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April 21, 2005

VIA EMAIL: shelby@niehs.nih.gov

Dr. Michael D. Shelby
CERHR Director
CERHR
79 T.W. Alexander Drive
Building 4401, Room103
Research Triangle Park, NC 27709.

Dear Dr. Shelby:

The American Chemistry Council Phthalate Esters Panel (PE Panel) is submitting the attached comments to assist the new CERHR Expert Panel in its review of di(2-ethylhexyl) phthalate (DEHP). 70 Fed. Reg. 6024 (Feb. 4, 2005). The comments address much of the new information that has become available on DEHP since the first Expert Panel review in 2000.

As explained in the attached comments, new information indicates that general population exposures to DEHP are about 10-fold lower than estimated by the first Expert Panel, while the appropriate no observed adverse effect level is about 10-fold higher. Therefore, the new information indicates that concern for adverse effects of DEHP exposure on human reproduction is much less than that expressed by the first Expert Panel.

The PE Panel requests that these comments be made directly available to the members of the new Expert Panel. These comments were written primarily by PE Panel toxicologists who are very familiar with the science for DEHP, and they include detail and perspective on the various studies that should be helpful to the Expert Panel members as they review the data on DEHP.

If you have any questions, or if you need any further information, please call Marian K. Stanley, Senior Director and Manager of the Phthalate Esters Panel, at (703) 741-5623, email her at Marian_Stanley@americanchemistry.com, or write her at the address below.

Sincerely yours,

Courtney M. Price

Attachment

Recent Information on Exposure to and Toxicology of Di(2-ethylhexyl) Phthalate (DEHP)

American Chemistry Council Phthalate Esters Panel
April 21, 2005

I. OVERVIEW

In 1999 and 2000, the first Expert Panel of the National Toxicology Program (NTP) Center for Evaluation of Risks to Human Reproduction (CERHR) evaluated seven phthalates, including di(2-ethylhexyl) phthalate (DEHP). The Expert Panel's report on DEHP was published on the NTP website in October 2000 (CERHR, 2000), and was subsequently published in the peer-reviewed literature (Kavlock et al., 2002).

The first Expert Panel chose a no observed adverse effect level (NOAEL) for DEHP reproductive toxicity of 3.7-14 mg/kg/day, based on rodent data. It estimated that general population exposures to DEHP were 3-30 µg/kg/day. For DEHP, the first Expert Panel expressed "minimal concern" for the general adult population. It expressed "concern" that, if

infant and toddler exposure is several-fold higher than adults, exposure might adversely affect male reproductive tract development. It expressed "concern" that ambient oral DEHP exposures to pregnant or lactating women might adversely affect development of their offspring. And it expressed "serious concern" that exposure of critically ill infants receiving intensive therapeutic treatments might adversely affect male reproductive tract development. The Expert Panel also recognized that the benefits of medical procedures could outweigh any risks.

Since the time of the first Expert Panel deliberations, a good deal of new information has become available on DEHP exposures and toxicology. Much of this new information is summarized in McKee et al. (2004). This document contains additional detail and information.

The following overall points can be drawn from the new information.

- New reproductive toxicology studies indicate that the appropriate oral NOAEL for DEHP is 46 mg/kg/day or more, about 10-fold higher than the lower NOAEL used by the first Expert Panel. The appropriate NOAEL for intravenous exposures from medical devices is 60 mg/kg/day or more.

- Biomonitoring data demonstrate that general population exposures are about 10-fold lower than the estimate used by the first Expert Panel.

- A reproductive toxicity study in marmosets and additional pharmacokinetic data provide additional support for the first Expert Panel's finding that "blood-MEHP levels associated with the ability of high-dose oral exposure to induce reproductive toxicity in rodents may never be achieved from oral exposure in most humans" (NTP CERHR, 2000, p. 100).

Taken together, the new information indicates that concern for reproductive toxicity in humans from DEHP is lower than expressed by the first Expert Panel.

II. EXPOSURE TO DEHP

A. Deterministic Estimates

Exposure of the general population to DEHP has been under study for a number of years. Early exposure estimates were based on measured levels of DEHP in various environmental media and rates of human contact with these media. Indirect estimates of this type (Doull et al., 1999; Huber et al., 1996) were relied on by the first Expert Panel to estimate that the general population received an estimated DEHP exposure in the range of 3–30 µg/kg-day.

More recently, Clark et al. (2003a; 2003b) compiled data on DEHP levels in various media and estimated exposure of the general population using a probabilistic model. The median estimates of 5.0 – 26 µg/kg-day were age dependent but comparable to the exposure range used by the first Expert Panel. The estimated total daily intake of DEHP by age group was: Adult – 8.2 µg/kg/day; Teen – 10.0 µg/kg/day; Child – 18.9 µg/kg/day; Toddler – 25.8 µg/kg/day; Formula-

fed Infant – 5.0 µg/kg/day; Breast-fed Infant – 7.3 µg/kg/day (Clark et al. 2003b). The Clark et al. investigations indicated that more than 90% of the DEHP exposure comes from food.

B. Biomonitoring Data

Biomarker procedures developed by the U.S. Centers for Disease Control and Prevention (CDC) allow DEHP exposures to be measured directly by non-invasively measuring DEHP metabolites in urine. The first such study (Blount et al., 2000) measured the level of mono(2-ethylhexyl) phthalate (MEHP) in the urine of 289 individuals. The reference sample was not representative of the U.S. population as it had an age distribution of 20-60 years, contained 56% women, and was weighted towards minority groups (Koo et al., 2002).

The Blount et al. urinary MEHP levels were used to calculate ambient exposures to DEHP, using two separate methodologies (David, 2000; Kohn et al., 2000). Both methodologies yielded similar DEHP exposures that were about 10-fold lower than the indirect exposure estimates used by the first Expert Panel. The method of David (David 2000), as expressed by Koch et al. (2003) is thus:

$$DI\ (\mu g/kg\ bw/day) = [UE\ (\mu g/g) \times CE\ (mg/kg/day)]/[Fue \times 1000\ (mg/g)] \times MWd/MWm$$

Where DI is the daily intake; UE is the level of monoester (creatinine-corrected) in the urine; CE is the creatinine clearance rate, normalized for body weight; Fue is the molar conversion factor which relates urinary excretion of monoester to diester ingested; and MWd and MWm are the molecular weights of the diester and monoester, respectively.

Based on the Blount et al. data, the mean and 95th percentile exposures to the general adult population were estimated to be 0.6 and 3.0 µg/kg-day by David (2000) and 0.71 and 3.6 µg/kg-day by Kohn et al. (2000).

The CDC subsequently collected biomarker data from a much larger population (over 2500), designed to be representative of the U.S. population (CDC, 2001; CDC 2003). The CDC data were also published in the scientific literature (Silva et al., 2004). The results were similar to those of the reference group (Blount et al., 2000). The mean and 95th percentile DEHP exposures were: adults (n=1456), 0.6 and 3.5 µg/kg-day; adolescents 12 to 19 years old (n=752), 0.5 and 2.4 µg/kg-day; older children 6 to 11 years old (n=328), 0.6 and 4.6 µg/kg-day.

Although these CDC studies provide information on the population at large, they do not directly address exposures to young children who may experience higher exposures as a result of disproportionate exposures due to dietary, exposure pattern and physiological differences. A pilot study of 19 children 12 to 18 months of age was conducted by Brock et al. (2002). The urinary MEHP results indicated that DEHP exposures in young children were somewhat higher than adults, with a mean value of 2.6 µg/kg/day. Koch et al. (2004a) measured three DEHP metabolites in German nursery school children, aged 2-6, and estimated the DEHP dose for children to be about twice as high as that taken up by adults.

Adibi et al. (2003) reported phthalate metabolite levels in 25 pregnant women described as Dominican or African-American, 18-35 years of age, low income, and living in Manhattan or the

South Bronx, New York. Median levels for MEHP were similar to those seen in the CDC general population data; mean levels were approximately 10 times higher, indicating that a few of the women had relatively high levels of exposure at the time of sampling.

Hoppin et al. (2002) measured phthalate metabolites in urine specimens from 46 African-American women, ages 35–49 years, residing in the Washington, DC area in 1996–1997. The median level for MEHP was about twice as high as in the general CDC population data.

Koch et al. (2003) evaluated DEHP levels in 86 members of the German population. Using the formula of David (2000) to calculate exposures, they reported significantly higher exposures than indicated by the CDC data. However, it appears that was due to their use of data from Schmid and Schlatter (1985) on excretion of metabolites, rather than data from Anderson et al. (2001). Schmid and Schlatter collected data from only 2 individuals with some inter-individual variability. The molar excretion fraction for MEHP from their data is 2.4%, far lower than the 13% calculated by Anderson et al. (2001) using groups of 8 individuals for each dose level. As summarized above, both Kohn et al. and David (2000) calculated exposures from the urinary excretion data set of Blount et al. (2000). The exposure level calculated by Kohn et al. did not incorporate a molar excretion fraction, and the exposure level is nearly identical to the value calculated by David using the 13% molar excretion fraction from Anderson et al. This would imply that 13% is an accurate value for MEHP rather than 2.4%. Furthermore, the percentage of excreted MEHP from Schmid and Schlatter differs from that noted by Dirven et al. (1993), who measured the urinary metabolite profiles of workers exposed to DEHP, and others who measured excretion from non-human primates. Koch et al. themselves subsequently measured a molar conversion factor for MEHP that is approximately a factor of 3 higher than that reported by Schmid and Schlatter (Koch et al., 2004b). Using the Anderson et al. molar excretion fraction, the calculated median exposure for DEHP from the Koch et al. data would be 1.76 µg/kg/day rather than 10.5 µg/kg/day as calculated by the authors.

Results of the biomarker studies by the CDC provide detailed information on the exposure of the general population, including young children, to DEHP. Because the exposures derived from the CDC data are less than those given by previous deterministic methods, the CDC biomarker results indicate that there are no unidentified sources of DEHP that contribute significantly to exposures of the general population. DEHP exposures calculated from the biomonitoring data are about 10-fold lower than the indirect exposure estimates used by the first Expert Panel to conclude that DEHP posed minimal concerns for the general population.

C. Medical Device Exposures

DEHP is the primary plasticizer used in flexible PVC medical devices. Medical device exposures were a primary concern of the first Expert Panel; however, information on such exposures was somewhat limited. Since the first DEHP review, new information has become available on the potential for exposure to DEHP from medical devices.

The Food and Drug Administration published a safety assessment on DEHP in medical devices in 2001 (FDA, 2001). The assessment included exposure estimates for a number of medical procedures involving DEHP-plasticized medical devices. Table 1 is a summary of the FDA

exposure estimates. *In using these exposures estimates for risk assessment, it is important to note that 1) the values in Table are the upper end of the exposure range estimated by FDA, and 2) with the exception of dialysis, the exposures are acute exposures*. Similar exposure estimates have been developed by Health Canada (2002) and the European Commission (SCMPMD, 2002).

The potential for exposure to DEHP from medical devices is greatest for premature infants in neonatal intensive care units, since they may receive those treatments with the highest exposure estimates (e.g., total transfusion and extra-corporeal membrane oxygenation (ECMO)). Calafat et al. (2004) measured DEHP metabolites in urine from 6 premature infants who underwent intensive therapeutic interventions. The geometric mean urinary concentration of MEHP was 100 ng/mL, compared to 4.6 ng/L in Brock et al. (2000; infants aged 12-18 months) and 3.43 ng/L in the general U.S. population (Silva et al.; persons aged 6 and up).

Table 1. Dose of DEHP received by adult and neonatal patients undergoing various medical procedures.

	Adult[1]	Neonate[2]
	DEHP dose (mg/kg/day)	**DEHP dose (mg/kg/day)**
Infusion of crystalloid IV solutions	0.005	0.03
IV infusion of drugs requiring pharmaceutical vehicles for solubilization	0.15	0.03
TPN administration		
Without added lipid	0.03	0.03
With added lipid	0.13	2.5
EVA bag with PVC tubing	0.06	
Blood transfusion		
Trauma patient	8.5	
Transfusion/ECMO pts.	3.0	
Exchange transfusion		22.6
Replacement transfusion - Neonate in NICU		0.3
Replacement transfusion - Correction of anemia in patients receiving chemotherapy and patients with sickle cell disease	0.09	
Replacement transfusion - Surgical patient undergoing CABG	0.28	
Treatment of clotting disorders with cryoprecipitate	0.03	
Cardiopulmonary bypass		
CABG	1	
Orthotopic heart transplant	0.3	
Artificial heart transplant	2.4	
ECMO		14
Apheresis	0.03	
Hemodialysis	0.36	
Peritoneal dialysis	<0.01	
Enteral nutrition	0.14	0.14

[1] 70 kg body weight
[2] 4 kg body weight
Source: FDA (2001), Table 4-1.

III. TOXICOLOGY

A. Human Data

While there are no definitive human data on the potential reproductive effects of DEHP, there are several human studies that were not available or not considered in the first CERHR review.

Several studies have evaluated reproductive and developmental effects in dialysis patients, a class of persons receiving higher-than-average exposure to DEHP over extended periods of time. Studies of pregnant women exposed to DEHP during dialysis do not indicate an increase in malformed offspring (Reister et al., 1999; Chan et al., 1998; Toma et al., 1999; Blowey and Warady, 1998). While some testicular effects have been seen in men with chronic renal insufficiency who underwent hemodialysis or peritoneal dialysis (Nistal et al., 1996), such effects cannot be attributed to DEHP, because they may be due to uremia, which is common in dialysis patients (FDA, 2001; Handelsman and Dong, 1993).

Colon et al. (2000) reported that DEHP levels in the blood of 41 girls experiencing premature thelarche were higher than those of 35 controls. McKee (2004) critiqued the Colon et al. study and showed that the reported DEHP levels do not appear plausible, and that an association between phthalates and early thelarche is not supported by the toxicological data.

Modigh et al. (2002) assessed paternal occupational exposure to DEHP and fertility. Men working in three plants with DEHP exposure were studied retrospectively. Information on time to pregnancy was available for 326 pregnancies fathered by 193 men. Male exposure to DEHP during every month of the time to pregnancy was classified into one of three exposure categories in which inhalation exposures ranged from <0.1 to 2.1 mg/m^3. The pregnancies of employed women with unexposed partners or pregnancies of employed men unexposed during the time to pregnancy formed the reference group. The fecundity ratio for time to pregnancy was 1.07 for those with low exposure and 0.97 for the highly exposed, after adjustment for the father's age, mother's age, and length of recall. When the analyses were restricted to first pregnancy, the fecundity ratio was 1.13 for low exposure and 1.02 for high exposure. The authors concluded that time to pregnancy was not prolonged among couples with paternal exposure to DEHP.

Hack et al. (2002) investigated various parameters in young adults who had been very-low-birth-weight infants (and thus probably had relatively high DEHP exposure due to intensive medical interventions). They found no difference in the rates of sexual intercourse, pregnancy, or live births between normal birth-weight men and men who had very-low-birth-weight. This suggests that probable high exposures to DEHP as neonates had no adverse effect on male reproductive function in these men. There was a significant difference in these parameters between very-low-birth-weight and normal-weight women, which could be due to a variety of factors.

Latini et al. (2003) measured DEHP and MEHP in cord blood of 84 newborns, and found that MEHP-positive newborns showed a significantly lower gestational age than MEHP-negative newborns (approximately one week on average). A review of the data suggests that this association may reflect greater use of medical procedures with shorter pregnancies. DEHP has a urinary excretion half-life of about 6 hours (Peck and Albro, 1982). Latini et al. reported mean concentrations of DEHP and MEHP of 1.19 and 0.52 μg/ml respectively. Because DEHP is

converted to MEHP in plasma with a half-time of 30 minutes (Peck and Albro, 1982), the only situation in which one would expect the DEHP concentration to exceed the MEHP concentration is if the sample collection had been immediately after the dose administration. Looking at the data in Table 1 of the paper, for 10 of the pregnancies there was no DEHP or MEHP detected. All newborns in this group were full term and there were no small-for-gestational-age infants. The absence of DEHP or MEHP may reflect that in this group there were no medical procedures, or they took place long before sample collection. There were 9 pregnancies for which DEHP but not MEHP were detected. Again, all were full term and there were no small for gestational age children. So, for these two groups, the blood data suggest that medical procedures were minimal and limited to those administered near the time of birth. There were 56 pregnancies for which both DEHP and MEHP were detected. Of these, 8 were preterm, 3 of the pre-terms were < 1500 g, and 2 were small for gestational age. This suggests that some of these pregnancies may have been more problematic, so that more medical procedures may have been employed, over a longer period of time. Finally, there were 9 pregnancies in which only MEHP was detected, of which 6 were full term and 3 were preterm. In this case, all 3 of the pre-terms were > 1500 g although 2 were considered to be small for gestational age. The presence of only MEHP suggests that medical procedures were employed but not at the time of delivery. Perhaps as the preterms in this group were not as small as those in the DEHP+/MEHP+ group, less needed to be done at the time of delivery. In any event, because of the short half-life of DEHP in the body, the cord blood DEHP and MEHP levels would reflect exposure within a day or two of the time of delivery, and therefore cannot explain the nature of the pregnancies.

Cobellis et al. (2003) measured DEHP and MEHP in plasma and interperitoneal fluid of 55 women with endometriosis and 24 controls. They found that the endometriotic women showed significantly higher levels of plasma DEHP than the controls. As noted above, DEHP is converted to MEHP in plasma with a half-time of 30 minutes (Peck and Albro, 1982). Because MEHP levels were not elevated in the endometriotic women, it appears the high DEHP levels either reflected recent medical intervention (not unlikely for patients undergoing treatment) or analytical error (DEHP is known to be a common laboratory contaminant – see Blount et al., 2000; Kessler et al., 2001).

Duty and associates have published three papers investigating phthalate metabolite levels and sperm parameters in men who were part of a subfertile couple presenting at an infertility clinic (Duty et al., 2003a; 2003b; 2004). No association between MEHP concentrations and the measured sperm parameters were seen in two of the studies (Duty et al., 2003a; 2003b). There was a suggestion of a negative association between MEHP and two computer-aided sperm analysis parameters, but the association was not statistically significant (Duty et al. 2004). The value of the Duty et al. data for assessing DEHP exposure and fertility is limited, because the samples would reflect exposures only over the previous day or two, and there were no study controls. Within these limitations, the weight of evidence of the Duty et al. papers is that there is no relationship between DEHP exposure and sperm quality.

Rais-Bahrami and coworkers (2004) studied the reproductive development of neonates (13 males and 6 females) who had undergone extracorporeal membrane oxygenation (ECMO) therapy as newborns. These results are particularly relevant to the first Expert Panel's concern regarding medical device exposures because ECMO treatment is considered to involve the highest DEHP

exposures (FDA, 2001; Health Canada, 2002). The authors reported no significant effects on physical growth and pubertal maturity. Thyroid, liver, and renal functions as well as male and female gonadal functions were tested and found to be within the normal range for age and sex distribution. While this study is preliminary, it suggests that DEHP does not have adverse reproductive effects in humans even at relatively high exposure levels during a sensitive lifestage.

B. Animal Data

1. Pharmacokinetics and Metabolism of DEHP in Primates vs. Rodents

There are significant differences between rodents and primates in the pharmacokinetics and metabolism of DEHP. Following oral administration to rats, DEHP is efficiently transformed in the gut by nonspecific pancreatic lipase and mucosal esterase to its rapidly absorbed monoester, MEHP (White et al., 1980; Albro et al., 1982; Albro and Thomas, 1983; Albro and Lavenhar, 1989), the toxicologically relevant metabolite of DEHP (Sjoeberg et al., 1986a, 1986b; Teirlynck et al., 1988; Richburg and Boekelheide, 1996; Li et al., 2000; Daalgard et al., 2001). As a result of this efficient metabolism and absorption, at least 50% of orally administered DEHP is absorbed by rats across a broad range of doses (Rhodes et al., 1986; Astill, 1989).

In contrast, absorption of orally administered DEHP by primates is more limited. For example, Rhodes et al. (1986) reported that marmosets dosed with dietary DEHP at 2,500 mg/kg/day achieved a maximum absorbed dose that was 10 to 25-fold lower than that of equally dosed rats. Similar results were obtained in studies in cynomologus monkeys (Astill, 1989). Both findings are supported by results of a recent study (Kurata et al., 2005) in which juvenile rats and marmosets were gavaged with 100 mg/kg DEHP. Plasma radioactivity measurements taken up to 24 hr post-dosing indicated that rats absorbed 20 to 100-fold more DEHP than marmosets. While this radiolabel study could not differentiate between DEHP and its metabolites, results of another recent study (Kessler et al., 2004) bear on this issue. In Kessler et al., pregnant and nonpregnant rats and marmosets were given oral doses of 30 or 500 mg/kg/day DEHP. In both species, MEHP was present in the blood at much higher levels than DEHP. In rats, the normalized areas under the concentration-time curves (AUCs) for MEHP were 100-fold higher than the normalized AUCs for DEHP; in marmosets, however, this difference was only about 10-fold. There was also a significant interspecies difference in plasma MEHP levels. Peak blood levels of MEHP in rats were 2 to 4-fold higher than those in marmosets, while AUC measurements indicated that MEHP levels in rats were 4 to 12-fold higher than those of marmosets. Thus, current evidence indicates that, when exposed to similar levels of DEHP, rats experience much higher levels of the toxicologically relevant metabolite, MEHP, than do primates.

The mouse may be even more sensitive than the rat to the reproductive effects of DEHP. Comparative pharmacokinetic data between pregnant and non-pregnant rats and mice from Laignelet and Lhuguenot (2000a-d) provide evidence that the increased sensitivity of mice can, at least in part, be explained by pharmacokinetic differences. These data demonstrate that peak blood levels (Cmax) are significantly higher in mice than rats administered comparable dose levels. The peak blood level in pregnant mice receiving a single dose of 200 mg/kg DEHP was

91 nmol DEHP equivalents/g blood, while the peak level in pregnant rats was 58 nmol DEHP equivalents/g blood. Peak levels of MEHP were 84 nmol DEHP equivalents/g blood in mice compared with 36.4 nmol DEHP equivalents/g blood in rats. These mouse data, combined with those above for rats, indicate that the systemic dose for primates is less that that for rodents at equivalent exposure levels.

2. Animal Reproductive Toxicity Data

a. Primate Data Since the First Expert Panel

The first Expert Panel, aware that phthalates have been shown to induce testicular atrophy in rodents, but not primates, discussed the need for studies of the effects of phthalates on male sexual development in juvenile, non-human primates. To address this need, Tomonari et al. (2003; and MCSI, 2003) conducted a repeated oral dose study of the effects of DEHP treatment on the development of the male reproductive tract in common marmoset monkeys (*Callithrix jacchus*). The animals were administered 0, 100, 500 or 2500 mg/kg/day by gavage on a daily basis for 65 weeks, from weaning (about three months) until about 18 months of age. This exposure period covered the entire sexual maturation phase as marmosets reach sexual maturity at about 400 to 450 days (57-65 weeks). During the treatment period, the testosterone levels in all treated groups were similar to those of control groups. At the end of the treatment period, the animals were examined for 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.

No treatment-related abnormalities were observed in microscopic and functional examinations of the marmosets' testes, and there were no treatment-related effects on sperm count. In addition, histochemical examination after 3β hydroxysteroid dehydrogenase staining did not reveal any alteration in steroid synthesis. The only significant effect observed, a dose-dependent increase in P450 content, was considered to be an adaptive change and not an adverse affect. Thus, this study demonstrated that daily administration of high doses of DEHP (up to 2,500 mg/kg/day) spanning the entire period of sexual maturation had no effect on male reproductive tract development in the marmoset. Consequently, the NOAEL from this study was 2,500 mg/kg/day.

The data for testosterone (T) levels in the plasma of control and treated marmosets in Tomonari et al. are variable over the sampling period. This variability is greater than that usually seen in rodent studies. However, a comparison of these data to published values indicates that such variability, even among untreated animals, is not uncommon for marmosets. McKinnell et al. (2001), Lunn et al. (1994), and Abbott and Hearn (1978) reported plasma T levels from marmosets that varied from detectable to 50 ng/ml over the course of 800 days post parturition. These fluctuations are within the values measured in Tomonari et al. Furthermore, the impact of serum T variability is questionable. Although Tanner scores for maturation of external male reproductive organs were not collected, the lack of differences in testes weight, sperm count, and histopathology suggests that these organs were not affected by continuous DEHP exposure. Thus, T levels and their variability in the recent marmoset study are not unique and do not appear to represent an adverse effect.

b. Rodent Data Since the First Expert Panel

The first Expert Panel noted that, although some studies were in progress,[1] no multigeneration studies of DEHP in rodents consistent with current guidelines were available for review at that time. Since then, four multigeneration studies of DEHP in rodents have been completed: (1) a continuous breeding study in rats (Wolfe and Layton, 2003); (2) a two-generation reproductive toxicity study in rats (Schilling et al., 2001); (3) a two-generation reproductive toxicity study in mice (Tanaka, 2002); and (4) a two-generation cross-mating reproductive toxicity study in mice (Tanaka, 2005). As discussed below, none of these studies reported male reproductive effects in rodents at dietary levels below 46 mg/kg/day, suggesting that reconsideration of the 3.7 – 14 mg/kg/day NOAEL range adopted by the first Expert Panel is warranted.

In a continuous breeding study sponsored by NTP, Wolfe and Layton (2003), administered DEHP in the diet of three generations of Sprague-Dawley rats at doses of 1.5, 10, 30, 100, 300, 1,000, 7,500 and 10,000 ppm. The authors reported that DEHP produced no male reproductive or developmental toxicity at doses lower than 7,500 ppm (about 360 mg/kg/day) except for a possible increase in aplastic testes and epididymides, small testis, hypoplasia of the seminal vesicles and small prostates in a low number of animals at 1,000 and 300 ppm (about 46 and 14 mg/kg/day, respectively). However, these minor effects were not statistically significant and were not accompanied by either histopathological changes to the reproductive organs (in particular there was no Sertoli cell vacuolation – see discussion of Poon et al. below), reduced organ weight, or lowered reproductive success. Because of their low incidence rate and the absence of other reproductive effects, the abnormalities in the male reproductive organs reported at 1,000 and 300 ppm should not be considered toxicologically significant. As such, the appropriate NOAEL for reproductive and developmental effects for this study is 1,000 ppm, or approximately 46 mg/kg/day.

In Schilling et al. (2001), DEHP was administered in the diet to groups of male and female Wistar rats at concentrations of 1,000, 3,000, or 9,000 ppm (about 113, 340, and 1,090 mg/kg/day respectively) continuously throughout two generations. As part of the overall evaluation of reproductive toxicity, which included assessment for gross pathology, testes from F_0 and F_1 parental males were subjected to extensive histopathological examination. DEHP had no effect on F_0 or F_1 fertility at any dose. Reproductive effects were observed only at the 1,090 mg/kg/day dose and developmental effects, including mild seminiferous tubular atrophy in F_1 males, were observed only at the 340 and 1,090 mg/kg/day doses. While there was a change in pup liver weights at all doses, this effect was considered to be an adaptive response rather than an adverse toxic effect. In rats, liver effects such as weight change are associated with PPARα induction, which is not relevant to humans (e.g., Klaunig et al., 2003). Therefore, the overall NOAEL from this study for pathological changes in the testes was about 113 mg/kg/day.

In Tanaka (2002), DEHP was administered in the diet of male and female mice for two generations, from five weeks of age for the F_0 generation (four weeks before mating) to nine weeks of age for the F_1 generation, at dietary levels of 0.01, 0.03, or 0.09% (about 15, 50 or 150

[1] The first Expert Panel report stated that two of these studies (Schilling et al. and Wolfe and Layton) "should provide additional data from which to establish a LOAEL and NOAEL."

mg/kg, respectively). The F_1 generation was assessed for litter size and weight and offspring survival and growth; no histopathological observations were made. The only effect observed was a slight depression in male offspring weight at the low dose, which was correlated to litter size, and not considered a treatment effect. Thus, there were no adverse reproductive effects at any of the dietary levels tested, and a reasonably conservative reproductive toxicity NOAEL for this study is 150 mg/kg.

In Tanaka (2005), DEHP was administered in the diet of male and female mice for two generations, from five weeks of age for the F_0 generation (four weeks before mating) to nine weeks of age for the F_1 generation, at a single dietary level of 0.03 %. This dietary level corresponded to varying DEHP doses in different experiments, depending upon food consumption. The lowest dose any experimental group received was about 42 mg/kg/day and the highest dose was about 171 mg/kg/day. Male and Female F_0 mice were cross-mated as: control males-control females; control males-DEHP females; DEHP males-control females; DEHP males-DEHP females. The F_1 generation was assessed for litter size and weight and offspring survival and growth; no histopathological observations were made. The only adverse affect observed was a depression in female offspring body weight at PND 14. As in Tanaka (2002), the author did not consider this a treatment effect. Thus, no reproductive effects were seen at doses between 42 and 171 mg/kg/day.

The lowest overall NOAEL identified by these new multigeneration studies, 46 mg/kg/day, is considerably higher than the NOAEL range adopted by the first Expert Panel, 3.7 – 14 mg/kg/day. The first Expert Panel's low-end NOAEL, 3.7 mg/kg/day, was derived from Poon et al. (1997), a study in which Sprague-Dawley rats were exposed to dietary DEHP levels of 0, 50, 500 or 5,000 ppm (about 0.4, 3.7, 38 and 375 mg/kg/day) for 13 weeks, beginning at puberty. The authors reported cytoplasmic vacuolation in the Sertoli (testicular) cells of male rats at concentrations as low as 38 mg/kg/day (the LOAEL), establishing the NOAEL as 3.7 mg/kg/day. The effect at the LOAEL was very mild – the Sertoli cell vacuolation was described by the authors as minimal – and is of questionable significance for the reproductive function of the rat. The high-end NOAEL, 14 mg/kg/day, was derived from reproductive toxicity experiments reported by Reel et al. (1985) and Lamb et al. (1987). In these experiments, 11 week old CD-1 Swiss mice were administered diets containing 0.0, 0.01, 0.1 or 0.3% DEHP (about 0, 14, 141 and 425 mg/kg/day) for a total of 15 weeks – individually for a 7-day premating period and for 14 weeks as breeding pairs. The authors reported reductions in litter size and proportions of pairs having litters at concentrations as low as 141 mg/kg/day (the LOAEL), establishing the NOAEL as 14 mg/kg/day. However, the first Expert Panel stated that, because the dose groups were not evaluated at necropsy, and because reproductive development and performance of the second generation were not assessed, it had moderate-to-low confidence that the doses represented the true LOAEL and NOAEL for reproductive toxicity.

The validity of the low-end NOAEL adopted by the first Expert Panel is questionable, as several subsequent studies have failed to replicate the testicular histopathology reported by Poon et al. As discussed above, neither Schilling et al. nor Wolfe and Layton reported any statistically significant evidence of testicular lesions in male rats despite continuous exposure to DEHP for two or more generations at doses approximating 113 and 46 mg/kg/day, respectively; nor did they find cytoplasmic vacuolation to be a sensitive indicator of testicular toxicity. In addition, a

subsequent independent review of the Wolfe and Layton testicular slides by a pathology working group (Peckham, 2003) 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 at the two highest doses, 7,500 and 10,000 ppm (359 and 543 mg/kg/day, respectively). There were, however, no treatment related lesions in animals exposed to 1,000 ppm (46 mg/kg/day) or less. Moreover, Sertoli cell vacuolation was not observed at any dose in any generation. Unlike the single-generation studies available to the first Expert Panel, these new multigeneration studies included assessments of testicular toxicity in response to continuous DEHP exposure from conception to termination, and are therefore particularly relevant in the determination of the overall NOAEL for reproductive toxicity.

In addition to the new multigeneration studies, two new single-generation studies reported no testicular pathology below 200 mg/kg/day. Dalgaard et al. (2000) investigated the testicular effects on juvenile Wistar rats of subchronic exposures to DEHP via gavage. Testicular atrophy was found in animals exposed to 5,000 or 10,000 mg/kg/day, but there were no pathological changes in animals exposed to 1,000 mg/kg/day. Akingbemi et al. (2001) investigated the effects of DEHP administered either *in utero* or to prepubertal Long-Evans rats. Although changes in testosterone levels, which are not themselves a proper endpoint for risk assessment,[2] were observed when the DEHP was given during *in utero* development, there were no effects on testicular weight and no pathological changes at levels up to 200 mg/kg/day. Thus, the report of testicular pathology by Poon et al., upon which the first Expert Panel based its low-end NOAEL, has not been replicated by four independent groups of investigators. In light of this, and the minimal nature of the effects reported at the lowest effect level in Poon et al., the 3.7 mg/kg/day NOAEL suggested by the first Expert Panel should not be regarded as sufficiently reliable for risk assessment.

Similarly, the litter effects reported in Reel et al. and Lamb et al., upon which the first Expert Panel based the high-end NOAEL (14 mg/kg/day) have not been replicated by two newer studies.. As described above, Tanaka (2002; 2005) found no litter effects in mice at any dose, including the maximum doses of 150 and 171 mg/kg/day. The combined results of these studies suggest that a reasonably conservative reproductive NOAEL in mice is about 100 mg/kg/day.

It could be argued that the Poon et al. NOEL of 3.7 mg/kg/day is supported by the results of David et al. (2000), who reported a NOAEL of 29 mg/kg/day based on a 104-week chronic exposure study in Fischer 344 rats (LOAEL of 147 mg/kg/day). The first Expert Panel suggested that the David et al. NOAEL should be even lower, 5.8 mg/kg/day, based on aspermia seen at 29 mg/kg/day at 104 weeks. However, the relevance of this end point for human risk assessment is

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[2] As stated by Moore et al. (1995): "Various [biochemical] markers of [reproductive] exposure and effect have been investigated in male reproductive toxicology, including ... androgens ... currently, however, they cannot be considered evidence of male reproductive toxicity." Similarly, in its assessment of DEHP, the FDA (2001) stated: ""Only studies with effects broadly considered to be adverse (histopathological or functional changes) will serve as the basis for TI derivation." Akingbemi et al. identified a "subclinical" or "precursor" effect, but this does not seem to be associated with either pathological or functional changes, as shown by their own data, and corroborated by Dalgaard (2000) and Schilling et al. (2001).

doubtful. First, aspermia is a normal occurrence in aging rats, as evidenced by the fact that 58% of the control group was affected at 104 weeks in David et al. The enhancement of this 'background' lesion at levels of 29 mg/kg/day and above likely reflects an acceleration of an aging process that may be unique to peroxisome proliferation, which is a rat-specific phenomenon (Ward et al., 1998; Youseff and Badr, 1998).[3] Second, the aspermia was very late appearing; there was evidence of aspermia in the high dose group (789 mg/kg/day) at the 78 week interim sacrifice, but it was not present in lower dose groups at 78 weeks. Third, although the aspermia increased modestly with dose, the differences were of doubtful statistical significance. Thus, it is questionable whether the 5.8 mg/kg/day dose in David et al. should be considered a NOAEL for purposes of setting a level protective of human health.

Based on the multigeneration studies completed since the first Expert panel, a conservative overall NOAEL for DEHP reproductive effects in male rats is 46 mg/kg/day, the NOAEL reported in Wolfe and Layton. This level is conservative because, applying a weight of evidence approach, the data from the new multigeneration studies would support an even higher NOAEL. The LOAELs reported by Wolfe and Layton and Schilling et al., 359 and 340 mg/kg/day, respectively, are substantially higher than the NOAEL of 113 mg/kg/day reported by Schilling et al., indicating that 113 mg/kg/day is a plausible overall NOAEL in itself. When combined with the 150 mg/kg/day NOAEL reported by Tanaka, the data suggest that a reasonably conservative NOAEL for oral DEHP exposure lies in the range of 100 to 150 mg/kg/day. Thus, the 46 mg/kg/day NOAEL reported by Wolfe and Layton is conservative.

In summary, new, high quality multigeneration exposure data suggest that a reasonably conservative NOAEL for DEHP reproductive toxicity in male rats is 113 mg/kg/day, and that the most conservative NOAEL is 46 mg/kg/day. These NOAELs are 7 – 25, and 3 – 12 fold higher, respectively, than the range of NOAELs suggested by the first Expert Panel. In addition, the recent studies in primates provide evidence that postnatal exposures, even at very high levels, do not affect male sexual maturation and thus that primates are likely less sensitive than rodents to DEHP. This provides additional confidence that adopting a NOAEL higher than that suggested by the first Expert Panel is scientifically appropriate and protective of human health.

c. Medical Device Exposures

As discussed above, medical device exposures to DEHP, particularly exposures to critically ill infants undergoing intensive therapeutic interventions, were a primary concern of the first Expert Panel. Since the first Expert Panel review, in addition to new exposure information, new data has become available on the toxicity of intravenous exposures to DEHP. In particular, in a recent study by Cammack et al. (2003), DEHP was administered to 3 to 5-day-old male Sprague-Dawley rats by daily intravenous injections of 60, 300, or 600 mg/kg/day or by daily oral gavage of 300 or 600 mg/kg/day for 21 days. Histopathological evaluation and organ weight measurements were performed on some animals after 21 days of dosing (primary group) and at a later date on the recovery group animals that were held without further treatment until sexual maturity at approximately 90 days of age. No effects of any type were observed in animals

[3] The hypophyseal-gonadal axis of hormone triggers and feedback breaks down in older rats, a condition unique to that species (Alison et al., 1994).

treated intravenously with 60 mg/kg/day. Testicular changes, consisting of a partial depletion of the germinal epithelium and/or decrease in diameter of seminiferous tubules, were present in all animals of the 300 and 600 mg/kg/day groups after the 21-day dosing period. Testes weight decreased and liver weight increased in these animals. Testes changes were dose-related and generally more severe among animals dosed orally versus intravenously. In the recovery animals, a residual DEHP-induced decrease in seminiferous tubule diameter was present in the testis of several animals dosed orally at 300 and 600 mg/kg/day, but not in animals dosed intravenously. There was no germinal cell depletion or Sertoli cell alteration observed in any dose group at any time. Notably, no effects on sperm count, sperm morphology, or sperm motility were observed at 90 days of age in any of the groups.

Thus, the NOEL for intravenous administration of DEHP in Cammack et al. was 60 mg/kg/day. This was the NOEL used by the FDA to develop its intravenous tolerable intake for DEHP (FDA, 2001). This should be considered a conservative NOEL because the effects observed at the lowest observed effect level of 300 mg/kg/day were relatively mild and nearly all reversible, indicating that the "true" NOEL is probably closer to 300 mg/kg/day than to 60 mg/kg/day. As with the rodent oral toxicity studies discussed above, the marmoset data indicating that primates are likely much less sensitive to DEHP than rodents provide additional confidence that the 60 mg/kg/day intravenous exposure NOEL is conservative, and protective of human health.

d. Suitability of the Marmoset as a Model

The available science indicates that there are significant differences between rodents and primates that call into question the relevance of rodent reproductive toxicity data to human health risk assessment. In the most recent primate study designed to evaluate the effect of prolonged exposure during a sensitive period of development, marmosets showed no adverse testicular toxicity after exposure to 2,500 mg/kg/day DEHP from weaning to adulthood (Tomonari et al., 2003). The low sensitivity of primates to DEHP is supported by the results of several other studies. Rhodes et al. (1986) reported a lack of testicular atrophy or histopathology in adult marmosets treated with 2,000 mg/kg DEHP for 14 days. A similar lack of effect was noted by Kurata et al. (1998) in adult marmosets treated with 2,500 mg/kg for 13 weeks, and by Pugh et al. (2000) in adolescent cynomolgus monkeys treated with 500 mg/kg DEHP for 14 days. These data contrast with studies of adult and periadolescent rodents, which reportedly demonstrate reproductive effects from DEHP exposures as low as 3.7 mg/kg/day (Reel et al., 1985; Agarwal et al., 1986; Lamb et al., 1987; Dostal et al., 1989; Poon et al., 1997). The testicular effects observed in rodents are absent in primates following exposure to DEHP.

The FDA and others have proposed that the marmoset is an appropriate model for human health assessment, and may be a more appropriate model than the rat for evaluation of reproductive toxicity hazard to humans. For example, the FDA Safety Assessment of DEHP states:

> Spermatogenesis in the marmoset is organizationally similar to the process that occurs in humans, with regard to length of the spermatogenic cycle, duration of spermatogenesis, and number of mitotic divisions (Millar et al., 2000; Weinbauer et al., 2001). Consequently, the marmoset has been described as an appropriate model for experimental studies of human spermatogenesis. By analogy, it can be

assumed that DEHP-induced effects on this process seen in marmosets would be applicable for humans.

(FDA, 2001, p. 35.)

Available science provides several reasons for favoring the marmoset model. First, the architecture of the testis in the marmoset is similar to human testis (Millar et al., 2000). Millar et al. found that the organization of seminiferous tubules in the marmoset was similar to humans; that DNA binding protamine-2 had similar localization in marmoset and human testes; and that cell proliferation, as measured by PCNA, was localized in the same cells in marmosets and humans, but in different cells in rats. Second, the stages and the organization of spermatogenesis in marmosets are more similar to humans than to rats (Weinbauer et al., 2001; Millar et al., 2000). For example, marmosets have nine and humans have six different stages of spermatogenesis, whereas rats have 14 stages. Also, spermatogenesis in humans and marmosets is more "efficient" in that each Sertoli cell supports a germ cell volume that is five times less than that supported by rat Sertoli cells. Third, Sharpe et al. (2000) reported that Sertoli cell numbers and postnatal development are similar in marmosets and humans. In both marmosets and humans, Sertoli cells proliferate during the fetal/neonatal period, and again during the peripubertal period. In rats, Sertoli cell proliferation may also follow this pattern, but because the fetal/neonatal and peripubescent periods overlap, it appears as a single burst of proliferation (Sharpe et al., 2003b). Furthermore, maturation of Sertoli cells may be under different endocrine control for marmosets and humans than for rats (Lunn et al., 1994; Sharpe et al., 2000; 2003a). In rats, thyroid hormones alone and in combination with FSH trigger expression of androgen receptor (AR) and the maturation of Sertoli cells. In marmosets and humans, no AR expression has been found during the neonatal period even though FSH levels are high, and thyroid hormones appear to have little impact on Sertoli cells maturation. Since the Sertoli cell is a target for DEHP, these observations suggest that the marmoset may represent a particularly good model for assessing likely effects of DEHP on humans (Sharpe et al., 2003a).

Endocrine control of testicular events in the marmoset is different than for rats, and may be more like humans (Sharpe et al., 2003a). As described above, neonatal expression of androgen hormone receptors in marmoset testis is similar to that in human testis (McKinnell et al., 2001), which is different from rat testis. Furthermore, low testosterone levels in marmosets during the neonatal period have little impact on sexual behavior, and may have little relevance for cryptorchidism in human males (Sharpe et al., 2003a), unlike the postulated MOA for testicular dysgenesis in rats (Parks et al., 2000).

(1) Effect of Vitamin C in the Marmoset Diet

As already indicated, Tomonari et al. observed no effects of DEHP on marmoset reproductive development at doses up to 2,500 mg/kg/day. Although Tomonari et al. is a high quality study, concerns have been raised suggesting that the lack of observed effects in primates at this dose was in fact due to the protective action of high doses of vitamin C, and not indicative of a difference in the effects of DEHP between rodents and primates. These concerns, however, are not well founded because: 1) the levels of vitamin C used in Tomonari et al. are not high relative to the marmoset's requirements and 2) based on the available science, it is not clear that vitamin C affords any protection to primates from DEHP exposure. Moreover, if the level of vitamin C

in the marmosets' diet in Tomonari et al. in fact provided the degree of protection necessary to be responsible for the observed lack of effects, then the level of vitamin C in the average human diet would be protective of any possible exposure to DEHP. In other words, the vitamin C levels in the marmoset diet were similar to normal levels in the human diet and, consequently, whether vitamin C had a protective effect in Tomonari et al. is not directly relevant to a risk assessment.

Marmosets, like all primates, require that their diet be supplemented with vitamin C (ascorbic acid) (NRC, 2003). Flurer et al. (1987) reported that marmosets need more vitamin C than do humans, suggesting that a minimum of 20 mg/kg/day (the same amount cited by NRC, 2003) should be provided in the diet. Flurer et al. also stated that they consider the optimal vitamin C content in the diet of the marmoset to be 2,000 ppm. The diet used in Tomonari et al. provided 1g vitamin C per 1,000 g feed (0.1%, or 1,000 ppm, or about 80 mg/day), an amount recommended in the published literature (Layne and Power, 2003), and one-half that recommended by Flurer et al. Thus, the amount of vitamin C used in the Tomonari et al. study was not excessive relative to the marmoset's dietary requirements and any potential protection conferred by the vitamin C would not be out of line with the degree of protection afforded the marmoset by its natural diet.

Moreover, it is questionable whether a vitamin C supplemented diet even impacts DEHP-induced testicular effects. Ishihara et al. (2000) demonstrated that rats given vitamins C and E in drinking water (about 450-500 mg/kg/day vitamin C) exhibited reduced testicular effects, relative to animals not receiving vitamins, from exposure to 20,000 ppm (1,000 – 1,500 mg/kg/day) DEHP in the diet. The absolute testes weights of DEHP/vitamin treated animals were significantly lower than controls (although testes-to-body weight ratios were comparable to controls), but significantly higher than DEHP-exposed rats that did not receive vitamins C and E. In addition, testicular pathology of DEHP/vitamin rats was improved relative to DEHP rats, though not entirely normal (spermatogenesis was present, but not at control levels; severe aspermatogenesis was not observed in DEHP/vitamin animals). Thus, the combination of vitamins C and E afforded some protection to the rats against the reproductive toxicity of high doses of DEHP. However, the potential protective effect of vitamin C cannot be distinguished from that of vitamin E because the two were provided together. Verma and Nair (2001) showed that mice pretreated with vitamin E showed little or no signs of testicular toxicity following treatment with aflatoxin. On the other hand, Cave and Foster (1990) reported that very high levels of vitamin C (2 mM) were required for any protective effect against *m*-dinitrobenzene or *m*-nitrosonitrobenzene toxicity on Sertoli cells *in vitro*. Hence, it is possible that vitamin C had little impact on testicular toxicity, and that vitamin E played the larger role in the protective effect observed by Ishihara et al. in rats.

Even if vitamin C does protect rats against the effects of DEHP exposure, the protective effect of dietary vitamin C in primates would have to be much greater than in rodents to account for the results of Tomonari et al. Because rats, unlike primates, produce about 150 mg/kg/day of their own vitamin C (Chatterjee, 1973), the rats in the Ishihara et al. study were effectively exposed to a total vitamin C dose of about 600 – 650 mg/kg/day. Comparing the results of Ishihara et al. to Tomonari et al., rats given about 600 mg/kg vitamin C (plus 225 mg/kg vitamin E) exhibited smaller testes and reduced spermatogenesis after exposure to 1,000 mg/kg/day DEHP whereas marmosets given only about 400 mg/kg vitamin C had normal-sized testes and comparable

spermatogenesis to controls (based on sperm counts) when ingesting 2,500 mg/kg/day DEHP. Thus, if the hypothesis is that dietary vitamin C accounted for the lack of effects seen in Tomonari et al., as opposed to a difference in the marmosets' sensitivity to DEHP, then a much smaller dose of vitamin C (50 – 66% of the amount given to the rats) would have to have protected the marmosets against 2 – 2.5 times the amount of DEHP given to rats. Put another way, vitamin C would have to be about 3 – 5 times more protective in primates than rodents to account for the results of Tomonari et al.

Indeed, if such a small amount of vitamin C in the diet had a complete protective effect against the high doses of DEHP given the marmosets, one might question the possible impact of DEHP exposure on human health. The RDA for vitamin C is 75 mg/person/day for women and 90 mg/person/day for men (NRC, 2003), although the mean daily intake is about 100 mg/day based on NHANES III and CSF II surveys (NRC, 2003). If 80 mg/day was as protective to primates as suggested, then the risk to humans would appear quite low since human exposures to DEHP are at least 100,000 times lower than the amount received by the marmosets (McKee et al., 2004), and the human diet contains higher levels of vitamin C. Even if one were to calculate the protective potential of that much vitamin C on a mg/kg body weight basis, the 360 mg/kg/day dose of vitamin C (hypothetically) protected the marmosets from testicular effects at 2,500 mg/kg DEHP (roughly a 7-fold protection factor). Applying this protection factor to an average human intake of 1.3 – 1.4 mg/kg/day vitamin C (90 – 100 mg/day for a 70 kg person), humans would be at no risk of testicular effects from exposures up to 6 mg/kg/day or roughly 10,000 times the mean exposures as determined by the CDC (Blount et al., 2000; CDC, 2001; CDC, 2003).

Thus, it seems unlikely that the amount of vitamin C provided the marmosets in Tomonari et al. invalidates the study's findings of no effect. Further, even if vitamin C had a protective effect, it is unlikely that any human except one severely deficient in vitamin C would be at risk of adverse effects from exposure to the amounts of DEHP found in the environment.

In conclusion, the need for supplemental vitamin C in primate and human diets reinforces the similarity between the two primate species, and since the amount of vitamin C administered in the marmoset study was in line with medical recommendations, there is no reason to question the results of the study, and no reason to consider the results not relevant to assessing potential health effects in humans. The administration of medically appropriate amounts of vitamin C to the marmosets certainly would not appear to provide any scientific reason for using rodent data over primate data for human hazard and risk assessment. Further, one might question whether it would have been scientifically appropriate, or even ethical, to withhold vitamin C from the marmosets. Indeed, had vitamin C been withheld or administered in artificially low doses, interpretation of any adverse findings would be difficult at best.

(2) Hormonal Differences between Primates and Rodents

A concern about the use of marmoset data has been expressed thus:

> [S]perm production and androgen synthesis in humans, macaque monkeys, and rodents are under regulation by hormones produced in the pituitary, such as follicle stimulating hormone (FSH) and luteinizing hormone (LH). However, the

> pituitary of the common marmoset does not produce LH. Instead, it produces chorionic gonadotropin (CG), which is only produced in the placenta of humans or rodents (Muller et al., 2004). Both CG and LH in mammals act on the same receptor, the LH receptor. The gene for this receptor in common marmoset is lacking one segment called exon 10. Lack of exon 10 in the LH receptor causes androgen deficiency and hypogonadism in humans (Zhang et al., 1998; Gromoll et al., 2000).

(OEHHA, 2004).

This difference in the hormone that initiates testosterone synthesis between the common marmoset and humans (Muller, et al. 2004) does not provide a sufficient basis for rejecting the marmoset as a model for human testicular development and function. The organization of spermatogenesis in the marmoset has been demonstrated to be similar to the human (Millar et al., 2000). Similarities have also been demonstrated between rats and marmosets in which the administration of a GnRH antagonist showed similar effects on spermatogenesis in both species (Sharpe et al., 2000). As the relevance of the difference in signaling peptide hormone with respect to the proposed mode of action for DEHP postnatal reproductive toxicity is unknown, it should not be the basis for discounting the significance of the marmoset data.

DEHP and its metabolite MEHP have been shown to have no affinity for the androgen receptor (Parks et al., 2000). Competition or interference with LH for the androgen receptor is therefore not a proposed site of action for DEHP and the effect of DEHP is most likely not an alteration of hypothalamic-pituitary function (McKee et al., 2004).

In rats exposed to DEHP for 14 days, DEHP was shown to impair prepubertal Leydig cell testosterone (T) production, a result associated with inhibition of steroidogenic enzyme activity ($P450_{scc}$, 3β-HSD, $P450_{17\alpha}$, and 17β-HSD) and cholesterol transport, rather than hormone interaction with the receptor (Akingbemi et al., 2001). In rats exposed to DEHP from 21 to 90 days, T biosynthesis was also decreased, while serum LH and T levels were increased due to hyperplasia of Leydig cells (Akingbemi et al., 2004), a compensatory response to decreased T production or to altered Sertoli cell paracrine secretions, not LH signaling. In the pubertal and neonatal rat, DEHP exposure directly affects Sertoli cell function (Li et al., 1998). Disturbances in testicular steroidogenesis and Leydig cell hyperplasia have also been reported in phthalate exposed transgenic mice overexpressing human CG, an analogue of LH (Matzuk et al, 2003; Rulli et al., 2002), again suggesting that LH signaling is not part of the mode of action (MOA). Current research also suggests that DEHP effects on spermatogenesis occur as a direct effect on the Sertoli cells and/or via alterations in T production and not as a result of androgen receptor-dependent mechanisms. These data indicate that the marmoset may be used as a model for human male testicular development and function despite the species differences in androgen hormones.

In addition, the presumption, based on evolutionary biology, is that non-human primate data are much more likely to reflect the response of a human primate to a chemical than are rodent data. At the very least, the marmoset data provide a basis for the Expert Panel to acknowledge that the use of rat data for human risk assessment is likely very conservative – that is, health protective.

e. Information on the Mechanism of DEHP Reproductive Toxicity

Although not specifically identified as a data need by the first Expert Panel, knowledge of the mechanism(s) underlying the effects of DEHP on male reproductive development is obviously helpful. Substantial progress has been made in understanding the mode of action, although there is still much to learn.

While one early report (Sohoni and Sumpter, 1998) suggested that some phthalates might interact with androgen receptors (AR), further studies indicated that the effects of phthalates are not receptor-mediated (e.g., Knudsen and Pottinger, 1999; Paganetto et al., 2000; Parks et al., 2000; Foster et al., 2001; Satoh et al., 2001; Sultan et al., 2001). To further test whether phthalates, including DEHP, were capable of producing androgen receptor-mediated effects, all of the commercially important phthalates and their corresponding monoesters, including MEHP, were tested for agonist and antagonist effects on the androgen receptor. These tests were performed in the yeast human androgen receptor assay (Gaido et al., 1997) and the HepG2 AR Reporter Gene Assay (Gaido et al., 2000). 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.

As indicated above, there are pharmacokinetic differences providing evidence that, at equivalent external exposures levels, primates have significantly lower internal doses than rodents. In addition, pharmacodynamic differences between humans and rodents may also be important. Based on a study that compared wild-type versus PPARα-null mice, Ward et al. (1998) concluded that the "results provide evidence that PPARα-dependent processes played a role in the testicular effects but that PPARα-independent processes were also involved." 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. PPARα 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 (Gazouli et al., 2002). There are other aspects of cholesterol uptake and androgen biosynthesis that may be inhibited by some phthalates (Akingbemi et al., 2001; Gazouli et al. 2002; Shultz et al. 2001) by processes that may be unrelated to PPARα induction. However, PPARα activation also appears to stimulate aromatase activity in rodent liver, and this may affect the balance between testosterone and β-estradiol (Biegel, et al. 2001). The extent to which PPARα induction is involved in the production of testicular atrophy in rodents is very pertinent to the overall assessment of human risk and certainly merits further study. (Corton et al., 2005).

The first Expert Panel stated that "[T]he presence of testicular effects in PPARα knockout mice and in guinea pigs exposed to DEHP indicates that the mechanism of action does not involve peroxisome proliferation." This conclusion may not be an accurate reflection of the data relating to the potential role of PPARα in the testicular atrophy effects of DEHP in juvenile and adult animals. This critique of the first Expert Panel's conclusion is based on three points:

(1) the Expert Panel did not accurately reflect the conclusions of the authors of the principal study on which they relied (Ward et al., 1998);

(2) the Expert Panel did not consider data from other substances suggesting a general relationship between peroxisomal proliferation and testicular effects; and,

(3) mechanistic information published since the completion of the first Expert Panel review suggests specific ways in which the reproductive effects could be a consequence of peroxisomal proliferation.

As discussed above, Ward et al. concluded that there most likely was a PPARα-dependent component to the testicular effects although it appeared that other, PPARα-independent factors might also be involved. The Expert Panel did not explain why its interpretation of these data, i.e. that PPARα activation was not involved, differed from that of the original authors

There is additional evidence suggesting a role for peroxisomal proliferation (or more specifically PPARα agonism) in the development of testicular effects in rodents; but it is possible that the first Expert Panel may have overlooked the relevant citations because none evaluated phthalates specifically. Cook et al. (1992) 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 (Biegel et al., 2001). This work was extended to other peroxisomal proliferating agents (Liu et al., 1996a; 1996b). 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 (Liu, 1996b). 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 (Cappon, 1996). In contrast, in the rat Wy 14,643 can increase hepatic aromatase levels as much as 16-fold. These studies provide clear evidence that a range of peroxisomal proliferating agents affect reproductive function in rodents through processes related to PPARα agonism. As humans seem much less sensitive to other PPARα-related phenomena, it seems likely that PPARα agonists would produce substantially less profound effects in primates than in rodents.

Finally, there are now reports that DEHP may influence the expression of gene functions related to steroid biosynthesis (e.g. Gazouli et al., 2002; Shultz et al. 2001, Wong and Gill, 2002). The Gazouli et al. study is particularly informative as it compared gene expression in wild-type and PPARα-null mice. The work of Gazouli et al. provided evidence that PPARα 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 may be PPARα-independent. Thus, there is a body of evidence showing that the testicular effects of DEHP in rodents are at least partially the consequence of PPARα activation. As humans and non-human primates do not exhibit other changes associated with PPARα activation, these data may provide at least a partial explanation for the empirical evidence of species differences provided by the non-human primate studies.

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