed negligible concern for adult reproductive toxicity and minimal concern about effects to human development and development of the reproductive system. However, the CERHR also indicated that this conclusion was only supported if exposures were similar to the estimate of 2–10  $\mu$ g/kg bw per day. As the most current data from the CDC indicate urinary metabolite levels at the 95th percentile for all segments of the population equate to exposures below 10  $\mu$ g/kg per day, the Expert Panel conclusions are well supported.

### 2.3. DnHP/DnOP

The Expert Panel noted that there was little if any commercial production of pure DnHP or DnOP and suggested that future assessments should focus on more complex phthalates containing these substances as constituents rather than on the substances themselves [8,9]. It should be noted, however, that exposure to DnOP has been assessed by the urinary metabolite method and found to be below the level of detection in most individuals [20]. The available toxicology data suggested that these phthalates are not as effective as, for example, DEHP, in causing reproductive effects in rodents. The urinary metabolite data suggest that exposures are well below those of the other, more widely used phthalates. Thus, it is reasonable to conclude that exposure to these phthalates is not problematic as long as exposures remain at current, low levels. As the CDC plans to continue to measure urinary metabolite levels of phthalates for the foreseeable future, it should be possible to monitor exposures, and perhaps devote more resource to risk characterization

and assessment for these substances if exposures seem to be significantly increasing.

#### 2.4. DEHP

DEHP has been the most intensely studied of the phthalate esters, and has by far the largest database of the seven esters considered. Given the extent of the database, a considerable number of issues were debated by the Expert Panel, and, ultimately a number of data gaps were identified [5]. A complicating issue, unique to DEHP, concerns its use in medical devices. Some individuals undergoing medical treatment may receive doses of DEHP which are higher than those of the population at large [82]. Further, because medical device use results in exposure by the parenteral route, this is the one situation in which significant amounts of DEHP may be introduced into the body in the diester rather than the monoester form.

The critical data needs for DEHP in general included hazard identification studies in rodents to assess reproductive effects and characterize dose–response relationships; hazard identification studies in non-rodent species to assess species specificity; extension of PBPK models to include pregnant humans; and several other issues listed as “timing, PPAR, metabolism” without further discussion. Additionally, because of the specific concerns related to medical devices, the Expert Panel suggested epidemiology studies to examine the consequence of medical device use, particularly associated with perinatal treatment; better studies of the consequences of parenteral as opposed to enteral administration; and inclusion of parenteral administration in the PBPK models.

##### 2.4.1. Hazard identification studies of DEHP in rodents

The Expert Panel noted that, although some studies were in progress, a multigeneration study of DEHP consistent with current guidelines was not available for review. Since then three studies have been completed, a continuous breeding study in rats [83], a two-generation reproductive toxicity study in rats [84], and a two-generation reproductive toxicity study in mice [85]. Although effects in rats were reported, there were no reproductive effects at dietary levels below 1000 ppm (approximately 100 mg/kg) in either study. Similarly, there were no reproductive effects in mice given DEHP by dietary administration at levels of 0.01, 0.03, or 0.09% (approximately 15, 50 or 150 mg/kg). The overall NOAEL identified by these studies was approximately 100 mg/kg per day. Parenthetically, Schilling et al. [84] and Tanaka [85] also found that prenatal exposure did not produce neurobehavioral effects. Thus, there is now evidence that DEHP is not a developmental neurotoxicant.

These new data suggest that reconsideration of the NOAELs may be warranted. The Expert Panel concluded that the lowest NOAEL, assigned for testis/developmental effects was 3.7 mg/kg bw per day. This value was derived from a study [86] in which cytoplasmic vacuolation was reported in testes from male rats given DEHP by dietary

administration at levels ranging from 0.4 to 375 mg/kg per day. The LOAEL from that study was 38 mg/kg bw per day. The Expert Panel also relied on a NOAEL of 14 mg/kg per day (with a corresponding LOAEL of 141 mg/kg per day) based on data from a continuous breeding study in Swiss mice reported by Reel et al. [87] and Lamb et al. [88]. The more recent multigeneration studies included an assessment of testicular toxicity and should be regarded as particularly relevant in the determination of the overall NOAEL for reproductive toxicity as exposure in these studies was continuous from conception to termination. The study by Poon et al. [86] in contrast, utilized subchronic administration only. Neither Schilling nor Wolfe reported any statistically significant evidence of testicular lesions in male rats given DEHP from conception to sacrifice at doses approximating 100 mg/kg bw per day; nor did they find cytoplasmic vacuolation to be a sensitive indicator of testicular toxicity [83,84]. A subsequent review of the testicular slides from the Wolfe study by a pathology working group confirmed the conclusions of the study pathologist. There was minimal to marked testicular atrophy of the seminiferous tubules characterized by loss of germ cells, the presence of Sertoli cell-only tubules and occasional failure of sperm release in the 7500 and 10,000 ppm groups. There were no treatment related lesions in animals exposed to 1000 ppm DEHP or less. However, Sertoli cell vacuolation was not reported at any dose in any generation [89]. Similarly, no testicular lesions were found in studies in which DEHP was given by subchronic administration of 1000 mg/kg bw day to juvenile rats [90] or when given at 200 mg/kg per day during the lactational phase [63]. Thus, the findings of Poon have not been replicated by four independent groups of investigators and should not be regarded as sufficiently reliable for risk assessment. Based on these new findings and using a weight of evidence approach, the NOAEL for reproductive effects in male rats is approximately 100 mg/kg per day.

##### 2.4.2. Studies of DEHP in non-rodent species

A second and related question was the relevance to humans of the effects in rodents. As discussed previously, a study was conducted to assess the effects of DEHP on male reproductive tract in the marmoset (*C. jacchus*). Treatment at levels up to 2500 mg/kg per day had no effects on male reproductive development [49], whereas administration of DEHP at similar levels produced testicular atrophy in rats [43–45]. This study provided additional evidence that primates are less sensitive than rodents to the testicular effects of phthalates. The greatest value of this study, however, may be in a risk assessment context. The Expert Panel expressed concerns about reproductive system development in young boys as a consequence of potentially high levels of exposure to DEHP as might occur for critically ill infants due to exposure from medical devices. That concern was directly addressed by this study; testicular development was unaffected even though the treatment spanned the entire period of male sexual maturation. Further, the doses used

were well in excess of those that might be experienced by individuals undergoing medical treatment. The U.S. FDA indicated that the exposures of greatest concern, those experienced by certain young children undergoing critical medical procedures, could be as high as 12 mg/kg per day [82]. By comparison, however, treatment of marmosets at levels up to 2500 mg/kg per day had no effects on male reproductive tract development.

#### 2.4.3. Extension of PBPK models

As noted previously in this report, the refinement of PBPK models to incorporate the new human and non-human primate data is ongoing but not yet complete. Clearly this remains a critical data need.

#### 2.4.4. Timing, PPAR and metabolism

Although the Expert Panel did not elaborate on these specific DEHP issues, one might assume that “timing” referred to the “critical window” for male reproductive effects; PPAR to the possibility that PPAR (i.e. PPAR $\alpha$ ) has a role in the reproductive effects associated with DEHP, and “metabolism” to the pharmacokinetic differences between species. The “critical window” and “metabolism” issues are discussed elsewhere in this document and will not be repeated here. As regards the role of PPAR $\alpha$ , the Expert Panel stated that “[T]he presence of testicular effects in PPAR-alpha knockout mice and in guinea pigs exposed to DEHP indicates that the mechanism of action does not involve peroxisome proliferation”. It is our view that this conclusion is not an accurate reflection of the data relating to the potential role of PPAR $\alpha$  in the testicular effects of DEHP and by extension other phthalates. This criticism of the Expert Panel conclusion is based on three points:

- (a) the Expert Panel did not correctly reflect the conclusions of the authors of the principal study on which they relied [68];
- (b) the Expert Panel did not consider data from other substances suggesting a general relationship between peroxisomal proliferation and testicular effects; and,
- (c) mechanistic information published since the completion of the Expert Panel review suggests specific ways in which the reproductive effects could be a consequence of peroxisomal proliferation.

The study by Ward et al. [68] compared the effects of DEHP treatment on wild-type mice to those lacking a PPAR $\alpha$  receptor. They found that the knockout mice developed testicular lesions but more slowly and to a lesser degree than did the wild-type mice. Based on these observations, the investigators concluded that there most likely was a PPAR $\alpha$ -dependent component to the testicular effects although it appeared that other, PPAR $\alpha$ -independent factors might also be involved. The Expert Panel did not explain why its interpretation of these data, i.e. that PPAR $\alpha$  activation was not involved, differed from that of the original authors.

There is other evidence suggesting a role for peroxisomal proliferation (or more specifically PPAR $\alpha$  agonism) in the development of testicular effects in rodents; but the Expert Panel may have overlooked the relevant citations as none evaluated phthalates specifically. Cook et al. [91] reported that another peroxisomal proliferating agent, ammonium perfluorooctonate (C8), affected the testosterone/estradiol balance in treated rats. Subsequent work revealed that C8 inhibited testosterone production by Leydig cells and that the inhibition was reversible [69]. This work was extended to other peroxisomal proliferating agents [92,93]. It was further shown that peroxisomal proliferating agents induced synthesis of aromatase (cytochrome P450-19A1) which converts testosterone to estradiol in rat liver, thus perturbing the testosterone/estradiol balance [93]. Interestingly, in the goat, a species which shows only a very modest response to peroxisomal proliferating agents, the very potent inducer of peroxisomal proliferation Wy 14,643 induced a 41% increase in hepatic aromatase levels and did not significantly affect estradiol levels [94]. In contrast, in the rat Wy 14,643 can increase hepatic aromatase levels as much as 16-fold. These papers provide clear evidence that a range of peroxisomal proliferating agents affect reproductive function in rodents through processes related to PPAR $\alpha$  agonism. As humans seem much less sensitive to other PPAR $\alpha$ -related phenomena, it seems likely that PPAR $\alpha$  agonists would produce substantially less profound effects in primates than in rodents.

Finally, there are now reports that phthalates may influence the expression of gene functions related to steroid biosynthesis (e.g. [64,65,95]). The Gazouli study is particularly informative as it compared gene expression in wild-type and PPAR $\alpha$ -null mice. The work by Gazouli et al. provided evidence that PPAR $\alpha$  induction reduced cholesterol and fatty acid availability to the Leydig cells, but that the subsequent steps relating to cholesterol uptake by the mitochondria and steroid biosynthesis might be PPAR $\alpha$ -independent [64]. Thus, there is a body of evidence showing that the testicular effects of DEHP in rodents, and by extension of other phthalates which produce testicular toxicity in rodents, are at least partially the consequence of PPAR $\alpha$  activation. As humans and non-human primates do not exhibit other changes associated with PPAR $\alpha$  activation, these data may provide at least a partial explanation for the empirical evidence of species differences provided by the non-human primate studies.

#### 2.4.5. Potential risks from medical devices

One issue on which the Expert Panel focused was the potential for effects on male reproductive development as a consequence of exposure to DEHP from medical devices by children undergoing certain specific intensive therapies. The Expert Panel had recommended further assessment of exposure as a consequence of these treatments as well as follow-up evaluations of individuals who underwent such treatments as children. The Expert Panel identified this as a critical issue since the data then available suggested that exposures could approach levels associated with effects in animals.

Although neither of these specific recommendations has been fully addressed, there have been further assessments of exposure, and there has been one study of reproductive development in children who had undergone extracorporeal membrane oxygenation (ECMO) therapy as newborns [96]. This group of individuals is particularly interesting as ECMO support is considered to involve the highest exposures to DEHP. The authors reported no significant adverse effects of DEHP on physical growth or pubertal maturity. Thyroid, liver, renal, and male and female gonadal functions were within normal range for age and sex distribution when compared with known reference data. There has also been a recent study that assessed the potential association of paternal occupational exposure and reduced fertility [97]. The investigators found no differences between the exposed and control populations. Finally, additional toxicology studies have addressed both dose–response and species-specificity.

One of the uncertainties identified by the Expert Panel related to the use of NOAELs from oral studies (in which the phthalate is absorbed from the gut as monoester which is the putative toxic metabolite) in the assessment of risk in situations in which exposure is intravenous (and the phthalate is introduced systemically in the diester form). To develop more appropriate NOAELs for this specific use, reproductive tract development in rodents was assessed in studies in which DEHP was given by intravenous administration. These studies [98,99] referenced in the FDA medical device risk assessment ([82]; see also [100]) provided evidence that the parenteral NOAEL in rats was approximately 60 mg/kg per day, very much in line with the oral NOAEL of approximately 100 mg/kg per day derived from the two-generation studies described above. In contrast, the CERHR Expert Panel used the NOAEL range of 3.7–14 mg/kg per day, based on oral studies, as the basis for its evaluation. With these new data, the margin of exposure may actually be more than an order of magnitude greater than previously estimated.

The Expert Panel identified a number of other issues related specifically to the medical device use of DEHP. These included the significance of perinatal exposure, the relevant animal model, extension of the PBPK model to include pregnant women and a better assessment of metabolism. As indicated above, some of these issues have been addressed whereas others have not. There are now better studies of dose–response in rodents, including studies conducted by parenteral administration. Thus, dose–response has been substantially addressed. There are also now studies that demonstrate that postnatal exposure to DEHP does not affect male reproductive tract development in primates. However, questions remain regarding the potential effects on humans of in utero exposure to DEHP. PBPK models are under development but further work is needed.

In this context, however, it should be noted that several groups have assessed pregnancy outcome in women undergoing dialysis. Developmental effects were not elevated in offspring from these women [101–104].

In summary, the data now available address many of the general questions raised by the Expert Panel [5]. In particular, there is a much fuller characterization of hazards and much better information on exposure. These data provide sufficient information to better define the degree of concern and substantially increase the level of confidence in the overall assessment. There were no apparent effects on female rats in any of the multi-generation studies or in other studies of reproductive effects. Thus, the NOAEL for reproductive effects in female rats is >600 mg/kg per day. There were effects on male reproductive development, with a NOAEL of ~100 mg/kg per day defined by the two studies in rat and one in the mouse. As the mean exposure to DEHP in the general population is less than 1 µg/kg per day, the margin of exposure in most circumstances is ~100,000. There were also no effects on male reproductive tract development in primates, and this information, along with other mechanistic data raises questions about the relevance of the rodent data to human risk.

The Expert Panel expressed minimal concern that ambient human exposures adversely affect adult human reproduction. However, concern was expressed over the potential for adverse effects on male reproductive tract development if exposures of infants were significantly greater than adult exposures. Concerns were also expressed over the potential effects from in utero exposures. As discussed above, the ambient exposures are actually lower than the Expert Panel's estimates, and children's exposures, although higher than those of adults, were still well within the range estimated by the Expert Panel and used in its assessment. As the data also indicated that the lowest NOAELs used by the Expert Panel in its analysis are of questionable validity, an overall conclusion of minimal concern seems appropriate.

Some issues related to medical device use have also been addressed, although some questions remain. If the medical device risk assessments are to be based on rodent data, developmental studies in rodents via the parenteral route provide a better data set than the previously used oral studies (and also obviate concerns related to "route to route" extrapolation). Marmoset studies provide evidence that DEHP is unlikely to affect male reproductive tract development in humans. Exposures from medical devices have not been as well established as those for the general public, but more rigorous assessments have been carried out. All of these data suggest that the concerns over exposures from medical devices may not be as serious as the Expert Panel had expressed. There are, however, still uncertainties over exposures from some uses, and the development of better PBPK models which incorporate fetal compartments remains a need.

### 2.5. *Di-isononyl phthalate (DINP)*

The principal issues raised by the CERHR with respect to DINP related to assessment of male reproductive development and a better definition of exposure [7]. The Expert Panel also discussed the use of DINP in children's toys and

indicated that a better assessment of DINP exposure as a consequence of toy use was warranted if DINP were to continue to be used in toys. Although adult reproductive toxicity and effects on the developing reproductive system had been evaluated in previous (older) studies and found to be unaffected at levels exceeding 600 mg/kg per day [105], the Expert Panel noted that some parameters including nipple retention had not been specifically addressed. They recommended “a perinatal developmental study in orally exposed rats that addresses landmarks of sexual maturation such as nipple retention, AGD, age at testes descent, age at prepuce separation, and structure of the developing reproductive system in pubertal or adult animals exposed through development”. The Expert Panel further recommended that the effective dose levels be compared to those that humans might experience, and, if there were remaining concerns, that further studies be conducted to assess the potential for species-specific effects.

### 2.5.1. Exposure

To begin with exposure, studies of urinary metabolites by the CDC [15,19,20] provide evidence that average ambient exposures to DINP for the US population are well below 1 µg/kg per day [17,18]. Additionally, there is no evidence that women of child-bearing age are exposed to DINP at higher levels than the population at large [20,27], and preliminary studies did not detect DINP metabolites in urine from infants [21]. The Consumer Product Safety Commission (CPSC) assessed the potential for DINP exposure as a consequence of mouthing toys. Based on its own recently conducted mouthing study and new migration data, the CPSC staff determined that previous estimates of exposure from toy use had been substantially exaggerated. The new CPSC estimates of DINP exposure from toy use ranged from <1 to 1–3 µg/kg per day with approximately 11 µg/kg per day as an extreme case [106]. Thus, the questions relating to DINP exposure have been addressed. The overall estimates of children’s exposure to DINP, an average of approximately 1 µg/kg per day and an extreme case of 11 µg/kg per day, are consistent with the estimate of “<3–30 µg/kg per day” used by the Expert Panel in its evaluation.

### 2.5.2. Hazard assessment studies in rodents

Questions about landmarks of sexual maturation were at least partially addressed by a publication by Gray et al. [40]. DINP was administered orally at 750 mg/kg per day from GD 14 to PND 3. The offspring were examined at various times until terminal sacrifice at ages ranging from 3 to 7 months of age. There were no effects on AGD or time to preputial separation. Some (22%) of the male offspring had areolae whereas none were found in the controls ( $P < 0.01$ ). However, this was apparently at least partially reversible as only 2 (of 52) males had retained nipples at terminal sacrifice. Gray also reported that there were also 2 (of 52) male offspring with testicular abnormalities; one with bilateral testicular atrophy and another with hyposper-

mia and fluid filled testes. The authors regarded this as a treatment-related effect as no testicular abnormalities were found in the control group. There were no effects on weights of testicular organs; nor was there evidence of cleft phallus, vaginal pouch, or hypospadias, and none of the males had undescended testes, prostatic or vesicular agenesis, or abnormalities of the gubernacular cord.

In a subsequent study of similar design, reported only in an abstract [107], DINP was given at 1000 or 1500 mg/kg per day. AGD was reported as reduced in the high-dose group. The percentage of males with areolae was also reported as increasing in a dose-related fashion. However, the incidence of areolae in the negative control group was given as 14%, in contrast to the previous study in which the incidence was zero. Results of the pathologic examination were not provided in the abstract.

Thus, the questions raised by the Expert Panel with respect to hazard characterization were substantially addressed by the Gray study. DINP increased areola retention at 750 mg/kg per day although the statistical (and biological) significance of that observation remains unclear given the range in the control groups. AGD was significantly decreased at 1500 mg/kg per day, but was not significantly affected at lower levels. There was a reported increase in testicular lesions, but the toxicological significance was difficult to assess without better characterization of the background response, particularly as Waterman et al. ([105] as further elaborated in the subsequent publication by McKee [108]) found no evidence of treatment-related pathological effects in the testes. In other respects, the results from Gray were very consistent with the earlier data from Waterman, particularly the evidence that weights of testes and accessory sexual organs were not affected by treatment in any substantial way. Inasmuch as the relatively minimal effects reported by Gray and co-workers were at levels well above those used by the Expert Panel in its determination of degree of concern, additional testing in a non-rodent species, a potential second tier test, seems unwarranted.

There was one other point raised by the Expert Panel that also merits comment. In the two-generation study [105], offspring body weight was significantly reduced at 0.2% in the first generation (the lowest dietary level) and at 0.4% during the second generation. The Expert Panel reported [incorrectly] that body weights were significantly reduced at the 0.2% level in both generations. On this basis, the Expert Panel regarded 0.2% as a LOAEL, and, as they felt that that this could have been related to either prenatal or lactational exposures, considered this to be equivalent to 143–285 mg/kg bw per day. In a subsequent study with a similar high molecular weight phthalate, DIDP, Hushka et al. [109] used cross-fostering techniques to demonstrate that the effects on weight were due to lactational rather than in utero exposures. Using statistical procedures (the polynomial model with ICF Kaiser software package THC), the 95% lower confidence for a 5% reduction in the predicted body weights in the DINP study ranged from 0.16 to 0.21%

in the diet, or approximately 200–260 mg/kg bw per day. The 5% level was utilized because it is a difference that is too small to be statistically distinguished from control values. The prediction was consistent with the data as the significant difference in the first generation was 9–10% below control values whereas the non-significant difference in the second generation was 7% below control values. An overall conclusion by the Expert Panel was that the lowest NOAELs for all effects reviewed were in the range of 100–200 mg/kg per day. As the “derived NOAEL” for offspring body weight is in the range of 200–260 mg/kg per day, the use of 100–200 mg/kg per day as a conservative starting point for risk assessment seems fully justified. Further, the effect on body weights may have been secondary to induction of peroxisomal proliferation. A similar reduction in body weight, reported in a study of HCFC-123 [110] was attributed to peroxisome proliferation [111].

### 2.5.3. *Exposure from children’s toys*

One substance-specific issue related to the uses of DINP in children’s toys and the potential for exposure as a consequence of toy use. The Expert Panel reviewed assessments of the potential risks to children as a consequence of the use of DINP in children’s toys by the U.S. Consumer Product Safety Commission [112], Europe [113], Canada [114] and The Netherlands [115]. These various assessments resulted in the removal of DINP from devices intended to be mouthed such as pacifiers, teething and bite rings. The use of DINP in other toys has not been restricted.

The Expert Panel suggested that if DINP were to continue to be used in toys, “[S]alivary extraction of DINP and better estimates of mouthing behavior, especially within the potentially highest risk group of 3–12-month-old children, using data from more children, should be carried out”. There have been several studies to more precisely define children’s exposure as a consequence of toy use. With respect to salivary extraction, the CERHR was aware of three studies [112,115,116] that reported results of salivary extraction in adult volunteers. There has been one additional study [117]. Although the number of individuals in each study was relatively small (10–20 individuals), they were all in good agreement. Thus, there are now four independent studies (see above) that have reached similar conclusions, and provide a good basis to assess the potential for phthalate extraction from these devices.

A second issue, mouthing behavior, i.e. studies to define the length of time spent mouthing toys, has been much more intensely studied. When the CERHR completed its review, estimates of children’s exposure were based primarily on a single behavioral study involving 19 children aged 3–12 months along with 23 more aged 12–36 months [115]. The younger children spent more time engaging in mouthing behavior with total mouthing times (for all objects including fingers except for pacifiers which do not contain phthalates) ranged from approximately 2 min to 3 h with an average of about 40 min. Two much larger studies

have now been completed [106,118]. These studies which focused on toys, indicated that children actually spend less time mouthing than had been previously believed. Based on a study of 169 children, the mean mouthing time for children 12–24 months of age (the age group with the highest mouthing time) was 1.9 min (1.2–2.6) per day. The CPSC reviewed the potential for exposure to DINP in light of this new information, along with an independent hazard evaluation conducted by a Chronic Hazard Advisory Panel (CHAP) [119], and determined that exposure to DINP from mouthing toys and other DINP-plasticized items does not pose a risk to children [106,120,121].

Parenthetically it should be noted that the evaluation of the safety of DINP in children’s toys was ultimately based on liver effects in rodents rather than any potential reproductive or developmental effects. The CHAP specifically was asked “Is DINP a developmental or reproductive toxicant and would the exposures from consumer products result in developmental or reproductive risks”? They concluded that “because of the large margin between doses to pregnant women and those expected to be without effect in the animal assays, the risk to reproductive and developmental processes in humans due to DINP exposure is extremely low or non-existent [119]”. The Expert Panel expressed “low concern” for potential health effects in children as a consequence of exposure to DINP in toys and other objects that children may mouth [7]. In light of the new CPSC data, indicating that exposures are much lower than previously believed, perhaps “minimal” or even “negligible” could be considered as the appropriate level of concern.

There was one other issue relating to the potential for effects by DINP although it was not specifically related to reproductive or developmental toxicity. It was noted that DINP produced liver tumors in rats and mice by a process consistent with a PPAR $\alpha$ -agonist mode of action. However, it was also noted that the criteria for peroxisomal proliferation had not been demonstrated at all of the doses associated with excess liver tumors in mice. Subsequently, a study of the dose–response relationships of markers of PPAR $\alpha$  induction, specifically liver weight, peroxisomal enzyme induction, peroxisomal proliferation and cell proliferation was carried out in the mouse. The data [122,123] provided evidence of peroxisomal proliferation at all of the doses associated with tumor formation—more firmly establishing peroxisomal proliferation as the mode of action. Parenthetically, peroxisomal enzyme induction was detected at levels which were not associated with tumor formation whereas peroxisomal volume and cell proliferation were only significantly elevated at tumorigenic doses.

Additionally, it may be of interest to note that the rodent pharmacokinetic data, referenced to original laboratory reports in the CERHR monograph [7], has now been published [124].

In summary, the Expert Panel had previously expressed minimal concerns for unborn children based on maternal exposure to DINP and for reproductive toxicity in general.

This was based on a NOAEL of 100 mg/kg bw day and an assumed exposure level of “<3–30  $\mu\text{g}/\text{kg}$  per day”. The more recent studies did not identify effects at levels lower than those used by the Expert Panel in its evaluation, and the CDC data indicated that ambient exposures at even the 95th percentile were below 1  $\mu\text{g}/\text{kg}$  per day. Thus, the overall conclusions of minimal concerns are now supported with greater certainty. Based on an overall NOAEL of 100 mg/kg per day, the margin of exposure is >100,000. Perhaps, given the increased precision in the exposure information, negligible might be more appropriate than minimal. With respect to the use of DINP in children’s toys, the more recent data by the CPSC have shown that exposures were lower than previously anticipated. After resolving most of the uncertainty the CPSC concluded that the use of DINP in toys is not associated with risks to children. Thus, questions related to toy use have been resolved.

### 2.6. Di-isodecyl phthalate (DIDP)

Very few DIDP-specific issues were raised by the CERHR Expert Panel [6]. As described above, the Expert Panel laid out a sequential testing strategy that focused on the most critical data first and defined the principal need as a perinatal developmental study in a non-rodent species to determine whether the rat is an appropriate model for assessing human risk. The Expert Panel also identified exposure characterization as a critical need. They noted the lack of data and recommended that additional information be collected. In particular they recommended a better assessment of children’s exposure.

As described above, a study of the effects of DEHP on male reproductive development in the marmoset has been carried out. As DEHP did not affect male reproductive parameters in this study, it seems unlikely that studies of other phthalates would be profitable. In particular, as DIDP did not affect male reproductive development or function in rodents [109], further studies of DIDP in primates seem unwarranted.

As for the uncertainties related to exposure, DIDP was not included in the urinary metabolite studies conducted by the CDC, and, therefore, human exposure has not been directly quantified. However, Stock et al. [23] did not detect DIDP metabolites in their study, and the studies of DINP by the CDC revealed that ambient exposures were below 1  $\mu\text{g}/\text{kg}$  per day. Aside from children’s toys in which DIDP is not used, DINP and DIDP have similar use patterns. Further, DIDP has a higher molecular weight, and is both less volatile and less water-soluble than DINP. Thus, it seems likely that exposures to DIDP would be similar to or lower than DINP exposures. For purposes of risk characterization, the more recent estimates of DINP exposure seem more relevant than the DEHP-based estimates used by the Expert Panel.

One issue raised in the monograph concerned the NOAEL for offspring survival in the two-generation study. The issue is important because it defined the lowest NOAEL that

the Expert Panel identified for DIDP. In the two-generation studies survival was significantly reduced at postnatal days 1 and 4, but live birth index was unaffected. Based on these observations, the exposure data which seem most relevant in the assessment of the NOAEL are the doses to the dams during the first week of lactation. As documented in the publication describing these studies [109], the experimentally defined NOAEL was 0.06% in the diet (or approximately 50 mg/kg per day), and the theoretical NOAEL, based on bench mark dose procedures was 108 mg/kg per day with a 95th percentile lower bound value of 86 mg/kg per day. (This paper had not been published at the time the Expert Panel completed its review.)

In summary, the Expert Panel expressed minimal concerns for developmental and reproductive effects resulting from DIDP exposure based on a NOAEL of  $\geq 38$  mg/kg per day and an estimated exposure of <3–30  $\mu\text{g}/\text{kg}$  per day. The authors of the paper describing the two-generation reproduction studies have proposed a somewhat higher NOAEL. Urinary metabolite information on DIDP has not been reported, other than a preliminary report by Stock et al. [23]; however, it seems unlikely that exposure to DIDP exceeds that of DINP, i.e.  $\leq 1$   $\mu\text{g}/\text{kg}$  per day. This is based on the belief that as DIDP is similar in both physical/chemical properties and use patterns to DINP, but is not used in toys, it would also present similar exposure opportunities to the general population. Thus, the conclusion of minimal concern for DIDP still appears justified. The Expert Panel had reserved judgment on two issues, exposures of children as a consequence of the use of DIDP in toys and exposures of pregnant women in the workplace. The new data on child behavior indicate that exposures to DINP from toys are much lower than previously anticipated. As DIDP is apparently not used in toys, children’s exposures as a consequence of this use are unlikely to be problematic. Occupational exposures to DIDP have not been better defined.

### 3. Overall conclusions

In the three years since the completion of the review of reproductive hazards by the NTP-CERHR, phthalate exposures to the general population have been much better characterized due to the urinary metabolite studies by the CDC and others. Additionally, a number of reproductive toxicity studies have been completed, and these, along with other information, have better defined the NOAELs in rodents (Table 6). The potential for effects in non-rodent species has been further studied, and the new information suggests that humans are unlikely to be more sensitive than rodents to the effects of phthalates and may in fact be substantially less sensitive. There is still some work that remains including completion of a PBPK model and, perhaps, further assessment of exposures of selected subgroups. But, overall, the general and phthalate-specific issues raised by the Expert Panel have been substantially addressed, and there has

Table 6  
Comparisons of NTP-CERHR consensus views to new exposure and hazard data

Phthalate	Exposed group	Exposure estimate (CERHR) ( $\mu\text{g}/\text{kg}$ per day)	More recent exposure estimates ( $\mu\text{g}/\text{kg}$ per day)	NOAEL (CERHR) ( $\text{mg}/\text{kg}$ per day)	NOAEL new data ( $\text{mg}/\text{kg}$ per day)	MOE <sup>a</sup>	
						CERHR <sup>b</sup>	New data
BBP	Adult	2	0.43 (2.08) <sup>c</sup>	Reproductive NOAEL (not defined)	50	Not defined	$\sim 10^5$
	Young child	$\leq 6$	1.64 <sup>d</sup>	Developmental NOAEL (182)	No change	$9 \times 10^4$	$4 \times 10^5$
DBP	Adult	2–10	0.86 (3.86) <sup>c</sup>	Reproductive LOAEL M:52, F:80	NOAEL M:60, F:600	Not defined	$6 \times 10^4$
	Young child	Not provided	2.65 <sup>d</sup>	Developmental (50)	No change	$\sim 5000$ – $25000$	$19 \times 10^3$
DEHP	Adult	3–30	0.61 (3.51) <sup>c</sup>	Reproductive (3.7–14)	$\sim 100$	$\sim 100$	$\sim 39 \times 10^3$
	Healthy infant	10–20 <sup>e</sup>	2.57 <sup>d</sup>	Developmental ( $\sim 40$ mg)	No change	Not defined	$\sim 65 \times 10^3$
DEHP—medical	Critically ill infant	1800–3300	12000 <sup>f</sup>	3.7–14	60	$\sim 1$	$\sim 5$
Device use							
DINP	Adult	$<3$ – $30$	$<\text{LOD}$ (0.73) <sup>c</sup>	Reproductive ( $>600$ )	No change	$2 \times 10^5$	$\sim 6 \times 10^5$
	Children using toys	Mean $<320$	$<\text{LOD}$ (1–11) <sup>g</sup>	Developmental (100–200)	No change	Not defined	$10^4$ to $10^5$
DIDP	Adult	$<3$ – $30$	DINP is best analogue <sup>h</sup>	Reproductive (427–929)	No change	$>14000$	$>4 \times 10^5$
	Children	$>3$ – $30$ (?)		Developmental ( $\geq 38$ $\text{mg}/\text{kg}$ per day)	50	$>1000$	$\sim 50 \times 10^3$

<sup>a</sup> MOE: margin of exposure, the ratio between the no effect level in rodent studies and the estimated human exposure.

<sup>b</sup> Calculations based on data provided in the CERHR monographs.

<sup>c</sup> Mean and 95th percentile values for the general population based on the CDC urinary metabolite data [20].

<sup>d</sup> Mean value based on urinary metabolite data [21].

<sup>e</sup> Estimated exposure by infants reported in the CERHR monograph on DEHP [5].

<sup>f</sup> The estimate of 12,000  $\mu\text{g}/\text{kg}$  per day for critically ill infants is from an FDA assessment of the potential total DEHP contribution from medical treatments and feeding tubes [82].

<sup>g</sup> As reported by CPSC [106] “for ‘all toys, teething and rattles’ exposure was greatest among 3–12-month-old children”. The mean exposure was 2.91  $\mu\text{g}/\text{kg}$  per day, the median was 1.45  $\mu\text{g}/\text{kg}$  per day and the 95th percentile value was 10.71  $\mu\text{g}/\text{kg}$  per day. Lower values were found for children of other ages.

<sup>h</sup> Based on very limited urinary metabolite data [23] as well as information on exposure sources, exposures to DIDP in the general population are believed to be similar to or less than those to DINP.

also been progress on many of the substance-specific issues. These new data provide additional support for the overall view by the Expert Panel of minimal or negligible concern for most phthalates and most uses. Additionally, the higher degrees of concerns related to some specific uses may be unwarranted due to evidence of higher NOAELs for critical effects, lower exposures related to these specific uses, and differences in species responsiveness during the periods at which exposures might occur.

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