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# **Follow-Up Study of Adolescents Exposed to di-2-Ethylhexyl Phthalate (DEHP) as Neonates on Extracorporeal Membrane Oxygenation (ECMO) Support**

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## **Abbreviations:**

DEHP: di-2-ethylhexyl phthalate

PVC: Polyvinyl chloride

ECMO: Extracorporeal membrane oxygenation

NICU: Neonatal intensive care unit

IRB: Institutional review board

LH: Luteinizing hormone

FSH: Follicle stimulating hormone

T4: Thyroxine

TSH: Thyroid stimulating hormone

AST: Aspartate aminotransferase

ALT: Alanine aminotransferase

GGT: Gamma-glutamyl transpeptidase

BUN: Blood urea nitrogen

MEHP: Mono-ethylhexyl phthalate

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**Abstract:**

di-2-ethylhexyl phthalate (DEHP) is used to make polyvinyl chloride (PVC) plastic tubing soft and flexible. Based on animal data, adverse effects of DEHP exposure may include reduced fertility, and sperm production in males and ovarian dysfunction in females. Known treatments that involve high DEHP exposures are blood exchange transfusions, ECMO and cardiovascular surgery. Although potential exposure to DEHP in ECMO patients is significant, it has not been associated with short-term toxicity. To evaluate long-term toxicity, we undertook a study of neonatal ECMO survivors to assess their onset of puberty and sexual maturity. Thirteen male and 6 female subjects at age 14-16 years who had undergone ECMO in the neonatal period were evaluated. All subjects had a complete physical examination including measurements for height, weight, head circumference and pubertal assessment by Tanner staging. The testicular volume and the phallic length were measured in male participants. Laboratory tests included thyroid, liver and renal function as well as measurements of LH, FSH, testosterone for males and estradiol for females. With the exception of one patient with Marfan syndrome, the rest had normal growth percentile for age and sex. All had normal values for thyroid, liver and renal functions. Sexual hormones were appropriate for the stage of pubertal maturity. Our results indicates that adolescents exposed to significant quantities of DEHP as neonates showed no significant adverse effects on their physical growth and pubertal maturity. Thyroid, liver, renal, male and female gonadal functions tested were within normal range for age and sex distribution.

## **Introduction:**

Human exposure to di-2-ethylhexyl phthalate (DEHP) occurs throughout life. Of particular concern is the exposure of fetuses, preterm infants and babies, because the developing human reproductive system may be affected when the metabolic pathways of detoxification are immature. DEHP has been shown to damage the male and female reproductive systems in newborn animals. Animal studies have shown DEHP to be particularly harmful to developing fetuses. Adverse effects in the reproductive system include changes in the testes, specifically the Sertoli cell, leading to reduced fertility and changes in sperm production in males (Foster et al. 2001; Park et al. 2002; Poon et al. 1997) and ovarian dysfunction and decreased hormone production in females (Davis et al. 1994; Lovekamp-Swan and Davis 2003). Respiratory distress, and changes in kidney and liver function have also been linked to DEHP exposure (Crocker et al. 1988; Kevy and Jacobson 1982; Latini 2000; Rock et al. 1987 Roth et al. 1988; Ward et al. 1998).

DEHP is a family of chemicals called phthalates. These chemicals are used to make polyvinyl chloride (PVC) plastic tubing soft and flexible. Because DEHP does not bind to the plastic, it can leach out of the PVC products. DEHP is widely used in PVC disposable medical devices. As in other products, DEHP can leach out of flexible PVC medical devices into the solution or medication it contains and subsequently into the patient (Rubin and Schiffer 1976).

Species differences in toxicity and metabolism of DEHP have created considerable debate about the relevance of studies in rodents to human health. However exposure in neonatal intensive care units (NICU) are potentially at or in excess of levels known to cause adverse health effects in

relevant animal studies. For infants requiring intensive care, DEHP exposure can occur at three order of magnitude greater than average adult exposures and at or above levels shown to cause adverse reproductive effects in animals.

DEHP concentrations in blood and blood products are of particular concern for neonates who receive regular blood transfusions. The most commonly used blood products, packed red blood cells and plasma are typically stored in DEHP plasticized bags and administered to patients through DEHP plasticized intravenous tubes. Less common treatments that involve potentially high DEHP exposures are blood exchange transfusions and extracorporeal membrane oxygenation (ECMO). Although potential exposure to DEHP in ECMO patients is significant, it has not been associated with short-term toxicity. To evaluate long-term toxicity, we undertook a study of adolescents who had previously undergone ECMO treatment in the neonatal period to assess their onset of puberty and sexual maturation in comparison to an age and sex matched reference population.

## **Methods:**

The study was approved by the Institutional Review Board (IRB) at Children's National Medical Center. After obtaining informed consent and assent, 19 (13 male and 6 female) adolescents age 14-16 years who had undergone ECMO in the neonatal period were evaluated in this prospective study. All subjects had a complete physical examination including measurements for height, weight, head circumference and pubertal staging according to the method of Tanner (Morris and Udry 1980; Tanner 1975). In addition, the testicular volume and the phallic length were measured in all male participants. Laboratory tests included measurements of thyroid function (TSH, Free T4 by dialysis, T4), liver function (AST, ALT, GGT, total and direct bilirubin), renal

function (BUN and Creatinine) as well as measurements of LH, FSH, testosterone for males and estradiol for females.

### **Results:**

With the exception of one female participant with diagnosis of Marfan syndrome, the rest had normal growth percentiles for age and sex. All the participants had normal laboratory values for thyroid, liver and renal functions. The levels of LH, FSH, testosterone in males and estradiol in females were normal and appropriate for the degree of pubertal development. Results of the sex hormones related to pubertal maturation are shown in tables 1 & 2 as mean values (normal ranges).

### **Discussion:**

Our study did not show long-term adverse outcome related to physical growth and pubertal development in adolescents previously exposed to DEHP in the neonatal period. This is in contrast to the animal data in multiple species which show a variety of reproductive and developmental toxicities when this plasticizer is administered both orally and parenterally.

Individuals that have among the highest exposures to DEHP are those undergoing certain medical treatments or procedures such as dialysis, exchange transfusion, ECMO and cardiovascular surgery. Sheinder, et al. have shown that babies undergoing extracorporeal membrane oxygenation (ECMO), in which the blood is circulating through PVC tubing, are exposed to 42 to 140 mg DEHP/kg body weight over a treatment period of three to ten days (Shnieder et al. 1989). Karle, et al. (1997) reported a lower level of exposure that ranged from non-detectable to 34.9 mg/kg/treatment period. The non-detectable level resulted from the use of



a DEHP plasticized PVC circuit that was coated with heparin. In addition to the heparin coated tubing, Karle, et al. attributed the difference between their study and Shneider's to the smaller surface area of the newer ECMO configurations and varying percentage of DEHP composition in each type of tubing.

Although intravenous exposure to DEHP through ECMO circuit or other intravenous routes exceeds recommended oral exposure limits, direct comparison between the two are difficult because of both temporal, an assumed lifetime daily oral exposure in contrast to acute temporary exposure during ECMO therapy and route of exposure, oral versus IV (Doull et al. 1999). Because the human exposure overlaps the doses that are toxic in rodents, there is an ongoing concern that exposure to DEHP in neonatal intensive care units may adversely affect the developing reproductive organs in these infants (Huber et al. 1996). The most sensitive system appears to be the immature male reproductive tract, especially the Sertoli cell (Parks et al. 2000; Poon et al. 1997).

When DEHP enters the human body, the compound is metabolized into various substances that are more rapidly excreted. The most important of these metabolites, mono-ethylhexyl phthalate (MEHP) is thought to be responsible for much of DEHP's toxicity. The enzymes that break down DEHP into MEHP are found mainly in the intestines but also occur in the liver, kidney, lungs, pancreas, and plasma. Because conversion of DEHP to MEHP occurs primarily in the intestinal tract, exposures to DEHP by ingestion may be more hazardous than by intravenous exposure, which largely bypasses the intestinal tract (Huber et al. 1996; Lewandowski et al. 1980; Thomas et al. 1979).

In conclusion our study of adolescents exposed to significant quantities of DEHP as neonates showed no significant adverse effects of DEHP on their physical growth and pubertal maturity. Thyroid, liver, renal, and male and female gonadal functions tested were within normal range for age and sex distribution. We hypothesize that the acute and short-term exposure to DEHP in an intravenous form and lack of significant conversion of DEHP to MEHP may be protective against its long term side effects

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**Table 1:** Results of sexual hormones in female subjects matched for Tanner stage. Mean value (normal reference range)

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<b>Females (n)</b>	<b>Tanner Stage</b>	<b>LH (IU/L)</b>	<b>FSH (IU/L)</b>	<b>Estradiol (pg/mL)</b>
<b>4</b>	<b>4</b>	<b>6.05 (0.72-15.01)</b>	<b>4.58 (1.26-7.37)</b>	<b>48.75 (25-345)</b>
<b>2</b>	<b>5</b>	<b>3.7 (0.30-29.38)</b>	<b>2.65 (1.02-9.24)</b>	<b>118.5 (25-410)</b>

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LH; Luteinizing hormone, FSH; Follicle stimulating hormone, IU/L; International unit per liter, Pg/mL; Picogram per milliliter.

**Table 2:** Results of sexual hormones, testicular volume and phallic length in male subjects matched for Tanner stage. Mean value (normal reference range)

Male (n)	Tanner Staging	LH (IU/L)	FSH (IU/L)	Testosterone (ng/dL)	Testicular Volume (ml)	Phallic length (cm)
4	2-3	1.83 (0.26-3.74)	2.40 (0.72-10.37)	119 (15-280)	11 (5-10)	8.0 (6.3-8.6)
9	4-5	3.02 (0.55-7.00)	3.61 (1.70-7.00)	387 (105-800)	22 (20-29)	11.2 (8.6-9.9)

LH; Luteinizing hormone, FSH; Follicle stimulating hormone, IU/L; International unit per liter, ng/dL; Nanogram per deciliter, ml; Milliliter, cm; Centimeter.