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## ASSESSMENT OF THE HEALTH RISKS OF THE EXPOSURE TO ORGANOPHOSPHATE AMONG MALES

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Albokhary, M. S.<sup>(1)</sup>; Radwan, O. M.<sup>(1)</sup>; Awad Allah, Hala, I.<sup>(1)</sup>;  
Sallam, Nancy, M.<sup>(1)</sup> and Sultan, Eman, A.<sup>(2)</sup>

1) Institute of Environmental Studies and Research, Ain Shams University

2) Department of Clinical Nutrition, National Nutrition Institute

### ABSTRACT

Over the past 20 years, a great deal of attention has focused on the impact of endocrine disruptors released in the environment on animal and human health. Generally, endocrine disruptors have estrogenic activity. These products interfere with hormone biosynthesis, metabolism, or action resulting in a deviation from normal homeostatic control or reproduction. Our primary objective was to evaluate the possible role of environmental estrogen on sexual disturbance among males.

**Methods:** A cross-sectional study recruited 26 apparently healthy males characteristics of life style, symptoms covering various systems, general examination including, body mass index, waist line, resting blood pressure were done. Venous blood samples were used for detection of both total testosterone & plasma (Butyryl choline esterase (BuChE) activity.

**Results:** the mean age was  $(36.54 \pm 6.04)$  years with mean BMI of  $(28.98 \pm 5.16)$  kg/m<sup>2</sup>. , mean waist  $(102.78 \pm 11.18)$  cm. 66.7% of the cases were married with 13.3% of them have fertility problem & 20% of sample have sexual problem. 66.7% of the studied group use pesticide at home. The main findings of this study were, regarding hormonal profile; nearly all studied group had hormonal values and acetylcholine esterase (AChE) within the reference range but there is a negative association between organophosphate (OP) pesticides exposure assessed by the AchE as indicator and serum total testosterone levels. AchE was also negatively associated with fruit & vegetables consumption/week; whereas there is a positive association with age.

**Key words:** Organophosphate, xenoestrogen, Acetyl choline esterase (AChE), male hormones, total testosterone .

## INTRODUCTION

A major challenge for life scientists in the 21st century is to understand how a changing environment impacts all life on earth. Exposure to environmental chemicals which have major risks for human by targeting different organs in the body has significant impacts on biological systems. For several years there have been a great amount of interest on the environmental endocrine disruptors (EEDs) and their relation with human health (Li *et al.*, 2016).

Endocrine-disrupting compounds (EDCs) are synthetic and naturally occurring chemicals that may interfere with endogenous endocrine action;

Exposure to EDCs has been associated with reproductive problems, obesity, diabetes, cancers, and behavioral and learning disorders (WHO/UNEP 2013). Several substances are thought to cause endocrine disruption; most EDCs are classified as xenoestrogens whereas others inhibit androgen production and function ([Meeker, 2012](#)).

The potential for deleterious effects of EDC must be considered relative to the regulation of hormone synthesis, secretion, and actions and the variability in regulation of these events across the life cycle. Because endocrine systems exhibit tissue-, cell-, and receptor-specific actions during the life cycle, EDC can produce complex, mosaic effects. This complexity causes difficulty when a static approach to toxicity through endocrine mechanisms driven by rigid guidelines is used to identify EDC and manage risk to human and wildlife populations (Zoeller *et al.*, 2012).

The mechanisms of EDCs involve divergent pathways among them including (but not limited to) estrogenic, antiandrogenic, steroidogenic enzymes; neurotransmitter receptors and systems; and many other pathways that are highly conserved in wildlife and humans (Diamanti-Kandarakis *et al.*, 2009).

One class of EDCs is the environmental pesticides that broadly classified in to organophosphorus (OPs), organochlorine, carbamates (CBs) and pyrethroids (Haque *et al.*, 2012). OPs are one of the most widely used classes of pesticides that being employed for both agricultural and landscape pest control. The use of OP has increased considerably due to their low toxicity and low persistence in the mammalian system compared to organochlorine pesticides (Kamath&Rajini, 2007). Due to the similarity with human physiological function, these chemicals can harm normal human body (Makelarski *et al.*, 2014). OP pesticides are esters of phosphoric or thiophosphoric acid, whose toxicity has been related to their ability to inhibit acetylcholinesterase activity causing acetylcholine accumulation at the nerve synapse level (Ecobichon & Klaassen 2001) and this enzyme, has been used the most in enzymatic detection of these pesticides.

Because of existing concerns about the anti-androgenic activity of environmental OPs, we evaluated the possible role of environmental estrogen on sexual disturbance among males.

**Aim of the Work:** To evaluate the association between environmental exposure to organophosphate (OP) measured by AchE and evaluating its possible role on sexual disturbance among males.

**Methods:** A cross-sectional study that recruited 26 apparently healthy males that agreed to participate in the study after explained details about the study and investigation that will be done and signed the informed consent for the study.

**Inclusion Criteria:**

- Apparently healthy males aged from 20-45 years.
- Ability to provide written informed consent.
- Able to comply with the procedures of the study protocol.

**Exclusion Criteria:**

- Has been diagnosed with diabetes or endocrinal problems such as hypogonadism, pituitary problems, hypothalamic or thyroid problems.
- Poorly controlled hypertension.
- Subjects with major illness such as cancer, hepatic, psychiatric or cardiac diseases.

**Methods:** This study carried on apparently healthy males from relatives and males attending courses in national nutrition institute from 12/2016 till 2/2017.

**1-Medical Assessments** including family history of chronic non-communicable diseases, symptoms covering various systems, general examination including blood pressure measurements, pulse, chest, heart and abdominal examination.

**a. Anthropometric Measurements:** Weight and height: Body weight was measured with scale to the nearest 0.1 kg, with the subject wearing only light clothing. Height was measured to the nearest 0.5 cm with the subject

standing on the floor without shoes with the back straight against the wall (Jelliffe & Jelliffe, 1989). BMI was calculated as the ratio of the weight and height squared (kg/m<sup>2</sup>) and assessment of BMI was done using categories reported by (WHO, 1995).

**Waist circumference:** Waist was measured with the subject in standing position at the top of the iliac crest to the nearest 0.5 cm (NHLBI Obesity Education Initiative, 2000) and hip circumference was measured as the maximum circumference over the buttocks to the nearest 0.5 cm (WHO, 2008).

**B-Biochemical Measurements:** Venous blood samples were drawn into heparinized tubes and plasma was separated by centrifugation of blood samples at 3000 rpm for 10 minutes.

- Plasma BuChE activity was measured with a Test-mate ChE Randox kit from the hydrolysis of butyrylcholine iodide, and data were expressed as micromoles per minute per milliliter of plasma (U/ml). Evaluation of Test-mate ChE results was based on AChE and BuChE inhibition associated with different degrees.
- Quantitative determination of Testosterone (ng/ml) in human serum by enzyme immunoassay using commercially available kit DRG® Testosterone ELISA (EIA-1559), the DRG® testosterone ELISA Kit is a solid phase enzyme-linked immunosorbent assay (ELISA), based on the principle of competitive binding normal range (3-11 ng/ml) (Tietz, 1986) .

**Statistical Analysis:** Number and percent (%) for categorized or numerical data. Statistical analysis Continuous variables were expressed as means + SD. To establish differences between continuous variables, The Student's t test

was applied to detect differences between the means of two normally distributed populations. The degree of association between variables was evaluated based on Pearson's or Spearman's correlation coefficient. Statistical analysis was performed with Statistical Package for the Social Sciences (SPSS) (Version 13.2, 2003, Echsoft Corp., USA). A level of ( $P < 0.05$ ) was accepted as statistically significant, and a level of ( $P < 0.001$ ) was accepted as statistically highly significant.

### RESULTS

Table (1) & (2) present the general characteristics of the study population, the mean age was ( $36.54 \pm 6.04$ ) years with mean BMI of ( $28.98 \pm 5.16$ )  $\text{kg/m}^2$ , mean waist ( $102.78 \pm 11.18$ ) cm. 66.7% of the cases were married and 13.3% of those married have fertility problem & 20% with sexual problem. 66.7% of the studied group use pesticide at home. The mean serving intake of fruit & vegetables /week were ( $2.65 \pm 2.90$ ) serving. Regarding hormonal profile, nearly all studied group had total testosterone & AchE values within the reference range with mean of ( $11.54 \pm 2.62$ ) & ( $8847.58 \pm 1908.13$ ) (U/ml) respectively. When correlated AchE levels with other variables there was a significant positive correlation with age ( $P = 0.008^{**}$ ) but, a significant inverse correlation with total testosterone fig (1) with ( $P = 0.010^*$ ) & with fruit & vegetables consumption/week ( $P = 0.024^*$ ) table (3).

**Table (1):** Characteristics of the study population

Variable	Mean± SD
Age/years	36.54 ± 6.04
Weight/kg	86.17 ± 15.12
Height/cm	172.57 ± 6.15
BMI kg/m <sup>2</sup>	28.98 ± 5.16
Waist/ cm	102.78 ± 11.18
Hip/cm	106.53 ± 8.44
Waist /Hip Ratio	0.94 ± 0.05
Systolic blood pressure/mmHg	133.00 ± 12.84
Diastolic blood pressure/mmHg	84.70 ± 9.58
Fruit & vegetables consumption/week	2.65 ± 2.90
Hemoglobin(gm/dl)	13.86 ± 1.03
Random blood glucose (mg/dl)	90.0 ± 14.31
Acetylcholine esterase (U/ml)	8847.58 ± 1908.13
Total testosterone(ng/ml )	11.54 ± 2.62

**Table (2):** Characteristics of the study population

Variable		%
Married		66.7
Fertility problem		13.3
Number of Shaving/months	1	4.2
	3	12.5
	4	16.7
	5	20.8
	11	45.8
Week Muscle power		9.1
Sexual problems		20.0
Use of pesticide		66.7
Fruit & vegetable consumption /week	0.5	8.7
	1.0	30.4
	2.0	30.4
	3.0	8.7
	4.0	13.0
	7.0	4.3
	14.0	4.3

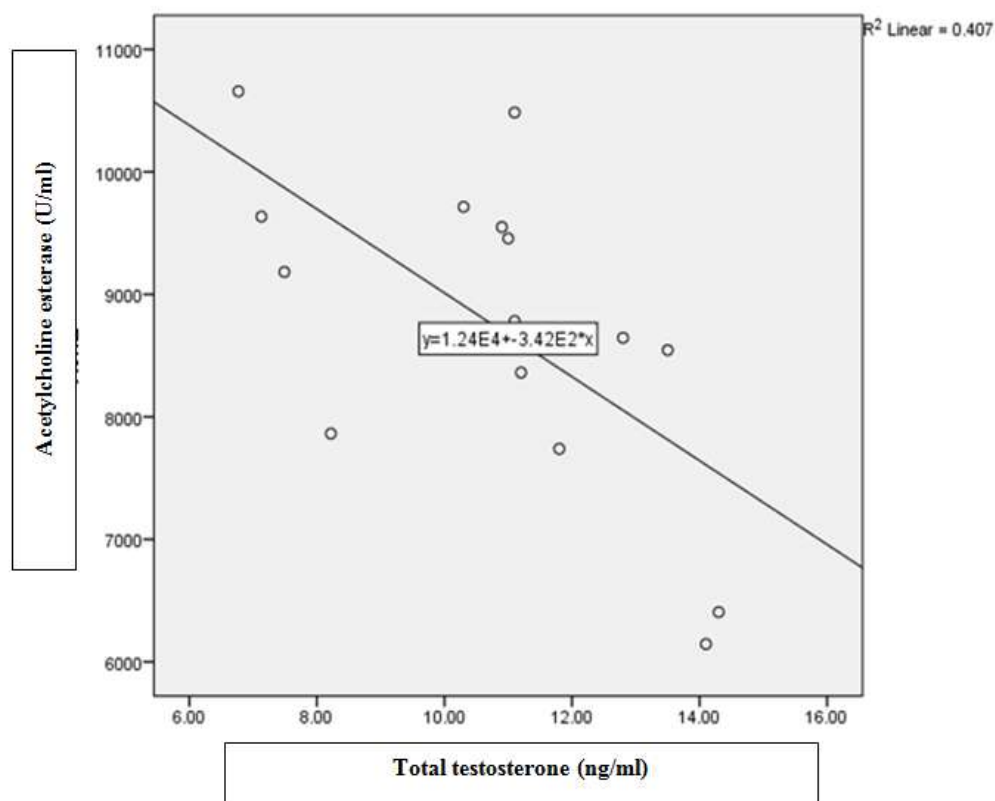
**Table (3):** Correlations between AchE and other variables

Variable	r	P
Age/years	0.576**	0.008**
Weight/kg	0.332	0.165
Height/cm	-0.326-	0.173
BMI kg/m2	0.423	0.071
Waist/ cm	0.359	0.131
Hip/cm	0.375	0.152
Waist /Hip Ratio	0.037	0.892
Systolic blood pressure/mmHg	0.319	0.182
Diastolic blood pressure/mmHg	0.229	0.345
Hemoglobin/gm/dl	-0.020	0.933
Random blood glucose (mg/dl)	0.101	0.672
Total testosterone(ng/ml)	-0.638 *	0.010*
Number of shaving/month	-0.058	0.813
Fruit & vegetables consumption/week	-0.514*	0.024*

\*A level of (P<0.05) was accepted as statistically significant,

\*\*A level of (P<0.001) was accepted as statistically highly significant\*



**DISCUSSION**

**Figure (1):** Correlation between testosterone and AchE

There is substantial toxicological evidence that repeated low-level exposure to organophosphate (OP) pesticides may have potential adverse health effects (Eskenazi *et al.*, 1999). In the past years, there has been increased interest in assessing the relationship between impaired male fertility and environmental factors. Human male fertility is a complex process and therefore a great variety of sites may be affected by exogenous harmful mediators. Lifestyle factors as well as various environmental and

occupational agents may impair male fertility. Many studies have been published reporting on reproductive dysfunctions in male animals and humans (Pflieger-Bruss *et al.*, 2004). However, relevant epidemiology studies in men are limited.

In our study nearly all studied group had total testosterone values and AChE within the reference range but there is a negative association between OP pesticides exposure assessed by the AchE as indicator and serum total testosterone levels. The same reported by, Straube *et al.* (1999), who showed that testosterone levels decrease during acute exposure whereas its levels are increased during chronic exposure. These findings are also consistent with studies on rats, in which subchronic exposure associated with an increase in serum testosterone (Sarkar *et al.*, 2000), another study that evaluated the effect of exposure to organophosphate pesticides on male hormone profile showed that most hormonal values fell within the wide normal range and recommends the need for further investigation to elucidate their biological and clinical relevance (Blanco-Munoz *et al.*, 2010). But Soliman *et al* (2008) & Arafa *et al.*, (2013) in their studies among Egyptians farmers& workers showed that chronic exposure to organophosphate can cause endocrine disrupting effects with low testosterone in exposed group. On the other hand, Kamijima *et al* (2004) found a significantly increased concentration than normal of serum testosterone concentrations after organo- phosphorous exposure during the off-season

Altered serum reproductive hormone levels have also been reported in epidemiology studies of OP insecticide exposure conducted in men (Meeker *et al.*, 2008) ,for example, serum testosterone levels were inversely related to urinary biomarkers of OP insecticide exposure in cohorts of men from a U.S. fertility clinic (Meeker *et al.*, 2006).

In a recent study evaluated urinary concentrations of OP in relation to serum concentrations of testosterone among 356 men aged 20-55 years old from the U.S. National Health and Nutrition Examination Survey. Biomarkers were detected in greater than 50% of the samples. In adjusted regression models, they observed a statistically significant inverse relationship between OP and total serum testosterone levels (Omoike *et al.*, 2015) .Studies conducted in rodent species have also demonstrated decreases in T following exposure to the OP. This analysis represents a unique contribution to the state-of-the-science on the potential link between exposure to OP insecticides and male reproductive hormones (Aguilar-Garduño *et al.*, 2013).These findings add to the limited evidence that exposure to certain OP insecticides is linked to altered T in men, which may have important implications for male health (Omoike *et al.*, 2015).

There is a growing concern about the endocrine effects of long-term, low-level exposure to organophosphate (OP) compounds. Studies on experimental animals have found that OP pesticides have an impact on the endocrine system and a few clinical and epidemiological studies have also shown that OPs may affect the male hormone profile, although results are inconsistent. OP pesticides may have an impact on the endocrine function because of their potential to modify the male hormone profile as a function of

the type of pesticide used as well as the magnitude of exposure (Aguilar-Garduño *et al.*, 2013).

Although many studies suggest the need to put into effect regulations regarding the proper use of pesticides on vegetables due to the high levels and wide assortment of pesticide residues found in vegetables (Armah, 2011) & (Akan *et al.*, 2013).

As the primary route of exposure to OPs is via ingestion of contaminated food (Lu *et al.*, 2008); in contrast to our expectation, AchE was also negatively associated with fruit & vegetables consumption/week.

In a study evaluating the pesticide residues in some Egyptian fruits, the most detected pesticide groups were Pyrethroids, 27.8% followed by Organophosphates (OP's) 24.6%, On the other hand, OP's are the most

violated group. Data showed that pesticides which not recommended for using in tested fruits were detected in frequency of 76.1% of the findings as well as pesticides revoked by Egyptian authorization (Gad Alla *et al.*, 2015).

In a study on the pesticide residues in food from Egyptian local market none of the detected organophosphorus pesticides found in citrus fruits exceeded their maximum residue level (MRL) (Dogheim *et al.*, 1996).

Available evidence suggest that there is a possibility of adverse effects occurring below OP compounds concentrations that are generally considered to be safe based on measurement of AchE inhibition (Singh & Sharma, 2000 and Salvi *et al.*, 2003). Delayed effects of pesticide are illnesses or injuries that do not appear immediately (within 24 hours) after exposure to a pesticide. Adverse effects may be delayed for weeks, months or compound,

dosage and exposure time (Jors *et al.*, 2006) and the health effects of low dose pesticides exposure are very difficult to evaluate mostly when pesticides mixtures are used (Lewalter &Leng, 1999 and Carpy *et al.*, 2000).

Although we did not directly assess consumption of food, organically grown foods have been reported to have significantly lower pesticide residues than conventionally grown foods (Baker *et al.*, 2002; Lu *et al.*, 2006). A study conducted among the members of a Danish association of organic farmers reported that sperm concentration was 43.1% higher among men eating organically produced food (Jensen *et al.*, 1996).

There is substantial evidence that dietary antioxidant supplementation and/or increased ingestion of fruit and vegetable may play a role in neutralizing or buffering the effects of pollutants. In vitro and in vivo studies suggest that antioxidant nutrients and related bioactive compounds common in fruits and vegetables can protect against environmental toxic insults (Poljšak &Fink, 2014). A study carried out by Zheng *et al.*, 2015 shown that fruit-vegetable dietary pattern may reverse oxidative stress and even genetic damage. The key mechanism of pesticides' cytotoxicity may be linked to their pro-oxidative potential, including damage by free radical oxidation of cellular components such as membrane lipids, protein and DNA and antioxidants may attenuate the pesticides-induced cell toxicity (Ilboudoa *et al.* 2014; Poljšak and Fink 2014).However, because our assessment of pesticide based on AChE, rather than on direct measurement of pesticides, further confirmation of these findings is warranted. Thus, we are unable to test the role that this potential bias may play in our findings.

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## تقييم المخاطر الصحية الناجمة عن التعرض للفوسفات العضوي في الرجال

[٢]

محمود سري البخاري<sup>(١)</sup> - أسامة محمد رضوان<sup>(١)</sup> - هالة إبراهيم عوض الله<sup>(١)</sup>  
نانسي محمد سلام<sup>(١)</sup> - إيمان أحمد سلطان<sup>(٢)</sup>

(١) معهد الدراسات والبحوث البيئية، جامعة عين شمس ٢) المعهد القومي للتغذية

### المستخلص

على مدى العشرين سنة الماضية أولى العلماء قدرا كبيرا من الاهتمام لتأثير المواد المسببة لاختلال الغدد الصماء المنتشرة في البيئة على صحة الإنسان والحيوان وتتميز هذه المواد بان لها نشاطا استروجيني فهي تتداخل مع التركيب الحيوي الهرمون أو التمثيل أو العمل مما يؤدي إلى الانحراف عن السيطرة الطبيعيه أو اعاده إنتاج الهرمون. وكان الهدف الأساسي لدينا هو تقييم الدور المحتمل لهرمون الاستروجين البيئي على الاضطراب الجنسي لدى الذكور.

الطريقة: دراسة مستعرضة استهدفت ٢٦ من الذكور الأصحاء وقد استطلعنا خصائص نمط الحياة، والأعراض التي تغطي مختلف الاجهزه، والفحص العام بما في ذلك قياس ضغط الدم، ومؤشر كتلة الجسم، محيط الخصر واستخدمت عينات من الدم الوريدي للكشف عن كل من هرمون التستوستيرون الكلي (T testosterone) و الاستيلكولينسترز (AChE) في البلازما .

النتائج: كان متوسط العمر (٣٦,٥٤ ± ٦,٠٤) سنة مع متوسط مؤشر كتلة الجسم (٢٨,٩٨ ± ٥,١٦) كجم / م٢ ، محيط الخصر (١٠٢,٧٨ ± ١١,١٨) سم. كانت ٦٦,٧٪ من الحالات متزوجين و ١٣,٣٪ لديهم مشكلة في الخصوبة و ٢٠٪ يعانون من مشاكل جنسية مرتبطة باختلال الهرمونات. ٦٦,٧٪ من العينة يستخدمون المبيدات الحشرية في المنزل. وكان متوسط تناول الفواكه والخضروات / الأسبوع (٢,٦٥ ± ٢,٩٠) وحده. وكانت أهم نتائج هذه الدراسة، هي ان مستويات الهرمونات الذكرية كانت في المعدلات الطبيعيه وكذلك الأستيلكولينسترز ولكن كان هناك ارتباط سلبي بين التعرض المبيدات (الأستيلكولينسترز كمؤشر) ومستوى هرمون التستوستيرون في الدم. وكما ارتبط الأستيلكولينسترز سلبا مع معدل تناول الفاكهة والخضار؛ في حين أن هناك علاقة إيجابية مع التقدم في السن.

الكلمات الدالة: الفوسفات العضوي، الإستيروجنات الصناعية، أستيلكولينسترز، الهرمونات الذكرية، هرمون التستوستيرون الكلي.