Di (2-ethylhexyl) phthalate (dehp) hormone levels in males

Introduction

Di (2-ethylhexyl) phthalate (DEHP) is one of phthalate ester and endocrine-disrupting chemical. It is used as plasticizer in Polyvinyl chloride (PVC) plastics tubing to make PVC plastic tubing more soft and flexible. Animal data show that target organs of exposure to DEHP are the liver, kidneys, lungs, and reproductive system. DEHP may cause adverse effects of animal's reproductive system such as reduced fertility, decreased spermatogenesis in males (Rais-Bahrami, 2004). Phthalate monoesters including mono-2-ethylhexyl phthalate (MEHP) is the DEHP metabolites. The primary targets of Phthalate monoesters toxicity are Leydig cells (LCs) and sertoli cells (SCs). The function of LCs and SCs is spermatogenesis and testosterone production. The toxicant will damage the LCs to decrease the secretion of testosterone, which will affect spermatogenesis and decrease the sperm count.

Food is one of the sources of exposure to DEHP for public (U. S. EPA, 2013). DEHP has been detected in many foods such as meat, cheese, milk, eggs, cereal products and fish. There is more and more evidence showing that DEHP induce many adverse effects on the reproductive system such as reproductive hormones included luteinizing hormone (LH), follicle-stimulating hormone (FSH) and free testosterone (Giuliana G. 2009). As DEHP can cause birth defects or reproductive harm, the government of California listed DEHP as reproductive toxin in 2003 (Chemical Market Reporter, 2003).

In addition to the food intake exposure to DEHP, occupational and environmental are the another sources of exposure to DEHP (Guowei Pan et. al., 2006). It is essential to concern whether high levels of DEHP exposure

would affect reproductive hormone levels in male. According to the studies of the United States Food and Drug Administration (FDA) and the National Institute of Occupational Safety and Health, they have found that DEHP can cause birth defects in laboratory rodents (Chemical Market Reporter, 2003). However, only limited studies focus on the relationship between human reproductive hormone and DEHP exposure (J. Mendiola et. al., 2011). Hence, there are limited evidences to conclude a correlation between human reproductive hormone and exposure to DEHP. It is important to find out what chemicals will influence human reproductive hormone because it decides the total number of our offspring.

Therefore, based on the related human studies, the effects of DEHP exposure on reproductive hormone levels in male would be discussed.

Method

The aim of the literature search was to find the associations between Di (2-ethylhexyl) phthalate (DEHP) and reproductive hormone levels in male. A board research was first conducted by Medline (EBSCO HOST), Web of Science and ProQuest. The search term “ Di (2-ethylhexyl) phthalate/ DEHP” AND “ reproductive hormones” was used and excluded the term “ animal.”

The criteria used to select the studies were as follows:

- The Studies were primary sources and published within 10 years, 2004-2014.
- The Studies must be human study for adult and NOT animal study.

- The Studies should address the associations between DEHP and reproductive hormone levels in male.
- The studies were in good quality, such as in high impact factors of 2013.

3 main studies met the above criteria for the topic examination. The impact factor on the 3 studies are 3. 206 (G. Pan et. al, 2011), 3. 206 (J. Mendiola et. al, 2010) and 7. 029 (Guowei Pan et. al, 2006). Totally 3 main studies at the end of literature has been searched. The three chosen studies were Cross-sectional study since there are limited long-term studies such as cohort studies and randomized controlled trial (RCT) fulfilled the above criteria.

Result

G. Pan et. al. (2011) carried a cross-sectional study to find the dose-response relationship between dose-response relationships between cumulative phthalate exposure and reproductive hormones in human. 74 male workers occupationally exposed to high levels of DEHP and 63 male construction workers as comparison group matched for age and smoking status. The urine and blood were collected from each patient on the same day. The authors of study observed a significantly negative correlated between hazard indices (HI) and serum concentrations of free testosterone (fT) in exposed workers (r = -0. 195, p = 0. 096), but not in unexposed workers.

J. Mendiola et. al. (2011) carried a cross-sectional study to find the association between exposures to Di (2-ethylhexyl) phthalate and reproductive hormone levels in men. 425 men were partners of pregnant women who were selected to participant in the study for Future Families

(SFF). All men completed a questionnaire and received a physical examination. The questionnaire included the question about demographics, recent fever, history of sexually transmitted disease, diet and as well as lifestyle factors such as smoking, alcohol and caffeine consumption. The urine sample was collected within 15 min after the blood was collected. The result was found that Free Androgen Index (FAI) was significantly associated with the concentration of DEHP metabolites in urine. However, total testosterone levels were no associated with urinary concentration of DEHP metabolites.

Guowei Pan et. al. (2006) carried a cross-sectional study to find the effect of occupational exposure to high levels of phthalate esters on the balance of gonadotropin and gonadal hormones, which including LH, FSH, free testosterone (fT), and estradiol. 74 male workers at a factory producing unfoamed PVC flooring exposed to di-n-butyl phthalate (DBP) and di-2-ethylhexyl phthalate (DEHP) as exposure group and compared them with samples from 63 male workers from a construction company as comparison group matched for age and smoking status. Urine and blood samples were collected from each workers between 800 and 1100 hours on the same day, but not on the first day of the worker’s work week or the day after a night work shift. The authors of study observed there is significant reducing of serum fT in workers with higher levels of urinary MEHP (DEHP metabolites) compared with unexposed workers. fT was negative association to MEHP (r = -0. 19, p = 0. 095) in the exposed group.

Discussion

Study Design

The three studies are a cross-sectional study. The limitations of cross-sectional studies are it cannot establish a temporal relationship and reflect the causal relationship between high levels of DEHP and the reproductive hormone levels in male. It only can provide a possible risk factor for reproductive hormones for further study.

The three studies both have different advantages and disadvantages. The sample size of the study of Guowei Pan et. al. (2006) and G. Pan et. al. (2011) is relative small. They are all less than 100 people in exposed group and comparison group. The study of J. Mendiola et. al. (2011) has relative large sample size (N= 425) but it does not contain a comparison group.

The study of Guowei Pan et. al. (2006) and G. Pan et. al. (2011) only investigated the confounding effect of the influence of occupational exposure to DEHP. The study of J. Mendiola et. al. (2011) also only investigated the confounding effect of the influence of environmental exposure to DEHP. They did not considered the confounding effect of the influence of food intake exposure to DEHP.

Study population

Randomization is a basic and essential technique to avoid selection bias in Epidemiology Study and It can show better association between exposure and outcome is true or not.

However, the participants of three studies are not strictly random. It may cause false association between high exposure of DEHP and male

reproductive hormone. In the study of Guowei Pan et. al. (2006) and G. Pan et. al. (2011), the authors wanted to compare the reproductive hormone levels between exposed group and comparison group. Therefore, they selected exposed group in a factory producing unfoamed PVC flooring and using DEHP as plasticizers and comparison group in a construction company. The authors can ensure the exposed group was exposed to DEHP by dermal contact and/ or through dust inhalation in their work. A comparison group who without occupational exposure to DEHP and matched for age and smoking status to exposed group. The comparison group can help authors to exclude other factors that may influence reproductive hormone levels in male in addition to DEHP exposure.

The study of J. Mendiola et. al. (2011) did not contain a comparison group. All the participants were conducted in the study for Future Families and at prenatal clinics affiliated with university hospital in five United States cities.

Exposure assessment

The concentration of MEHP and reproductive hormone were determined by using different methods. Both methods have advantages and limitations.

Table 1: The methods used to determine the concentration of MEHP

Study	Methods (the concentration of MEHP)

Guowei Pan et. al, (2006)	liquid chromatography-MS/MS (LC-MS/MS)
G. Pan et. al. (2011)	liquid chromatography-electrospray-ionization tandem mass spectrometry (LC-ESI-MS/MS)
J. Mendiola et. al. 2011	high-performance liquid chromatography with tandem mass spectrometry detector (HPLC-MS/MS)

Three studies used similar method to determine the urinary concentrations of DEHP metabolites. Thus, the urinary DEHP metabolite concentration of three studies will not have significant difference.

Table 2: The methods used to measure the levels of reproductive hormone

Study	Reproductive Hormones	Method
Guowei Pan et. al, (2006)	Plasma levels of LH, FSH, fT, and oestradiol (E_2)	Radioimmunoassay
J.	• Serum	• Time-resolved

Mendiola et. al. (2011)	levels of FSH and LH • Testosterone • E_2	immunofluorometric assay (TR-IFMA) • Time-resolved fluoroimmunoassay • Radioimmunoassay
G. Pan et. al. (2011)	• Plasma fT • Plasma LH, FSH and fT • Plasma E_2	• Radioimmunoassay with in-tube solid phase microexcretion technique • Chemiluminescence enzyme immunoassay (CLIA) • Electrochemilumines cence enzyme immunoassay (ECLIA)

The advantages of using radioimmunoassay are high sensitivity and highly specific. However, using radioimmunoassay method is very dangerous because it uses radiolabelled reagents. Thus, the labs require specially trained person and special license to handle radioactive material. The following methods have less dangerous than radioimmunoassay.

Chemiluminescent immunoassay (CLIA) is it has been applied broadly to the clinical diagnosis and environmental analysis. It can show the result quickly

because it does not require long incubation time (Ayio, K. 2010). Also, CLIA has high specificity and relatively simple and inexpensive instrumentation (J. L. S. ARAÚJO-FILHO et. al 2011). An electrochemiluminescence immunoassay (ECLIA) procedure was based on solid-phase sandwich immunoassay method. Thus, ECLIA has the superior sensitivity to determine plasma and urine sample (Xiao-yan, C. 2013). The advantages of TR-IFMA are higher sensitivity due to lower background values, higher specificity as only intact molecules of FSH and LH can be measured. (Van Casteren JI et. al 2000)

Result analysis

The result of J. Mendiola et. al. (2011) in addition to showing the relationship between reproductive hormone in male and the concentration of DEHP metabolite (N= 425), it also found some factors such as age, Body Mass Index (BMI) and smoking etc may influence the reproductive hormone in male. Therefore, J. Mendiola et. al. (2011) had done multivariate analysis for reproductive hormone in male and the concentration of DEHP metabolite (N= 363) to control for factors such as age, BMI and smoking states etc. It can increase the accuracy of the studies. Although the study did not contain a comparison group, J. Mendiola et. al. (2011) tried consistent the factors found in the questionnaire of participants to minimize the influence of confounding factors to the data analysis.

G. Pan et. al. (2011) was using hazard indices (HI) to show the result. The hazard index is the sum of hazard quotients. The ratio of hazard index is compared to 1. 0. If the ratio is less than 1, then the systemic effects are assumed not to be of concern; if the hazard quotient is greater than 1, then

the systemic effects are assumed to be of concern (U. S. EPA, 2012). It is more easily to show the result and indicate whether DEHP exposure as the concerned factors for reproductive hormone to public and epidemiology.

In addition to showing the relationships between serums free Testosterone in workers and the exposure to high levels of DEHP, Guowei Pan et. al. (2006) also compared the demographic characteristics including age, marriage status, smoking and alcohol consumption and plastic material contact of exposed group (N= 74), comparison group (N= 63) and all the workers (N= 137). Also, all the results of this study were compared to exposed group and comparison group. The result can be shown the different of reproductive hormone levels between exposed group and comparison group easily.

Limitation of stud ies

The data and result of the three studies were limited by using a single urine and blood/serum samples to determine the levels of DEHP and hormone. It may cause a bias in spot sampling because the result is not reflecting average hormone levels. The endogenous serum LH and FSH concentration had significant change by assessing in different time. The urine samples only reflect the recent exposure to DEHP because phthalates have short half-lives.

Also, the concentration of DEHP in three studies was based on the concentration of urinary metabolites of DEHP (MEHP). Therefore, the concentration of DEHP may be not the real concentration of participants.

Conclusion

The result of three study indicate that there are adverse effects of high levels of DEHP exposure on reproductive hormone levels in male. However, there is no clear evidence to prove a causal relationship between high levels of DEHP and the reproductive hormone levels in male because cross-sectional studies cannot reflect the causal relationship between them. High level of DEHP can as an investigative target included in long term studies such as cohort study and randomized controlled trial of reproductive hormone levels in male. The further research is required to prove that high levels of DEHP exposure will decrease the reproductive hormone levels in human.