2. RELEVANCE TO PUBLIC HEALTH

2.1 BACKGROUND AND ENVIRONMENTAL EXPOSURES TO DI(2-ETHYLHEXYL)-PHTHALATE IN THE UNITED STATES

Di(2-ethylhexyl)phthalate, commonly referred to as DEHP, is predominantly used as a plasticizer in the production of flexible polyvinyl chloride (PVC) products. At least 95% of DEHP produced is used as a plasticizer for PVC. PVC is made flexible by addition of plasticizers and is used in many common items such as wall coverings, tablecloths, floor tiles, furniture upholstery, shower curtains, garden hoses, swimming pool liners, rainwear, baby pants, dolls, toys, shoes, automobile upholstery and tops, packaging film and sheet, sheathing for wire and cable, medical tubing, and blood storage bags. Numerous nonplasticizer uses of DEHP have been reported; however, it is not clear to what extent these uses are, or have ever been, important. Because of concerns regarding potential health effects from DEHP exposure, many toy manufacturers have discontinued use of DEHP in their products. The use of DEHP in domestically produced baby teethers and rattles has been discontinued, and DEHP is also no longer used as a plasticizer in plastic food wrap products.

DEHP is a widely used chemical that enters the environment predominantly through disposal of industrial and municipal wastes in landfills and, to a much lesser extent, volatilization into air (from industrial and end uses of DEHP), carried in waste water from industrial sources of DEHP, and within effluent from municipal waste water treatment plants. It tends to sorb strongly to soils and sediments and to bioconcentrate in aquatic organisms. Biodegradation is expected to occur under aerobic conditions. Sorption, bioaccumulation, and biodegradation are likely to be competing processes, with the dominant fate being determined by local environmental conditions. When DEHP is present in the environment, it is usually at very low levels. It is very difficult to determine these low levels accurately since DEHP is a ubiquitous laboratory contaminant, and laboratory contamination may cause false positives to be reported in the literature.

The principal route of human exposure to DEHP is oral. Most of the available monitoring data are old and may not represent current exposures, especially since the uses of DEHP have changed over the last 20 years. Even so, some recent estimates for the average total daily individual ambient exposures to DEHP of 3–30 Fg/kg/day (in a 70-kg adult) have been proposed. These intake approximations indicate that the general population is exposed to DEHP at levels that are 3–4 orders of magnitude lower than those observed to cause adverse health effects in animal studies (see Section 2.2). Occupational

exposures may be significant, but the highest exposures to DEHP result from medical procedures such as blood transfusions (upper bound limit of 8.5 mg/kg/day) or hemodialysis (upper bound limit of 0.36 mg/kg/day), during which DEHP may leach from plastic equipment into biological fluids. Exposures of neonatal children to DEHP can be especially high as a result of some medical procedures. For example, upper-bound doses of DEHP have been estimated to be as high as 2.5 mg/kg/day during total parenteral nutrition (TPN) administration and 14 mg/kg/day during extracorporeal membrane oxygenation (ECMO) procedures.

People residing near hazardous waste disposal sites or municipal landfills may be subject to higher than average levels of DEHP in ambient air and drinking water. Even so, the concentrations of DEHP in these media will be greatly limited by the low volatility and water solubility of DEHP, and subpopulations living in the vicinity of hazardous waste sites are much less highly exposed than those exposed to DEHP during medical procedures. DEHP is included in the priority list of hazardous substances identified by ATSDR and the EPA, and has been found in at least 733 of the 1,613 current or former NPL sites. However, the number of NPL sites evaluated for DEHP is not known. As more sites are evaluated, the sites at which DEHP is found may increase.

2.2 SUMMARY OF HEALTH EFFECTS

The health effects of DEHP are well studied in animals, particularly by the oral route, but there is a paucity of data in humans by any route of exposure. Information on the oral toxicity of DEHP in humans is limited to gastrointestinal symptoms (mild abdominal pain and diarrhea) in two individuals who ingested a single large dose of the compound. Studies in rats, mice, and other rodent species show that DEHP has a low order of acute oral toxicity, with some data indicating that young animals are more susceptible than adults. Numerous repeated dose oral studies in rats and mice have clearly established that the main targets of DEHP toxicity are the liver and testes. Toxicity of DEHP in other tissues is less well characterized, although effects in the thyroid, ovaries, kidneys, and blood have been reported in a few studies. In contrast to the findings in rats and mice, non-human primates appear to be relatively insensitive to the hepatic and testicular effects of DEHP. Manifestations of testicular toxicity in rats and mice include loss of spermatogenesis and decreased fertility. A more limited data set indicates that long-term oral exposure to DEHP can also cause adverse reproductive effects in female rats and mice. There is sufficient evidence showing that DEHP is fetotoxic and teratogenic in rodents, inducing a range of effects that includes abnormal development of the male reproductive tract following perinatal exposure. DEHP has been extensively tested for genotoxicity in a variety of *in vitro* and *in vivo* microbial and mammalian

assay systems with results that are predominately negative or misinterpreted as positive. The weight of evidence indicates that DEHP is not genotoxic, but exerts multiple effects by an epigenetic mechanism that can alter the expression of genes in cells. Sustained long-term oral exposure to DEHP is hepatocarcinogenic in rats and mice, but the mechanism by which liver cancer (and liver toxicity) is induced in these species does not appear to be operative in humans.

Limited information is available on the health effects of DEHP in humans or animals following inhalation or dermal exposure. Regarding effects of inhalation, lung disorders resembling hyaline membrane disease were observed during the fourth week of life in three children who had received respiratory ventilation via PVC tubing as preterm infants. Although interpretation of these findings is complicated by confounding variables such as compromised health status of the preterm infants, the available information suggests that the lung disorders were related to DEHP released from the walls of the respiratory tubing. One inhalation study in rats that were intermittently exposed to DEHP aerosol for 28 days reported increased lung and liver weights and histological changes in the lungs that were reversed following cessation of exposure. Two inhalation studies found no evidence of DEHP-induced reproductive or developmental toxicity in rats. One dermal study found no indications of skin irritation or sensitization in humans, skin irritation in rabbits, or ocular irritation in rabbits.

Additional information on the main health effects of DEHP is discussed below. Because its effects are exerted in animals in a dose-related manner, exhibit threshold responses, and are not necessarily relevant to humans due to rodent-specific mechanisms, and concentrations of DEHP in the environment are almost certain to be well below effect thresholds, ambient levels exposure are not expected to be toxicologically significant, even in the vicinity of hazardous waste sites. DEHP therefore is not expected to pose a serious public health concern for the vast majority of the population.

Hepatotoxicity and Liver Cancer. Acute, intermediate, and chronic oral exposures to DEHP have profound effects in the rodent liver. Characteristic hepatic effects in rats and mice observed in numerous studies include hypertrophy and hyperplasia, beginning within 24 hours of exposure as reflected by induction of DNA synthesis and mitosis; proliferation of hepatic peroxisomes and, to a lesser extent, mitochondria and lysosomes; induction of peroxisome β-oxidation enzymes, with an accompanying increase in mitochondrial fatty acid oxidation; decreased cholesterol synthesis and degradation; induction of microsomal CYP4A-associated enzymes; altered concentrations of membrane proteins and lipids; increased production of H_2O_2 and reduction of H_2O_2-degrading enzymes; decreased liver glycogen; alterations in the morphology of the bile ducts; increases in malondialdehyde, conjugated dienes, and

lipofuscin deposits, indicating increased cellular concentration of free radical oxygen due to insufficient catalase; possible increased 8-hydroxydeoxy-guanosine in hepatic DNA; and eventual appearance of precancerous altered cell foci, nodules, and tumors.

It is generally believed that many of these liver effects are mediated through transcriptional activation of the peroxisome proliferator-activated receptor α (PPARα). The conclusion that the hepatic effects of DEHP are largely due to peroxisome proliferation is supported by findings that increased cell proliferation, hypertrophy, induction of peroxisomal and microsomal fatty acid-oxidizing enzymes, increased fatty acyl-CoA oxidase activity, excess production of hydrogen peroxide, decreased plasma lipid levels, and expression and activation of PPARα are common effects among structurally unrelated chemicals and drugs inducing peroxisome proliferation. As discussed in Section 3.5.2 Mechanisms of Toxicity, there are marked species differences in hepatic peroxisome proliferation. In particular, although human liver has low expression of PPARα, the characteristic effects of rodent peroxisome proliferators have not been observed in humans, either in liver biopsies from humans exposed to peroxisome proliferators, or in human hepatocytes exposed to peroxisome proliferators *in vitro*. The overall evidence indicates that most of the hepatic effects observed in DEHP-exposed rodents, including liver cancer, result from a mechanism that does not operate in humans.

It is well documented that long-term oral exposure to DEHP causes cancer of the liver in both rats and mice. There is no evidence that DEHP is genotoxic or a liver tumor initiator in rats and mice, although it does appear to have tumor promotion activity. Based on the findings from one of the cancer studies, an NTP bioassay, EPA classified DEHP in Group B2 (probable human carcinogen) and derived a cancer risk value (q_1*) of 1.4×10^{-2} $(mg/kg/day)^{-1}$. Based largely on the same findings, the U.S. Department of Health and Human Services suggests that it is reasonable to consider DEHP as a human carcinogen. IARC recently (2001) updated its cancer classification of DEHP from Group 2B (possibly carcinogenic to humans) to Group 3 (not classifiable as to its carcinogenicity to humans). In making its overall evaluation of the carcinogenicity of DEHP to humans, IARC took into consideration that (1) DEHP produces liver tumors in rats and mice by a non-DNA-reactive mechanism involving peroxisome proliferation, (2) peroxisome proliferation and hepatocellular proliferation have been demonstrated under the conditions of the carcinogenicity studies of DEHP in rats and mice, and (3) peroxisome proliferation has not been documented either in human hepatocyte cultures exposed to DEHP or in the liver of nonhuman primates. Based on these three lines of evidence, IARC concluded that the mechanism by which DEHP increases the incidence of hepatocellular tumors in rats and mice is not relevant to humans. This conclusion is based on the assumption that peroxisome proliferation is the mechanism causing liver

cancer. The peroxisome proliferation mechanism of DEHP hepatocarcingenicity in rodents seems to be threshold-based and the NOEL for peroxisome proliferation in rats and mice is in the range of 20–25 mg/kg/day. Although there is a real difference in the induction of peroxisome proliferation ability between rats/mice and humans, peroxisome proliferation might only be correlated with, but not be the actual mechanism of, tumor promotion.

Even though studies have shown that DEHP can cause liver cancer in rats and mice, the mechanism data suggests that these findings may not be relevant to the probability of DEHP causing cancer in humans.

Reproductive and Developmental Toxicity. No studies were located regarding reproductive effects of DEHP in humans. There are multiple studies in adult rats in which oral exposure to DEHP decreased the weights of the testes, prostate, seminal vesicles, and epididymis, and caused atrophy and degeneration of the seminiferous tubules with consequent altered sperm measures and reduced fertility. Testicular effects were induced in rats at doses as low as 37.6 mg/kg/day for 13 weeks, 50 mg/kg/day for 30 days, and 14 mg/kg/day for 102 weeks. The testicular damage was more severe in young male rats than in older rats, and appeared to be reversible if DEHP was withdrawn from the diet before sexual maturity was reached. Oral exposure to DEHP similarly induced testicular atrophy in mice, and mating of exposed male and female mice in a continuous breeding study resulted in significantly reduced number of litters and live births. Few reproductive studies of DEHP have been conducted in nonrodent species. Available data in monkeys suggest that non-human primates are less sensitive than rodents to the testicular effects of DEHP. As discussed in Section 2.3, decreased fertility and testicular toxicity are the bases of the intermediate- and chronic-duration minimal risk levels (MRLs) for oral exposure to DEHP.

Few studies have investigated the reproductive toxicity of DEHP in female animals. When female mice were exposed to a dietary dose of 420 mg/kg/day DEHP for 105 days and mated with unexposed males, combined weights of the ovaries, oviducts, and uterus were reduced, and no litters were produced. When female mice exposed to 14 or 140 mg DEHP/kg/day were mated with males given these same doses, there was a dose-related decline in the number of litters, live pups per litter, and live pup weight. Short-term gavage exposure to a very high level of DEHP (2,000 mg/kg/day), particularly with respect to possible human exposure, had clear effects on estradiol synthesis, manifested as decreased serum estradiol levels and anovulatory cycles and polycystic ovaries, in female rats. These data indicate that oral exposure to DEHP can affect reproductive processes in female rodents.

No studies were located regarding developmental toxicity in humans exposed to DEHP. Oral exposure to doses as low as 375 mg DEHP/kg/day during gestation and lactation altered development of the reproductive system in male rat offspring. A variety of effects were observed in androgen-sensitive tissues of young male rats, including reduced (female-like) anogenital distance and permanent nipples, vaginal pouch, penile morphological abnormalities, hemorrhagic and undescended testes, testicular and epididymal atrophy or agenesis, and small to absent sex accessory glands. These morphological effects, as well as reduced fetal and neonatal testosterone levels and adult sexual behavioral changes in male rats following gestational and lactational exposure, are consistent with an antiandrogenic action of DEHP. Function as well as development of the reproductive system were adversely affected in male offspring of rats that were orally exposed to DEHP in a two-generation study. The changes in the development, structure, and function of the male reproductive tract observed in various studies indicate that effects of DEHP on reproduction and development are interrelated.

Most of the developmental toxicity evaluations of DEHP are traditionally designed studies in which physical development was evaluated just prior to birth in pups of rodents that were orally exposed during gestation only. These studies clearly show that gestational exposure to DEHP was embryotoxic and teratogenic in rats and mice. A range of effects were observed including intrauterine deaths, skeletal and cardiovascular malformations, neural tube closure defects, increased perinatal mortality, and developmental delays.

2.3 MINIMAL RISK LEVELS

A minimal risk level (MRL) is an estimate of the daily human exposure to a hazardous substance that is likely to be without appreciable risk of adverse noncancer health effects over a specified duration of exposure. MRLs are based on noncancer health effects only and are not based on a consideration of cancer effects. These substance-specific estimates, which are intended to serve as screening levels, are used by ATSDR health assessors to identify contaminants and potential health effects that may be of concern at hazardous waste sites. MRLs are not intended to define clean-up or action levels. Additional background information on MRLs is provided in Appendix A.

Inhalation MRLs

No inhalation MRLs were derived for DEHP due to inadequate data for this route of exposure. As summarized in Section 2.2, the inhalation database for DEHP is essentially limited to two studies in rats that found some reversible effects in the lungs and liver following exposure for 28 days and no evidence for reproductive or developmental toxicity (Klimisch et al. 1991; Merkle et al. 1988).

Oral MRLs

An MRL was not derived for acute-duration oral exposure (#14 days) to DEHP due to insufficient data on male reproductive effects, a known critical end point based on longer duration studies. In particular, derivation of an acute oral MRL is precluded by a lack of dose-response information on development of the male reproductive system in offspring acutely exposed during gestation and/or lactation. As previously discussed in Reproductive and Developmental Toxicity (Section 2.2, Summary of Health Effects), morphological and other effects in androgen-sensitive tissues, as well as reduced fetal and neonatal testosterone levels and adult sexual behavioral changes, have been observed in male rat offspring exposed to DEHP during gestation and lactation for intermediate durations of exposure.

- An MRL of 0.1 mg/kg/day was derived for intermediate-duration oral exposure (15–364 days) to DEHP.

This MRL is lower than the previous intermediate-duration MRL derived in the 1993 profile but is based on a more appropriate end point. Refer to Chapter 8 for additional information.

The intermediate MRL is based on a no-observed-adverse-effects level (NOAEL) of 14 mg/kg/day for decreased fertility in a mouse reproductive toxicity study (Lamb et al. 1987). A continuous breeding protocol was used in which pairs of mice were exposed to DEHP in the diet at doses of 0, 14, 140, or 420 mg/kg/day for up to 126 days. There were 20 breeding pairs in each exposed group and 40 pairs in the control group. No reproductive effects were observed at 14 mg/kg/day. Fertility was reduced at 140 mg/kg/day, as shown by reductions in number of litters per pair, number of live pups per litter, and proportion of live pups, indicating that this dose is the lowest-observed-adverse effects level (LOAEL). Exposure to 420 mg/kg/day caused complete infertility during the continuous breeding part of the study (0/18 fertile pairs). Fertility was also profoundly reduced in crossover mating trials conducted in the 420 mg/kg/day mice (lower doses not tested) at the end of the continuous breeding phase of the study. The crossover study involved mating high dose mice of each sex to unexposed mice of the opposite sex to

determine the affected sex; near to complete infertility occurred in both sexes (0/16 fertile females and 4/20 fertile males). Other effects included reduced combined testis, epididymis, and prostate weights, reduced percentages of motile sperm and abnormal sperm, and reduced sperm concentration in the males, and reduced combined weight of ovaries, oviducts, and uterus in the females. Essentially all of the high-dose males had some degree of bilateral atrophy of the seminiferous tubules, but no exposure-related reproductive histopathology was observed in the females. Considering the reduced fertility and reproductive organ weights in the high-dose females, there is evidence that reproductive performance was impaired in both sexes at 420 mg/kg/day. Because the crossover mating study was only conducted at the high dose level, the reduced fertility observed at the 140 mg/kg/day LOAEL is not necessarily due to reproductive toxicity in both sexes.

Other studies have established that testicular toxicity is a critical effect of DEHP. It is well documented that oral exposure to DEHP in adult rats and mice causes decreased weights of the testes, prostate, seminal vesicles, and epididymis, atrophy and degeneration of the seminiferous tubules, and/or altered sperm measures and reduced fertility (David et al. 2000a; Dostal et al. 1988; Ganning et al. 1991; Gray and Butterworth 1980; Gray and Gangolli 1986; Kluwe et al. 1982a; Lamb et al. 1987; Oishi 1986, 1994; Parmar et al. 1987, 1995; Price et al. 1987; Sjoberg et al. 1986a, 1986b). The lowest reproductive effect levels in these studies are a NOAEL and LOAEL for testicular histopathology of 3.7 and 38 mg/kg/day, respectively, in rats exposed for 90 days (Poon et al. 1997), and 5.8 and 29 mg/kg/day, respectively, in rats exposed for 104 weeks (David et al. 2000a). Because the 14 mg/kg/day NOAEL in the critical study (Lamb et al. 1987) is higher than the NOAELs of 3.7 and 5.9 mg/kg/day (David et al. 2000a; Poon et al. 1997), and is based on an assessment of fertility rather than histological examination without evaluation of reproductive function, the 14 mg/kg/day NOAEL is the most appropriate basis for derivation of the intermediate-duration MRL.

Gestational and lactational exposure to DEHP has adversely affected the morphological development of the reproductive system, as well as caused reduced fetal and neonatal testosterone levels and adult sexual behavioral changes, in male rat offspring (Arcadi et al. 1998; Gray et al. 1999, 2000; Moore et al. 2001; Parks et al. 2000). One of these studies (Arcadi et al. 1998) was used as the basis of a provisional intermediate-duration oral MRL in a previous draft of the DEHP toxicological profile (i.e., the Draft for Public Comment). In the Arcadi et al. (1998) study, severe testicular histopathological changes were observed at 21–56 days of age in male offspring of rats that were exposed to DEHP in the drinking water at reported estimated doses of 3.3 or 33 mg/kg/day throughout pregnancy and continuing during postnatal days 1–21. The 3.3 mg/kg/day dose was classified as a serious LOAEL and was used to derive a MRL of

0.01 mg/kg/day by using an uncertainty factor of 300 (10 for the use of a LOAEL, 10 for interspecies extrapolation, and 3 for human variability). A component factor of 3 was used for human variability because DEHP was administered during the most sensitive period during development. The MRL was provisional because it was derived from a serious LOAEL, which is not conventional ATSDR methodology. The Arcadi et al. (1998) study is now judged to be inadequate for MRL derivation because the NTP-CERHR Expert Panel on DEHP (NTP 2000) concluded that the effect levels are reliable and are unsuitable for identifying a LOAEL. In particular, NTP (2000) found that (1) the methods used to verify and characterize the administered doses were not clearly described or completely reported, and could not be resolved, and (2) the study authors did not reconcile their blood DEHP concentration data with other studies.

The intermediate MRL of 0.1 mg/kg/day was derived by dividing the 14 mg/kg/day reproductive NOAEL by an uncertainty factor of 100 (10 for extrapolation from animals to humans and 10 for human variability). Additional information regarding the derivation of this MRL, including evidence supporting selection of the uncertainty factor, is provided in Appendix A.

- An MRL of 0.06 mg/kg/day was derived for chronic-duration oral exposure ($365 days) to DEHP.

The chronic MRL is based on a NOAEL of 5.8 mg/kg/day for testicular pathology in male rats from a comprehensive chronic toxicity study (David et al. 2000a). Groups of 50–80 rats of both sexes were fed DEHP in the diet for up to 104 weeks. Reported average daily doses were 0, 5.8, 29, 147, or 789 mg/kg/day in males and 0, 7.3, 36, 182, or 939 mg/kg/day in females. End points evaluated in all dose groups included clinical observations, food consumption, body and organ weights, and clinical pathology indices. Necropsy and histological examinations included the control and two highest dose groups after 78 weeks, the control and high-dose groups after 104 weeks, and target tissues and gross lesions from the remaining dose groups after 104 weeks. No exposure-related effects were observed at 5.8 mg/kg/day in the males or 7.3 mg/kg/day in the females. Bilateral aspermatogenesis was significantly (p#0.05) increased in the higher dose male groups, indicating that the LOAEL for testicular effects is 29 mg/kg/day. The incidences of bilateral spermatogenesis were 37/64 (58%), 34/50 (64%), 43/55 (78%), 48/65 (74%), and 62/64 (97%), showing a dose-related increase and consistency with a significant reduction in relative testes weight observed at 789 mg/kg/day (59% less than controls). Examinations at week 78 showed aspermatogenesis at 789,but not 147 mg/kg/day (no interim exams were performed in the lower dose groups), suggesting the possibility that the lesion was age- rather than treatment-related at 29 and 147 mg/kg/day. Also observed in the high dose males was an increased incidence of castration cells in

the pituitary gland, which are promoted by reduced testosterone secretions from the testes. Other significant changes were essentially limited to the liver, including increased liver weights accompanied by increased peroxisome proliferation in both sexes at $147 mg/kg/day, spongiosis hepatis in males at $147 mg/kg/day, and hepatocellular neoplasms in males at $147 mg/kg/day and in females at 939 mg/kg/day, but the mechanism for these hepatic effects is probably not relevant to humans as previously discussed in Hepatotoxicity and Liver Cancer (Section 2.2, Summary of Health Effects). A variety of renal changes were observed in all dose groups (e.g., increases in kidney weight, incidence and severity of mineralization of the renal papilla, and severity of normally occurring chronic progressive nephropathy and renal tubule pigmentation), but are unlikely to be toxicologically significant because they appeared to be age-related and/or species-specific. Body weight gain was significantly reduced throughout the study only in the high dose males and females (approximately 15% lower than controls at the end of the study).

The chronic MRL of 0.06 mg/kg/day was derived by dividing the 5.8 mg/kg/day testicular NOAEL by an uncertainty factor of 100 (10 for extrapolation from animals to humans and 10 for human variability). Additional information regarding the derivation of this MRL, including evidence supporting selection of the uncertainty factor, is provided in Appendix A.