NOMINATION FOR TOXICOLOGICAL TESTING:
NEONATAL/JUVENILE EXPOSURE DI-(2-ETHYLHEXYL)PHTHALATE (DEHP)

NOMINATING CENTER/OFFICE/BRANCH:

U.S. Food & Drug Administration
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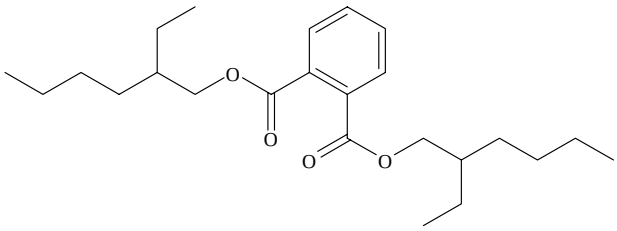
CHEMICAL/AGENT NAME:

Di-(2-ethylhexyl)phthalate; bis(2-ethylhexyl)phthalate; dioctylphthalate; C_6H_4-1,2-$[CO_2CH_2CH(C_2H_5)(CH_3)_3CH_3]_2$; $C_{24}H_{38}O_4$; FW, 390.56



CAS NUMBER:

117-81-7

SOURCE OF DRUG/COMPOUND:

There is widespread exposure of the general population to DEHP through food, water and air, although the major sources of high level human exposure are blood-contact medical

devices including blood storage bags and tubing used in devices for cardiopulmonary bypass and hemodialysis.

DEHP is a plasticizer used in the manufacture of polyvinyl chloride (PVC) plastic. The production volume of DEHP has been reported to be approximately 2 million tons (NTP-CERHR, 2000). Unplasticized PVC is hard and brittle at room temperature; therefore, the addition of a plasticizer is necessary to make it pliable. Various plasticizers have been used in manufacturing plastics for medical devices, but the plasticizer of choice to date is DEHP due to its suitability for imparting the properties necessary for the performance demands of medical materials. The PVC used in medical devices contains from 20-40% DEHP by weight. The DEHP is not covalently bound to the PVC polymer and thus can be readily leached from the material during use with the extent of leaching dependent upon many factors, including the temperature, volume, and lipophilicity of the fluid in contact with the PVC and the flow rate or extent of shaking of the fluid (Health Canada, 2002). While superior gas diffusion properties have led to the use of non DEHP-containing materials for the storage of platelets, bags made of PVC-DEHP are still used for platelet storage, and remain the predominant storage and administration containers for whole blood, red blood cells, plasma, and stem cells.

MAGNITUDE OF HUMAN EXPOSURE:

The issues of the magnitude and sources of human exposure to DEHP as well as the potential for these exposures to cause toxicity have been exhaustively and critically reviewed in recent years (ATSDR, 2002; FDA, 2002; Health Canada, 2002; National Chemicals Inspectorate, 2001; NTP-CERHR, 2000). The issue of exposures and toxicity concerns associated with exposures from medical devices were major foci of the evaluations produced by the Center for Devices and Radiological Health of the FDA (FDA, 2002), the Medical Device Bureau of Health Canada (Health Canada, 2002), and the Center for Evaluation of Risks to Human Reproduction of the National Toxicology Program (NTP, 2000). For the most part, with the exception of very recent data that have appeared since their publication, these reviews rather than primary sources will be cited in this nomination. All of the reviews were in general agreement that, while there were no existing human studies that implicate DEHP with toxicity, the extensive body of animal toxicity data raises concerns that need to be resolved. In particular, the subpopulation at greatest possible risk was identified as males exposed to multiple sources of DEHP in neonatal intensive care units (possible exposure approximately 3 mg/kg/day over weeks or months; FDA, 2002).

The introduction of vinyl plastic blood bags and associated medical devices into blood banking practice has led to enormous advantages in the collection, processing, storing, and dispensing of human blood and blood components. It has been shown that the DEHP leached out of storage bags is incorporated into the membrane of red blood cells and increases osmotic stability and, thus, storage life (Horowitz *et al*., 1984; Rock *et al*., 1986). Currently, medical devices made of DEHP-plasticized PVC are widely used in the US and worldwide and, as mentioned previously, bags made of this material are

currently the preferred containers for whole blood, red blood cells, plasma, and stem cells. According to the National Blood Data Resource Center (www.nbdrc.org), in 2001 (the most recent year for which data are available) U.S. hospitals transfused nearly 14 million units of whole blood and red blood cells to 4.9 million patients, and the volume of blood transfused was increasing at a rate of approximately 6% per year.

To calculate incidence rates of the transfusion of blood and blood components in the general population and in age- and gender-specific groups, all residents of a United States county who received transfusion(s) from 1989 through 1992 were studied (Vamuakas and Taswell, 1994). The incidence of red blood cell transfusion was 42.88 units per 1000 population per year in both men and women and varied from 12.08 units per 1000 population per year in those less than 41 years old to 245.24 units per 1000 population per year in the group aged more than 65. A random resident's probability of receiving transfusion(s) in any year was 0.89 percent (0.83% for men and 0.94% for women) and varied from 0.26 to 5.17 percent among the three age groups. The incidence of platelet and fresh-frozen plasma transfusion was 21.24 units per 1000 population per year and 8.64 units per 1000 population per year, respectively.

Since DEHP is not bound chemically to the vinyl plastic but is merely mixed in it, the plasticizer migrates from the plastic film to the stored blood. Both FDA (2002) and Health Canada (2002) reviewed the available data on the level of DEHP contamination of blood and blood products (plasma, platelet rich plasma, platelet poor plasma, leukocyte poor plasma, fresh frozen plasma, and platelet or red blood cell concentrates) collected and stored in DEHP-containing PVC containers and the resulting level of exposure in human adults and neonates. Levels were highly variable (range from approximately 4 – 1230 mg per L; FDA, 2002, Table A-8) due in part to factors such as duration and temperature of storage, differences in lipid levels (higher lipid levels extract more DEHP), and the analytical techniques used. Doses received by patients also vary widely depending on the medical procedure used. The estimated upper bound doses of DEHP received by patients receiving transfusions were 3 mg/kg/day for an adult (8.5 mg/kg/day for a trauma patient) and 22.6 mg/kg/day for a neonate receiving an exchange transfusion although doses in infants receiving exchange transfusions in one report were 0.84-4.22 mg/kg (FDA, 2002, Table 2-2). For infants requiring extracorporeal membrane oxygenation (ECMO), information is unavailable to accurately estimate the dose of DEHP received by these patients on a mg/kg/day basis, since the exposure period is represented as a range (3-10 days). However, if we assume that the larger DEHP doses were received by patients undergoing this procedure for 10 days, the time averaged dose of DEHP received by these neonates is expected to be 3.5 to 14 mg/kg/day (FDA, 2002; Health Canada, 2002). From a chronic dosing standpoint, the greatest exposure (0.36 mg/kg/day) is from hemodialysis (FDA, 2002; Health Canada, 2002). It should also be noted that a metabolite of DEHP, 2-(monoethylhexyl) phthalate (MEHP), which is produced by the action of lipases, has been implicated as the toxic metabolite of DEHP. MEHP can be produced from DEHP on storage by the action of plasma lipases, with the extent of production higher with longer storage time and at higher storage temperatures (FDA, 2002; Health Canada, 2002; NTP, 2000). MEHP is also produced *in vivo* by the

action of intestinal and plasma lipases. As discussed below, the extent of this conversion is thought to be a critical factor in the toxicity elicited by DEHP exposure.

EVIDENCE FOR CENTER/OFFICE CONCERN:

While data on humans are lacking, there have been numerous studies, including NTP studies, of the effects of DEHP treatment on rodents and non-rodents (reviewed in ATSDR, 2002; FDA, 2002; Health Canada, 2002; National Chemicals Inspectorate, 2001; NTP-CERHR, 2000). Liver and testes have been identified as the major target organs, but other targets, including the female reproductive tract, kidneys, lung, heart, and thyroid have been identified in some studies. The most sensitive endpoint, and the endpoint that has been identified as being of the highest concern for human exposures, is testicular toxicity. In particular, developing animals have been shown to be more sensitive to the testicular toxicity of DEHP than older animals. This, combined with the data demonstrating that critically ill neonates can be exposed to doses of DEHP near the NOAELs reported in rodent studies from medical procedures has led to particular concern for this sensitive subpopulation (FDA, 2002; Health Canada, 2002; NTP-CERHR, 2000).

The number of low birth weight infants (<2,500g) has been on the increase in recent decades (Martin *et al*., 2003). In 2002, there were approximately 314,077 (7.8% of all births) low birth weight infants born in the United States with approximately 58, 544 (1.5% of all births) of these being in the very low birth weight (<1,500g) category (Martin *et al*., 2003). Very low birth weight and moderately low birth weight infants have, respectively, a 100 – and 5-fold increased risk of dying in the first year relative to normal birth weight infants (Martin *et al*., 2003). Critically ill neonates often require replacement transfusions, and Levy *et al*. (1993) reported that 80% of low birth weight infants in the U.S. would receive multiple transfusions. While advances in medical technology are gradually reducing the number of these neonatal transfusion procedures (Maier *et al*, 2000), they remain a common required treatment in the neonatal intensive care unit (NICU). The blood drawn up for this use is typically stored up to 42 days at 4 ^{o}C in a storage bags made of DEHP-plasticized PVC materials. The FDA has also pointed out that these critically ill neonates have multiple sources of exposure other than the blood bags during medical procedures and estimated a potential exposure of approximately 3 mg/kg/day from commonly used NICU procedures (FDA, 2002). Greater exposures of up to 22 mg/kg/day were possible from the more rarely used exchange transfusions (FDA, 2002). Fetal exchange transfusions due to red blood cell alloimmunization were estimated by Schmacher and Moise (1996) to be needed in less than 500 cases per year in the United States, and neonatal exchange transfusions indicated for treatment of infants with bilirubin levels greater than 30 mg/dL were required in about 1 in 10,000 term births in a large California managed care organization (Newman *et al*., 2003).

In addition to the *in utero* exposure from fetal transfusions mentioned above, the fetus might be exposed to DEHP in mothers undergoing hemodialysis. Following delivery, nursing infants would also be exposed to DEHP and MEHP through milk (Health

Canada, 2002). It has been estimated that 1-7% of women undergoing chronic hemodialysis become pregnant (Holley and Reddy, 2003). Based on the number of female hemodialysis patients in 2001 from United States Renal Data System (www.usrds.org), this would translate to between 1,200 and 8,600 pregnancies per year in this population. In rare situations, such in a subpopulation of sickle cell anemia patients, maternal blood transfusions would also be a possible source of fetal exposure to DEHP (Koshi, 1995).

It is known that prenatal testosterone is critical in the development of male reproductive tract and behaviors in primates (Herman *et al*., 2000). While there is clear evidence from many studies that a major testicular target of DEHP in the testis is the Sertoli cell (reviewed in NTP-CERHR, 2000), several studies in recent years have indicated that DEHP affects the production of testosterone and insulin-like growth factor 3 by Leydig cells *in utero* and in neonates (Akingbemi *et al*., 2001, 2004; Kim *et al*., 2003; Parks *et al*., 2000; Wilson *et al*., 2004).

While the rodent DEHP toxicity data have been used in risk evaluations for human exposures, there have been questions raised about the relevance of those data for humans. This is particularly true for exposures from medical devices, which occur primarily via the intravenous route in contrast to the oral exposures used in the vast majority of rodent toxicity studies. Route of administration and species differences in the uptake and metabolism of DEHP have been clearly established (reviewed in ATSDR, 2002; FDA, 2002; Health Canada, 2002; National Chemicals Inspectorate, 2001; NTP-CERHR, 2000) and lack of toxicity of orally administered DEHP in nonhuman primates at doses above those producing toxicity in rodents has been reported (Kurata *et al*., 1998; Pugh *et al*., 2000; Rhodes *et al*., 1986; Tomonari *et al*., 2003). As mentioned previously, MEHP has been implicated as the toxic metabolite of DEHP. On oral ingestion, DEHP is rapidly converted to MEHP by pancreatic lipase and mucosal esterase in the gut and MEHP and its metabolites predominate over DEHP in blood and excreta after oral administration of DEHP. In the marmoset, lipase activity in the gut appears to be lower, and thus conversion to MEHP is much lower (Rhodes *et al*., 1986; Kessler *et al*., 2004). For example, a recent study examined blood levels of DEHP and MEHP after equivalent doses of DEHP administered by gavage to pregnant and non-pregnant rats and marmosets (Kessler et al., 2004). Regardless of whether the animals were pregnant, C_{max} for MEHP was on average 3.2 times higher in rats than in marmosets, while the AUC was on average 7.3-fold higher in rats. MEHP can be further oxidized, and over 30 metabolites have been identified (reviewed in ATSDR, 2002). While tests of MEHP *in vitro* and *in vivo* have shown it to be more potent than DEHP, the oxidized metabolites tested have not shown activity (Gray and Beamond, 1984; Sjoberg *et al*., 1986; Li *et al*., 1998, 2000). The pattern of urinary metabolites of DEHP is qualitatively similar in rats, nonhuman primates, humans, and other species (summarized in National Chemical Inspectorate, 2001, Table 4.2.1.2). Unlike other species, including humans, rats do not glucuronidate MEHP or its metabolites (summarized in National Chemical Inspectorate, 2001, Table 4.2.1.8). The only data on the glucuronidation of DEHP metabolites in neonatal humans reported thus far is from a single neonate who received an exchange transfusion due to hyperbilirubinemia (Egestad *et al*., 1996). Quantitative data was not reported for this

infant, but several glucuronidated oxidized metabolites of MEHP, but not MEHP itself, were detected. More recently, Calafat *et al*. (2004) did report MEHP in the urine of infants in an NICU (discussed below).

While plasma and hepatic lipases can cleave DEHP to MEHP, these activities are much lower than in the gut so that the ratio of DEHP to MEHP is greater after intravenous administration than after oral administration. However, the levels of hepatic and plasma lipases are reported to be higher in infants than in adults, pre-term infants have higher lipoprotein lipase than at term infants, and both heparin and DEHP induce plasma lipase activity (reviewed in Health Canada, 2002), all of which are consistent with higher levels of MEHP production in infants exposed to DEHP in the NICU. Data on the kinetics of the metabolism of DEHP to MEHP *in utero* are lacking, and data in infants are limited. Sjoberg *et al*. (1985a, b) detected DEHP and MEHP in the plasma of infants receiving exchange transfusions. A slow elimination phase with a half-life of 10 hours was reported, and one pre-term infant appeared to eliminate MEHP more slowly than a full term infant (Sjoberg, 1985b). A more recent report examined levels of MEHP and two further oxidation products of MEHP in the urine of six premature neonates in a NICU and found levels approximately an order of magnitude higher than levels detected in reference populations of children and adults (Calafat *et al*., 2004). Due to sample size limitations, it was not possible to determine what proportion of the excreted metabolites was glucuronidated in the infants. While the data from this initial study are limited, they do support the high exposure of NICU patients to high levels of DEHP and confirm that conversion to the active metabolite, MEHP, does occur.

It should be noted that the metabolism of DEHP to MEHP can also occur in stored blood products or in blood that is in contact with PVC tubing during such procedures as ECMO or hemodialysis (FDA, 2002; Health Canada, 2002). In stored blood products, storage time and temperature of storage are important factors in determining the level of MEHP (Cole *et al*., 1981), although the kinetics of this conversion to MEHP have not been thoroughly investigated.

While the expected lower exposure to MEHP after intravenous exposure than oral exposure and the apparent insensitivity of adult primates to the hepatic and testicular effects of orally administered DEHP have been demonstrated, reviews of existing data by expert panels and regulatory agencies have concluded that the risks of DEHP exposure to infants exposed to medical procedures involving PVC/DEHP-containing medical devices remain uncertain (ATSDR, 2002; FDA, 2002; Health Canada, 2002; National Chemicals Inspectorate, 2001; NTP-CERHR, 2000). Since the publication of these reports, several studies have been conducted to address this uncertainty. The results of these studies will be briefly reviewed below, and the degree to which they resolve the issue of the sensitivity of infants to DEHP exposure will be considered.

Recent studies addressing DEHP exposure and risk of exposure of neonates. The study of Calafat *et al*. (2004), which was mentioned above, confirmed high levels of exposure to MEHP in a limited set of NICU infants. Rais-Bahrami *et al*. (2004) reported a follow-up study on 13 male and 6 female subjects who had undergone extracorporeal

membrane oxygenation (ECMO) as neonates. ECMO results in high exposures of up to 35-140 mg/kg body weight over a period of 3-10 days (FDA, 2002; Health Canada, 2002). The adolescents examined in the study of Rais-Bahrami *et al*. showed normal growth and development and had sex hormone levels appropriate to the stage of puberty, that is, there was no evidence of adverse effects of exposure to DEHP in this set of patients.

In a study designed to assess the toxicity of intravenous administration of DEHP to neonatal rats, a sensitive species at a sensitive developmental time period, Cammack *et al*. (2003) administered DEHP intravenously (60, 300, and 600 mg/kg/day) daily from postnatal day 3-5 for 21 days. A second set of rats was treated with DEHP (300, and 600 mg/kg/day) by gavage. Animals were examined at the end of the treatment period and after 90 days of recovery from treatment. No effects of the 60 mg/kg/day intravenous doses were observed. Testis weights decreased, liver weights increased, and microscopic lesions were observed in the testes (depletion of the germinal epithelium, decreased diameter of the seminiferous tubules) in the animals treated with 300 and 600 mg/day, with more severe effects in the orally dosed groups. While some effects persisted in the testes of the orally dosed rats after the 90 day recovery period, no effects persisted in the intravenously dosed rats. No Sertoli cell damage was observed in any dose group, oral or intravenous.

Finally, a study of the effects of orally administered DEHP (100, 500, and 2500 mg/kg/day) on juvenile marmosets has been reported in abstract form (Tomonari *et al*., 2003; also discussed in McKee *et al*., 2004). Previous studies in non-human primates had utilized animals that were sexually mature; beyond the stage of maximum sensitivity demonstrated in rodent studies. Tomonari *et al*. administered daily oral doses from 3 months of age for 15 months. Dosing was started at 3 months rather than neonatally due to practical issues relating to daily dosing of the neonates (McKee *et al*., 2004). The focus of the study was on the male reproductive tract, and no significant adverse effects were observed. A related study on the blood and tissue distribution was also reported in abstract form (Kurata *et al*., 2003). Among the findings reported was that repeated oral dosing with DEHP in the marmoset resulted in lower pharmacokinetic parameters. A potentially important issue that was not addressed in the abstract was the extent of glucuronidation of the metabolites in the young marmosets. In humans, the glucuronidation capacity of neonates is significantly lower than in adults although, as mentioned previously, limited evidence of glucuronidation of MEHP metabolites has been reported in one infant (Egestad *et al*., 1996). While the marmoset has been proposed to be a good model for human glucuronidation in adults (Soars *et al*., 2001), limited data were found in the literature on neonatal marmosets. One study (Hall and James, 1980) reported that the glucuronidation of p-aminobenzoic acid did not change significantly with age in the marmoset, in contrast with humans where glucuronidation capacity increases with age.

The recent studies discussed above all address to some extent the critical question of the risk to neonates of exposure to DEHP from medical devices, but they do not resolve the question. The neonatal rat study of Cammack *et al*. (2003) was available in

prepublication form to both the FDA (2002) and Health Canada (2002) prior to the publication of their reports. Both Agencies agreed that these data were the best available for setting a rodent NOAEL (60 mg/kg) for intravenous DEHP. However, intravenous dosing can be an error prone procedure (Groman and Reinhardt, 2004), and the administration of daily intravenous doses to a young rat as conducted by Cammack *et al*. (2003) is a particular technical challenge. The Health Canada report (2002) noted both the strengths and weaknesses of the study. Weaknesses included the facts that the bolus injection of DEHP is not equivalent to the continuous leaching of DEHP that occurs during medical procedures and, perhaps more relevant to the current discussion, the occasional leaking of dosing solution after injection mentioned in the original study report so that the lack of blood level data to confirm administered dosing somewhat reduced confidence in the exactness of the NOAEL (Health Canada, 2002).

The study of Rais-Bahrami *et al*. (2004) is an important study in that it addresses the issue of the toxic effects of DEHP in the population of interest rather than in a surrogate test system. The actual exposure levels in the subjects are not known, but they did undergo the procedure, ECMO, associated with the highest exposures to DEHP. It is reassuring that no effects were observed in growth or pubertal development, but this study raises a question of what effects would actually be expected in human infants exposed in the neonatal period. Rats exposed to DEHP during the perinatal period suffer severe and lasting testicular effects to the point of atrophy. However, the perinatal rat is not at an equivalent stage of reproductive tract development as the perinatal human. For example, in rodents, proliferation of Sertoli cells, a major target of DEHP, is confined to the neonatal period, while in primates, Sertoli cell proliferation occurs beyond this point (Sharpe *et al*., 2000; Simorangkir *et al*., 2003) so that, unlike the situation in the rat, sperm production can recover from a reduction in Sertoli cell proliferation confined to the neonatal period (Sharpe *et al*., 2000). Furthermore, in primates, unlike rodents, there is a prolonged (several months) elevation of testosterone in neonatal males (Mann and Fraser, 1996). The physiological significance of this period of high neonatal testosterone in primates is not yet understood. In nonhuman primates, complete block of this testosterone surge through the use of gonadotropin releasing hormone antagonists or agonists does not result in significant effects on sperm counts or fertility in adults and little or no effect on sexual behavior has been reported (Eisler *et al*., 1993; Lunn *et al*., 1994, 1997; Mann *et al*., 1998; Nevison *et al*., 1997; Wallen *et al*., 1995), although delays or prolongation of the pubertal increase in testosterone levels have been reported (Lunn *et al*., 1994) as well as alterations in testicular cell composition (Sharpe *et al*., 2000) and transient retardation of penis growth (Wallen *et al*., 1995). In addition, in both the rhesus monkey and the marmoset, interfering with the neonatal testosterone surge in males has been reported to result in immune system alterations (Mann and Fraser, 1996; Lunn *et al*., 1997; Gould *et al*., 1997; Mann *et al*., 2000). Little data exists related to possible immune system effects of DEHP. Kurata *et al*. (1998) did report a dose-dependent reduction in spleen weight in male marmosets dosed with 100, 500, and 2500 mg/kg/day for 13 weeks, but this effect was dismissed as insignificant since no microscopic lesions were seen and the control spleen weight was greater than expected. The abstract of the prepubertal exposure of Tomonari *et al*. (2003) indicates that spleen was examined, but that no treatment-related changes were noted in any organ. At any

rate, the data from nonhuman primates suggests that if DEHP exposure inhibits testosterone production in neonatal primates as it does in rats, the effects in might be more subtle than those observed in the rat.

The marmoset study of Tomonari *et al*. (2003) utilized doses from 100 – 2,500 mg/kg/day, a high dose well above any expected human exposure to DEHP in male marmosets from 3 months of age (approximately 90 days old) to 18 months of age. Abbott and Hearn (1978) reported that the male marmoset exhibited a neonatal testosterone surge from 15-100 days of age, and more recently McKinnell *et al*. (2001) found the highest levels of testosterone from 1 to 7 weeks of age, with decreasing levels starting at 8 weeks and reaching the lowest levels by 16 weeks. Sharpe *et al*. (2000) have found that the Sertoli cell number in marmosets reaches adult numbers by weeks 18-24. Thus, while the study of Tomonari *et al*. does cover the pre and peri adolescent periods in the marmoset, the exposure period does not cover the early peak surge of testosterone. Comparing the data of Sharpe's group on Sertoli cell counts from 5-6 week-old animals (Sharpe *et al*., 2002) to those from 18-24 week animals (Sharpe *et al*., 2000) indicates an approximately 2-fold increase in numbers between those times, although the rate of increase is not known. It is likely that the dosing period does cover a portion of the increase in Sertoli cell proliferation, but perhaps not the peak. Sharpe and colleagues (Sharpe *et al*., 2000, 2002) have argued that the marmoset provides a good model for human male reproductive development because several aspects of this process, including testicular descent prior to birth, the organization of spermatogenesis (Millar *et al*., 2000; Weinbauer *et al*., 2001), and substantial Sertoli cell proliferation in the neonatal period (Sharpe *et al*., 2000). In contrast, the testes of the rhesus monkey descend after birth and the organization of spermatogenesis differs from that in the human. Simorangkir *et al.* (2003) have questioned whether the marmoset provides a better model of Sertoli cell development and proliferation than the rhesus monkey for the human. While the Sertoli cells of rhesus monkeys and humans show a proliferative peak near puberty, this has not been demonstrated in the marmoset where, as mentioned previously, near adult levels of Sertoli cells are found in early infancy (Sharpe *et al*., 2000). The extent of proliferation of Sertoli cells in neonatal humans is unclear (reviewed in Simorangkir *et al*., 2003) and may, according to Simorangkir *et al*. (2003), be better reflected by the pattern in the rhesus monkey. This is an important issue to be considered in designing further experiments to address the issue of DEHP exposure and toxicity in neonates, since a model that is as close as possible to the human in both metabolism of DEHP and in reproductive development is desirable. The rhesus monkey may be more suited to examination of the critical issues requiring resolution, since the diminutive size of the marmoset makes its use less practical for addressing the toxicokinetics of transplacental transfer following intravenous exposure or for multiple blood samplings from the same animal during continuous intravenous infusion.

As discussed above, the potential for DEHP-induced testicular toxicity in young developing males is of concern. Due to many advantages of DEHP-plasticized PVC in the blood banking industry, i.e., cost and clarity of the plastic film, the plastic is favored by the industry; therefore, the sensitivity of humans to the adverse effects of DEHP needs to be established through studies in a relevant model in terms of species, timing of

exposure, and route of administration. It is thus recommended that further studies be conducted with intravenous exposures in developing (*in utero* and neonatal) nonhuman primates (rhesus or cynomolgus monkey) or another suitable species, if one can be identified.

RECOMMENDED STUDIES:

STUDY 1: Existing data demonstrate that the toxicokinetics of DEHP differ following oral and intravenous administration and between young animals and adults, but quantitative data on toxicokinetics and biotransformation of DEHP following intravenous administration to neonatal and pregnant female primates is lacking. It is proposed that data on these endpoints, including the extent of conversion to MEHP and the extent of glucuronide formation, be obtained in neonatal male and pregnant/fetal nonhuman primates (rhesus or cynomolgus monkeys). Blood and urine should be analyzed so that urine data can be compared with those recently obtained in NICU patients (Calafat *et al*., 2004).

STUDY 2: If warranted by the results of study 1 (significant formation of MEHP after intravenous DEHP), acute and subchronic intravenous dosing during times of peak testosterone production in neonatal males should be conducted. Blood and urine levels of DEHP should be monitored, as well as markers of Sertoli cell function (inhibin B and androgen binding protein) and Leydig cell function (testosterone and insulin-like growth factor 3). Levels of inhibin B have been demonstrated to correlate with Sertoli cell numbers in nonhuman primates (Mann *et al*., 1997; Ramaswamy *et al*., 1999; Winters and Plant, 1999). Investigation of markers of immune function (e.g. peripheral lymphocyte subsets) should also be considered in the subchronic study.

STUDY 3: A neonatal intravenous study in rats following the protocol of Cammack *et al*. (2003) should be considered that would include determination of blood levels of DEHP metabolites and measurement of effects on immune system parameters.

SCIENTIFIC ISSUES:

Studies in rodent models have clearly established the perinatal period as the time of greatest sensitivity to the adverse effects of DEHP, particularly testicular atrophy. It is also clearly established that the levels of exposure of critically ill infants to DEHP are considerably greater than those of the general population. The medical use of PVC devices containing DEHP has clearly provided great benefit to patients and has contributed to the improved survival of low birth weight infants. However, despite decades of research, the long-term risks associated with exposures of infants to DEHP have not been clearly elucidated, and there are significant knowledge gaps on the toxicokinetics and effects in fetal and neonatal primates of intravenous DEHP. The requested studies will help to better define these risks so that the costs and benefits of

seeking and utilizing non-DEHP-containing devices with lower toxicity can be better assessed.

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