

**Proposition 65 Maximum Allowable Dose Level (MADL) for
Reproductive Toxicity for
Di(2-ethylhexyl)phthalate (DEHP) by Oral Exposure**

April~~June~~ 2006~~5~~

**Office of Environmental Health Hazard Assessment (OEHHA)
Reproductive and Cancer Hazard Assessment Section**

SUMMARY

The maximum allowable dose levels (MADL) for di(2-ethylhexyl)phthalate (DEHP) by the oral route of exposure are **410 micrograms/day ($\mu\text{g}/\text{day}$)** for adults, **58 $\mu\text{g}/\text{day}$** for infant boys (29 days to 24 months of age) and **20 $\mu\text{g}/\text{day}$** for neonatal infant boys (birth to 28 days of age). These values are based on the male reproductive effects of DEHP observed in the study by David et al. (2000a). As specified in regulation, when the applicable reproductive effect is upon the male, the MADL is calculated based on a human body weight of 70 kg (Title 22, California Code of Regulations, section 12803(b))¹. In the case of DEHP, however, developing animals are sensitive to the testicular effects of DEHP (e.g., Sjoberg et al., 1985; 1986; Li et al., 2000; CERHR, 2000; U.S. FDA, 2001; Borch et al., 2004). Bodyweights of infants and neonatal infants ~~or neonates~~ are markedly lower than that of an adult. Accordingly, age-specific MADLs have been calculated for infant and neonatal infant boys based on bodyweights of 10 and 3.5 kg, respectively (Sections 12801(a) and 12803(a)(6)). Children and adolescents also differ in bodyweight from adults. Age-specific MADLs for males in those age groups can be calculated by application of the default bodyweights specified in Section 12703(a)(8).

BACKGROUND

This report describes the derivation of a maximum allowable dose level (MADL) for DEHP (CAS No. 117-81-7) by the oral route of exposure.

DEHP is mainly used as a plasticizer of polyvinyl chloride (PVC) in the manufacture of a wide variety of consumer products for building construction, automobiles, clothing, toys and medical devices (OEHHA, 1997; CERHR, 2000). DEHP was listed under the Safe Drinking Water and Toxic Enforcement Act of 1986 (commonly known as Proposition 65, codified at Health and Safety Code section 25249.5 et seq.) as known to the State to cause reproductive toxicity (developmental and male reproductive toxicity), effective October 24, 2003. This listing was based on formal identification of DEHP as causing developmental and male reproductive toxicity, by the National Institute for Occupational

¹ All further references to regulations are to Title 22 of the California Code of Regulations, unless otherwise noted

Safety and Health (NIOSH, 1990) and the U.S. Food and Drug Administration (U.S. FDA, 2001). NIOSH and U.S. FDA are authoritative bodies under Proposition 65 for identification of chemicals as causing reproductive toxicity (Section 12306(l)).

Procedures for the development of Proposition 65 MADLs are provided in Sections 12801 and 12803. Exposure at a level 1,000 times greater than the MADL is expected to have no observable effect. As defined in regulation, a MADL is derived from a No Observable Effect Level (NOEL) based on the most sensitive study deemed to be of sufficient quality (Section 12803). This document addresses the oral route of exposure for DEHP to assist in the implementation of Proposition 65 relative to the widespread human exposures by this route.

STUDY SELECTION

Relevant studies or reports that provide information on the developmental or male reproductive toxicity of DEHP have been identified through literature searches and through reviewing documents produced by authoritative bodies or other expert groups. These documents include the two reports by the authoritative bodies that provided the primary support for the Proposition 65 listing of DEHP as a chemical known to cause reproductive toxicity – the U.S. FDA (2001) document *Safety Assessment of Di (2-ethylhexyl)phthalate (DEHP) Released from PVC Medical Devices*, and the NIOSH (1990) document *NIOH and NIOSH basis for an Occupational Health Standard: Di (2-ethylhexyl) phthalate (DEHP)*. In addition, the detailed review by an expert panel convened by the National Toxicology Program’s Center for the Evaluation of Risks to Human Reproduction (CERHR, 2000) entitled *NTP-CERHR Expert Panel Report on Di (2-ethylhexyl) Phthalate* was consulted. OEHHA staff have reviewed the relevant studies or reports cited in those documents. OEHHA staff have also reviewed additional studies that are not cited in those documents. Studies and documents reviewed by OEHHA and cited in the text of this report are listed in the References section. Studies reviewed by OEHHA but not cited in the present document or in the documents by NIOSH (1990), CERHR (2000), or U.S. FDA (2001) are listed in the Bibliography.

There are numerous studies or review reports providing relevant information on the developmental or male reproductive toxicity of DEHP. A majority of the studies published prior to 2000 were already included in the reviews by NIOSH (1990), CERHR (2000), and U.S. FDA (2001). After briefly reviewing all studies or reports available to OEHHA staff, OEHHA focused on studies that appear to be sensitive studies in order to identify “the most sensitive study deemed to be of sufficient quality.”

Developmental or Male Reproductive Toxicity in Humans

There are a few epidemiological studies investigating possible associations of exposure to DEHP and other phthalates with developmental or reproductive effects in humans (Modigh et al., 2002; Latini et al., 2003; Duty et al., 2003a; 2003b; 2004; Rais-Bahrami et al., 2004). One study used human sperm to study possible direct effects of DEHP and other phthalates on sperm motility in vitro (Fredricsson et al., 1993).

Latini et al. (2003) investigated the possible association of concentrations of DEHP and its main metabolite, mono (2-ethylhexyl) phthalate (MEHP), in the cord blood of 84 newborns to birth outcomes including weight, gestational age, and other endpoints. All 84 newborns were born at a general-practice hospital in Italy; there were 82 singleton births, one set of twins and 39 male and 45 female offspring. Eleven were preterm, and three of them had very low birth weight. The maternal age range was from 18 to 24 years. The authors found that DEHP or MEHP were present in 74 (88.1%) of the 84 examined cord serum samples at mean concentrations of 1.19 ± 1.15 $\mu\text{g/ml}$ and 0.52 ± 0.61 $\mu\text{g/ml}$ (mean \pm standard deviation), respectively. MEHP-positive newborns (65 or 77.4%) had a significantly lower gestational age (38.16 ± 2.34 weeks) compared with MEHP-negative infants (39.35 ± 1.35 weeks, $p < 0.05$). Logistic regression analysis also indicated a positive correlation between absence of MEHP in cord blood and gestational age at delivery (odds ratio = 1.50, 95% confidence interval = 1.013-2.21). No other statistically significant relationships were observed between DEHP or MEHP concentrations and other birth outcomes (e.g., birth weight). The authors concluded that phthalate exposure is significantly associated with shortened pregnancy duration. Altered gestation length associated with maternal exposure to a chemical can be an indicator of female reproductive toxicity (U.S. EPA, 1996); however, female reproductive toxicity is not among the bases for the Proposition 65 listing of DEHP. Therefore, this study cannot be used as the basis for MADL development (Section 12803(a)(1)).

Fredricsson et al. (1993) studied effects of DEHP at concentrations of 0.001-100 mM on human sperm motility in vitro. The authors found that incubation of human sperm with DEHP caused a statistically significant decrease in sperm motility, with a 25% reduction of motility at 1 mM. The authors did not study the effect of DEHP metabolites (e.g., MEHP) on sperm motility.

A series of recent studies by Duty et al. (2003a; 2003b; 2004) investigated the relationship of sperm parameters to phthalate exposure among male partners of subfertile couples who presented to the Andrology Laboratory at the Massachusetts General Hospital in Boston for semen analysis as part of an infertility work-up. The authors found sperm DNA damage, decreased sperm motility, and/or reduced sperm concentration in semen samples to be associated with increased urinary levels of mono-ethyl phthalate or mono-butyl phthalate (metabolites of diethyl phthalate or dibutyl phthalate, respectively), but not with urine levels of DEHP or MEHP.

Modigh et al. (2002) found no effect of DEHP at a mean exposure level of < 0.5 mg/m^3 on time to pregnancy among partners of 193 men who were occupationally exposed to DEHP in air at three plants either producing DEHP or processing polyvinyl chloride (PVC) plastic. A recent clinical study investigated testicular volume, phallic length, and the serum levels of luteinizing hormone (LH), follicle-stimulating hormone (FSH), and testosterone of 13 adolescent boys (14-16 years old) who were exposed to DEHP as neonates on extracorporeal membrane oxygenation (ECMO) support (Rais-Bahrami et al. (2004). Mean values for these parameters were within the appropriate range for the degree of pubertal development. Detailed information (e.g., time and duration on

ECMO, range of the values for sexual hormones or testicular volumes) was not reported; no control group was included in the study. The route of exposure in these two studies was either inhalation or intravenous injection, not oral.

All the studies in humans discussed above included relatively small numbers of subjects and have other important limitations (e.g., lack of appropriate control group, phthalate levels based on a single spot urine samples, selection of subjects from men who were part of subfertile couples visiting an andrology clinic in the studies by Duty et al.). Although some findings in the human studies reported by Fredricsson et al. (1993) or by Duty et al. (2003a; 2003b; 2004) provide limited evidence on an association between exposure to phthalates and damaged sperm quality, none of the human studies on the developmental or male reproductive effects of phthalates is “of sufficient quality” for MADL development for the purposes of Proposition 65. Thus, the MADL is necessarily based on animal studies.

Male Reproductive Toxicity in Animals

The male reproductive toxicity of DEHP has been studied in many species including rats, mice, hamsters, ferrets, and non-human primates. Findings from the majority of these studies have been well reviewed and summarized in many documents or review reports (e.g., CERHR, 2000; U.S. FDA, 2001). Therefore, detailed findings from each individual study are not discussed in this document. Instead, this document focuses on a number of studies that can be potentially identified as the most sensitive study of sufficient quality for the purpose of Proposition 65 and on relevant mechanistic data (e.g., metabolism, cellular and/or molecular targets, and biochemical pathways) that are critical for determining the relevance of rodent data to humans.

Studies in Rats. The majority of studies on the male reproductive toxicity of DEHP were conducted in rats using oral administration (gavage, feed, or drinking water). Depending on the doses, dosing duration, age of animals, and endpoints included, it has been shown that oral treatment with DEHP causes reduced fertility, decreased weights of male reproductive organs, and histopathological changes in the testis of juvenile and adult rats (CERHR, 2000; U.S. FDA, 2001). Characteristics of histopathological changes include vacuolation and rarefaction of the cytoplasm, disruption of cytoskeletons, destruction of intercellular specializations (e.g., ectoplasmic specialization, occluding junctions) in Sertoli cells, followed by degeneration of spermatocytes by apoptosis and/or sloughing of germ cells into the lumen of seminiferous tubules (e.g., Saitoh et al, 1997; Park et al., 2002; Boekelheide, 2004). Different groups of germ cells in the testis of rats are organized in an orderly manner along the length of seminiferous tubules. A defined group of germ cells is called a stage. Along the length of a seminiferous tubule, there is a distinct ordering of stages, namely from stage I to XIV. Sertoli cells undergo morphological and functional fluctuation from stages I to XIV (Russell and Griswold, 1993). In the testis of young rats (8-week-old), Sertoli cells and the spermatocytes associated with them in seminiferous tubules at stages IX-XIV and I are most sensitive to the testicular effects of DEHP (Saitoh et al., 1997).

Oral administration of DEHP to rats during the perinatal period results in severe permanent abnormalities in the male reproductive system of male offspring (Tandon et al., 1991; Arcadi et al., 1998; Gray et al., 1999; Schilling et al., 1999; Moore et al., 2001). Neonatal or young rats have been found to be more sensitive to the male reproductive effects of DEHP than are adults (Gray and Butterworth, 1980; Sjoberg et al., 1985; 1986; Dostal et al., 1988; Li et al., 2000; CERHR, 2000; U.S. FDA, 2001; Cammack et al., 2003; Akingbemi et al., 2001; 2004; Borch et al., 2004). The testis at early developmental stages (late gestation and early days after birth in rats) is more sensitive to DEHP than that of juvenile or adult animals (Gray et al., 1999; 2000; Moore et al., 2001; CERHR, 2000). Thus, the NOELs and/or LOELs for the male reproductive effects of DEHP observed in studies that treated rats either perinatally or in the early weeks of the postnatal period are in general lower than those observed in young or adult animals.

Table 1 summarizes a list of studies that observed relatively low values of LOELs and/or NOELs in rats. The animals used in these studies received DEHP treatment either perinatally (Arcadi et al., 1998) or as juveniles (three-four weeks old; Poon et al., 1997; David et al., 2000a; Akingbemi et al., 2001; 2004). Manifestation of DEHP-caused testicular damage takes different forms, depending on the age of animals, dosing levels, and dosing durations. For example, as stated in the document by CERHR (2000), “during the time of Sertoli cell divisions (before pnd [post natal day] 15 in rats), phthalate exposure apparently inhibits cell division. In animals older than pnd 15, toxicity is manifest as vacuoles, followed by germ cell sloughing.” Therefore, when comparing different studies to identify “the most sensitive study”, OEHHA considered different endpoints used in different studies and attempted to compare different studies based on the same or similar endpoints. In addition, the clear difference in sensitivity to the testicular effects of DEHP between developing and adult rats suggests that a NOEL observed in adult animals should be compared to those observed in developing animals in order to determine if a NOEL in adult animals has no observable effects in developing animals.

The study by Arcadi et al. (1998) observed the lowest LOEL (32.5 µl/L in drinking water) in rats for the male reproductive effects of DEHP in male offspring exposed to DEHP from gestational day 1 to postnatal day 21. The authors stated this dose was roughly equivalent to 3.0-3.5 mg/kg-day, but assumptions of body weights and water consumption for their estimate were not reported. This study has some limitations. For example, DEHP is essentially insoluble in water (3 µg/L or approximately 0.003 µl/L; CERHR, 2000). The concentrations of DEHP used in the study were 32.5 and 325 µl/L. The authors stated that “the suspension was prepared daily by adding DEHP to mineral water and then sonicating for 30 min.” However, actual concentrations of DEHP in the drinking water were not verified. Daily water consumption was not recorded. Maternal body weights were not reported. Therefore, for purposes of MADL development, this study is not “of sufficient quality” for identification of a NOEL or LOEL, although this study provided important evidence on the adverse effects of DEHP on rat testicular development during the perinatal period.

Among other studies listed in Table 1, the studies by Akingbemi et al. (2001; 2004) observed an oral LOEL of 10 mg/kg-day, based on abnormal changes in testosterone production and altered Leydig cell proliferation in the testes of prepubertal rats. This LOEL is markedly lower than those based on histopathological changes in adult animals following long-term treatment with DEHP (29 mg/kg-day as observed by David et al. (2000a) or 38 mg/kg-day by Poon et al., 1997). It should be noted that the NOELs observed in adult animals by Poon et al. or David et al., respectively, are lower than the LOEL of 10 mg/kg-day observed in juvenile animals by Akingbemi et al. (2001; 2004). Therefore, based on endpoints indicative of morphological or functional changes, there is no observed effect of DEHP on the testis at doses lower than 10 mg/kg-day following oral administration, regardless of the age of rats used in the studies. The highest dose below 10 mg/kg-day used in the studies listed in Table 1 is the NOEL (5.8 mg/kg-day) observed by David et al. (2000a). Thus, this NOEL (5.8 mg/kg-day) has no observable testicular effects in rats of different ages. The mg/kg dose resulting from exposure to DEHP at a MADL based on this NOEL can therefore be expected to be protective against the testicular effects of DEHP for both developing and adult humans. As noted above, the apparently lower LOEL in the study by Arcadi et al. (1998) cannot be taken into account because the study is not of sufficient quality.

Table 1. Oral studies that observed relatively low values of LOEL or NOEL for the male reproductive toxicity of DEHP in rats.

Study Reference	Animals	Treatment	General Toxicity	Male repro effects and LOEL	NOEL
Poon et al., 1997	Sprague-Dawley rats, about 6 wks old at the beginning, 10 rats per group.	Feed, 0, 5, 50, 500, 5,000 ppm for 13 wks.	Increased liver and kidney weights, histopathological changes in the liver at 5,000 ppm.	Sertoli cell vacuolation and seminiferous tubular atrophy at 5000 ppm. Minimal Sertoli cell vacuolation in 7/10 rats at 500 ppm. LOEL: 500 ppm (38 mg/kg-day)	50 ppm (3.7 mg/kg-day).
Arcadi et al., 1998	Long-Evans rats, 12 pregnant rats per group	Drinking water, 0, 32.5, 325 µL/L DEHP, from gestational day 1 to postnatal day (PND) 21. Pups examined on PND 21, 28, 35, 42, and 56.	No effects on body weight gains of dams or pups. Changed weights and pathology in the kidney and liver of pups at both doses.	Reduced testis weights and histopathological changes in the testes of male pups at both doses. LOEL: 32.5 µL/L (3.0-3.5 mg/kg-day, estimated by the study authors; water consumption not reported)	Not observed.
David et al., 2000a	Fischer 344 rats, about six-wk-old at the start, 55-80 rats per group.	Feed, 0, 100, 500, 2,500, or 12,500 ppm DEHP for 104 wks.	Reduced survival rates, reduced body weights, adverse effects in the liver, kidney, and pituitary at ≥2,500 ppm.	Significantly increased incidence of aspermatogenesis at ≥ 500 ppm at Week 105. LOEL: 500 ppm (29 mg/kg-day)	100 ppm (5.8 mg/kg-day)
Akingbemi et al., 2001	Male Long-Evans rats, 21, 35, or 62 days of age; ten rats per group	Gavage, 0, 1, 10, 100 or 200 mg/kg-day, PND 21-34, 35-48, 21-48, or 62-89.	No obvious general toxicity.	Decreased testosterone (T) production by Leydig cells at ≥10 mg/kg-day at PND 21-34; increased T production when exposed at PND 21-48. LOEL: 10 mg/kg-day	1 mg/kg-day
Akingbemi et al., 2004	Male Long-Evans rats, 21 day of age, ten rats per group	Gavage, 0, 10, 100 mg/kg-day, from postnatal day (PND) 21 to PND 48, 90 or 120.	No obvious general toxicity.	Reduced T production in Leydig cells. Increased numbers and proliferating activity of Leydig cells at ≥ 10 mg/kg-day: LOEL: 10 mg/kg-day	Not found.

Studies in Other Species. The male reproductive effects of DEHP following oral administration have also been studied in mice, guinea pigs, hamster, ferrets, and non-human primates. There is clear evidence indicating that oral administration of DEHP causes adverse effects in the male reproductive systems of mice, guinea pigs, hamsters, and ferrets (e.g., Lake et al., 1976; Gray et al., 1982; Gangolli, 1982; Lamb et al., 1987; David et al., 2000b; CERHR, 2000). The LOELs and/or NOELs for the male reproductive effects of DEHP observed in mice are generally higher than those in rats. Syrian hamsters are much less sensitive to the testicular effects of DEHP than rats (Gray et al., 1982). The LOEL for the testicular effects of DEHP administered in diet for 14 months in mature albino ferrets were 1200 mg/kg-day, which again is much higher than

that in rats (e.g., David et al., 2000a). The studies in mice, hamsters, and ferrets clearly demonstrated that male reproductive effects occur in these species. However, these species are less sensitive to the testicular effects of DEHP than is the rat, based on similar endpoints indicative of testicular damage under similar treatment regimes. Therefore, for the purpose of Proposition 65, studies conducted in these species are not considered as “the most sensitive study” for male reproductive effects of DEHP.

In addition to rats, mice, hamsters, and ferrets, non-human primates have been used in several oral studies of the toxic effects of DEHP (Rhodes et al., 1986; Kurata et al., 1998; Pugh et al., 2000; MCSI et al., 2003). The studies by Rhodes et al. (1986), Kurata et al. (1998), and MCSI et al. (2003), were conducted in common marmosets (*Callithrix jacchus*), a New World primate. In the study by Pugh et al. (2000), cynomolgus monkeys (*Macaca fascicularis*), an Old World primate, were used. Because results from these primate studies have been suggested as basis for determining the relevance of rodent data to humans (e.g., McKee et al., 2004), details of these four primate studies are discussed below.

In the study by Rhodes et al. (1986), groups of five adult male marmosets (weighing 250-400 g) were treated by gavage with 0 or 2,000 mg/kg-day for 14 days. Body weight gain in the DEHP-treated group was significantly lower than that in the control (body weights in the DEHP-treated group are approximately 70% of those in the control group, $p < 0.05$), but no effect on testicular weights was observed. The histopathological findings were not reported, although the authors reported that they included testes for histopathological evaluation by light microscopy.

In the study by Kurata et al. (1998), groups of four adult male marmosets (body weights at the end of 13-week treatment averaged about 330 g) were treated orally with 0, 100, 500, and 2,500 mg/kg-day DEHP for 13 weeks. Body weight gain was significantly reduced in males treated with 2,500 mg/kg-day, but no significant effect on blood testosterone levels, testis weights or morphology at light and electron microscopic levels was observed.

The final report of a recent study in juvenile marmosets was submitted to OEHHA by the American Chemistry Council (ACC). In this study, sponsored by the Japanese Plasticizer Industry Association, conducted by Kurata et al. at the Mitsubishi Chemical Safety Institute Ltd. (MCSI, 2003), groups of male marmosets (8-10 animals per group) aged from 90 to 110 days were treated by gavage for 65 weeks with 0, 100, 500, or 2,500 mg/kg-day DEHP. The authors stated that there was no treatment-related effect on body weights or weights of reproductive organs including testes and epididymides. No apparent histopathological changes in the testis were observed in DEHP-treated animals. Epididymal sperm count in DEHP-treated animals was not different from that in the control animals. There was no significant difference in mean levels of blood testosterone in blood samples collected at intervals during the treatment between DEHP-treated and control animals. No treatment-related changes in histochemical and biochemical examinations for testicular functions were observed.

The findings from three studies conducted in common marmosets indicate that DEHP, even at very high dose levels, does not cause testicular damage in this species. Because the seminiferous epithelium in the testis of common marmoset is organized similarly to that in humans, some have suggested the common marmoset to be a good model to predict the potential testicular effects of chemicals in humans (Millar et al., 2000; Sharpe et al., 2000; ACC, 2004), while others have noted fundamental species differences and have concluded otherwise (Zuhkle and Weinbauer, 2003). Based on relevant information regarding the male reproductive system of common marmosets that OEHHA staff has reviewed, the testis of the common marmoset indeed has some unique characteristics that are dramatically different from other mammals including rats, cynomolgus macaques, and humans. For example, sperm production and androgen synthesis in humans, macaque monkeys, and rodents are regulated 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 use 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). Recent studies using transplanting techniques have also shown that the conditions needed for initiation of spermatogenesis in the marmoset are remarkably different from those present in most other mammals (e.g., Wistuba et al., 2004). Because of fundamental differences in the testis between common marmosets and humans, it has been suggested that “the use of this animal model cannot be recommended for reproductive toxicology assessment” (Zuhkle and Weinbauer, 2003). In addition, vitamins C and E are protective against the testicular effects of DEHP in rats or mice (Ishihara et al., 2000; Ablake et al., 2004). Common marmosets require high levels of dietary vitamin C so regular diets for this species usually contain high levels of vitamin C supplements (e.g., MCSI, 2003). Serum levels of vitamin C in common marmosets are markedly higher (2.56 mg/100ml in average) than most other mammals (0.63 mg/100 ml in average in humans; Flurer and Zucker, 1987; 1989; Hampl et al., 2004), creating the possibility of reduced sensitivity to DEHP in this species. Based on the facts discussed above, OEHHA has determined that the data from studies in common marmosets should not be used as the basis for MADL development for DEHP.

In addition to the three studies in common marmosets discussed above, there is one study in cynomolgus monkeys reported by Pugh et al. (2000). In this study, male monkeys of about two years of age (weighing 1977-2921 g), four animals per group, were treated by gavage with 0, 500 mg/kg-day DEHP, 500 mg/kg-day di-isononyl phthalate (DINP), or 250 mg/kg-day clofibrate for 14 days. The overall objective of this study was to assess the effects of DEHP, DINP, and clofibrate on peroxisome proliferation in the cynomolgus monkey. The initial body weights for each group were not reported. The final body weight of monkeys in the DEHP-treated group (2378±194 g; mean ± standard deviation) was lower than that of the control group (2590±138 g), but the difference was not statistically significant (determined by ANOVA followed by a Dunnet’s test, as reported by the authors). With regard to testicular effects of DEHP, absolute testis or epididymis weights were not reported. Relative weight (%) of testes/epididymides in the DEHP

group (0.069 ± 0.005 ; mean \pm standard deviation) was approximately 83% of that of the control animals (0.083 ± 0.018), indicating a 17% decrease, but the difference is not statistically significant. It is unclear whether the relative weight of testes/epididymides as reported was a combined weight of testes and epididymides or testes only. The authors stated that there was no treatment-related histopathological change in the testes, but detailed information on histopathological observations was not reported. No effect on liver or kidney weight, hepatic peroxisomal beta-oxidation, or replicative DNA synthesis and gap junctional intercellular communication in the liver was observed. The authors concluded primates were unresponsive to the induction of DNA synthesis and peroxisomal beta-oxidation, but did not make any conclusion regarding their observations on the possible testicular effects of DEHP.

The study by Pugh et al. (2000) used four monkeys per group. The sample size is small and thus has limited statistical power to reveal treatment related effects among DEHP-treated animals. Statistical power is the probability of detecting an effect if there really is one. It is highly influenced by the size of a study (the number of subjects per group). A statistical power of 0.8 or higher is generally used (Schwetz et al., 1980; Lenth, 2001; Festing and Altman, 2002). Based on reported means and standard deviations of relative testis/epididymis weights, the sample size only provides a statistical power of 0.2 – 0.3. Thus, the study by Pugh et al. (2000) has only approximately a 20-30% chance to detect a difference in testicular weights between the control and DEHP-treated monkeys if a real difference exists. In order to detect a statistically significant difference (at a significance level of 0.05) in body weights or relative testis/epididymis weight with a statistical power of 0.8 (i.e., an 80% likelihood of detecting the effect), at least 10-14 animals per group are required (Stata Corporation, 2003). Thus, the study by Pugh et al. (2000) does not have sufficient power to detect a statistically significant difference in the relative weight of testis/epididymis in cynomolgus monkeys between the control and treated group under the experimental designs used in the study.

Cynomolgus monkeys used in the Pugh et al. (2000) study were approximately two years of age weighing 1977-2921 g. The testis in two-three year old cynomolgus monkeys is immature and relatively quiescent (e.g., Cho et al., 1975; Kluin et al., 1983; Liang et al., 2001; Smedley et al., 2002). Tightly-packed, small-diameter seminiferous cords consist of Sertoli cells with few interspersed spermatogonia. There are no spermatocytes or spermatids since meiosis does not occur until puberty around 3.5-4 years of age (Kluin et al., 1983; Smedley et al., 2002). Therefore, degenerative changes in spermatocytes, which are seen in young or adult rat testis following DEHP treatment, may not be expected in the testis of cynomolgus monkeys two-three years of age. Sertoli cell proliferation remains at very low levels; with only approximately 0.3 % of Sertoli cells in the S-phase of the cell cycle in cynomolgus monkeys two-three years of age, as compared to approximately 10-20% in rats during the first two weeks after birth (Orth, 1982; Kluin et al., 1983; Liang et al., 2001). This cellular event (i.e., Sertoli cell proliferation) is critical for establishing normal testis size in the adult (e.g., Orth et al., 1988) and has been shown to be targeted by DEHP in the developing testis (Li et al., 1998; 2000; Li and Kim, 2003). Based on the physiological characteristics of the testis (e.g., slow growth in the testis, low proliferating activity in Sertoli cells, low testosterone production in Leydig

cells) in two-to-three years old cynomolgus monkeys, it appears that the age of two-to-three years may represent a window of relatively low sensitivity to the testicular effects of DEHP. Because proliferative activity of Sertoli cells is low, any possible change in testis weight resulting from inhibition of Sertoli cell proliferation by DEHP treatment as seen in neonatal rat testis may not be dramatic in cynomolgus monkeys two-three years of age. Nevertheless, a decrease (by approximately 17%) in relative weight of testes/epididymides (assuming combined weights) was observed in the DEHP-treated monkeys by Pugh et al. (2000).

Based on considerations discussed above, OEHHA concludes that testicular damages caused by DEHP in cynomolgus monkeys of 2-3 years of age cannot be ruled out. Because of the low statistical power of the study and the use of only one dose level (500 mg/kg-day) of DEHP, this study does not provide a sufficient basis for establishing a NOEL for the testicular effects.

Most Sensitive Study for the Male Reproductive Effects. Based on the findings from all relevant studies reviewed and the discussions presented above, for the purpose of Proposition 65, OEHHA has determined that the study in rats by David et al. (2000a) is “the most sensitive study of sufficient quality” for the male reproductive toxicity of DEHP following oral treatment.

Developmental Toxicity in Animals

The developmental toxicity of DEHP in laboratory animals has been extensively studied. In traditional developmental toxicity studies, DEHP has been found to cause intrauterine death, developmental delay, and structural malformations and variations (CERHR, 2000). Based on the relevant data available, the CD-1 mouse appears to be the species most sensitive to the developmental effects of DEHP following oral treatment. The lowest LOEL for the developmental toxicity of DEHP via the oral route of exposure was 0.05% in feed as observed in the studies reported by Tyl et al. (1984; 1988) and Price et al. (1988). Major findings from these two studies were presented in Table 2. The estimated doses, expressed as mg/kg-day, of DEHP used in the study by Price et al. (1988) were slightly higher (95 mg/kg-day for 0.05%; 48 mg/kg-day for 0.025%) than those in the study by Tyl et al., (1984; 1988; 91 mg/kg-day for 0.05%; 44 mg/kg-day for 0.025%). The NOEL (48 mg/kg-day) for the developmental toxicity of DEHP observed in the study by Price et al. (1988) is slightly higher than that (44 mg/kg-day) in the study by Tyl et al. (1984) and is lower than the LOEL from either study (91 or 95 mg/kg-day). Therefore, for the purpose of Proposition 65, the study by Price et al. (1988) is identified by OEHHA as the most sensitive study for the developmental toxicity of DEHP following oral treatment.

Table 2. Major findings in the studies by Tyl et al. (1984; 1988) and by Price et al. (1988)

Study Reference	Animals	Treatment	Maternal Toxicity	Developmental or male repro effects and LOEL	NOEL
Tyl et al., 1984 ; 1988	CD-1 mice, 30-31 pregnant mice per group.	Diet, 0, 0.025, 0.05, 0.10, 0.15% DEHP, GD 0-17; examined on GD 17.	Reduced maternal body weights at \geq 0.10%.	Increased number and percentage of resorptions; reduced live litter size; increased malformations. LOEL: 0.05% (91 mg/kg-day)	0.025% (44 mg/kg-day)
Price et al. (1988)	CD-1 mice, 28-29 pregnant mice per group.	Diet, 0, 0.01, 0.025, or 0.05% DEHP, GD 0-17. Examined postnatally.	No obvious maternal or general effects.	Increased prenatal mortality and reduced live litter size at 0.05% on PND 1. LOEL: 0.05% (95 mg/kg-day)	0.025% (48 mg/kg-day)

In addition to the assessment of standard endpoints of developmental toxicity, effects of DEHP following gestational exposure on development of the male reproductive system have been investigated in recent years. It has been found that DEHP administered during gestation causes adverse changes in testosterone production, Leydig cell proliferation, prostate development, or expression of genes for insulin-like hormone 3 (Insl3) in the testes of male fetuses or male offspring in rats (Akingbemi et al., 2001; 2004; Banerjee et al., 2002; Borch et al., 2004; Wilson et al., 2004). Insl3 is considered to be a biomarker of Leydig cell maturation in fetal and pubertal rats; disruption in this gene causes cryptorchidism in mice (Teerds et al., 1999; Nef and Parade, 1999; Ivell and Bathgate, 2002). Thus, alteration in expression of Insl3 gene by DEHP may represent one of the potential molecular pathways underlying DEHP-caused damage in testicular development.

There are also several studies that observed adverse effects of DEHP on development of the male reproductive system in rats exposed to DEHP during the perinatal period (e.g., Arcadi et al., 1998; Gray et al., 1999; 2000; 2001; Moore et al., 2001). These studies are discussed above in the section on male reproductive toxicity. Among all the studies that either used gestational or perinatal treatment, all of them observed developmental effects of DEHP on the male reproductive system at the single does used or the lowest dose included in the study. Except for in the study by Arcadi et al. (1998; see discussions above on this study), effects were seen at the lowest dose tested in these studies. The lowest dose used in these studies is 93.5 mg/kg-day as used in the study by Banerjee et al. (2002) reported in an abstract, which is nearly 20-fold greater than the NOAEL observed in the study by David et al. (2000a) discussed above. Thus, none of these studies is more sensitive than David et al. (2000a). Therefore, none of these studies can be identified as “the most sensitive study deemed to be of sufficient quality” (Section 12803) and thus be used for MADL development for the purposes of Proposition 65.

Identification of the Most Sensitive Study

The NOEL is based on the most sensitive study deemed to be of sufficient quality and is the highest dose level which results in no observable reproductive effect, expressed in milligrams of chemical per kilogram of bodyweight per day (Section 12803(a)).

The controlling regulation also specifies that “where multiple reproductive effects provide the basis for the determination that a chemical is known to the state to cause reproductive toxicity, the reproductive effect for which studies produce the lowest NOEL shall be utilized for the determination of the NOEL” (Section 12803(a)(1)). The NOEL (5.8 mg/kg-day) for the male reproductive toxicity as observed by David et al. (2000a) is lower than the NOEL (48 mg/kg-day) for the developmental toxicity of DEHP as observed by Price et al. (1988). Therefore, the oral study in rats reported by David et al. (2000a) was used as basis for establishing the MADL for DEHP via the oral route of exposure.

In the study reported by David et al. (2000a), groups of Fischer 344 rats (55-80 animals per sex per group) were treated with 0, 100, 500, 2,500, or 12,500 ppm DEHP in the diet for up to 104 weeks. The animals were about six weeks old at the beginning of treatment. The doses of DEHP were 0, 5.8, 28.9, 146.6, and 789.0 mg/kg-day for the five groups, respectively, as estimated by the study authors based on the average daily feed consumption. Reduced mean body weights, abnormal changes in serum chemistry, hematology parameters, increased liver and kidney weights, and histopathological changes in the liver, kidney, pancreas, and pituitary glands were observed in rats exposed to 12,500 ppm DEHP. Increased weights of liver and kidney and histopathological changes in the livers and kidneys were also observed in rats treated with 2,500 ppm DEHP for 104 weeks. Testis weights were significantly decreased in male rates treated with 12,500 DEHP for 104 weeks. Bilateral aspermatogenesis was observed in 10 out of 10 animals treated with 12,500 ppm DEHP, but not in any of 10 rats treated with 2,500 ppm DEHP when examined at Week 78. After exposure for 104 weeks, the incidence of bilateral aspermatogenesis was observed in 37/64 (58%) in the control group and 34/50 (64%), 43/55 (78%), 48/65 (74%), 62/64 (97%) in groups treated with 100, 500, 2,500, or 12,500 ppm DEHP, respectively. The increase in the incidence of bilateral aspermatogenesis was statistically significant in groups treated with ≥ 500 ppm DEHP. Thus, 500 ppm, equivalent to 28.9 mg/kg-day, is identified as the LOEL. The NOEL observed in this study, 100 ppm (equivalent to 5.8 mg/kg-day) is used as basis for establishing a MADL for DEHP by the oral route of exposure.-

Relevance of the Testicular Effects in Rats to Humans

For the purpose of Proposition 65, the study in rats reported by David et al. (2000a) is identified as “the most sensitive study deemed to be sufficient quality” and the NOEL for the testicular effects (indicative of the male reproductive toxicity) of DEHP observed in this study is used by OEHHA as the basis for establishing a MADL for DEHP by the oral route of exposure. The relevance of testicular effects of DEHP in rats to humans was taken into account by OEHHA.

It is generally accepted that “an agent that produces an adverse reproductive effect in experimental animal studies is assumed to pose a potential reproductive threat to humans” (U.S. EPA, 1996). However, in the case of DEHP, because DEHP does not cause obvious testicular damages in the common marmoset, a non-human primate (Rhodes et al., 1986; Kurata et al., 1998; MCSI, 2003) and there are known inter-species differences in the testicular toxicity of DEHP (e.g., CERHR, 2000; U.S. FDA, 2001), there have been questions raised regarding the relevance of testicular effects in rats to humans. To determine if the testicular effects of DEHP observed in rats are relevant to humans, OEHHA has reviewed relevant data on pharmacokinetics, metabolism, and mode(s) of action underlying the testicular effects of DEHP. In particular, OEHHA focused on similarities and differences in pharmacokinetics, metabolism, and mode(s) of action between rats and humans.

Pharmacokinetics and Metabolism. This subsection briefly summarize major pharmacokinetic characteristics of DEHP in rats, non-human primates, and humans. There are numerous studies and comprehensive reviews on the absorption, disposition, metabolism, and excretion of DEHP following oral administration in rats, non-human primates, and humans. Discussions below are based on experimental data that have been repeatedly reviewed and summarized in published reviews (e.g., Albro, 1986; Albro and Lavenhar, 1989; Astill, 1989) or regulatory or expert reports (e.g., CERHR, 2000; U.S. FDA, 2001). References cited in the text are exemplary, not comprehensive. In addition, several recent unpublished studies (Laignelet and Lhuguenot, 2000a; 2000b; 2000c; 2000d; 2001) submitted to OEHHA by the ACC were also included for review. Since exposure levels of DEHP in humans are generally low (CERHR, 2000), special attention has been paid to data obtained from studies using relatively low doses of DEHP (e.g., below 500 mg/kg-day).

Pharmacokinetic characteristics of DEHP are qualitatively similar among rats, non-human primates, and humans. Briefly, orally-administered DEHP is rapidly hydrolyzed to mono(2-ethylhexyl) phthalate (MEHP) and 2-ethylhexanol (2-EH) by ester hydrolases mainly in the gastrointestinal tract (GI). High levels of hydrolytic activity on DEHP have been found in the pancreatic juice, intestinal contents or tissues, and liver tissues of a wide variety of species including rats, non-human primates, and humans (Albro and Thomas, 1973; Albro and Lavenhar, 1989). Trace amounts or no intact DEHP have been found in the blood or liver tissues of rats or primates treated orally (either by gavage or in diet) with DEHP at levels below 500 mg/kg (Albro et al., 1982a; Albro, 1986; Astill, 1989; MCSI, 2003; Kessler et al., 2004). In rats following oral administration in diet, more than 90% or almost complete absorption as DEHP or its metabolites has been reported (Albro and Lavenhar, 1989; Astill, 1989). Rapid and near complete absorption of DEHP or its metabolites has also been observed in adult common marmosets treated with 100 mg/kg-day DEHP in diet (MCSI, 2003). However, the exact extent of DEHP absorption in the GI tract in humans is not clear.

Concentrations and kinetics of DEHP metabolites in the blood have been studied in rats and common marmosets. In general, blood concentrations of DEHP metabolites reach

maximal levels within 4-8 hr after dosing in both species. Maximal concentrations of DEHP metabolites in common marmosets are 1.3 to 10-fold lower than that in rats, depending on the dose level (Rhodes et al., 1986; Kessler et al., 2004). However, clearance of DEHP metabolites from the blood circulation appears to be slower in common marmosets than in rats (e.g., Albro and Lavenhar, 1989; Rhodes et al., 1986; MCSI, 2003). Thus, the difference in exposure, based on blood concentration, may be less than 1.3 to 10-fold.

Following primary metabolism and absorption in the GI tract, the primary metabolite of DEHP (i.e., MEHP) is further metabolized by one of three pathways: hydrolysis to phthalic acid and 2-ethylhexonal, conjugation to form glucuronide ester followed by rapid excretion, and hydroxylation at various sites on the ethylhexyl chain by cytochrome P450 enzymes. Hydroxylation of MEHP at ω - or ω 1-position on the ethylhexyl chain is the major pathway and generates a variety of metabolites (Albro et al., 1983; Lhuguenot et al., 1985; Albro and Lavenhar, 1989). Hydroxylation products are further metabolized by alcohol dehydrogenase and aldehyde dehydrogenase enzymes to yield keto-metabolites or dicarboxylic acids. Dicarboxylic acids can then undergo α - or β -oxidation reaction. In addition to MEHP itself, more than 20 other metabolites of DEHP have been identified. Major metabolites that have been found in the urine or fecal samples of rats have also been detected in the urine or fecal samples of non-human primates or humans. There are a few metabolites that have been frequently analyzed in the urine or fecal samples from rodents, non-human primates, and humans. These include *metabolite V* [mono(2-ethyl-5-carboxypentyl) phthalate, product generated from hydroxylation at the ω - position on the hexyl branch], *metabolite IX* [mono(2-ethyl-5-hydroxy-hexyl) phthalate, product of hydroxylation at the ω 1- position on the hexyl branch], and *metabolite VI* [mono(2-ethyl-5-oxo-hexyl) phthalate, keto-metabolite of metabolite IX].

Orally administered DEHP is quickly excreted from the body in urine as metabolites or in feces as either intact DEHP or metabolites, with a near complete clearance from the body within two-four days in rats, non-human primates, and humans. In addition to excretion in the urine and feces, absorbed DEHP metabolites can be excreted in the bile and subsequently excreted in feces or re-absorbed into blood circulation via entero-hepatic circulation. In rats and common marmosets, approximately 40-50% of the metabolites of DEHP administered by intravenous injection can be excreted in the bile, but only approximately 10-20% of the administered dose can be found in fecal samples, suggesting that entero-hepatic circulation of DEHP metabolites is significant (Daniel and Bratt, 1974; Chu et al., 1978; Rhodes et al., 1986; MCSI, 2003).

MEHP and all of its major metabolites can be conjugated to glucuronide via glucuronyl transferase. Glucuronide and MEHP or its metabolites in the conjugates can also be disassociated via β -glucuronidase. In rats, all metabolites excreted in the urine are in free (non-conjugated) form. In non-human primates and humans, glucuronide conjugates of DEHP metabolites account for 15-95% of the metabolites excreted in the urine, depending on the chemical structure of metabolites, route of exposure and individual primate or human subject (inter-individual variation). It has been shown that glucuronyl transferases from the rat are as active on MEHP as those from the mouse, but high

activity of β -glucuronidase activity in the rat results in absence of glucuronide-conjugates of MEHP or its metabolites in the urine of rats (Albro et al., 1981; 1982a; Albro, 1986; Albro and Lavenhar, 1989). In addition, predominant excretion of certain forms of glucuronide conjugates in the bile can also result in apparent absence of glucuronides in the urine (Chiu and Huskey, 1998). Therefore, lack of glucuronide conjugates in the urine may not reflect the inability of an animal species to form such conjugates.

Urinary excretion of DEHP metabolites accounts for about 30%- 70% (approximately 50% on average) of DEHP orally administered in rats, non-human primates, and humans (Albro et al., 1982a; Astill, 1989; CERHR, 2000). Only approximately 30% of the administered dose is excreted in the urine as MEHP, metabolite V, metabolite VI, and metabolite IX in rats. These findings clearly indicate that the extent of oral absorption of DEHP and/or its immediate metabolite MEHP, at least 90% in rats, is far higher than that excreted in the urine as the four metabolites discussed above. Thus, actual absorption rate or extent of DEHP in the GI tract is markedly higher than the extent of urinary excretion of major DEHP metabolites (approximately 30% of the dose). In humans, urinary excretion of DEHP metabolites has been investigated in several studies (Peck and Albro, 1982; Schmid & Schlatter; 1985; Dirven et al., 1993; Anderson et al., 2001; Koch et al. 2004a; Koch et al., 2004b). Following oral administration, up to approximately 70% of the administered dose of DEHP can be excreted in urine within the first 48 hours after administration (Koch et al., 2003; 2004), while Schmid and Schlatter (1985) reported that approximately 10-13% of the administered dose was excreted in the urine as MEHP, metabolite XI, IX, and V. None of the human studies determined levels of DEHP in feces or the possible extent of enterohepatic circulation. The absolute or relative amount of DEHP metabolites excreted in urine samples is a clear indicator of human exposure to DEHP. However, based on the data observed in rats as discussed above, the extent of absorption of DEHP or its metabolites in the GI tract in humans may well exceed the extent of urinary excretion of DEHP metabolites (i.e., more than up to 70% of orally administered DEHP can be expected to be absorbed in humans). In this regard, the possible difference in the absorption rate or extent of DEHP or its metabolites between rats and humans may not be significant.

Among the metabolites excreted in the urine in rats, metabolite V accounts for approximately 10-25% of the dose administered. MEHP, metabolite VI and IX excretion accounts for approximately 8-10% of the dose administered (Albro et al., 1981; 1982a; 1982b; Astill, 1989). Compared to the profile of DEHP metabolites in rats, relatively less metabolite V (approximately 5% of the dose) and more metabolites of ω 1-oxidation (metabolite IX and VI; 14-40% of the dose) are excreted in the urine in non-human primates or humans (Rhodes et al., 1986; Astill 1989; Schmid and Schlatter, 1985; Koch et al., 2003). Because of substantial biliary and fecal excretion of DEHP metabolites, difference in the profile of DEHP metabolites in the urine may not reflect actual status of oxidative metabolism of MEHP. For example, Short et al. (1987) compared DEHP metabolism and urinary excretion between rats and cynomolgus monkeys. They reported that 8.4% and 2.2% of the dose administered to Fischer 344 rats was excreted as metabolite V in the urine and feces, respectively. In cynomolgus monkeys, 5.7% and 5.3% of the dose administered was excreted as metabolite V in the urine and feces,

respectively, suggesting that there was relatively less metabolite V excreted in the urine and more of it in the feces in cynomolgus monkeys than that in rats. However, if the relative amount of metabolite V excreted in the urine and feces is combined, both species excreted about 11% of the dose as metabolites, indicating that generation of metabolite V in both species may be quantitatively similar, even though the relative amount of this metabolite excreted in the urine is different. This example clearly suggests that difference in the relative amount of metabolite V in the urine between rats and cynomolgus monkeys may not reflect actual status of oxidative metabolite of MEHP at organ levels. It may also suggest that differences in the relative amount of metabolite V in urine samples between rats, non-human primates, and humans may not indicate actual differences in the oxidative metabolism of MEHP in the target organs of DEHP (e.g., testis or liver) between different species.

From the data discussed above, it is clear that pharmacokinetic characteristics and metabolism of DEHP in rats, non-human primates, and humans are both qualitatively similar in many aspects and quantitatively similar to a large extent at relatively low exposure levels (e.g., below 500 mg/kg-day). There are some quantitative differences in the blood concentration of DEHP or its metabolites and in the profiles of DEHP metabolites in the urine among rats, non-human primates, and humans, but these differences may not reflect actual extent of absorption of DEHP in the GI tract and actual status of oxidative metabolism of MEHP; they may also play little role in the dramatic difference in testicular response to DEHP between rats and common marmosets. As stated in the study report by MCSI (2003), "it can no longer be assumed that this is due to poor absorption. This difference is thought to arise from a difference in target organs physiology between the two animal species rather than from any significant differences in metabolic kinetics." Similarly, Kessler et al. (2004) found that toxicokinetics alone could not account for the observed differences in toxicity, suggesting that toxicodynamic factors (possibly interactions of MEHP with receptor-mediated processes) may also contribute to this pronounced difference between the rodent and the marmoset. Physiological features of the testis in common marmosets that are fundamentally different from those in rats, cynomolgus monkeys, and humans may explain, at least in part, the lack of testicular effects of DEHP in the common marmoset.

In conclusion, similarities in pharmacokinetics and metabolism of DEHP between rats and humans strongly suggest that the testicular effects of DEHP observed in rats are relevant to humans. Quantitative, not qualitative, difference in blood burdens of DEHP metabolites and in the profiles of DEHP metabolites in the urine between rats and non-human primates or humans do not explain the lack of testicular effects in common marmosets.

Active Metabolite(s) Responsible for the Testicular Effects of DEHP. The active metabolite(s) responsible for the testicular effects of DEHP has been studied in rats using both in vivo and in vitro approaches. MEHP mimics the testicular effects of DEHP both in vivo and in vitro in juvenile rats, but not 2-ethylhexanol or any of three major oxidative metabolites including metabolite V, VI, and IX (Gangolli, 1982; Gray and Ganagolli, 1986; Sjoberg et al., 1986; Albro et al., 1989; Grasso et al., 1993; Jones et al.,

1993). DEHP, but not 2-ethylhexanol, causes reduction in Sertoli cell proliferation in neonatal rats (Li et al., 2000). MEHP, but not DEHP itself, also causes decreased proliferation of cultured Sertoli cells isolated from neonatal rats (Li et al., 1998; Li and Kim, 2003). These data clearly indicate that MEHP is the proximal metabolite for DEHP-induced testicular damage in rats.

Distribution of DEHP metabolites to the testis has been reported in rats and common marmosets (Williams and Blanchfield, 1974; Tanaka et al., 1975; Rhodes et al., 1986; MCSI, 2003; Ono et al., 2004). In rats, radioactivity of DEHP ³H-labeled at the phthalic acid moiety was found in the basal area of seminiferous tubules at the stages IX-XIV and I of the spermatogenic cycle, within six hours after a single oral dose. As discussed in the subsection of “Male Reproductive Toxicity in Animals”, seminiferous tubules at the stages IX to I of the spermatogenic cycle have been shown to be more sensitive to the testicular effects of DEHP than those at other stages (e.g., Saitoh et al, 1997; CERHR, 2000). Within the seminiferous epithelium, high levels of DEHP metabolites were mainly found in Sertoli cells and in the cytoplasm of spermatocytes. The Sertoli cell has been shown to be the initial target testicular cell of DEHP in juvenile and adult rats (see discussions below). By 24 hours after dosing, DEHP metabolites in the testis decreased to approximately 50% of the level observed 6 hours after dosing, suggesting rapid clearance of DEHP metabolites from the testis in rats (Ono et al., 2004). These data suggest not only that MEHP and/or MEHP metabolites reach the testis after oral administration, they are also distributed to the seminiferous tubules that have been shown to be targeted by DEHP in young or adult animals. In this regard, it should be noted that the Sertoli cells in common marmosets are morphologically uniform, i.e., there is no morphological variation along the eight stages of seminiferous tubules in this species (Rune et al., 1992). This feature of Sertoli cells in marmosets is different from these cells in most other mammals including humans, indicating another difference in the physiological features of the testis between common marmosets and humans.

Potential Modes of Actions. Using both in vivo and in vitro approaches, it has been repeatedly shown that the Sertoli cell and the Leydig cell appear to be the initial target cells of MEHP in the testis (e.g., Gray and Beaman, 1984; Heindel and Powell, 1992; Li et al., 1998; Akingbemi et al. 2001; CERHR, 2000). The Sertoli cell is the somatic cell that provides a supportive role in spermatogenesis in adult animals and whose population established during proliferating periods determines the size of the testis and the volume of daily sperm production in the adult. Maintenance and development of germ cells into functionally normal spermatozoa depend on a permissive milieu provided by the Sertoli cells (Russell and Griswold, 1993; Boekelheide et al., 2000). The Leydig cell produces androgen that regulates development of the male reproductive system and plays a critical role in spermatogenesis in the adult (Payne et al., 1996). In the adult, the effect of DEHP/MEHP on the Leydig cells at high doses probably plays a minimal role in the overall testicular toxicity of DEHP, even though there is clear evidence that DEHP in vivo and MEHP in vitro damages the Leydig cells in rats (e.g., Jones et al., 1993; CERHR, 2000). On the other hand, both DEHP in vivo and MEHP in vitro damage both Sertoli cells and Leydig cells in fetal or neonatal testes from rats at doses that have no

obvious effects on the testis in young or adult animals (e.g., Dostal et al., 1988; Li et al., 2000; Akingbemi et al., 2004; Boekelheide, 2004).

The exact biochemical or molecular mechanism(s) underlying the testicular effects of DEHP remains unclear. Several hypotheses have been proposed, including: (1) alterations in testicular zinc levels or zinc-dependent enzymatic activities, (2) oxidative stress in the testis; (3) FSH receptor-dependent pathways, (4) estrogenic activity or interactions with estrogen receptors, (5) peroxisome proliferator-activated receptor (PPAR)-dependent pathways, and (6) other cellular or molecular events or pathways (e.g., alterations in Sertoli-germ cell interactions or changed expression of genes critical for germ cell survival or functions of Sertoli cells or Leydig cells). OEHHA has reviewed a large amount of the relevant mechanistic data that are available. For the purposes of this document, the discussion below focuses on the role of PPAR in the male reproductive effects of DEHP. This issue is critical for determining the relevance of the rodent data to humans, since it has been proposed that induction of liver tumors by DEHP via PPAR α -mediated mechanism(s) as observed in rodents is not relevant to humans (Klaunig et al., 2003). It has been suggested that PPAR α may also play an important role in the testicular effects of DEHP and thus PPAR α -mediated testicular effects in rats are also not relevant to humans (ACC, 2004; McKee et al., 2004).

Two lines of evidence have been cited to support an active role of PPAR- α in the testicular effects of DEHP (ACC, 2004; McKee et al., 2004). One is the finding from the study by Ward et al. (1998) that compared the toxicity including testicular lesions caused by oral administration of DEHP at 12,000 ppm in diet for up to 24 weeks between wild-type (normal) mice and those lacking PPAR α receptors (knock-out mice). The authors found that DEHP-induced testicular lesions in knock-out mice were less severe and required longer treatment than in the wild-type animals. The authors suggested that both PPAR α -dependent and -independent pathways are involved in the testicular effects of DEHP. In discussing the presence of DEHP-induced kidney toxicity in PPAR α knock-out mice, the authors stated that “it is possible that other receptor subtypes (PPAR δ or γ) may play a role in the observed delayed kidney toxicity or the high dose of DEHP may modify the pharmacokinetics of DEHP in these mice.”

The other line of evidence cited to support an active role of PPAR in DEHP-caused testicular damage primarily comes from studies that investigated the roles of PPAR α in induction of Leydig cell tumors (LCTs) by peroxisome proliferators (PPs) in rodents (e.g., Cook et al., 1992; Klaunig et al., 2003). DEHP has been shown to cause Leydig cell hyperplasia and tumors in rats (e.g., Akingbemi et al., 2004; Voss et al., 2005). Cook and his co-workers have found that ammonium perfluorooctonate (C8), a peroxisome proliferating agent causing Leydig cell tumors, causes imbalance between testosterone and estradiol by directly inhibiting testosterone production in Leydig cells and/or by inducing synthesis of aromatase (which converts testosterone to estradiol) in the liver (Cook et al., 1992; Biegel et al., 1995, 2001; Liu et al., 1996a, 1996b).

In addition to the studies on C8, the study by Gazouli et al. (2002) investigated the effects of several PPs (including DEHP, bezafibrate, WY-14,643) on steroid synthesis in Leydig

cells and the mechanism underlying these effects. The authors found that the anti-androgenic effects of some PPs are mediated by suppression of PPAR α -mediated transcription of peripheral-type benzodiazepine receptor (PBR) gene. This gene encodes a high-affinity mitochondrial cholesterol-binding protein which plays an important role in transportation of cholesterol into mitochondria, a hormone-induced rate-determining step in steroid synthesis. The authors also reported several other important findings. For example, the circulating testosterone levels in PPAR α knock out mice were significantly lower than that in the wild-type mice, suggesting that PPAR α may play a positive role in maintaining the balance of circulating testosterone levels. When the animals were treated with 1 g/kg/day DEHP or 50 mg/kg/day WY-14,643 for eight days, circulating testosterone levels were significantly decreased in the wild-type mice. However, circulating testosterone levels were markedly increased to a level significantly higher than the knock-out controls and even slightly higher than that in the wild-type control animals, indicating some PPs like DEHP may act through PPAR β or other unknown mechanisms to disrupt the balance of circulating testosterone levels. In addition, the authors found that bezafibrate acts mainly on the step of cholesterol transportation in steroid formation, while MEHP acts on many steps of steroidogenesis. Other than cholesterol transportation, the role of PPARs in many steps in the biochemical cascades of steroidogenesis in Leydig cells remains unclear.

In spite of the arguments discussed above, there are numerous data suggesting that PPAR α plays a minimal role, if any, in the testicular effects of DEHP. First of all, the testicular toxicity of DEHP is characterized by disruption in Sertoli cell function or proliferation followed by apoptosis in spermatocytes and alterations in Leydig cell function and/or proliferation with subsequent disruption in androgen-dependent development of the male reproductive system. There is no evidence indicating that C8 causes similar testicular damage (e.g., Kennedy et al., 2004). The findings by Gazouli et al. (2002) also clearly indicate that the disruptive effects of DEHP and/or MEHP may be mediated by both PPAR α -dependent and -independent mechanism(s). Therefore, there are differences in the mechanism(s) underlying the disruptive effects on testosterone synthesis or balance among different PPs. Moreover, even if DEHP and other PPs (e.g., C8) cause LCTs via similar mechanism(s), a mode of action for the non-cancer testicular toxicity of DEHP based on evidence from studies on a chemical that does not cause similar non-cancer testicular damage is not a valid comparison.

Secondly, two modes of actions (MOAs) have been postulated by Klaunig et al. (2003) to describe the etiology of Leydig cell tumors in PPAR α agonist-treated rats. The authors have concluded that “the weight of evidence available to date to support virtually all of the postulated key events is weak overall, and moderate at best for only two or three of the postulated events.” Furthermore, Klaunig et al. (2003) concluded that “the proposed animal MOAs - induction of aromatase secondary to liver induction (Pathway 1) and the direct inhibition of testosterone biosynthesis (Pathway 2) - are plausible mechanisms and could occur in humans. If PPAR α is mediating the induction of aromatase, this mechanism could occur in humans due to the expression of PPAR α in human liver. The inhibition of testosterone biosynthesis by PPAR agonists is better established than the induction of aromatase and is also plausible, as PPAR α is present in human Leydig cells.

The pathways for the regulation of the HPT [hypothalamic-pituitary-testicular] axis of rats and humans also are similar, in that compounds that decrease testosterone will increase LH levels. Hence, compounds that induce LCTs in rats by disruption of the HPT axis pose a potential risk to human health.”

Thirdly, as stated by the Phthalate Expert Panel of CERHR (2000), “in contrast to hepatic toxicity, testicular toxicity is noted in PPAR-alpha knockout mice exposed to DEHP, albeit that appearance of the testicular effects was delayed compared to wild-type mice. In addition, the guinea pig, a non-responding species to the peroxisomal-proliferating effects of DEHP, is susceptible to the testicular effects of this agent.” The Phthalate Expert Panel of CERHR concluded that “Overall, the Panel believes that the reproductive toxicity of DEHP appears independent of PPAR-alpha. However, other members of the PPAR family (beta or delta and gamma) have not been extensively studied with regard to activation by phthalates. PPAR-gamma has been found in human testis, ovary, placenta, and embryo. MEHP (but not DEHP, 2-EH, or 2-EHA) has been shown to activate PPAR-gamma receptor in a transcription reporter assay [Maloney and Waxman, 1999].”

Therefore, the weight of evidence does not indicate that the non-cancer testicular effects of DEHP are mainly mediated by PPAR α . Even if PPARs including PPAR α , β , and γ play any important role in DEHP-induced damage in testicular development and functions, as suggested by evidence summarized in a recent comprehensive review by Corton and Lapinskas (2004), PPARs are expressed in human male reproductive organs (e.g., Elbrecht et al., 1996; Schultz et al., 1999; Collett et al., 2000; Hase et al., 2002). Therefore, PPAR-mediated testicular effects of DEHP in rats are relevant to humans. Possible modes of actions underlying the induction of Leydig cell tumors in rodents, including those involving PPARs, are also plausible in humans.

With regard to the other hypotheses proposed for the testicular effects of DEHP, the male reproductive system in humans has capabilities to carry out all of them. There is no evidence to indicate otherwise.

Conclusion on Relevance to Humans. Based on the data that are available to OEHHA, orally administered DEHP at doses relatively low (<500 mg/kg-day) but still markedly higher than the LOEL for testicular effects in rats (10 – 40 mg/kg-day) is absorbed and metabolized in humans in ways in general qualitatively and quantitatively similar to those in rats and non-human primates. Lack of testicular effects in common marmosets may be due to fundamental differences in testicular physiology between this species and other mammals including cynomolgus monkeys and humans. Potential testicular effects of DEHP at a relatively low dose observed by Pugh et al. (2000) in late-infantile cynomolgus monkey and similarities in the testicular physiology between cynomolgus monkeys and humans indicate that DEHP may cause testicular damages in humans. All potential modes of actions or mechanisms underlying the testicular effects of DEHP in rats are also plausible in humans. Therefore, OEHHA concludes that the weight of the evidence supports a finding that the testicular effects of DEHP observed in rodents are relevant to humans.

MADL CALCULATION

The NOEL is the highest dose level that results in no observable reproductive effect, expressed in milligrams of chemical per kilogram of bodyweight per day. The NOEL is converted to a milligram per day dose level by multiplying the assumed human body weight by the NOEL (Section 12803(b)). When the applicable reproductive effect is upon the male, the MADL is generally calculated based on a human body weight of 70 kg (Section 12803(b)). As already noted, however, developing animals are sensitive to the testicular effects of DEHP (e.g., Sjoberg et al., 1985; 1986; Li et al., 2000; CERHR, 2000; U.S. FDA, 2001; Borch et al., 2004). The bodyweights of infants and ~~neonates~~ neonatal infants are approximately 7-20 fold lower than that of an adult (National Center for Health Statistics, 2005). Thus, exposure of an infant ~~or neonate~~ to DEHP at a MADL calculated on the basis of an adult body weight of 70 kg would result in a dose up to 20-fold higher than the corresponding dose in adults. Accordingly, age-specific MADLs have been calculated for infant boys of age 29 days to 24 months and for infant boys during the neonatal period (0-28 days of age) ~~and neonatal boys based on bodyweights of 10 and 3.5 kg, respectively,~~ as also allowed by regulation (Sections 12801(a) and 12803(a)(6)). The neonatal period is “the period immediately succeeding birth and continuing through the first 28 days of extrauterine life,” as defined by Stedman’s Medical Dictionary (27th Edition). For neonatal infants, the 50th percentile birthweight for boys of 3.5 kg is used (National Center for Health Statistics, 2005). For purposes of this regulation the body weight of 10 kg for infants aged 0-2 years of age specified in Section 12703(a)(8) is applied to infants to 29 days-24 months of age. Boy children and adolescents also have lower body weights than do the adult. If males in those age groups are exposed to DEHP by the oral route of exposure, age-specific MADLs can be calculated by application of the corresponding default bodyweights specified in Section 12703(a)(8).

The following calculations were performed to derive the MADLs for DEHP via the oral route of exposure, based on a NOEL of 5.8 mg/kg-day found in rats by David et al (2000a).

For Adults:

When the applicable reproductive effect is upon the male, human body weight of 70 kilograms shall be assumed (Section 12803(b)).

Calculation of the NOEL for a 70 kg man:

$$5.8 \text{ mg/kg-day} \times 70 \text{ kg} = 406.0 \text{ mg/day}$$

The MADL is derived by dividing the NOEL by one thousand (Section 12801(b)(1)). Thus, the adjusted NOEL was divided by 1,000 to obtain the MADL.

$$\text{MADL}_{\text{adult oral}} = 406 \text{ mg/day} \div 1000 = 406 \text{ } \mu\text{g/day} \text{ or } \mathbf{410 \text{ } \mu\text{g/day}}$$
 after rounding.

For ~~Neonates and Infants~~ Neonatal Infant Boys:

Assuming a body weight of 10 kg for a one-year-old infant (National Center for Health Statistics, 2000), an exposure of an infant to DEHP at the level of the MADL_{adult} (410 µg/day) is equivalent to 41 µg/kg-day. In order to derive a MADL for infants of 410 µg/day, it would require a NOEL of 41 mg/kg-day ($410 \mu\text{g}/\text{day} \div 10 \text{ kg} \times 1000 = 41 \text{ mg}/\text{kg}\text{-day}$). This estimated infant NOEL would be seven-fold higher than the NOEL for the adult (5.8 mg/kg-day), indicating that application of the adult-derived MADL would result in a 7-fold higher dose in infants and a higher dose in ~~neonates~~ neonatal infants. It is even higher than the LOELs observed by Poon et al. (1997) (38 mg/kg-day) in rats treated for 13 weeks beginning 6 weeks postnatal, and by Akingbemi et al. (2001, 2004) (10 mg/kg-day) in rats treated for various periods of time beginning 21 days postnatal. Therefore, a MADL based on the body weight of an adult human may not be protective against male reproductive effects in a neonatal or infant boy.

Section 12801(a) specifies that “nothing in this article shall preclude a person from using evidence, standards, assessment methodologies, principles, assumptions or levels not described in this article to establish that a level of exposure has no observable effect at one thousand (1,000) times the level in question,” while Section 12803(a)(6) specifies that “when available data are of such quality that anatomic, physiologic, pharmacokinetic and metabolic considerations can be taken into account with confidence, they may be used in the assessment.” In this case, the anatomic and physiologic differences between an infant boy and an adult man can be taken into account with much confidence. Therefore, MADLs specific to infants and ~~neonates~~ neonatal infant boys are developed as follows:

The infant period extends from birth to age 24 months and an average body weight of 10 kg over this developmental period is assumed (Section 12703(a)(8); OEHHA, 2000; National Center for Health Statistics, 2005). For purposes of this regulation this same average body weight of 10 kg is used to apply to those infants 29 days-24 months of age. ~~For infants 0-2 years of age, the average body weight of 10 kg over this developmental period is used (Section 12703(a)(8); OEHHA, 2000; National Center for Health Statistics, 2005).~~

Calculation of the NOEL for a 10 kg infant:

$$5.8 \text{ mg}/\text{kg}\text{-day} \times 10 \text{ kg} = 58 \text{ mg}/\text{day}$$

$$\text{MADL}_{\text{infant oral}} = 58 \text{ mg}/\text{day} \div 1000 = \mathbf{58 \mu\text{g}/\text{day}}.$$

The neonatal period consists of the first 28 days of the infant period (Stedman's Medical Dictionary (27th Edition)). For infants 0-28 days of age (i.e., neonatal infants), the 50th percentile birthweight of 3.5 kg for boys is used (National Center for Health Statistics, 2005). ~~For neonates, the 50th percentile birthweight for boys of 3.5 kg is used (National Center for Health Statistics, 2005).~~

Calculation of the NOEL for a 3.5 kg neonate:

$$5.8 \text{ mg}/\text{kg}\text{-day} \times 3.5 \text{ kg} = 20.3 \text{ mg}/\text{day} = 20 \text{ mg}/\text{day} \text{ (rounded)}$$

MADL_{neonate neonatal infant oral} = 20 mg/day ÷ 1000 = **20 µg/day**.

All the MADLs derived above (410 µg/day for adults, 58 µg/day for infant boys and 20 µg/day for neonatal infant boys) apply to exposure to DEHP by the oral route.

REFERENCES

Ablake M, Itoh M, Terayama H, Hayashi S, Shoji S, Naito M, Takahashi K, Suna S, Jitsunari F (2004). Di-(2-ethylhexyl) phthalate induces severe aspermatogenesis in mice, however, subsequent antioxidant vitamins supplementation accelerates regeneration of the seminiferous epithelium. *Int J Androl* **27**, 274-81.

Akingbemi BT, Ge R, Klinefelter GR, Zirkin BR, Hardy MP (2004). Phthalate-induced Leydig cell hyperplasia is associated with multiple endocrine disturbances. *Proc Natl Acad Sci U S A* **101**, 775-80.

Akingbemi BT, Youker RT, Sottas CM, Ge R, Katz E, Klinefelter GR, Zirkin BR, Hardy MP (2001). Modulation of rat Leydig cell steroidogenic function by di-(2-ethylhexyl)phthalate. *Biol Reprod* **65**, 1252-9.

Albro PW (1986). Absorption, metabolism, and excretion of di(2-ethylhexyl) phthalate by rats and mice. *Environ Health Perspect* **65**, 293-8.

Albro PW, Chapin RE, Corbett JT, Schroeder J, Phelps JL (1989). Mono-2-ethylhexyl phthalate, a metabolite of di-(2-ethylhexyl) phthalate, causally linked to testicular atrophy in rats. *Toxicol Appl Pharmacol* **100**, 193-200.

Albro PW, Corbett JT, Schroeder JL, Jordan S, Matthews HB (1982a). Pharmacokinetics, interactions with macromolecules and species differences in metabolism of DEHP. *Environ Health Perspect* **45**, 19-25.

Albro PW, Hass JR, Peck CC, Jordan ST, Corbett JT, Schroeder J (1982b). Applications of isotope differentiation for metabolic studies with di-(2-ethylhexyl) phthalate. *J Environ Sci Health B* **17**, 701-14.

Albro PW, Hass JR, Peck CC, Odam DG, Corbett JT, Bailey FJ, Blatt HE, Barrett BB (1981). Identification of the metabolites of di-(2-ethylhexyl) phthalate in urine from the African green monkey. *Drug Metab Dispos* **9**, 223-5.

Albro PW, Lavenhar SR (1989). Metabolism of di(2-ethylhexyl)phthalate. *Drug Metab Rev* **21**, 13-34.

Albro PW, Thomas RO (1973). Enzymatic hydrolysis of di-(2-ethylhexyl) phthalate by lipases. *Biochim Biophys Acta* **306**, 380-90.

Albro PW, Tondeur I, Marbury D, Jordan S, Schroeder J, Corbett JT (1983). Polar metabolites of di-(2-ethylhexyl)phthalate in the rat. *Biochim Biophys Acta* **760**, 283-92.

American Chemistry Council (ACC, 2004). Information pertaining to development of a maximum allowable dose level for di(2-ethylhexyl) phthalate. Submitted to the Office of Environmental Health Hazard Assessment, California Environmental Protection Agency, Sacramento, on May 12, 2004.

Anderson WA, Castle L, Scotter MJ, Massey RC, Springall C (2001). A biomarker approach to measuring human dietary exposure to certain phthalate diesters. *Food Addit Contam* **18**, 1068-74.

Arcadi FA, Costa C, Imperatore C, Marchese A, Rapisarda A, Salemi M, Trimarchi GR, Costa G (1998). Oral toxicity of bis(2-ethylhexyl) phthalate during pregnancy and suckling in the Long-Evans rat. *Food Chem Toxicol* **36**, 963-70.

~~Aslan AR, Kogan BA, and Gondos B (2003). Testicular development. In: Polin RA, Fox WW, and Abman SH eds. Fetal and Neonatal Physiology. Saunders, Philadelphia, PA. 3rd ed. Volume 2, Chapter 191, pp1950-1960.~~

Astill BD (1989). Metabolism of DEHP: effects of prefeeding and dose variation, and comparative studies in rodents and the cynomolgus monkey (CMA studies). *Drug Metab Rev* **21**, 35-53.

Banerjee S, Thuillier R, Culty M, Papadopoulos V, Brown TR, Banerjee PP (2002). In utero exposure to di(2-ethylhexyl) phthalate alters growth, tissue organization, and the expression of androgen receptor protein of rat prostate. *Biol Reprod* **66(Suppl 1)**, 200.

Biegel LB, Hurtt ME, Frame SR, O'Connor JC, Cook JC (2001). Mechanisms of extrahepatic tumor induction by peroxisome proliferators in male CD rats. *Toxicol Sci* **60**, 44-55.

Biegel LB, Liu RC, Hurtt ME, Cook JC (1995). Effects of ammonium perfluorooctanoate on Leydig cell function: in vitro, in vivo, and ex vivo studies. *Toxicol Appl Pharmacol* **134**, 18-25.

Boekelheide K (2004). Cracking the nut. *Toxicol Sci* **81**, 1-2 .

Boekelheide K, Fleming SL, Johnson KJ, Patel SR, Schoenfeld HA (2000). Role of Sertoli cells in injury-associated testicular germ cell apoptosis. *Proc Soc Exp Biol Med* **225**, 105-15.

Borch J, Ladefoged O, Hass U, Vinggaard AM (2004). Steroidogenesis in fetal male rats is reduced by DEHP and DINP, but endocrine effects of DEHP are not modulated by DEHA in fetal, prepubertal and adult male rats. *Reprod Toxicol* **18**, 53-61.

Cammack JN, White RD, Gordon D, Gass J, Hecker L, Conine D, Bruen US, Friedman M, Echols C, Yeh TY, Wilson DM (2003). Evaluation of reproductive development following intravenous and oral exposure to DEHP in male neonatal rats. *Int J Toxicol* **22**, 159-74.

Center for The Evaluation of Risks to Human Reproduction (CERHR, 2000). NTP-CERHR Expert Panel Report on Di (2-ethylhexyl) Phthalate. National Toxicology Program, U.S. Department of Health and Human Services, Research Triangle Park, NC, October.

Chiu SH, Huskey SW (1998). Species differences in N-glucuronidation. *Drug Metab Dispos* **26**, 838-47.

Cho F, Yabe M, Honjo S (1975). The weight of the reproductive organs, hypophysis and thyroid of male cynomolgus monkeys (*Macaca fascicularis*). *Jikken Dobutsu* **24**, 173-5.

Chu I, Villeneuve DC, Secours V, Franklin C, Rock G, Viau A (1978). Metabolism and tissue distribution of mono-2-ethylhexyl phthalate in the rat. *Drug Metab Dispos* **6**, 146-9.

Collett GP, Betts AM, Johnson MI, Pulimood AB, Cook S, Neal DE, Robson CN (2000). Peroxisome proliferator-activated receptor alpha is an androgen-responsive gene in human prostate and is highly expressed in prostatic adenocarcinoma. *Clin Cancer Res* **6**, 3241-8.

Cook JC, Murray SM, Frame SR, Hurtt ME (1992). Induction of Leydig cell adenomas by ammonium perfluorooctanoate: a possible endocrine-related mechanism. *Toxicol Appl Pharmacol* **113**, 209-17.

Corton JC, Lapinskas PJ (2004). Peroxisome Proliferator-Activated Receptors: Mediators of Phthalate Ester-Induced Effects in the Male Reproductive Tract? *Toxicol Sci*.

Daniel JW, Bratt H (1974). The absorption, metabolism and tissue distribution of di(2-ethylhexyl)phthalate in rats. *Toxicology* **2**, 51-65.

David RM, Moore MR, Finney DC, Guest D (2000a). Chronic toxicity of di(2-ethylhexyl)phthalate in rats. *Toxicol Sci* **55**, 433-43.

David RM, Moore MR, Finney DC, Guest D (2000b). Chronic toxicity of di(2-ethylhexyl)phthalate in mice. *Toxicol Sci* **58**, 377-85.

Dirven HA, van den Broek PH, Arends AM, Nordkamp HH, de Lepper AJ, Henderson PT, Jongeneelen FJ (1993). Metabolites of the plasticizer di(2-ethylhexyl)phthalate in urine samples of workers in polyvinylchloride processing industries. *Int Arch Occup Environ Health* **64**, 549-54.

Dostal LA, Chapin RE, Stefanski SA, Harris MW, Schwetz BA (1988). Testicular toxicity and reduced Sertoli cell numbers in neonatal rats by di(2-ethylhexyl)phthalate and the recovery of fertility as adults. *Toxicol Appl Pharmacol* **95**, 104-21.

Duty SM, Calafat AM, Silva MJ, Brock JW, Ryan L, Chen Z, Overstreet J, Hauser R (2004). The relationship between environmental exposure to phthalates and computer-aided sperm analysis motion parameters. *J Androl* **25**, 293-302.

Duty SM, Silva MJ, Barr DB, Brock JW, Ryan L, Chen Z, Herrick RF, Christiani DC, Hauser R (2003a). Phthalate exposure and human semen parameters. *Epidemiology* **14**, 269-77.

Duty SM, Singh NP, Silva MJ, Barr DB, Brock JW, Ryan L, Herrick RF, Christiani DC, Hauser R (2003b). The relationship between environmental exposures to phthalates and DNA damage in human sperm using the neutral comet assay. *Environ Health Perspect* **111**, 1164-9.

Elbrecht A, Chen Y, Cullinan CA, Hayes N, Leibowitz M, Moller DE, Berger J (1996). Molecular cloning, expression and characterization of human peroxisome proliferator activated receptors gamma 1 and gamma 2. *Biochem Biophys Res Commun* **224**, 431-7.

Festing MF, Altman DG (2002). Guidelines for the design and statistical analysis of experiments using laboratory animals. *ILAR J* **43**, 244-58.

Flurer CI, Zucker H (1987). Difference in serum ascorbate in two species of Callithricidae. *Int J Vitam Nutr Res* **57**, 297-8.

Flurer CI, Zucker H (1989). Ascorbic acid in a New World monkey family: species difference and influence of stressors on ascorbic acid metabolism. *Z Ernahrungswiss* **28**, 49-55.

Fredricsson B, Moller L, Pousette A, Westerholm R (1993). Human sperm motility is affected by plasticizers and diesel particle extracts. *Pharmacol Toxicol* **72**, 128-33.

Gangolli SD (1982). Testicular effects of phthalate esters. *Environ Health Perspect* **45**, 77-84.

Gazouli M, Yao ZX, Boujrad N, Corton JC, Culty M, Papadopoulos V (2002). Effect of peroxisome proliferators on Leydig cell peripheral-type benzodiazepine receptor gene expression, hormone-stimulated cholesterol transport, and steroidogenesis: role of the peroxisome proliferator-activator receptor alpha. *Endocrinology* **143**, 2571-83.

Grasso P, Heindel JJ, Powell CJ, Reichert LE Jr (1993). Effects of mono(2-ethylhexyl) phthalate, a testicular toxicant, on follicle-stimulating hormone binding to membranes from cultured rat Sertoli cells. *Biol Reprod* **48**, 454-9.

Gray LE, Hotchkiss AK, Price M, Wolf CJ, Furr J, Ostby J, Lambright C, Parks L, Wilson V, Bobseine K and others (2001). Adverse effects of antiandrogenic pesticides and toxic substances on reproductive development in the male. *Biol Reprod* **64(Suppl 1)**, 87-8

Gray LE Jr, Ostby J, Furr J, Price M, Veeramachaneni DN, Parks L (2000). Perinatal exposure to the phthalates DEHP, BBP, and DINP, but not DEP, DMP, or DOTP, alters sexual differentiation of the male rat. *Toxicol Sci* **58**, 350-65.

Gray LE Jr, Wolf C, Lambright C, Mann P, Price M, Cooper RL, Ostby J (1999). Administration of potentially antiandrogenic pesticides (procymidone, linuron, iprodione, chlozolate, p,p'-DDE, and ketoconazole) and toxic substances (dibutyl- and diethylhexyl phthalate, PCB 169, and ethane dimethane sulphonate) during sexual differentiation produces diverse profiles of reproductive malformations in the male rat. *Toxicol Ind Health* **15**, 94-118.

Gray TJ, Beaman JA (1984). Effect of some phthalate esters and other testicular toxins on primary cultures of testicular cells. *Food Chem Toxicol* **22**, 123-31.

Gray TJ, Butterworth KR (1980). Testicular atrophy produced by phthalate esters. *Arch Toxicol Suppl* **4**, 452-5.

Gray TJ, Gangolli SD (1986). Aspects of the testicular toxicity of phthalate esters. *Environ Health Perspect* **65**, 229-35.

Gray TJ, Rowland IR, Foster PM, Gangolli SD (1982). Species differences in the testicular toxicity of phthalate esters. *Toxicol Lett* **11**, 141-7.

Gromoll J, Eiholzer U, Nieschlag E, Simoni M (2000). Male hypogonadism caused by homozygous deletion of exon 10 of the luteinizing hormone (LH) receptor: differential action of human chorionic gonadotropin and LH. *J Clin Endocrinol Metab* **85**, 2281-6.

Hampl JS, Taylor CA, Johnston CS (2004). Vitamin C deficiency and depletion in the United States: the Third National Health and Nutrition Examination Survey, 1988 to 1994. *Am J Public Health* **94**, 870-5.

Hase T, Yoshimura R, Mitsuhashi M, Segawa Y, Kawahito Y, Wada S, Nakatani T, Sano H (2002). Expression of peroxisome proliferator-activated receptors in human testicular cancer and growth inhibition by its agonists. *Urology* **60**, 542-7.

Heindel JJ, Powell CJ (1992). Phthalate ester effects on rat Sertoli cell function in vitro: effects of phthalate side chain and age of animal. *Toxicol Appl Pharmacol* **115**, 116-23.

~~Hilscher B, Engemann A (1992). Histological and morphometric studies on the kinetics of germ cells and immature Sertoli cells during human prespermatogenesis. *Andrologia* **24**, 7-10.~~

Ishihara M, Itoh M, Miyamoto K, Suna S, Takeuchi Y, Takenaka I, Jitsunari F (2000). Spermatogenic disturbance induced by di-(2-ethylhexyl) phthalate is significantly prevented by treatment with antioxidant vitamins in the rat. *Int J Androl* **23**, 85-94.

Ivell R, Bathgate RA (2002). Reproductive biology of the relaxin-like factor (RLF/INSL3). *Biol Reprod* **67**, 699-705.

Jones HB, Garside DA, Liu R, Roberts JC (1993). The influence of phthalate esters on Leydig cell structure and function in vitro and in vivo. *Exp Mol Pathol* **58**, 179-93.

Kennedy GL Jr, Butenhoff JL, Olsen GW, O'Connor JC, Seacat AM, Perkins RG, Biegel LB, Murphy SR, Farrar DG (2004). The toxicology of perfluorooctanoate. *Crit Rev Toxicol* **34**, 351-84.

Kessler W, Numtip W, Grote K, Csanady GA, Chahoud I, Filser JG (2004). Blood burden of di(2-ethylhexyl) phthalate and its primary metabolite mono(2-ethylhexyl) phthalate in pregnant and nonpregnant rats and marmosets. *Toxicol Appl Pharmacol* **195**, 142-53.

Klaunig JE, Babich MA, Baetcke KP, Cook JC, Corton JC, David RM, DeLuca JG, Lai DY, McKee RH, Peters JM, Roberts RA, Fenner-Crisp PA (2003). PPARalpha agonist-induced rodent tumors: modes of action and human relevance. *Crit Rev Toxicol* **33**, 655-780.

Kluin PM, Kramer MF, de Rooij DG (1983). Testicular development in *Macaca irus* after birth. *Int J Androl* **6**, 25-43.

Koch HM, Bolt HM, Angerer J (2004a). Di(2-ethylhexyl)phthalate (DEHP) metabolites in human urine and serum after a single oral dose of deuterium-labelled DEHP. *Arch Toxicol* **78**, 123-30

Koch HM, Preuss R, Drexler H, Angerer J, Bolt HM (2004b). Biological monitoring of (DEHP) exposure: the relevance of the oxidative metabolites of Di(2-ethylhexyl)phthalate (DEHP) compared to the classical parameter MEHP. Annual Meeting of the International Society of Exposure Analysis, Philadelphia, Pennsylvania, USA, October 17-21, 2004. Abstract M1A-06.

Koch HM, Rossbach B, Drexler H, Angerer J (2003). Internal exposure of the general population to DEHP and other phthalates--determination of secondary and primary phthalate monoester metabolites in urine. *Environ Res* **93**, 177-85.

Kurata Y, Kidachi F, Yokoyama M, Toyota N, Tsuchitani M, Katoh M (1998). Subchronic toxicity of Di(2-ethylhexyl)phthalate in common marmosets: lack of hepatic peroxisome proliferation, testicular atrophy, or pancreatic acinar cell hyperplasia. *Toxicol Sci* **42**, 49-56.

Laignelet L, Lhuguenot JC (2000a). Di-(2-ethylhexyl)phthalate (DEHP) absorption, excretion, metabolism and pharmacokinetic profile in Wistar female rats. Report No. 1/99. Submitted by the American Chemistry Council to OEHHA, California EPA, on May 12, 2004.

Laignelet L, Lhuguenot JC (2000b). Di-(2-ethylhexyl)phthalate (DEHP) absorption, excretion, metabolism and pharmacokinetic profile in pregnant Wistar rats. Report No. 2/99. Submitted by the American Chemistry Council to OEHHA, California EPA, on May 12, 2004.

Laignelet L, Lhuguenot JC (2000c). I-(2-ethylhexyl)phthalate (DEHP) absorption, excretion, metabolism and pharmacokinetic profile in pregnant CD1 mice. Report No. 3/99. Submitted by the American Chemistry Council to OEHHA, California EPA, on May 12, 2004.

Laignelet L, Lhuguenot JC (2000d). Di-(2-ethylhexyl)phthalate (DEHP) absorption, excretion, metabolism and pharmacokinetic profile in female CD1 mice. Report No. 4/99. Submitted by the American Chemistry Council to OEHHA, California EPA, on May 12, 2004.

Laignelet L, Lhuguenot JC (2001). Di-(2-ethylhexyl)phthalate (DEHP) absorption, excretion, metabolism and pharmacokinetic profile in pregnant and non-pregnant rats and mice. Synthesis of Reports No. 1/99 to 4/99. Submitted by the American Chemistry Council to OEHHA, California EPA, on May 12, 2004.

Lake BG, Brantom PG, Gangolli SD, Butterworth KR, Grasso P (1976). Studies on the effects of orally administered Di-(2-ethylhexyl) phthalate in the ferret. *Toxicology* **6**, 341-56.

Lamb JC 4th, Chapin RE, Teague J, Lawton AD, Reel JR (1987). Reproductive effects of four phthalic acid esters in the mouse. *Toxicol Appl Pharmacol* **88**, 255-69.

Latini G, De Felice C, Presta G, Del Vecchio A, Paris I, Ruggieri F, Mazzeo P (2003). In utero exposure to di-(2-ethylhexyl)phthalate and duration of human pregnancy. *Environ Health Perspect* **111**, 1783-5.

Lenth RV (2001). Some practical guidelines for effective sample size determination. *Am Statist* **55**, 187-193.

Lhuguenot JC, Mitchell AM, Milner G, Lock EA, Elcombe CR (1985). The metabolism of di(2-ethylhexyl) phthalate (DEHP) and mono-(2-ethylhexyl) phthalate (MEHP) in rats: in vivo and in vitro dose and time dependency of metabolism. *Toxicol Appl Pharmacol* **80**, 11-22.

~~Li LH, Donald J, Golub M (2004). Testicular development, structure, function, and~~

~~regulation in common marmosets. Southern California/Northern California Chapters of Society of Toxicology 2004 Annual Meeting, September 30-October 1, 2004, San Diego, CA.~~

Li H, Kim KH (2003). Effects of mono-(2-ethylhexyl) phthalate on fetal and neonatal rat testis organ cultures. *Biol Reprod* **69**, 1964-72.

Li LH, Jester WF Jr, Laslett AL, Orth JM (2000). A single dose of Di-(2-ethylhexyl) phthalate in neonatal rats alters gonocytes, reduces sertoli cell proliferation, and decreases cyclin D2 expression. *Toxicol Appl Pharmacol* **166**, 222-9.

Li LH, Jester WF Jr, Orth JM (1998). Effects of relatively low levels of mono-(2-ethylhexyl) phthalate on cocultured Sertoli cells and gonocytes from neonatal rats. *Toxicol Appl Pharmacol* **153**, 258-65.

Liang JH, Sankai T, Yoshida T, Yoshikawa Y (2001). Immunolocalization of proliferating cell nuclear antigen (PCNA) in cynomolgus monkey (*Macaca fascicularis*) testes during postnatal development. *J Med Primatol* **30**, 107-11.

Liu RC, Hahn C, Hurtt ME (1996a). The direct effect of hepatic peroxisome proliferators on rat Leydig cell function in vitro. *Fundam Appl Toxicol* **30**, 102-8.

Liu RC, Hurtt ME, Cook JC, Biegel LB (1996b). Effect of the peroxisome proliferator, ammonium perfluorooctanoate (C8), on hepatic aromatase activity in adult male Crl:CD BR (CD) rats. *Fundam Appl Toxicol* **30**, 220-8.

Maloney EK, Waxman DJ (1999). Trans-activation of PPAR α and PPAR γ by structurally diverse environmental chemicals. *Toxicol Appl Pharmacol* **161**, 209-218.

McKee RH, Butala JH, David RM, Gans G (2004). NTP center for the evaluation of risks to human reproduction reports on phthalates: addressing the data gaps. *Reprod Toxicol* **18**, 1-22.

Millar MR, Sharpe RM, Weinbauer GF, Fraser HM, Saunders PT (2000). Marmoset spermatogenesis: organizational similarities to the human. *Int J Androl* **23**, 266-77.

Mitsubishi Chemical Safety Institute Ltd. (MCSI, 2003). Sixty-five week repeated oral dose toxicity study of di(2-ethylhexyl) phthalate (DEHP) in juvenile common marmosets. Mitsubishi Chemical Safety Institute Ltd., Study No. B000496. [A copy of the final study report was provided to OEHHA by the American Chemistry Council.]

Modigh CM, Bodin SL, Lillienberg L, Dahlman-Hoglund A, Akesson B, Axelsson G (2002). Time to pregnancy among partners of men exposed to di(2-ethylhexyl)phthalate. *Scand J Work Environ Health* **28**, 418-28.

Moore RW, Rudy TA, Lin TM, Ko K, Peterson RE (2001). Abnormalities of sexual

development in male rats with in utero and lactational exposure to the antiandrogenic plasticizer Di(2-ethylhexyl) phthalate. *Environ Health Perspect* **109**, 229-37.

Muller T, Simoni M, Pekel E, Luetjens CM, Chandolia R, Amato F, Norman RJ, Gromoll J (2004). Chorionic gonadotrophin beta subunit mRNA but not luteinising hormone beta subunit mRNA is expressed in the pituitary of the common marmoset (*Callithrix jacchus*). *J Mol Endocrinol* **32**, 115-28.

National Center for Health Statistics (2005). Clinical growth charts for infants. U.S. Department of Health and Human Services, Centers for Disease Control and Prevention, National Center for Health Statistics, Hyattsville, MD. Available at <http://www.cdc.gov/growthcharts/>.

National Institute for Occupational Safety and Health (NIOSH, 1990). *NIOSH and NIOSH basis for an Occupational Health Standard: Di (2-ethylhexyl) phthalate (DEHP)*. U.S. Department of Health and Human Services. Public Health Service. Centers for Disease Control. NIOSH.

Nef S, Parada LF (1999). Cryptorchidism in mice mutant for *Ins13*. *Nat Genet* **22**, 295-9.

Office of Environmental Health Hazard Assessment (OEHHA, 1997). Public Health Goal for Di(2-ethylhexyl) Phthalate (DEHP) in Drinking Water. OEHHA, California Environmental Protection Agency, Sacramento, California, February.

Office of Environmental Health Hazard Assessment (OEHHA, 2000). Air Toxics Hot Spots Program Risk Assessment Guidelines. Part IV. Technical Support Document for Exposure Assessment and Stochastic Analysis. OEHHA, California Environmental Protection Agency, Sacramento, California, September.

Ono H, Saito Y, Imai K, Kato M (2004). Subcellular distribution of di-(2-ethylhexyl)phthalate in rat testis. *J Toxicol Sci* **29**, 113-24.

Orth JM (1982). Proliferation of Sertoli cells in fetal and postnatal rats: a quantitative autoradiographic study. *Anat Rec* **203**, 485-92.

Orth JM, Gunsalus GL, Lamperti AA (1988). Evidence from Sertoli cell-depleted rats indicates that spermatid number in adults depends on numbers of Sertoli cells produced during perinatal development. *Endocrinology* **122**, 787-94.

Park JD, Habeebu SS, Klaassen CD (2002). Testicular toxicity of di-(2-ethylhexyl)phthalate in young Sprague-Dawley rats. *Toxicology* **171**, 105-15.

Payne AH, Hardy MP, and Russell LD (1996). *The Leydig Cell*. Cache River Press, Clearwater, FL.

Peck CC, Albro PW (1982). Toxic potential of the plasticizer Di(2-ethylhexyl) phthalate

in the context of its disposition and metabolism in primates and man. *Environ Health Perspect* **45**, 11-7.

Poon R, Lecavalier P, Mueller R, Valli VE, Procter BG, Chu I (1997). Subchronic oral toxicity of di-n-octyl phthalate and di(2-Ethylhexyl) phthalate in the rat. *Food Chem Toxicol* **35**, 225-39.

Price CJ, Tyl RW, Marr MC, Myers CB, Sadler BM, Kimmel CA (1988). Reproduction and fertility evaluation of diethylhexyl phthalate (CAS No. 117-81-7) in CD-1 mice exposed during gestation. Report No. NTP 88-092.

Pugh G Jr, Isenberg JS, Kamendulis LM, Ackley DC, Clare LJ, Brown R, Lington AW, Smith JH, Klaunig JE (2000). Effects of di-isononyl phthalate, di-2-ethylhexyl phthalate, and clofibrate in cynomolgus monkeys. *Toxicol Sci* **56**, 181-8.

Rais-Bahrami K, Nunez S, Revenis ME, Short BL, Luban NL (2004). Follow-up study of adolescents exposed to di-2-ethylhexyl phthalate (DEHP) as neonates on extracorporeal membrane oxygenation (ECMO) support. *Environ Health Persp* **112**, 1339-40.

Rhodes C, Orton TC, Pratt IS, Batten PL, Bratt H, Jackson SJ, Elcombe CR (1986). Comparative pharmacokinetics and subacute toxicity of di(2-ethylhexyl) phthalate (DEHP) in rats and marmosets: extrapolation of effects in rodents to man. *Environ Health Perspect* **65**, 299-307.

Rune GM, Pretzer D, De Souza P, Bollmann U, Merker HJ (1992). Ultrastructure of adult and juvenile marmoset (*Callithrix jacchus*) Sertoli cells in vivo and in vitro. *J Androl* **13**, 560-70.

Russell LD and Griswold MD (1993). *The Sertoli Cell*. Cache River Press, Clearwater, FL.

Saitoh Y, Usumi K, Nagata T, Marumo H, Imai K, Katoh M (1997). Early changes in the rat testis induced by di-(2-Ethylhexyl) phthalate and 2,5-hexanedione: ultrastructure and lanthanum trace study. *J Toxicol Pathol* **10**(1), 51-7

Schilling K, Gembardt C, Hellwig J (1999). Reproduction toxicity of di-2-ethylhexyl phthalate (DEHP). *Toxicologist* ;48(1-S):147-8

Schmid P, Schlatter C (1985). Excretion and metabolism of di(2-ethylhexyl)phthalate in man. *Xenobiotica* **15**, 251-6.

Schultz R, Yan W, Toppari J, Volkl A, Gustafsson JA, Pelto-Huikko M (1999). Expression of peroxisome proliferator-activated receptor alpha messenger ribonucleic acid and protein in human and rat testis. *Endocrinology* **140**, 2968-75.

Schwetz BA, Rao KS, Park CN (1980). Insensitivity of tests for reproductive problems. *J Environ Pathol Toxicol* **3**, 81-98.

Sharpe RM, Walker M, Millar MR, Atanassova N, Morris K, McKinnell C, Saunders PT, Fraser HM (2000). Effect of neonatal gonadotropin-releasing hormone antagonist administration on Sertoli cell number and testicular development in the marmoset: comparison with the rat. *Biol Reprod* **62**, 1685-93.

Short RD, Robinson EC, Lington AW, Chin AE (1987). Metabolic and peroxisome proliferation studies with di(2-ethylhexyl)phthalate in rats and monkeys. *Toxicol Ind Health* **3**, 185-95.

Sjoberg P, Bondesson U, Gray TJ, Ploen L (1986). Effects of di-(2-ethylhexyl) phthalate and five of its metabolites on rat testis in vivo and in vitro. *Acta Pharmacol Toxicol (Copenh)* **58**, 225-33.

Sjoberg P, Bondesson U, Kjellen L, Lindquist NG, Montin G, Ploen L (1985). Kinetics of di-(2-ethylhexyl) phthalate in immature and mature rats and effect on testis. *Acta Pharmacol Toxicol (Copenh)* **56**, 30-7.

Smedley JV, Bailey SA, Perry RW, O'Rourke CM (2002). Methods for predicting sexual maturity in male cynomolgus macaques on the basis of age, body weight, and histologic evaluation of the testes. *Contemp Top Lab Anim Sci* **41**, 18-20.

Stata Corporation (2003). Small Stata 8.0 for Windows. Stata Corporation, College Station, TX.

Stedman's Medical Dictionary, 27th edition (2000). Pub. Lippincott Williams & Wilkins. Accessible online at <http://www.stedmans.com>.

Tanaka A, Adachi T, Takahashi T, Yamaha T (1975). Biochemical studies on phthalic esters I. Elimination, distribution and metabolism of di-(2-ethylhexyl)phthalate in rats. *Toxicology* **4**, 253-64.

Tandon R, Seth PK, Srivastava SP (1991). Effect of in utero exposure to di(2-ethylhexyl)phthalate on rat testes. *Indian J Exp Biol* **29**, 1044-6.

Teerds KJ, de Boer-Brouwer M, Dorrington JH, Balvers M, Ivell R (1999). Identification of markers for precursor and Leydig cell differentiation in the adult rat testis following ethane dimethyl sulphonate administration. *Biol Reprod* **60**, 1437-45.

Tyl RW and Jones-Price C (1984). Teratological evaluation of diethylhexylphthalate (CAS No. 117-81-7) in Fischer 344 rats.: Jefferson, AR, National Center for Toxicological Research.

Tyl RW, Price CJ, Marr MC, Kimmel CA (1988). Developmental toxicity evaluation of

dietary di(2-ethylhexyl)phthalate in Fischer 344 rats and CD-1 mice. *Fundam Appl Toxicol* **10**, 395-412.

U.S. Environmental Protection Agency (U.S. EPA, 1996). Guidelines for reproductive toxicity risk assessment. *EPA/630/R-96/009* FRL-5630-6.

U.S. Food and Drug Administration (U.S. FDA, 2001). Safety Assessment of Di (2-ethylhexyl)phthalate (DEHP) Released from PVC Medical Devices. Centers for Devices and Radiological Health. U.S. Food and Drug Administration. Rockville, MD.

Voss C, Zerban H, Bannasch P, Berger MR (2005). Lifelong exposure to di-(2-ethylhexyl)-phthalate induces tumors in liver and testes of Sprague-Dawley rats. *Toxicology* **206**, 359-71.

Ward JM, Peters JM, Perella CM, Gonzalez FJ (1998). Receptor and nonreceptor-mediated organ-specific toxicity of di(2-ethylhexyl)phthalate (DEHP) in peroxisome proliferator-activated receptor alpha-null mice. *Toxicol Pathol* **26**, 240-6.

Williams DT, Blanchfield BJ (1974). Retention, excretion and metabolism of di-(2-ethylhexyl) phthalate administered orally to the rat. *Bull Environ Contam Toxicol* **11**, 371-8.

Wilson VS, Lambricht C, Furr J, Ostby J, Wood C, Held G, Gray LE Jr (2004). Phthalate ester-induced gubernacular lesions are associated with reduced insl3 gene expression in the fetal rat testis. *Toxicol Lett* **146**, 207-15.

Wistuba J, Mundry M, Luetjens CM, Schlatt S (2004). Co-grafting of hamster (*Phodopus sungorus*) and marmoset (*Callithrix jacchus*) testicular tissues into nude mice does not overcome blockade of early spermatogenic differentiation in primate grafts. *Biol Reprod* **71**, 2087-91.

Zhang FP, Kero J, Huhtaniemi I (1998). The unique exon 10 of the human luteinizing hormone receptor is necessary for expression of the receptor protein at the plasma membrane in the human luteinizing hormone receptor, but deleterious when inserted into the human follicle-stimulating hormone receptor. *Mol Cell Endocrinol* **142**, 165-74.

Zuhlke U, Weinbauer G (2003). The common marmoset (*Callithrix jacchus*) as a model in toxicology. *Toxicol Pathol* **31 Suppl**, 123-7.

BIBLIOGRAPHY

Additional Studies or Reports Reviewed by OEHHA

Abbott DH, Hearn JP (1978). Physical, hormonal and behavioural aspects of sexual development in the marmoset monkey, *Callithrix jacchus*. *J Reprod Fertil* **53**, 155-66.

Agarwal DK, Eustis S, Lamb JC 4th, Jameson CW, Kluwe WM (1986a). Influence of dietary zinc on di(2-ethylhexyl)phthalate-induced testicular atrophy and zinc depletion in adult rats. *Toxicol Appl Pharmacol* **84**, 12-24.

Aslan AR, Kogan BA, and Gondos B (2003). Testicular development. In: Polin RA, Fox WW, and Abman SH eds. Fetal and Neonatal Physiology. Saunders, Philadelphia, PA. 3rd edi. Volume 2, Chapter 191, pp1950-1960.

Awal MA, Kurohmaru M, Ishii M, Andriana BB, Kanai Y, Hayashi Y (2004). Mono-(2-ethylhexyl) phthalate (MEHP) induces spermatogenic cell apoptosis in guinea pig testes at prepubertal stage in vitro. *Int J Toxicol* **23**, 349-55.

Anderson WA, Barnes KA, Castle L, Damant AP, Scotter MJ (2002). Determination of isotopically labelled monoesterphthalates in urine by high performance liquid chromatography-mass spectrometry. *Analyst* **127**, 1193-7.

~~Anderson WA, Castle L, Scotter MJ, Massey RC, Springall C (2001). A biomarker approach to measuring human dietary exposure to certain phthalate diesters. *Food Addit Contam* **18**, 1068-74.~~

Arslan M, Weinbauer GF, Schlatt S, Shahab M, Nieschlag E (1993). FSH and testosterone, alone or in combination, initiate testicular growth and increase the number of spermatogonia and Sertoli cells in a juvenile non-human primate (*Macaca mulatta*). *J Endocrinol* **136**, 235-43.

Asaoka K, Hagihara K, Kabaya H, Sakamoto Y, Katayama H, Yano K (2000). Uptake of phthalate esters, di(n-butyl)phthalate and di(2-ethylhexyl)phthalate, as environmental chemicals in monkeys in Japan. *Bull Environ Contam Toxicol* **64**, 679-85.

Barr AB (1973). Timing of spermatogenesis in four nonhuman primate species. *Fertil Steril* **24**, 381-9.

Behr R, Hunt N, Ivell R, Wessels J, Weinbauer GF (2000). Cloning and expression analysis of testis-specific cyclic 3', 5'-adenosine monophosphate-responsive element modulator activators in the nonhuman primate (*Macaca fascicularis*): comparison with other primate and rodent species. *Biol Reprod* **62**, 1344-51.

Berensztein EB, Sciara MI, Rivarola MA, Belgorosky A (2002). Apoptosis and proliferation of human testicular somatic and germ cells during prepuberty: high rate of

testicular growth in newborns mediated by decreased apoptosis. *J Clin Endocrinol Metab* **87**, 5113-8.

Blair RM, Fang H, Branham WS, Hass BS, Dial SL, Moland CL, Tong W, Shi L, Perkins R, Sheehan DM (2000). The estrogen receptor relative binding affinities of 188 natural and xenochemicals: structural diversity of ligands. *Toxicol Sci* **54**, 138-53.

Bhattacharya N, Dufour JM, Vo MN, Okita J, Okita R, Kim KH (2005). Differential effects of phthalates on the testis and the liver. *Biol Reprod* **72**, 745-54.

Bility MT, Thompson JT, McKee RH, David RM, Butala JH, Vanden Heuvel JP, Peters JM (2004). Activation of mouse and human peroxisome proliferator-activated receptors (PPARs) by phthalate monoesters. *Toxicol Sci* **82**, 170-82.

Borch J, Dalgaard M, Ladefoged O (2005). Early testicular effects in rats perinatally exposed to DEHP in combination with DEHA-apoptosis assessment and immunohistochemical studies. *Reprod Toxicol* **19**, 517-25.

Borch J, Vinggaard AM, Ladefoged O (2002). The effect of combined prenatal exposure to di(2-ethylhexyl)phthalate and di(2-ethylhexyl)adipate on testosterone production in rats. *Reprod Toxicol* **16(4)**, 406.

Borch J, Vinggaard AM, Ladefoged O (2003). The effect of combined exposure to di(2-ethylhexyl)phthalate and diisononylphthalate on testosterone production in rats. *Reprod Toxicol* **17(4)**, 487-8.

Brock JW, Caudill SP, Silva MJ, Needham LL, Hilborn ED (2002). Phthalate monoesters levels in the urine of young children. *Bull Environ Contam Toxicol* **68**, 309-14.

Center for the Evaluation of Risks to Human Reproduction (CERHR, 2005). NTP-CERHR Expert Panel Update on the Reproductive and Developmental Toxicity of Di (2-ethylhexyl) Phthalate. National Toxicology Program, U.S. Department of Health and Human Services, Research Triangle Park, NC, November.

Chapin RE, Gray TJ, Phelps JL, Dutton SL (1988). The effects of mono-(2-ethylhexyl)-phthalate on rat Sertoli cell-enriched primary cultures. *Toxicol Appl Pharmacol* **92**, 467-79.

Chemes HE (2001). Infancy is not a quiescent period of testicular development. *Int J Androl* **24**, 2-7.

Clermont Y (1972). Kinetics of spermatogenesis in mammals: seminiferous epithelium cycle and spermatogonial renewal. *Physiol Rev* **52**, 198-236.

Colon I, Caro D, Bourdony CJ, Rosario O (2000). Identification of phthalate esters in the serum of young Puerto Rican girls with premature breast development. *Environ Health*

Perspect **108**, 895-900.

Cortes D, Muller J, Skakkebaek NE (1987). Proliferation of Sertoli cells during development of the human testis assessed by stereological methods. *Int J Androl* **10**, 589-96.

Corton JC, Lapinskas PJ (2005). Peroxisome proliferator-activated receptors: mediators of phthalate ester-induced effects in the male reproductive tract? *Toxicol Sci* **83**, 4-17.

Dalgaard M, Nellemann C, Lam HR, Sorensen IK, Ladefoged O (2001). The acute effects of mono(2-ethylhexyl)phthalate (MEHP) on testes of prepubertal Wistar rats. *Toxicol Lett* **122**, 69-79.

Dalgaard M, Ostergaard G, Lam HR, Hansen EV, Ladefoged O (2000). Toxicity study of di(2-ethylhexyl)phthalate (DEHP) in combination with acetone in rats. *Pharmacol Toxicol* **86**, 92-100.

David RM (2004). Commentary regarding the article by Koch et al.: an estimation of the daily intake of di(2-ethylhexyl)phthalate (DEHP) and other phthalates in the general population. *Int. J. Hyg. Environ. Health*, 206, 77-83 (2003). *Int J Hyg Environ Health* **207**, 75-6; author reply 77-8.

Dhanya CR, Gayathri NS, Mithra K, Nair KV, Kurup PA (2004). Vitamin E prevents deleterious effects of di (2-ethyl hexyl) phthalate, a plasticizer used in PVC blood storage bags. *Indian J Exp Biol* **42**, 871-5.

Dhanya CR, Indu AR, Deepadevi KV, Kurup PA (2003). Inhibition of membrane Na(+)-K+ Atpase of the brain, liver and RBC in rats administered di(2-ethyl hexyl) phthalate (DEHP) a plasticizer used in polyvinyl chloride (PVC) blood storage bags. *Indian J Exp Biol* **41**, 814-20.

Duty SM, Calafat AM, Silva MJ, Ryan L, Hauser R (2005). Phthalate exposure and reproductive hormones in adult men. *Hum Reprod* **20**, 604-610.

~~Dirven HA, van den Broek PH, Arends AM, Nordkamp HH, de Lepper AJ, Henderson PT, Jongeneelen FJ (1993). Metabolites of the plasticizer di(2-ethylhexyl)phthalate in urine samples of workers in polyvinylchloride processing industries. *Int Arch Occup Environ Health* **64**, 549-54.~~

Eisler JA, Tannenbaum PL, Mann DR, Wallen K (1993). Neonatal testicular suppression with a GnRH agonist in rhesus monkeys: effects on adult endocrine function and behavior. *Horm Behav* **27**, 551-67.

Elcombe CR, Mitchell AM (1986). Peroxisome proliferation due to di(2-ethylhexyl) phthalate (DEHP): species differences and possible mechanisms. *Environ Health Perspect* **70**, 211-9.

Fan LQ, You L, Brown-Borg H, Brown S, Edwards RJ, Corton JC (2004). Regulation of phase I and phase II steroid metabolism enzymes by PPARalpha activators. *Toxicology* **204**, 109-21.

Fisher JS, Millar MR, Majdic G, Saunders PT, Fraser HM, Sharpe RM (1997). Immunolocalisation of oestrogen receptor-alpha within the testis and excurrent ducts of the rat and marmoset monkey from perinatal life to adulthood. *J Endocrinol* **153**, 485-95.

Fisher JS, Turner KJ, Fraser HM, Saunders PT, Brown D, Sharpe RM (1998). Immunoexpression of aquaporin-1 in the efferent ducts of the rat and marmoset monkey during development, its modulation by estrogens, and its possible role in fluid resorption. *Endocrinology* **139**, 3935-45.

Fisher JS (2004). Environmental anti-androgens and male reproductive health: focus on phthalates and testicular dysgenesis syndrome. *Reproduction* **127**, 305-15.

Foster PM (2005). Mode of action: impaired fetal Leydig cell function--effects on male reproductive development produced by certain phthalate esters. *Crit Rev Toxicol* **35**, 713-9.

Foster PM, Mylchreest E, Gaido KW, Sar M (2001). Effects of phthalate esters on the developing reproductive tract of male rats. *Hum Reprod Update* **7**, 231-5.

Fouquet JP, Dang DC (1980). A comparative study of the development of the fetal testis and ovary in the monkey (*Macaca fascicularis*). *Reprod Nutr Dev* **20**, 1439-59.

Fritz IB (1994). Somatic cell-germ cell relationships in mammalian testes during development and spermatogenesis. *Ciba Found Symp* **182**, 271-4; discussion 274-81.

Fukuwatari T, Suzuki Y, Sugimoto E, Shibata K (2002). Elucidation of the toxic mechanism of the plasticizers, phthalic acid esters, putative endocrine disrupters: effects of dietary di(2-ethylhexyl)phthalate on the metabolism of tryptophan to niacin in rats. *Biosci Biotechnol Biochem* **66**, 705-10.

Garde SV, Sheth AR, Kulkarni SA (1991a). Cellular distribution of inhibin in marmoset testes during development. *Anat Rec* **229**, 334-8.

Garde SV, Sheth AR, Kulkarni SA (1991b). FSH in testes of marmosets during development: immunocytochemical localization and de novo biosynthesis. *Anat Rec* **231**, 119-24.

Giammona CJ, Sawhney P, Chandrasekaran Y, Richburg JH (2002). Death receptor response in rodent testis after mono-(2-ethylhexyl) phthalate exposure. *Toxicol Appl Pharmacol* **185**, 119-27.

Gray LE, Ostby J, Furr J, Wolf CJ, Lambright C, Parks L, Veeramachaneni DN, Wilson V, Price M, Hotchkiss A, Orlando E, Guillette L (2001). Effects of environmental antiandrogens on reproductive development in experimental animals. *Hum Reprod Update* **7**, 248-64.

Green R, Hauser R, Calafat AM, Weuve J, Schettler T, Ringer S, Huttner K, Hu H (2005). Use of di(2-ethylhexyl) phthalate-containing medical products and urinary levels of mono(2-ethylhexyl) phthalate in neonatal intensive care unit infants. *Environ Health Perspect* **113**, 1222-1225.

Gromoll J, Weinbauer GF, Skaletsky H, Schlatt S, Rocchietti-March M, Page DC, Nieschlag E (1999). The Old World monkey DAZ (Deleted in AZoospermia) gene yields insights into the evolution of the DAZ gene cluster on the human Y chromosome. *Hum Mol Genet* **8**, 2017-24.

Gromoll J, Wistuba J, Terwort N, Godmann M, Muller T, Simoni M (2003). A new subclass of the luteinizing hormone/chorionic gonadotropin receptor lacking exon 10 messenger RNA in the New World monkey (Platyrrhini) lineage. *Biol Reprod* **69**, 75-80.

Haider SG, Passia D, Treiber A, Milhorst S (1989). Description of eight phases of spermiogenesis in the marmoset testis. *Acta Anat (Basel)* **135**, 180-4.

Haishima Y, Matsuda R, Hayashi Y, Hasegawa C, Yagami T, Tsuchiya T (2004). Risk assessment of di(2-ethylhexyl)phthalate released from PVC blood circuits during hemodialysis and pump-oxygenation therapy. *Int J Pharm* **274**, 119-29.

Hasmall SC, James NH, Macdonald N, Soames AR, Roberts RA (2000). Species differences in response to diethylhexylphthalate: suppression of apoptosis, induction of DNA synthesis and peroxisome proliferator activated receptor alpha-mediated gene expression. *Arch Toxicol* **74**, 85-91.

Hauser R, Williams P, Altshul L, Calafat AM (2005). Evidence of interaction between polychlorinated biphenyls and phthalates in relation to human sperm motility. *Environ Health Perspect* **113**, 425-30.

Heyn R, Makabe S, Motta PM (1998). Ultrastructural dynamics of human testicular cords from 6 to 16 weeks of embryonic development. Study by transmission and high resolution scanning electron microscopy. *Ital J Anat Embryol* **103**, 17-29.

Heyn R, Makabe S, Motta PM (2001). Ultrastructural morphodynamics of human Sertoli cells during testicular differentiation. *Ital J Anat Embryol* **106**, 163-71.

Hilscher B, Engemann A (1992). Histological and morphometric studies on the kinetics of germ cells and immature Sertoli cells during human prespermatogenesis. *Andrologia* **24**, 7-10.

Hodges JK, Hearn JP (1977). Effects of immunisation against luteinising hormone releasing hormone on reproduction of the marmoset monkey *Callithrix jacchus*. *Nature* **265**, 746-8.

Holt WV, Moore HD (1984). Ultrastructural aspects of spermatogenesis in the common marmoset (*Callithrix jacchus*). *J Anat* **138 (Pt 1)**, 175-88.

Hoppin JA (2003). Male reproductive effects of phthalates: an emerging picture. *Epidemiology* **14**, 259-60.

Howarth JA, Price SC, Dobrota M, Kentish PA, Hinton RH (2001). Effects on male rats of di-(2-ethylhexyl) phthalate and di-n-hexylphthalate administered alone or in combination. *Toxicol Lett* **121**, 35-43.

Hurst CH, Waxman DJ (2003). Activation of PPARalpha and PPARgamma by environmental phthalate monoesters. *Toxicol Sci* **74**, 297-308.

Husen B, Giebel J, Rune G (1999). Expression of the integrin subunits alpha 5, alpha 6 and beta 1 in the testes of the common marmoset. *Int J Androl* **22**, 374-84.

Ito Y, Yokota H, Wang R, Yamanoshita O, Ichihara G, Wang H, Kurata Y, Takagi K, Nakajima T (2005). Species differences in the metabolism of di(2-ethylhexyl) phthalate (DEHP) in several organs of mice, rats, and marmosets. *Arch Toxicol* **79**, 147-54.

Jackh R, Rhodes C, Grasso P, Carter JT (1984). Genotoxicity studies on di-(2-ethylhexyl) phthalate and adipate and toxicity studies on di-(2-ethylhexyl) phthalate in the rat and marmoset. *Food Chem Toxicol* **22**, 151-5.

Jarfelt K, Dalgaard M, Hass U, Borch J, Jacobsen H, Ladefoged O (2005). Antiandrogenic effects in male rats perinatally exposed to a mixture of di(2-ethylhexyl) phthalate and di(2-ethylhexyl) adipate. *Reprod Toxicol* **19**, 505-15.

Jackson MR, Edmunds JG (1984). Morphological assessment of testicular maturity in marmosets (*Callithrix jacchus*). *Lab Anim* **18**, 173-8.

Johnson L, Chaturvedi PK, Williams JD (1992). Missing generations of spermatocytes and spermatids in seminiferous epithelium contribute to low efficiency of spermatogenesis in humans. *Biol Reprod* **47**, 1091-8.

Johnson L, McKenzie KS, Snell JR (1996). Partial wave in human seminiferous tubules appears to be a random occurrence. *Tissue Cell* **28**, 127-36.

Jonsson BA, Richthoff J, Rylander L, Giwercman A, Hagmar L (2005). Urinary phthalate metabolites and biomarkers of reproductive function in young men. *Epidemiology* **16**, 487-93.

Kang KS, Lee YS, Kim HS, Kim SH (2002). Di-(2-ethylhexyl) phthalate-induced cell proliferation is involved in the inhibition of gap junctional intercellular communication and blockage of apoptosis in mouse Sertoli cells. *J Toxicol Environ Health A* **65**, 447-59.

Kasahara E, Sato EF, Miyoshi M, Konaka R, Hiramoto K, Sasaki J, Tokuda M, Nakano Y, Inoue M (2002). Role of oxidative stress in germ cell apoptosis induced by di-(2-ethylhexyl)phthalate. *Biochem J* **365**, 849-56.

Kavlock R, Boekelheide K, Chapin R, Cunningham M, Faustman E, Foster P, Golub M, Henderson R, Hinberg I, Little R, Seed J, Shea K, Tabacova S, Tyl R, Williams P, Zacharewski T (2002). NTP Center for the Evaluation of Risks to Human Reproduction: phthalates expert panel report on the reproductive and developmental toxicity of di-(2-ethylhexyl) phthalate. *Reprod Toxicol* **16**, 529-653.

Kelnar CJ, McKinnell C, Walker M, Morris KD, Wallace WH, Saunders PT, Fraser HM, Sharpe RM (2002). Testicular changes during infantile 'quiescence' in the marmoset and their gonadotrophin dependence: a model for investigating susceptibility of the prepubertal human testis to cancer therapy? *Hum Reprod* **17**, 1367-78.

Keys DA, Wallace DG, Kepler TB, Conolly RB (2000). Quantitative evaluation of alternative mechanisms of blood disposition of di(n-butyl) phthalate and mono(n-butyl) phthalate in rats. *Toxicol Sci* **53**, 173-84.

Kholkute SD, Aitken RJ, Lunn SF (1983). Plasma testosterone response to hCG stimulation in the male marmoset monkey (*Callithrix jacchus jacchus*). *J Reprod Fertil* **67**, 457-63.

Kijima K, Toyosawa K, Yasuba M, Matsuoka N, Adachi T, Komiyama M, Mori C (2004). Gene expression analysis of the rat testis after treatment with di-(2-ethylhexyl) phthalate using cDNA microarray and real-time RT-PCR. *Toxicol Appl Pharmacol* **200**, 103-10.

Kim HS, Ishizuka M, Kazusaka A, Fujita S (2004). Alterations of activities of cytosolic phospholipase a(2) and arachidonic Acid-metabolizing enzymes in di-(2-ethylhexyl)phthalate-induced testicular atrophy. *J Vet Med Sci* **66**, 1119-24.

Kim HS, Saito K, Ishizuka M, Kazusaka A, Fujita S (2003). Short period exposure to di-(2-ethylhexyl) phthalate regulates testosterone metabolism in testis of prepubertal rats. *Arch Toxicol* **77**, 446-51.

~~Kluin PM, Kramer MF, de Rooij DG (1983). Testicular development in *Macaca irus* after birth. *Int J Androl* **6**, 25-43.~~

Kluwe WM (1982). Overview of phthalate ester pharmacokinetics in mammalian species. *Environ Health Perspect* **45**, 3-9.

Koch HM, Bolt HM, Preuss R, Angerer J (2005). New metabolites of di(2-ethylhexyl)phthalate (DEHP) in human urine and serum after single oral doses of deuterium-labelled DEHP. *Arch Toxicol* **79**, 367-76.

Kubota Y, Nef S, Farmer PJ, Temelcos C, Parada LF, Hutson JM (2001). Leydig insulin-like hormone, gubernacular development and testicular descent. *J Urol* **165**, 1673-5.

Kulkarni SA, Garde SV, Sheth AR (1992). Immunocytochemical localization of bioregulatory peptides in marmoset testes. *Arch Androl* **29**, 87-102.

Kumar RA, Phillips DM (1991). Spermiation and sperm maturation in the marmoset. *Anat Rec* **229**, 315-20.

Kurata Y, Makinodan F, Shimamura N, Okada M, Katoh M (2005). Metabolism of di(2-ethylhexyl) phthalate (DEHP) in juvenile and fetal marmoset and rat. *Toxicologist* **84 (S-1)**: 256.

Kuwada M, Kawashima R, Nakamura K, Kojima H, Hasumi H, Maki J, Sugano S (2002). Neonatal exposure to endocrine disruptors suppresses juvenile testis weight and steroidogenesis but spermatogenesis is considerably restored during puberty. *Biochem Biophys Res Commun* **295**, 193-7.

~~Laignelet L, Lhuguenot JC (2001). Di-(2-ethylhexyl)phthalate (DEHP) absorption, excretion, metabolism and pharmacokinetic profile in pregnant and non-pregnant rats and mice. Synthesis of Reports No. 1/99 to 4/99. Submitted by the American Chemistry Council to OEHHA, California EPA, on May 12, 2004.~~

Ljungvall K, Karlsson P, Hulten F, Madej A, Norrgren L, Einarsson S, Rodriguez-Martinez H, Magnusson U (2005). Delayed effects on plasma concentration of testosterone and testicular morphology by intramuscular low-dose di(2-ethylhexyl)phthalate or oestradiol benzoate in the prepubertal boar. *Theriogenology* **64**, 1170-84.

Lampen A, Zimnik S, Nau H (2003~~2~~). Teratogenic phthalates and metabolites activate the nuclear receptors PPARs and induce differentiation of F9 cells. *Tox Appl Pharmacol* **188**, 14-23.
~~*Reprod Toxicol* **16(4)**, 430.~~

Latini G, Gallo F, De Felice C (2004). Birth characteristics and hepatoblastoma risk in young children. *Cancer* **101**, 210.

Lee BC, Pineda JL, Spiliotis BE, Brown TJ, Bercu BB (1983). Male sexual development in the nonhuman primate. III. Sertoli cell culture and age-related differences. *Biol Reprod* **28**, 1207-15.

Lee J, Park J, Jang B, Knudsen TB (2004). Altered expression of genes related to zinc

homeostasis in early mouse embryos exposed to di-2-ethylhexyl phthalate. *Toxicol Lett* **152**, 1-10.

Li LH, Donald JM, Golub M (2004). Testicular development, structure, function, and regulation in common marmosets. Southern California/Northern California Chapters of Society of Toxicology 2004 Annual Meeting, September 30-October 1, 2004, San Diego, CA.

Li LH, Donald JM, Golub MS (2005). Review on testicular development, structure, function, and regulation in common marmoset. *Birth Defects Res B Dev Reprod Toxicol* 74, 450-69.

Liu K, Lehmann KP, Sar M, Young SS, Gaido KW (2005). Gene expression profiling following in utero exposure to phthalate esters reveals new gene targets in the etiology of testicular dysgenesis. *Biol Reprod* 73, 180-92.

Ljungvall K, Tienpont B, David F, Magnusson U, Torneke K (2004). Kinetics of orally administered di(2-ethylhexyl) phthalate and its metabolite, mono(2-ethylhexyl) phthalate, in male pigs. *Arch Toxicol* **78**, 384-9.

Lottrup G, Andersson AM, Leffers H, Mortensen GK, Toppari J, Skakkebaek NE, Main KM (2006). Possible impact of phthalates on infant reproductive health. *Int J Androl* 29, 172-80.

Lovekamp-Swan T, Davis BJ (2003). Mechanisms of phthalate ester toxicity in the female reproductive system. *Environ Health Perspect* **111**, 139-45.

Lovekamp TN, Davis BJ (2001). Mono-(2-ethylhexyl) phthalate suppresses aromatase transcript levels and estradiol production in cultured rat granulosa cells. *Toxicol Appl Pharmacol* **172**, 217-24.

Lunn SF, Cowen GM, Morris KD, Fraser HM (1992). Influence of the gonad on the degree of suppression induced by an LHRH agonist implant in the marmoset monkey. *J Endocrinol* **132**, 217-24.

Mann DR, Akinbami MA, Gould KG, Paul K, Wallen K (1998). Sexual maturation in male rhesus monkeys: importance of neonatal testosterone exposure and social rank. *J Endocrinol* **156**, 493-501.

Mann DR, Lunn SF, Akinbami MA, Samuel K, Waterfall M, Fraser HM (1999). Effect of neonatal treatment with a GnRH antagonist on development of the cell-mediated immune response in marmosets. *Am J Reprod Immunol* **42**, 175-86.

Manojkumar V, Padmakumaran Nair KG, Santhosh A, Deepadevi KV, Arun P, Lakshmi LR, Kurup PA (1998). Decrease in the concentration of vitamin E in blood and tissues caused by di(2-ethylhexyl) phthalate, a commonly used plasticizer in blood storage bags

and medical tubing. *Vox Sang* **75**, 139-44.

McKee RH (2004). Phthalate exposure and early thelarche. *Environ Health Perspect* **112**, A541-3.

McKinnell C, Saunders PT, Fraser HM, Kelnar CJ, Kivlin C, Morris KD, Sharpe RM (2001). Comparison of androgen receptor and oestrogen receptor beta immunoexpression in the testes of the common marmoset (*Callithrix jacchus*) from birth to adulthood: low androgen receptor immunoexpression in Sertoli cells during the neonatal increase in testosterone concentrations. *Reproduction* **122**, 419-29.

Merkle J, Klimisch HJ, Jackh R (1988). Developmental toxicity in rats after inhalation exposure of di-2-ethylhexylphthalate (DEHP). *Toxicol Lett* **42**, 215-23.

Miraglia T, Telles Filho M, Branco AL (1970). The male reproductive system of the common marmoset (*Callithrix jacchus*). *Acta Anat (Basel)* **76**, 594-611.

Moore NP (2000). The oestrogenic potential of the phthalate esters. *Reprod Toxicol* **14**, 183-92.

Morrissey RE, Harris MW, Schwetz BA (1989). Developmental toxicity screen: results of rat studies with diethylhexyl phthalate and ethylene glycol monomethyl ether. *Teratog Carcinog Mutagen* **9**, 119-29.

Muller T, Simoni M, Pekel E, Luetjens CM, Chandolia R, Amato F, Norman RJ, Gromoll J (2004). Chorionic gonadotrophin beta subunit mRNA but not luteinising hormone beta subunit mRNA is expressed in the pituitary of the common marmoset (*Callithrix jacchus*). *J Mol Endocrinol* **32**, 115-28.

Murature DA, Tang SY, Steinhardt G, Dougherty RC (1987). Phthalate esters and semen quality parameters. *Biomed Environ Mass Spectrom* **14**, 473-7.

Nagano M, McCarrey JR, Brinster RL (2001). Primate spermatogonial stem cells colonize mouse testes. *Biol Reprod* **64**, 1409-16.

Narotsky MG, Hamby BT, Mitchell DS, Weller E, Chinchilli VM, Kavlock RJ (1995). Non-additive developmental toxicity in mixtures of trichloroethylene (TCE), di(2-ethylhexyl)phthalate (DEHP), and heptachlor (HEPT). *Fundam Appl Toxicol/Teratol* **45(5)** **27**, 203-216:489-90.

Nunes S, Brown C, French JA (2002). Variation in circulating and excreted estradiol associated with testicular activity in male marmosets. *Am J Primatol* **56**, 27-42.

Ohlson CG, Hardell L (2000). Testicular cancer and occupational exposures with a focus on xenoestrogens in polyvinyl chloride plastics. *Chemosphere* **40**, 1277-82.

Oishi S (1989). Effects of co-administration of di(2-ethylhexyl)phthalate and testosterone on several parameters in the testis and pharmacokinetics of its mono-de-esterified metabolite. *Arch Toxicol* **63**, 289-95.

Oishi S (1990). Effects of phthalic acid esters on testicular mitochondrial functions in the rat. *Arch Toxicol* **64**, 143-7.

Oishi S (1993). Strain differences in susceptibility to di-2-ethylhexyl phthalate-induced testicular atrophy in mice. *Toxicol Lett* **66**, 47-52.

Oishi S, Hiraga K (1980a). Testicular atrophy induced by phthalic acid monoesters: effects of zinc and testosterone concentrations. *Toxicology* **15**, 197-202.

Oishi S, Hiraga K (1980b). Effect of phthalic acid esters on mouse testes. *Toxicol Lett* **5**, 413-6.

Oishi S, Hiraga K (1983). Testicular atrophy induced by di-2-ethylhexyl phthalate: effect of zinc supplement. *Toxicol Appl Pharmacol* **70**, 43-8.

Preslock JP, Steinberger E (1976). Pathway of testosterone biosynthesis in the testis of the marmoset *Saguinus oedipus*. *Steroids* **28**, 775-84.

Preslock JP, Steinberger E (1977a). Androgen biosynthesis by marmoset testes in vitro. *Gen Comp Endocrinol* **31**, 101-5.

Preslock JP, Steinberger E (1977b). Testicular steroidogenesis in the common marmoset, *Callithrix jacchus*. *Biol Reprod* **17**, 289-93.

Preslock JP, Steinberger E (1979). Metabolism of pregnenolone and progesterone by testicular microsomes of the baboon *Papio anubis* and the marmoset *Saguinus oedipus*. *J Steroid Biochem* **10**, 75-80.

Pretzer D, Ghaida JA, Rune GM (1994). Growth factors (EGF, IGF-I) modulate the morphological differentiation of adult marmoset (*Callithrix jacchus*) Sertoli cells in vitro. *J Androl* **15**, 398-409.

Prince FP, Mann DR, Fraser HM (1998). Blockade of the hypothalamic-pituitary-testicular axis with a GnRH antagonist in the neonatal marmoset monkey: changes in Leydig cell ultrastructure. *Tissue Cell* **30**, 651-61.

Reddy BS, Rozati R, Reddy S, Kodampur S, Reddy P, Reddy R (2006). High plasma concentrations of polychlorinated biphenyls and phthalate esters in women with endometriosis: a prospective case control study. *Fertil Steril* **85**, 775-9.

Rhodes C, Elcombe CR, Batten PL, Bratt H, Jackson SJ, Pratt IS, Orton TC (1983). The disposition of ¹⁴C-di-2-ethylhexylphthalate (DEHP) in the marmoset. *Dev Toxicol*

Environ Sci **11**, 579-81.

Richburg JH (2000). The relevance of spontaneous- and chemically-induced alterations in testicular germ cell apoptosis to toxicology. *Toxicol Lett* **112-113**, 79-86.

Richburg JH, Boekelheide K (1996). Mono-(2-ethylhexyl) phthalate rapidly alters both Sertoli cell vimentin filaments and germ cell apoptosis in young rat testes. *Toxicol Appl Pharmacol* **137**, 42-50.

Richburg JH, Johnson KJ, Schoenfeld HA, Meistrich ML, Dix DJ (2002). Defining the cellular and molecular mechanisms of toxicant action in the testis. *Toxicol Lett* **135**, 167-83.

Richburg JH, Nanez A, Gao H (1999). Participation of the Fas-signaling system in the initiation of germ cell apoptosis in young rat testes after exposure to mono-(2-ethylhexyl) phthalate. *Toxicol Appl Pharmacol* **160**, 271-8.

Richburg JH, Nanez A, Williams LR, Embree ME, Boekelheide K (2000). Sensitivity of testicular germ cells to toxicant-induced apoptosis in gld mice that express a nonfunctional form of Fas ligand. *Endocrinology* **141**, 787-93.

Roberts RA, Chevalier S, Hasmall SC, James NH, Cosulich SC, Macdonald N (2002). PPAR alpha and the regulation of cell division and apoptosis. *Toxicology* **181-182**, 167-70.

Rozati R, Reddy PP, Reddanna P, Mujtaba R (2002). Role of environmental estrogens in the deterioration of male factor fertility. *Fertil Steril* **78**, 1187-94.

Rune GM, de Souza P, Merker HJ (1991). Ultrastructural and histochemical characterization of marmoset (*Callithrix jacchus*) Leydig cells during postnatal development. *Anat Embryol (Berl)* **183**, 179-91.

Schlatt S, Kim SS, Gosden R (2002). Spermatogenesis and steroidogenesis in mouse, hamster and monkey testicular tissue after cryopreservation and heterotopic grafting to castrated hosts. *Reproduction* **124**, 339-46.

Schmezer P, Pool BL, Klein RG, Komitowski D, Schmahl D (1988). Various short-term assays and two long-term studies with the plasticizer di(2-ethylhexyl)phthalate in the Syrian golden hamster. *Carcinogenesis* **9**, 37-43.

Scientific Committee on Toxicity, Ecotoxicity, and the Environment (CSTEE, 2004). Opinion on the results of a second risk assessment of bis(2ethylhexyl) phthalate [DEHP]: human health part. Health and Consumer Protection Directorate-general, European Commission, Brussels.

Sharpe RM (2001). Hormones and testis development and the possible adverse effects of

environmental chemicals. *Toxicol Lett* **120**, 221-32.

Sharpe RM (2006). Pathways of endocrine disruption during male sexual differentiation and masculinisation. *Best Pract Res Clin Endocrinol Metab* **20**, 91-110.

Sharpe RM, Martin B, Morris K, Greig I, McKinnell C, McNeilly AS, Walker M (2002). Infant feeding with soy formula milk: effects on the testis and on blood testosterone levels in marmoset monkeys during the period of neonatal testicular activity. *Hum Reprod* **17**, 1692-703.

Sharpe RM, McKinnell C, Kivlin C, Fisher JS (2003). Proliferation and functional maturation of Sertoli cells, and their relevance to disorders of testis function in adulthood. *Reproduction* **125**, 769-84.

Sharpe RM, Fraser HM, Brougham MF, McKinnell C, Morris KD, Kelnar CJ, Wallace WH, Walker M (2003). Role of the neonatal period of pituitary-testicular activity in germ cell proliferation and differentiation in the primate testis. *Hum Reprod* **18**, 2110-7.

Shirota M, Saito Y, Imai K, Horiuchi S, Yoshimura S, Sato M, Nagao T, Ono H, Katoh M (2005). Influence of di-(2-ethylhexyl)phthalate on fetal testicular development by oral administration to pregnant rats. *J Toxicol Sci* **30**, 175-194.

Silva MJ, Barr DB, Reidy JA, Malek NA, Hodge CC, Caudill SP, Brock JW, Needham LL, Calafat AM (2004). Urinary levels of seven phthalate metabolites in the U.S. population from the National Health and Nutrition Examination Survey (NHANES) 1999-2000. *Environ Health Perspect* **112**, 331-8.

Smith D, Trennery P, Farningham D, Klapwijk J (2001). The selection of marmoset monkeys (*Callithrix jacchus*) in pharmaceutical toxicology. *Lab Anim* **35**, 117-30.

Sonmez M, Turk G, Yuce A. (2005). The effect of ascorbic acid supplementation on sperm quality, lipid peroxidation and testosterone levels of male Wistar rats. *Theriogenology* **63**, 2063-72.

Swan SH (2003). Do environmental agents affect semen quality? *Epidemiology* **14**, 261-2.

Swan SH, Main KM, Liu F, Stewart SL, Kruse RL, Calafat AM, Mao CS, Redmon JB, Ternand CL, Sullivan S, Teague JL; Study for Future Families Research Team (2005). Decrease in anogenital distance among male infants with prenatal phthalate exposure. *Environ Health Perspect* **113**, 1056-61.

Takeuchi S, Iida M, Kobayashi S, Jin K, Matsuda T, Kojima H (2005). Differential effects of phthalate esters on transcriptional activities via human estrogen receptors alpha and beta, and androgen receptor. *Toxicology* **210**, 223-33.

Tanaka T (2005). Reproductive and neurobehavioural effects of bis(2-ethylhexyl) phthalate (DEHP) in a cross-mating toxicity study of mice. *Food Chem Toxicol* **43**, 581-9.

Tanaka T (2002). Reproductive and neurobehavioural toxicity study of bis(2-ethylhexyl) phthalate (DEHP) administered to mice in the diet. *Food Chem Toxicol* **40**, 1499-506.

Tanaka T (2003). Effects of bis(2-ethylhexyl) phthalate (DEHP) on secondary sex ratio of mice in a cross-mating study. *Food Chem Toxicol* **41**, 1429-32.

Tickner JA, Schettler T, Guidotti T, McCally M, Rossi M (2001). Health risks posed by use of Di-2-ethylhexyl phthalate (DEHP) in PVC medical devices: a critical review. *Am J Ind Med* **39**, 100-11.

Toda C, Okamoto Y, Ueda K, Hashizume K, Itoh K, Kojima N (2004). Unequivocal estrogen receptor-binding affinity of phthalate esters featured with ring hydroxylation and proper alkyl chain size. *Arch Biochem Biophys* **431**, 16-21.

van Wezel AP, van Vlaardingen P, Posthumus R, Crommentuijn GH, Sijm DT (2000). Environmental risk limits for two phthalates, with special emphasis on endocrine disruptive properties. *Ecotoxicol Environ Saf* **46**, 305-21.

Veeramachaneni DN (2000). Deteriorating trends in male reproduction: idiopathic or environmental? *Anim Reprod Sci* **60-61**, 121-30.

Waliszewski M, Szymczynski GA (1990). Determination of phthalate esters in human semen. *Andrologia* **22**, 69-73.

Weinbauer GF, Schubert J, Yeung CH, Rosiepen G, Nieschlag E (1998). Gonadotrophin-releasing hormone antagonist arrests premeiotic germ cell proliferation but does not inhibit meiosis in the male monkey: a quantitative analysis using 5-bromodeoxyuridine and dual parameter flow cytometry. *J Endocrinol* **156**, 23-34.

Weinbauer GF, Aslam H, Krishnamurthy H, Brinkworth MH, Einspanier A, Hodges JK (2001). Quantitative analysis of spermatogenesis and apoptosis in the common marmoset (*Callithrix jacchus*) reveals high rates of spermatogonial turnover and high spermatogenic efficiency. *Biol Reprod* **64**, 120-6.

Wolfe GW, Layton K, Nehrebeckyj L, Wang Y, Chapin R, Rousselle SD, Bishop J (2002). Reproductive effects of diethylhexylphthalate (DEHP) in Sprague-Dawley rats when assessed by the continuous breeding protocol. *Toxicologist* **66(1-S)**, 234.

Wong JS, Ye X, Muhlenkamp CR, Gill SS (2002). Effect of a peroxisome proliferator on 3 beta-hydroxysteroid dehydrogenase. *Biochem Biophys Res Commun* **293**, 549-53.

Worrell NR, Cook WM, Thompson CA, Gray TJB (1989). Effect of mono-(2-ethlyhexyl) phthalate on the metabolism of energy-yielding substrates in rat Sertoli cell-enriched cultures. *Toxi in Vitro* **3**, 77-81.