



EFFECT OF PENTACHLOROPHENOL FROM SAW DUST ON CHOLESTEROL AND SOME REPRODUCTIVE HORMONE LEVELS

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ABSTRACT

Pentachlorophenol (PCP) is an industrial wood preservative. It can be absorbed through the lungs, skin and gastrointestinal tract, the skin being the most significant route. This study determined the serum testosterone, estradiol and cholesterol levels of sawmill workers exposed to PCP and sawdust. A total of 50 male subjects exposed to sawdust and PCP (test group) and 50 apparently healthy non-Sawmill workers and who do not have any contact with PCP were recruited as control. Testosterone and estradiol were assayed using Enzyme-linked immunosorbent assay (ELISA) method. Total Cholesterol level was determined using cholesterol enzymatic end-point method while Low density lipoprotein cholesterol (LDL-C) was determined using clearance method. Data obtained were analyzed using student t- test. The results showed that the mean values of serum testosterone levels of subjects exposed to sawdust and PCP was significantly lower ($P < 0.05$) when compared with the control. The mean serum estradiol level of subjects exposed to sawdust and PCP was significantly higher ($P < 0.05$) when compared with the control, while the mean values of cholesterol and LDL-C, respectively were significantly higher ($P < 0.05$) in test group when compared with the control. This study showed significantly increased levels of estradiol, total cholesterol, LDL-C and reduced level of testosterone on subjects exposed to sawdust and PCP. This suggests a predisposition to reproductive hormone imbalance and cardiovascular disease.

KEYWORDS: Pentachlorophenol, Saw dust, Testosterone, Estradiol, Total Cholesterol, Low density lipoprotein, Enzyme-linked immunosorbent assay, Sawmill workers, Nnewi.

INTRODUCTION

Pentachlorophenol (PCP) is a chlorinated aromatic fungicide that is commercially manufactured by direct chlorination of phenol with chlorine gas. It is a structurally halogenated hydrocarbon, composed of a benzene ring to which is attached a hydroxide radical making a chlorinated phenol. Over the years, PCP has been the prevalent industrial wood preservative (Colosio *et al.*, 1993). PCP can be absorbed through the lungs, skin and gastrointestinal tract, the skin being the most significant route (Kallioski and Kauppinen ACGIH, 2001). Absorbed PCP is excreted primarily in urine, with elimination half-life of between 4 and 72 days (Bingham and Bethany, 1991). The hypothesis has been put forward that exposure to certain persistent synthetic chemicals, which are known to interact with the mammalian endocrine system, may cause adverse effects on reproductive function in wildlife and humans (Colborn *et*

al., 1993; Sharpe and Skakkebaek, 1993). People working in sawmills are regularly exposed to the harmful effects of sawmill chemicals and carcinogens as well as other occupational risk factors that may have hazardous effects on some hormones such as estradiol, testosterone as well as on cholesterol levels. Estradiol is a sex hormone with two hydroxyl groups in its molecular structure. Estradiol is present in males, being produced as an active metabolic product of testosterone. It has a critical impact on reproductive and sexual functioning. It also affects other organs, like the bones. In males, it functions to prevent apoptosis of male sperm cells (Pentikäinen, 2000). Testosterone plays a key role in men in the development of male reproductive organs such as the testes and the prostate as well as promoting secondary sexual characteristics for example, increased muscle, bone mass and growth of body hair (Cox and John, 2005; Reed *et al.*, 2006). There is scarce

information as regards hormonal changes associated with exposure to sawdust and pentachlorophenol in the study area. Hence, the goal of this study is to determine the effect of exposure to sawdust and pentachlorophenol (PCP) on testosterone, estradiol and cholesterol levels in Nnewi, Anambra State, Nigeria.

MATERIAL AND METHODS

Study Area

This study was carried out in Nnewi, Nnewi North Local Government area of Anambra State, South Eastern, Nigeria. Nnewi is a commercial city with a large auto spare parts market and the residents are mainly business men and women as well as Health workers (Doctors, Medical Laboratory Scientists, Nurses, Imaging Scientists, Pharmacists, Physiotherapists etc.) who work at the Nnamdi Azikiwe University Teaching Hospital and other private and Mission Hospitals located in Nnewi.

Study Design

The study was an experimental study designed to assess the serum testosterone, estradiol, total cholesterol and low density lipoprotein cholesterol of subjects exposed to PCP and saw dust.

Subject Recruitment

This study was done among Sawmill workers in Nnewi Town, Anambra State, South-East, Nigeria. Control subjects were recruited from the students and staff of College of Health Sciences, Nnewi Campus, Anambra State, Nigeria. A total of 100 male subjects within the age range of 18years and 50years were recruited in this study. Fifty (50) Sawmill workers were recruited as test subject, while fifty (50) apparently healthy non-Sawmill workers and who do not have any contact with PCP were recruited as control subjects.

Informed Consent

The aim, benefit and purpose of this study was narrated to the participants, participation was voluntary and informed consent was obtained from all willing to participate, the participants were allowed at any time they desired to drop out. The information obtained from the participants was kept highly confidential in observance of the privacy act. The benefit of the findings was communicated to participants that made the request. This study had no risk attached to it since the investigations did not introduce any substance to the participants.

Ethical Approval

Ethical approval was obtained from the Ethics committee of the Faculty of Health Sciences and Technology, Nnamdi Azikiwe University, Nnewi Campus.

Sample Collection

Five milliliter (5ml) of whole blood sample was collected from each of the subjects in the morning between 9 to 11 am into plain specimen tube. The

samples were allowed to clot and retract, centrifuged at 3500 rpm for 5 minutes using Wisperfuge model 1384 centrifuge (Samson, Holland). Serum was separated from clot with Pasteur pipette into sterile serum sample tubes for the measurement of biochemical parameters and stored at -20°C till day of analysis.

Inclusion and Exclusion Criteria

Apparently healthy male sawmill workers aged between 18 and 50 years were recruited for the study whereas those outside the age range, ill-looking subjects, Smokers and alcoholics were excluded from this study.

Laboratory Method and Procedure

All reagents were commercially purchased and the manufacturers' standard operation procedures (SOP) were strictly followed.

Estimation of Total Testosterone

Testosterone was estimated by Competitive Enzyme Immunoassay (type 7) method as described by Accubind incl. USA, product code: 3725-300

Estimation of Estradiol

It was estimated by Delayed Competitive Enzyme Immunoassay (type 9) method as described by Accubind incl. USA, product code: 4925-300.

Estimation of Total Cholesterol

Total cholesterol was estimated by cholesterol enzymatic end-point method (Roeschlau *et al.*, 1974). The kit was purchased from randox diagnostic Ltd, cat. No. CH 200

Estimation of Low density lipoprotein cholesterol

(LDL-C) in mmol/l was estimated by a clearance method for the direct measurement of LDL Cholesterol using randox diagnostic Ltd kit. The assay consists of two distinct reaction steps; elimination of chylomicrons, VLDL-cholesterol and HDL-cholesterol after release of LDL-cholesterol by cholesterol enzymatic end point method (Trinder, 1969).

Statistical analysis

Statistical package for social sciences (SPSS) (version 20.0 for windows, SPSS Inc. Chicago, USA) was used to analyze the data. Data were expressed as Mean \pm SD. The differences in parameters studied between the test and control groups were evaluated using student t- test.

Statistical significance was set at p-value <0.05.

RESULTS

Table 1 shows that the mean value of Estradiol was significantly increased in the test group compared to the control group ($P < 0.05$), while Testosterone was significantly reduced in the test group compared to the control ($P < 0.05$).

Table 1: Comparison of Mean \pm SD Serum estradiol and testosterone levels of test and control group.

Parameter	Control group (N = 50)	Test Group (N = 50)	t-value	P-value
Estradiol (pg/ml)	46.12 \pm 26.20	72.31 \pm 46.39	3.168	0.003*
Testosterone (ng/mg)	9.07 \pm 2.49	7.63 \pm 2.54	-2.538	0.013*

*Statistically significant at $p < 0.05$.

Table 2 shows that the mean values of Total cholesterol and LDL-Cholesterol were significantly increased in the test group compared to the controls ($P < 0.05$).

Table 2: Comparison of Mean \pm SD of serum cholesterol and LDL-cholesterol levels of test and control group.

Parameter	Control group (N = 50)	Test Group (N = 50)	t-value	P-value
Cholesterol (mmol/L)	3.20 \pm 0.36	3.70 \pm 0.73	-3.895	0.000*
LDL-C (mmol/L)	1.44 \pm 0.23	2.06 \pm 0.89	-4.243	0.000*

*Statistically significant at $p < 0.05$.

DISCUSSION

This study reveals that there was significantly increased level of serum estradiol among Saw Mill workers (test group) when compared with non-Saw mill workers (control group) and significantly decreased level of testosterone on the test group when compared with the control. These effects may be due to the harmful exposure of Saw Mill workers to Pentachlorophenol (PCP) as it is widely used for the preservation of wood. Although studies on the effect of PCP on human reproduction is scarce, a study done on Japanese Medaka (*Oryzias latipes*) indicated that PCP exposure caused responses consistent with estrogen and aryl hydrocarbon receptor activation and also reproductive impairment at environmentally relevant concentrations (Zha *et al.*, 2006). An earlier study reported that PCP has adverse effects on reproduction in rats if given orally during pregnancy at very high concentrations, near lethal levels. He reported increased embryo death in rats that were given high concentrations of PCP during the gestational period (Wagner and Sheldon, 1983). PCP acts mainly by inhibiting natural hormones because of their strong affinity to estrogen or androgen receptors; hence they copy estradiol (Tabb and Blumberg, 2006). A theory has shown that most female reproductive endocrine disorders in both humans and wildlife may be due to exposures to estrogens, androgen excess or insufficiency, and/or by a lack of proportion between estrogens and androgens at sensitive times during the life cycle. Balanced hormonal levels at these times are essential to enhance reproductive health (Tabb and Blumberg, 2006). Some authors (Beard *et al.*, 1999) also observed from their study on Rams exposed to PCP that the effects of pentachlorophenol on the testis may be linked to a decrease in thyroxine levels, and decreased reproductive behavior in lindane-treated rams may be related to decreased testosterone levels (Beard *et al.*, 1999). A research on crucian carp (*Carassius carassius*) exposed to a sublethal level of pentachlorophenol, reported that serum testosterone is a sensitive endpoints to PCP and that PCP may have endocrine disorder activities and may affect the reproductive success of this species (Zhang *et al.*, 2008). They concluded that it is possible that the

changes of hepatic microsomal enzyme activities may result in alterations of serum testosterone levels in Crucian carp. This study also reveals significantly higher level of total cholesterol in Saw Mill workers when compared to control subjects. Similarly the mean serum LDL-C level of subjects exposed to saw mill facilities were statistically higher when compared to normal control (table 2). This may suggest a more likely predisposition to cardiovascular disorders by subjects exposed to PCP, however further studies are required to investigate this. This finding also correlates with an earlier study that showed a higher level of serum cholesterol and low density lipoprotein cholesterol (LDL-C) in experimental subjects when compared to the control (Lewington *et al.*, 2007).

CONCLUSION

This study reveals that exposure to pentachlorophenol (PCP) can predispose one to infertility as PCP has been shown to be an endocrine disruptor and also, exposure to PCP raises cholesterol level which predisposes to hypertension among other heart condition.

LIMITATION OF STUDY

This study was limited by our inability to collect data for the measurement of subjects' Weight, Body Mass Index, and duration of exposure of subjects to PCP as well as the use of small sample size. Inclusion of the above data would have further enhanced and unraveled the mechanism of action of PCP in the study subjects. Hence, the need for further studies in this respect.

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