Prenatal Exposure to Di(2-ethylhexyl) phthalate and Subsequent Infant and Child Health Effects

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Because fetuses are considered the most sensitive to di(2-ethylhexyl) phthalate (DEHP) exposure, the effects of maternal exposures during pregnancy on infant and child health were reviewed from recently reported studies. The major modes of exposure to this chemical are food and indoor air; hence, it remains important to decrease the risk of exposure from contaminated foods. Although DEHP metabolic profiles were not different between pregnant and non-pregnant women, metabolic rates appeared lower in late pregnancy. Maternal serum triglyceride, fatty acids, estradiol, and progesterone levels are normally higher in late pregnancy than in non-pregnancy and early pregnancy, whereas free triiodothyronine and thyroxin levels are low during late pregnancy. Previous epidemiological studies of the effects of maternal exposure to DEHP on the mother, infant, and child show that DEHP disrupts maternal lipid levels and influences infant and child endocrine hormone levels, infant birth parameters, child neurobehavioral development, maturation, and asthma. Because these studies are limited to children less than 10 or 13 years old, further longitudinal follow-up studies are warranted to determine the influences of prenatal DEHP exposures on lifestyle diseases. Additionally, animal studies are needed to reinforce associations between maternal exposure to DEHP and health effects in the subsequent generation, and to elucidate the corresponding mechanisms. The risk of exposure to DEHP during childhood is another important consideration because associations with thyroid function, puberty, and neurobehavioral development have also been observed.

Key words: Child health; di(2-ethylhexyl) phthalate; fetal malnutrition; infant health; prenatal exposure; pregnant women
1. Introduction

Di(2-ethylhexyl) phthalate (DEHP) is an excellent agent for improving plasticity and elasticity of plastic materials and is used globally. In Japan, the shipping volume of DEHP was 127,637 tons in 2013 and has varied little over the past five years, accounting for more than 50% of total phthalate esters. DEHP is widely used in various vinyl chloride products such as sheets, artificial leathers, wire coverings, agricultural vinyl films, pastes, coating materials, colorants, and adhesive agents. Because DEHP and other phthalates are contained in plastic materials without chemical bond, they can easily diffuse into the environment under high temperatures or during contact with hydrophobic materials, leading to ubiquitous environmental contamination.

Reportedly, estimated daily DEHP intake was 87%-89% from food, 9.5%-9.7% from indoor air, and 1.4%-2.0% from drinking water in a Canadian population, and the intake from ambient air and soil was considerably low. Thus, the general population is mainly exposed to DEHP via food, particularly fatty foods such as milk, including breast milk. Fish, fats, oils, and freeze-dried foods can also be highly contaminated with DEHP.

DEHP toxicities reportedly include hepatic carcinogenicity, nerve and immune toxicities, and disruptions of endocrine and reproductive systems. However, in vitro and in vivo genotoxicity assays were negative except for the induction of chromosomal aneuploidy and cellular transformation. Among these toxicities, hepatic carcinogenicity was the most important issue a decade and a half ago because the International Agency for Research on Cancer (IARC) classification of DEHP was downgraded from a group 2B carcinogen to a group 3 carcinogen. This decision reflected the absence of data showing effects on peroxisome proliferation in human hepatocyte cultures and non-human primate livers. However, the mechanisms behind the effects of DEHP on peroxisome proliferator-activated receptor (PPAR) α and subsequent carcinogenicity remain controversial. In particular, DEHP induced liver tumors were observed in PPARα-null mice, and the mechanism was different from that in wild-type mice. However, hepatic carcinogenicity of DEHP has been demonstrated in rats and mice, and carcinogenesis was observed in livers and other organs, such as pancreas and testis of DEHP-treated animals. Although little epidemiological evidence supports the association between DEHP and liver cancer in humans, IARC finally decided that there was sufficient evidence from experimental animals to consider DEHP as a carcinogen and up-graded DEHP to Group 2B in 2012.

In addition to carcinogenicity, recent studies of DEHP have been directed to its effects on reproduction and development, and infants are the most sensitive to this chemical because the detoxifying enzymes are not fully developed and hormonally active chemicals may affect childhood development. The Food Safety Commission of Japan assessed the risk associated with DEHP in 2013 and concluded that infants may be the most susceptible. The commission also estimated a tolerable daily intake of 0.03 mg/kg/day on the basis of no-observed-adverse-effect level (NOAEL) for shortening of anogenital distances (AGD) after exposure of pregnant rats to DEHP.

According to the Developmental Origins of Health and Disease (DOHaD) concept, nutritional status during early life, such as fetal, infant, and childhood environments, are considered effectors of later life health and disease risk. This concept was derived from the relationship between unbalanced nutrition in utero and increased risk of lifestyle diseases, such as coronary heart disease, hypertension, and type 2 diabetes. Accordingly, maternal undernutrition during pregnancy is associated with reduced birth weights, altered postnatal growth, and the development of hypertension in experimental animals. Moreover, maternal exposure to DEHP significantly decreased triglyceride (TG) and some fatty acid levels in the plasma of mice and led to decreased birth weights and plasma glucose and leptin levels in pups. Hence, the influence of maternal DEHP exposure on the health of offspring later in life remains an important area of investigation. Consequently, DEHP is a candidate chemical for investigation in the “Japan Environment and Children’s Study”.

In this review, we discuss the metabolism of DEHP during pregnancy and the effects of exposure during pregnancy on the mother, infant, and child health by referring to recent reports after the risk assessments in the IARC Monograph in 2012 and the Food Safety Commission of Japan in 2013.

2. Metabolism during Pregnancy

DEHP in the body is metabolized to mono(2-ethylhexyl) phthalate (MEHP) by lipase and is then oxidized by cytochrome P450 (CYP) and converted to various dicarboxylic acids by alcohol dehydrogenase (ADH) and aldehyde dehydrogenase (ALDH). In humans, orally ingested DEHP is metabolized by lipase in the small intestine and is absorbed as a form of MEHP. The main urinary metabolites in healthy adults include mono(2-ethyl-5-hydroxylhexyl)
phthalate (5OH-MEHP, 23.3%), followed by mono(2-ethyl-5-carboxypentyl) phthalate (5cx-MEPP, 18.5%), and mono(2-ethyl-5-carboxyl) phthalate (5oxo-MEHP, 15%), all of which are oxidative metabolites of MEHP. MEHP (5.9%) and the oxidative metabolite mono(2-carboxymethylhexyl) phthalate (2cx-MMHP; 4.2%) are excreted via urine as minor metabolites. Phthalic acid (PA) is also metabolized by the human CYP3A4 or rat CYP3A2, but information regarding PA detection in urine samples is not available.

Ito et al measured urinary metabolites in intact 129/SV male mice and reported that the major metabolite was 2cx-MMHP, which is an oxidative metabolite of the branched ethyl group. However, their analytical methods failed to distinguish 2cx-MMHP from PA, suggesting that PA was substituted for 2cx-MMHP. Choi et al investigated the in vitro metabolism of DEHP in human and rat tissues carrying various CYP isoforms. In their study, 5OH-MEHP, 5oxo-MEHP, and 5cx-MEPP were the major metabolites from MEHP in human liver microsomes, whereas only the first two were major metabolites in rat liver microsomes. These data indicate differing metabolic pathways in humans, mice and rats, and although MEHP is mainly oxidized at the fifth and sixth carbon position of the ethylhexyl ester side chain in humans, the sixth carbon position is rarely oxidized in rodent livers (Fig. 1). Human CYP2C9*1 and CYP2C19 and rat CYP2C6 are involved in oxidation of the fifth carbon position of the ethylhexyl ester group (5OH-MEHP formation), whereas human CYP2C9*1 and CYP2C9*2 oxidize the sixth carbon position (5cx-MEPP formation). Moreover, human CYP3A4 and rat CYP3A2 contribute to de-alkylation of MEHP to form PA in vitro.

Blood or urine metabolites are generally used as DEHP exposure biomarkers, and MEHP levels did not differ between the urine and serum in young Danish men. However, MEHP oxidative metabolites were present at considerably lower levels in the serum than in the urine. Recently, DEHP metabolite profiles were also investigated in the urine during pregnancy, and urinary metabolite profiles did not differ from those in healthy non-pregnant women in several countries. Braun et al also measured the urinary metabolites of DEHP in pre- and post-pregnancy samples from 113 women who gave live births and showed insignificant differences in total DEHP and MEHP oxidative metabolite concentrations (9% and 8%, respectively) between pre- and post-pregnancy. However, significant differences in MEHP concentrations were identified between pre- and post-pregnancy. Urinary MEHP, MEHP oxidative, and total DEHP metabolite concentrations all decreased over time during pregnancy. Arbuckle et al measured urinary DEHP metabolite concentrations in women in their first trimester of pregnancy and showed that urinary metabolite concentrations of DEHP were comparable or lower than those observed in a Canadian national population-based survey. Jia et al measured blood MEHP levels in 318 healthy pregnant women living in Sapporo, Japan, and reported significantly lower levels in women at 35–41 weeks of gestation than in those at 23–31 or 32–34 weeks. Ferguson et al also measured urinary DEHP metabolite concentrations at 4.71–16.1, 14.9–21.9, 22.9–29.3, and 33.1–38.9 weeks of gestation and showed significant decreases in specific gravity-corrected levels of all DEHP metabolites, including MEHP, 5OH-MEHP, 5oxo-MEHP, and 5cx-MEPP at later stages of pregnancy compared with those at 4.71–16.1 weeks. However, decreasing patterns differed slightly between the first two weeks and the last two weeks, and 5oxo-MEHP and 5cx-MEPP were only different at 22.9–29.3 weeks. Taken together, the urinary levels of DEHP metabolites in pregnant women were lower, particularly at the later stages of pregnancy, than those in non-pregnant women, and this finding should be considered when urinary metabolites are used as exposure biomarkers.

DEHP is mainly metabolized in the small intestine and liver, and is also metabolized by lung and kidney lipases. However, it remains unknown whether DEHP is metabolized to MEHP in the blood. A part of the formed MEHP is further metabolized by several CYP isoforms in human (CYP2C9*1, CYP2C9*2, and CYP2C19) and rat (CYP2C6). The remaining MEHP is conjugated by UDP-glucuronosyltransferase (UGT) and is excreted into the urine. Expression of CYP2C19 decreases during pregnancy, potentially contributing to decreases in urine 5OH-MEHP and 5oxo-MEHP. However, rat CYP2C6 expression did not change during pregnancy.

In pregnant mice, liver lipase activity was significantly higher than that in postpartum or non-pregnant mice, and it led to higher liver MEHP concentrations in pregnant mice exposed to DEHP than in postpartum mice. Conversely, as mentioned above, urinary DEHP metabolite levels were lower in pregnant women than those in non-pregnant women. Thus, the influence of pregnancy on DEHP metabolizing liver enzymes in mice may be different from that in humans.
3. Effects of Exposure to DEHP during Pregnancy on Maternal Health

3–1. Hormone Effects

Recent epidemiological studies show that maternal exposures to DEHP lead to disruptions of hormone or lipid levels. Moreover, Sathyanarayana et al.\textsuperscript{36} reported an inverse association between urinary total DEHP metabolite concentrations and serum levels of total or free testosterone in 180 pregnant women. It is of interest that this was observed even in 86 pregnant women with female fetuses. Johns et al.\textsuperscript{37} collected urine and serum samples at 16−20 and 24−28 gestational weeks and showed that serum progesterone, estradiol, and sex hormone-binding globulin concentrations were significantly higher at the latter time point, whereas free triiodothyronine (T3) and thyroxin (T4) concentrations were significantly lower. These data suggest that sex and thyroid hormone contents change during pregnancy. In addition, longitudinal associations showed a significant inverse relationship between urinary DEHP metabolites and serum-free T4 at the latter time point. Thus, DEHP exposure during pregnancy may influence maternal sex and thyroid hormones.

In a study of pregnant mice exposed to 0%, 0.01%, 0.05%, or 0.1% DEHP in the diet, progesterone and estradiol levels in ovaries were measured on gestational day 18.\textsuperscript{15} The numbers of live fetuses were significantly reduced in the 0.1% group. Moreover, the same dose tended to decrease estradiol levels in the ovary and increase progesterone levels. Accordingly, the highest dose significantly increased the ratio of progesterone to estradiol and may have reduced the number of live fetuses or altered the duration of the pregnancies as mentioned later.
3–2. Effects on Lipid Concentrations

Although no associations were found between blood MEHP concentrations and birth sizes\(^{30,38}\), maternal blood MEHP levels were inversely associated with maternal blood concentrations of TG, palmitic acid, oleic acid, linoleic acid, and \(\alpha\)-linoleic acid in 318 healthy pregnant women in Sapporo, Japan (Sapporo Cohort Study). Among these lipids, \(\alpha\)-linoleic acid was the most sensitive to the levels of MEHP, although blood concentrations of TG and these fatty acids were all increased with gestation. Similarly, Alvarez et al\(^{39}\) measured serum TG levels and lipoprotein lipase activity to hydrolyze TG in lipoproteins in 25 pregnant women at first (9–12 weeks), second (21–24), and third (32–35) trimesters, and at 2–6 weeks after childbirth. In this study, lipoprotein lipase activity decreased and TG concentrations increased from the second trimester, but both levels were restored to first-trimester levels at 2–6 weeks after childbirth. These studies suggest that serum TG levels, perhaps as with fatty acids, increase in pregnant women as pregnancy progresses. However, DEHP exposure during pregnancy disrupts these innate physiological changes in lipid levels.

Previous studies showed that serum TG and fatty acid concentrations were generally higher in pregnant mice and rats than in postpartum animals\(^{15,16,40}\). Moreover, exposure to 0.1\% dietary DEHP decreased TG, palmitic acid, and oleic acid levels, whereas exposure to 0.05\% and 0.1\% dietary DEHP decreased plasma linoleic acid and \(\alpha\)-linoleic acid levels, respectively, in pregnant wild-type mice at gestational day 18 and led to a decreased number of live pups at neonatal day 2\(^{15,16}\). In contrast, DEHP did not decrease these lipid levels or numbers of live pups in PPAR-null mice, suggesting a relationship between decreased maternal lipid levels and numbers of live pups. As shown in human studies\(^{39}\), \(\alpha\)-linoleic acid levels decreased in all mice fed 0.01\% DEHP-containing diets, whereas decreases in the concentrations of other fatty acids were only observed in the presence of 0.05\% dietary DEHP. DEHP decreased the number of live pups at a dose of 0.05\% and the number of live fetuses at a dose of 0.1\%, which were the lowest-observed-adverse-effect level (LOAEL) of live pups and fetuses, respectively, despite remaining significant effects on \(\alpha\)-linoleic acid levels at a lower dose (0.01\%) than the LOAEL. Further experiments indicated that decreased TG and fatty acid levels reflected suppression of the liver microsomal TG transporter protein following DEHP exposure\(^{15}\). Taken together, DEHP exposure leads to decreased concentrations of some fatty acids at lower exposure levels than are associated with adverse effects on fetuses, pups, and infants.

3–3. Effects on Gestational Period

Mother/infant studies indicate that the exposure to DEHP prolongs gestation\(^{24,41}\), although contrasting results have also been reported\(^{42–44}\). Specifically, Huang et al\(^{45}\) measured DEHP levels in cord bloods of 209 mother/child pairs and showed a positive association between DEHP concentrations and preterm births. In agreement, Ferguson et al\(^{46}\) recruited 130 pregnant women with preterm births (delivery before 37 gestational weeks) and made comparisons with 352 random control subjects. In this study, spontaneous preterm births were associated with total maternal DEHP metabolite concentrations at the beginning of the third trimester. These data conflicted with previous epidemiological relationships between DEHP exposure and gestation periods\(^{24,41}\), but showed positive associations between preterm births and DEHP exposure. Taken together, the recent two studies warrant investigations of DEHP-related changes in sex hormone levels in humans\(^{37}\) and mice, and assessments of the influences of placenta expression of nuclear receptors, such as PPAR\(\gamma\) and aromatic hydrocarbon receptors in experimental animals\(^{41}\), may offer important mechanistic insights.

4. Effects of Exposure to DEHP in utero on Infant Health

4–1. Effects on Birth Parameters

Multiple studies clarify relationships between prenatal maternal exposure to DEHP and infant health (Table 1). In particular, two prospective cohort studies reported no associations between DEHP exposure during pregnancy and infant weights, lengths, and head circumferences at birth\(^{24,47}\). Similarly, the Sapporo Cohort Study found that prenatal exposure to DEHP was not associated with infant size (length, weight, head, and chest circumference) at birth\(^{30}\). However, higher DEHP exposure during pregnancy was associated with low birth weights\(^{48}\). Moreover, in the study of 209 mother/child pairs by Huang et al\(^{45}\), DEHP concentrations in cord blood were inversely associated with fetal growth parameters and were positively correlated with birth weights of male infants and femur lengths of female infants. In this study, the mean DEHP concentration in cord blood was 187.16 \(\mu\)g/L (0.48 nmol/ml), which was 10-fold higher than maternal blood MEHP levels (0.028–0.046 nmol/ml) in the Sapporo Cohort Study, in which no association with infant
Table 1. Effects of DEHP exposure in utero on infant health

<table>
<thead>
<tr>
<th>Reference number</th>
<th>Authors</th>
<th>Publication</th>
<th>Population</th>
<th>Infant age</th>
<th>Subjects</th>
<th>Measurement of DEHP or its metabolites</th>
<th>End points</th>
<th>Infant health outcomes</th>
</tr>
</thead>
<tbody>
<tr>
<td>30</td>
<td>Jia et al.</td>
<td>2014</td>
<td>Sapporo, Japan</td>
<td>Infants</td>
<td>318 mother/infant pairs</td>
<td>Blood MEHP</td>
<td>Birth parameters</td>
<td>No significant association between MEHP and birth parameters</td>
</tr>
<tr>
<td>45</td>
<td>Huang et al.</td>
<td>2014</td>
<td>Chongqing, China</td>
<td>Infants</td>
<td>207 mother/child pairs</td>
<td>DEHP in cord blood</td>
<td>Fetal growth parameters</td>
<td>DEHP concentrations in cord blood were inversely associated with fetal growth parameters and were positively correlated with birth weight of male infants and femur lengths of female infants</td>
</tr>
<tr>
<td>51</td>
<td>Swan et al.</td>
<td>2015</td>
<td>USA</td>
<td>Infants</td>
<td>753 mother/infant pairs</td>
<td>MEHP, 5oxo-MEHP, 5OH-MEHP, 5cx-MEPP</td>
<td>AGD</td>
<td>Inverse association between maternal DEHP metabolites and AGD of boys</td>
</tr>
<tr>
<td>56</td>
<td>Araki et al.</td>
<td>2014</td>
<td>Sapporo, Japan</td>
<td>Infants</td>
<td>318 mother/infant pairs</td>
<td>Blood MEHP</td>
<td>Hormone in cord blood</td>
<td>Negative association between maternal MEHP levels and testosterone/estradiol, progesterone and inhibin B</td>
</tr>
<tr>
<td>57</td>
<td>Jensen et al.</td>
<td>2015</td>
<td>Denmark</td>
<td>Infants</td>
<td>270 cases of cryptorchidism, 75 cases of hypospadias and 300 controls</td>
<td>5cx-MEPP in amniotic fluid</td>
<td>Testosterone, insulin-like factor 3</td>
<td>No association between cryptorchidism or hypospadias and DEHP metabolites</td>
</tr>
<tr>
<td>60</td>
<td>Yolton et al.</td>
<td>2011</td>
<td>USA</td>
<td>5 weeks of age</td>
<td>350 mother/infant pairs</td>
<td>5cx-MEPP, 5OH-MEHP, 5oxo-MEHP, MEHP</td>
<td>Early infant neurobehavior</td>
<td>Positive association between DEHP metabolites and nonoptical reflexes in male infants</td>
</tr>
<tr>
<td>61</td>
<td>Kim et al.</td>
<td>2011</td>
<td>Korea</td>
<td>6 months of age</td>
<td>460 mother/infant pairs</td>
<td>5OH-MEHP, 5oxo-MEHP</td>
<td>MDI and PDI</td>
<td>Inverse association between MDI and maternal urinary SOH-MEHP and 5oxo-MEHP; PDI and 5OH-MEHP</td>
</tr>
</tbody>
</table>

AGD, anogenital distance; MDI, Mental Development Indices; PDI, Psychomotor Development Indices
size was reported\textsuperscript{30}. However maternal and cord blood MEHP levels (2.5 mg/L, 8.98 nmol/ml) were much higher in the study by Zhang et al\textsuperscript{48}, in which relationships with birth weights were observed. Taken together, high exposure to DEHP during pregnancy may influence birth weight and various birth parameters.

In addition to prenatal exposure to DEHP, premature neonates are highly exposed to DEHP from DEHP-containing endotracheal, orogastric and nasogastric tubes, continuous positive airway pressure devices, oxygen hoods, intravenous injections, blood transfusions, and umbilical venous catheterizations. Accordingly, Su et al\textsuperscript{49} collected urine samples from 32 premature neonates [20 with very low birth weights (<1500 g), 12 with low birth weights (<2500 g)] and 30 controls and showed significantly higher concentrations of MEHP, 5OH-MEHP, and 5oxo-MEHP in premature neonates treated with endotracheal, orogastric, or nasogastric tubes. In agreement, DEHP metabolite levels in premature neonates receiving intravenous injections were two-fold higher than those in healthy controls. Thus, the risks of adverse effects caused by DEHP in premature infants, particularly in those with nasogastric tubes, may be higher than those in patients with hemodialysis\textsuperscript{50}, for whom high DEHP exposures have been well documented.

4–2. Effects on Anogenital Distances (AGD)

Associations between in utero exposure to DEHP and shortened AGD have been reported in infants in the US and Japan\textsuperscript{51,52}. Although no such associations were observed in a Taiwanese study\textsuperscript{53}, Swan et al\textsuperscript{54} recently showed a significant inverse association between AGD of 753 infants and urine DEHP metabolite concentrations in their mothers living in the United States.

In pregnant Wistar rats, DEHP doses of 0, 3, 10, 30, and 100 mg/kg/day were orally administered on gestational days 7–16\textsuperscript{11}, and anti-androgenic effects, such as shortened AGD in male pups, were observed at doses of over 10 mg/kg/day, suggesting a NOAEL of 3 mg/kg/day. Thus, most epidemiological and animal studies show that in utero exposure to DEHP leads to shorter AGD.

4–3. Effects on Hormones and Related Organs

Because DEHP reduces androgen levels in men, women, and children\textsuperscript{55}, Araki et al\textsuperscript{56} investigated the association between maternal blood MEHP and reproductive hormone levels in cord blood in 514 pregnant women living in Sapporo, Japan. In this study, MEHP levels were significantly associated with reduced total testosterone and estradiol ratios, decreased inhibin B and progesterone levels in the cord blood, and with insulin-like factor 3 expressions in males. Hence, in utero DEHP exposure may have adverse effects on the development of both Sertoli and Leydig cells. Accordingly, Jensen et al\textsuperscript{57} studied 270 patients with cryptorchidism and 75 with hypospadias in comparison with 300 control infants. The determinations of the DEHP metabolite 5cx-MEPP in amniotic fluid during the second trimester in these cases revealed no association with the prevalence of cryptorchidism or hypospadias. However, higher testosterone and lower insulin-like factor 3 levels were observed in the highest 5cx-MEPP tertile compared with the lowest.

In animal studies, exposure to DEHP in utero also influenced fetal testis morphology and testosterone production. Specifically, rat fetuses that were exposed in utero to DEHP between gestational days 12 and 19 had aggregates or clusters of Leydig cells in interstitial spaces, multinucleated germ cells in seminiferous cords, and significantly reduced testosterone levels on gestational day 19\textsuperscript{58,59}. These data suggest that the testis is a target organ for phthalates in animals as well as humans.

4–4. Effects on Neurobehavioral Development

Prenatal exposure to DEHP has also been related to infant neurobehavioral development. Yolton et al\textsuperscript{60} recruited 350 mother/infant pairs and compared maternal urinary DEHP metabolites at 16 and 26 gestational weeks and NICU Network Neurobehavioral Scale (NNNS) scores of five week-old infants. In this study, prenatal exposure to DEHP at 26 weeks was associated with nonoptimal reflexes in male infants. In agreement, Kim et al\textsuperscript{61} recruited and measured maternal urinary DEHP metabolites at the third trimester in 460 expectant mothers, and calculated Mental and Psycho-motor Development Indices (MDI and PDI, respectively) of the Bayley Scales of Infants Development 2\textsuperscript{nd} edition at six months. In this study, MDI were inversely associated with the gestational urinary 5OH-MEHP and 5oxo-MEHP levels, and PDI were inversely associated with urinary 5OH-MEHP. Taken together, these observations indicate that prenatal exposure to DEHP influences neurobehavioral development in infants.

Although prenatal DEHP exposure influences birth parameters, hormones, and neurobehavioral development, the exposure was significantly lower in infants than in mothers in a human study\textsuperscript{62} and four-fold less in mouse pups than in their mothers\textsuperscript{35}. 

76

Nakajima T. et al: Prenatal DEHP Exposure and Child Health
4-5. Effects on Offspring Lipids, Glucose and Leptin

Although no epidemiological evidence is available, maternal lipid undernutrition in mice may lead to undernutrition in fetuses and offspring. Because 0.1% DEHP in the diet decreased total fatty acid concentrations in plasma to half of that in non-exposed pregnant mice, it likely induced maternal fatty acid undernutrition. However, maternal DEHP exposure did not reduce TG and fatty acid concentrations in fetuses and pups. Nonetheless, plasma glucose and leptin levels in offspring were significantly decreased in comparison with those in offspring of control mice with normal lipid levels, suggesting that maternal exposure to DEHP induces lipid deficiencies in mothers, but that it induces glucose and leptin deficiencies in offspring.

5. Effects of Exposure to DEHP in utero on Child Health

 Associations of prenatal exposure to DEHP with child health are listed in Table 2.

5–1. Effects of DEHP on Hormones and Sexual Maturation

DEHP is known to possess anti-androgenic properties that influence early development, and may affect the onset of puberty. Watkins et al. investigated the effects of in utero and peripubertal exposure to DEHP on female sexual maturation. Specifically, urine samples were collected from 113 mothers during the third trimester and subsequently from their 8–13 year-old children. Interquartile range increases of DEHP metabolites in maternal urine were positively associated with higher [95% confidence interval (CI); 9.2%–52.6%] dehydroepiandrosterone sulfate concentrations, which are an early indicator of adrenarche, and a 5.3-fold (95% CI; 1.13–24.9) higher odds ratio of a Tanner stage >1 for pubic hair development. However, no significant differences in associations between peripubertal DEHP metabolites and maturity indices were observed, suggesting that prenatal DEHP exposure influences child maturation.

5–2. Effects on Asthma

Whyatt et al. determined urine concentrations of the DEHP metabolite 5OH-MEHP in 300 pregnant inner-city women of Columbia during their third trimesters of pregnancy and assessed associations with asthma and asthma-like symptoms in 5–11 year-old offspring using a questionnaire survey. However, no significant associations were observed between asthma symptoms and maternal prenatal 5OH-MEHP concentrations. Gasscon et al. investigated the relationship between prenatal exposure to total DEHP metabolites in urine during first and third trimesters and the relative risks of wheeze, chest infections, and bronchitis in children of 6 and 14 months and 4 and 7 years of age using a questionnaire survey of mothers. Their data showed increasing relative risks of wheeze and bronchitis at four and seven years with the increase in maternal concentrations of these DEHP metabolites. However, cumulatively these studies are insufficient to conclude whether or not prenatal exposure to DEHP influences asthma in children.

5–3. Effects on Neurobehavioral and Mental Development

To determine associations between prenatal exposure to DEHP and neurodevelopment in children, Téllez-Rojo et al. measured maternal urinary DEHP metabolites during pregnancy in 136 mothers and assessed mental and psychomotor development in their 24–36 month old children. Negative associations between MDI and the DEHP metabolites MEHP, 5oxo-MEHP, and 5cx-MEPP were observed in girls but not in boys. Similarly, Polanska et al. determined 165 maternal urinary DEHP metabolites in women at 30–34 gestational weeks and evaluated neurodevelopment in two year-old children in a Polish mother/child cohort. In this study, child psychomotor development was assessed using the Bayley Scales of Infant and Toddler Development, third edition, and inverse associations with 5OH-MEHP, 5oxo-MEHP, and total DEHP metabolites were identified. Whyatt et al. also evaluated associations between DEHP metabolite concentrations in maternal prenatal urine from 319 women during the third trimester and MDI and PDI, and behavior problems were assessed from the maternal report using the Child Behavior Checklist. However, no associations were found between maternal DEHP metabolite concentrations and MDI, PDI, and behavioral problems in three year-old children.

Kobrosly et al. measured DEHP metabolites in 153 urine samples of pregnant women at a mean gestational age of 26.6 ± 7.2 weeks and applied Child Behavior Checklist to mothers when their children were 6–10 years old. These
<table>
<thead>
<tr>
<th>Reference numbers</th>
<th>Authors</th>
<th>Publication year</th>
<th>Population</th>
<th>Infant age</th>
<th>Subjects</th>
<th>Measurement of DEHP or its metabolites</th>
<th>End points</th>
<th>Child health outcomes</th>
</tr>
</thead>
<tbody>
<tr>
<td>68</td>
<td>Whyatt et al.</td>
<td>2012</td>
<td>Colombia</td>
<td>3 years of age</td>
<td>319 mother/child</td>
<td>MEHP, SOH-MEHP, 5oxo-MEHP, 5cx-MEPP</td>
<td>Mental, psychomotor, and behavioral development</td>
<td>No association between DEHP metabolite concentrations and MDI, PDI and behavior problems</td>
</tr>
<tr>
<td>63</td>
<td>Watkins et al.</td>
<td>2014</td>
<td>Mexico</td>
<td>8–13 years of age</td>
<td>113 mother/female child pairs</td>
<td>MEHP, SOH-MEHP, 5oxo-MEHP, 5cx-MEPP</td>
<td>Maturation (hormone and Tanner stage)</td>
<td>Positive association between all DEHP metabolites and higher dehydroepiandrosterone sulfate; higher odds of having Tanner stage &gt;1 for pubic hair development in MEHP</td>
</tr>
<tr>
<td>64</td>
<td>Whyatt et al.</td>
<td>2014</td>
<td>Colombia</td>
<td>5–11 years of age</td>
<td>300 mother/child</td>
<td>5OH-MEHP</td>
<td>Asthma</td>
<td>No association between diagnosis of asthma and 5OH-MEHP</td>
</tr>
<tr>
<td>65</td>
<td>Gascon et al.</td>
<td>2015</td>
<td>Spain</td>
<td>6 and 14 months, 4 and 7 years of age</td>
<td>657 mother/child pairs</td>
<td>MEHP, SOH-MEHP, 5oxo-MEHP, 5cx-MEPP</td>
<td>Wheeze, chest infections, bronchitis</td>
<td>Total of DEHP metabolites was associated with increased relative risks of wheezing and bronchitis</td>
</tr>
<tr>
<td>66</td>
<td>Tellez-Rojo et al.</td>
<td>2013</td>
<td>Mexico City, Mexico</td>
<td>24–36 months</td>
<td>136 mother/child</td>
<td>MEHP, SOH-MEHP, 5oxo-MEHP, 5cx-MEPP in urine</td>
<td>Mental and psychomotor development indexes (MDI and PDI) from a Bayley test</td>
<td>Negative association between MDI and DEHP metabolites in girls</td>
</tr>
<tr>
<td>67</td>
<td>Poianska et al.</td>
<td>2014</td>
<td>Poland</td>
<td>2 years of age</td>
<td>165 mother/child</td>
<td>MEHP, SOH-MEHP, 5oxo-MEHP</td>
<td>Psychomotor development</td>
<td>Negative associations between maternal 5OH-MEHP, 5oxo-MEHP and total DEHP metabolites and motor development</td>
</tr>
<tr>
<td>69</td>
<td>Kobrosly et al.</td>
<td>2014</td>
<td>USA</td>
<td>6–10 years of age</td>
<td>153 mother/child</td>
<td>MEHP, SOH-MEHP, 5oxo-MEHP, maternal urinary</td>
<td>Neurobehavioral development</td>
<td>Positive association between total of DEHP metabolites and scores of somatic problems</td>
</tr>
<tr>
<td>70</td>
<td>Lien et al.</td>
<td>2015</td>
<td>Taiwan</td>
<td>8 years of age</td>
<td>122 mother/child</td>
<td>MEHP, SOH-MEHP, 5oxo-MEHP</td>
<td>Behavior</td>
<td>Positive associations between DEHP metabolites and externalizing problem, delinquent behavior and aggressive behavior</td>
</tr>
</tbody>
</table>

CBCL: Child Behavior Checklist score; ADD: Attention deficit disorder; LD, Learning disability; MDI, Mental Development Indices; PDI, Psychomotor Development Indices
studies showed that total DEHP metabolite concentrations were associated with higher scores for somatic problems in boys. Similarly, Lien et al. recruited 122 mother/child (eight year-old) pairs from the general population in central Taiwan and measured MEHP, 5OH-MEHP, and 5oxo-MEHP in maternal urine that was collected during all three trimesters. Subsequently, behavioral syndromes in eight year-old children were evaluated using the Child Behavior Checklist. In this study, associations between chemical exposure and behavioral syndrome, such as withdrawal, somatic complaints, anxiety/depression, social problems, thought problems, attention problems, delinquent behaviors and aggressive behaviors, and intelligence quotients (IQ) were assessed using the Chinese version of the Child Behavior Checklist and the Wechsler Intelligence Scale for Children-version III. The ensuing analyses showed that externalizing problem scores were significantly increased with 1-unit increases in log10-transformed maternal MEHP and 5oxo-MEHP concentrations after adjustment by creatinine (µg/g). In contrast, no associations were identified between prenatal exposure to DEHP and child IQ at 7 years in a prospective study of 328 mother/child pairs, although inverse associations between child IQ and other phthalates, such as di-n-butyl phthalate and di-isobutyl phthalate, were identified. Taken together, in utero DEHP exposure may cause neurodevelopment deficits in young children.

6. Health Effects of Childhood Exposures to DEHP

6–1. Effects on Hormones

Several epidemiological studies suggest that prenatal and childhood exposures to DEHP may disturb normal development, because urinary levels of metabolites of DEHP in children are reportedly higher than those in adults. However, although five reports show effects of DEHP exposure on child health, the effects of prenatal exposures were not concomitantly investigated (Table 3). Boas et al. reported a negative association between total DEHP metabolites in 845 4–9 year-old children and serum T3 and T4 levels in girls and serum insulin-like growth factor 1 (IGF-1) expression in boys. They concluded that DEHP exposure negatively impacts child health. Similarly Wu et al. recruited 60 ≤ 10 year-old Taiwanese children with Tanner stage 1 and no pubic hair who were potentially exposed to phthalate-contaminated foodstuffs on May 31, 2011. Subjects were divided into high exposure (n = 29, exposure to >500 ppm of DEHP in contaminated foodstuffs), low exposure (n = 23, DEHP 1–500 ppm), and no exposure (n = 8, DEHP <1 ppm) groups, and endocrine hormone levels were determined in 22 children (13 high exposure, 6 low exposure, and 3 no exposure) six months after the exposure date. Among these children, serum thyroid-stimulating hormone levels were significantly lower in high and low exposure groups than in the no-exposure group. After six months, T3 levels were significantly lower in the high exposure group, and estradiol levels were also decreased in 10 of 13 children in the high exposure group. However, no changes were observed in low and no-exposure groups. In a similar longitudinal study of 168 healthy 5.9–12.8 year-old children (84 girls), serum adrenal androgen levels and urinary DEHP metabolite concentrations were examined every six months for five years. The authors also investigated pubertal development and showed that girls with the highest DEHP metabolite excretions had lower body weights and reduced expression of luteinizing hormone and IGF-1 at 10 years of age (girls, n = 47).

Wolff et al. enrolled 6–8 year-old girls and determined DEHP metabolite concentrations in urine samples from 1,170 subjects. They also assessed breast and pubic hair stage and body size once or twice a year to determine the age at transition from stage 1 to 2 for breast and pubic hair. Among normal-weight girls, age of pubic hair stage 2 was 9.5 months later in the fifth quintile than in the first quintile of total urinary DEHP metabolites.

6–2. Effects on Neurobehavioral Development

Chopra et al. measured phthalate concentrations in urine samples from 1,493 children with parent-reported information on psychosocial disorders. There were 112 subjects with attention deficit disorder (ADD), 173 subjects with learning disability (LD), and 56 subjects with both ADD and LD. Subsequent analyses showed higher rates of ADD but not LD in children with higher urinary DEHP concentrations, indicating an association between DEHP exposure and ADD. Although numbers of studies are limited, the report of Chopra et al indicates that DEHP exposure during childhood has significant effects on endocrine and sex hormones and on neurobehavioral development.
In this review, we summarized epidemiological findings and related animal studies to determine the health effects of exposure to DEHP on pregnant women, infants, and children. Taken together, the present studies referred to in this review show that DEHP exposures influence infant and child birth sizes, AGD, endocrine hormones, and neurodevelopment.

### Table 3. Effects on DEHP exposure in childhood on child health

<table>
<thead>
<tr>
<th>Reference numbers</th>
<th>Authors</th>
<th>Publication year</th>
<th>Population</th>
<th>Infant age</th>
<th>Subjects</th>
<th>Measurement of DEHP or its metabolites</th>
<th>End points</th>
<th>Infant health outcomes</th>
</tr>
</thead>
<tbody>
<tr>
<td>72</td>
<td>Boas et al.</td>
<td>2010</td>
<td>Denmark</td>
<td>4–9 years of age</td>
<td>845 children</td>
<td>DEHP-exposed group 29, 6 month follow-up group 23 and non-exposed group 8</td>
<td>Thyroid function, IGF-1 and growth</td>
<td>Negative association between DEHP metabolites and thyroid hormone or IGF-1</td>
</tr>
<tr>
<td>73</td>
<td>Wu et al.</td>
<td>2013</td>
<td>Taiwan</td>
<td>≤10 years</td>
<td>845 children</td>
<td>DEHP exposure by the phthalate-containing foodstuffs</td>
<td>Adrenal androgen levels</td>
<td>Lower serum TSH and T3</td>
</tr>
<tr>
<td>74</td>
<td>Mouritsen et al.</td>
<td>2013</td>
<td>Denmark</td>
<td>5.9–12.8 years of age</td>
<td>168 healthy children</td>
<td>Categorized DEHP exposure</td>
<td>Negative association between DEHP metabolites and androgen levels</td>
<td></td>
</tr>
<tr>
<td>75</td>
<td>Wolff et al.</td>
<td>2014</td>
<td>USA</td>
<td>6–8 years old</td>
<td>1239 girls</td>
<td>MEHP, 5ox-MEPP, SOH-MEHP, 5oxo-MEHP in urine</td>
<td>Breast and pubic hair stages, body size</td>
<td>Age at pubic hair stage 2 was 9.5 months later for 5 year-old girls compared with first quartile of urinary sum of DEHP metabolites.</td>
</tr>
<tr>
<td>76</td>
<td>Chopra et al.</td>
<td>2014</td>
<td>USA</td>
<td>6–15 years of age</td>
<td>1496 children</td>
<td>Urinary DEHP</td>
<td>Positive association between DEHP and ADD</td>
<td></td>
</tr>
</tbody>
</table>

IGF-1, insulin-like growth factor 1; TSH, thyroid-stimulating hormone; T3, triiodothyronine.
opment. In addition, phthalate exposures can compromise lipid variables, resulting in undernutrition of mothers and contrasting glucose-related malnutrition in offspring. Exposures to DEHP also disrupted endocrine hormone levels in mothers. However, no studies have assessed the effects of maternal DEHP exposures in the ensuing adult men and women. In future studies, the influences of exposures on adult diseases will be examined in the context of DOHaD, and direct or indirect effects of neonatal nutrition status will be elucidated. Further longitudinal epidemiological studies are required to reinforce the present associations, and further experimental studies using laboratory animals are warranted.

Conflict of Interest Statement

The authors declare that no conflicts of interest.

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