Journal of Zhejiang University SCIENCE
ISSN 1009-3095
http://www.zju.edu.cn/jzus
E-mail: jzus@zju.edu.cn

Effect of polychlorinated biphenyls on spermatogenesis and testosterone secretion in adult cocks*

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Received Sept. 3, 2002; revision accepted Jan. 8, 2003

Abstract: The effects of polychlorinated biphenyls (PCBs) on reproduction of adult cocks were studied by gavaging peanut oil or PCBs (Aroclor 1254, 50 mg/kg) once a week for six consecutive weeks. Physiological parameters were recorded and gonads were removed at the end of experiment for histological examination. The results showed that there was no significant difference between the control and treatment group in body weight, respiration rate, heart rate, body temperature, and the numbers of red and white blood cells. However, there was a marked decrease in the testicular weight and serum testosterone level after PCB treatment. Morphological studies manifested severe damage of the seminiferous tubules by PCB. The number of the germ cells at the different developmental stages was decreased and condensed nuclei were observed in most of these cells. This study revealed that the reproductive function of the adult cocks is sensitive to PCBs, which inhibited mainly spermatogenesis and testosterone secretion.

Key words: Chicken, Polychlorinated biphenyls (PCBs), Spermatogenesis, Testosterone
Document code: A **CLC number:** S859.83; S814.1

INTRODUCTION

Polychlorinated biphenyls (PCBs) are members of the halogenated hydrocarbon class of lipophilic environmental endocrine disrupters that includes the dibenzofurans and dioxins. Their appearance in the ecosystem and in the tissues from wildlife, as well as documented cases of accidental poisoning led to the banning of their manufacture in 1977 (Tilson and Kodavanti, 1997). The PCBs continue to be of concern to biologists due to reports that exposure to relatively low levels may be associated with reproductive failure in wild animals, especially in wild fish-eating birds. Common toxic effects most widely documented on mammals include immunotoxic effects, reproductive impairment, porphyria and related liver damage (Hoffman *et al*., 1996). Beside neural and immune toxicity, environmental disrupters with physiological effects similar to endogenous estrogens (xenoestrogens) disrupted reproductive processes in humans and other vertebrates. Reproductive abnormalities caused by these environmental pollutants have been reported in many vertebrates, such as inhibition of spermatogenesis in cod (Sangalang *et al*., 1981), decrease of 11-ketotestosterone and gonadotropin secretion in Atlantic croaker (Loomis and Thomas, 2000). Male rats born from dams treated with 2,3,7,8-tetrachlorodibenzo-*p*-dioxin during gestation suffered various types of damage in their reproductive function and androgenic situation (Mably *et al*., 1992).

We previously found that the adverse effects of

* Project supported by the National Natural Science Foundation of China (No. 39970535) and Zhejiang (No. 302352) and Beijing Natural Science Foundation (No. 8000001), China

PCBs on chicken gonadal and germ cell development were initiated during the early embryonic stage, and that PCBs induced degeneration of seminiferous tubules and decrease of testis size in newly hatched chickens (Fang *et al*., 2002; Zhang *et al*., 2002). However, the effects of PCBs on spermatogenesis and androgen secretion of adult cocks remain unknown. In the present study adult cocks were orally administered with PCBs to test the effects of PCBs on physiological parameters and reproductive functions.

MATERIALS AND METHODS

Animals

Ten-month-old Hyline cocks were reared under temperature of 18 ºC–24 ºC and 14 h light/10 h dark light cycle. They were given feed and tap water *ad libitum*. After 1-week acclimation, eight cocks were randomly divided into two groups: the control and PCB-treatment (n=4).

Treatment with PCBs

Aroclor 1254 (lot number 124-191-A, 99.99% pure, Accustandard Inc., New Haven, Connecticut, USA) was prepared in fresh sterilized peanut oil. The chemicals were administered by gavage at 50 mg/kg body weight between 14:00–15:00 every Monday for six consecutive weeks. The volume of peanut oil was 1 ml/kg body weight. The control received the same volume of vehicle (peanut oil).

Record of physiological parameters

Heart rate, respiration rate and body temperature were recorded with a multi-channel physiological recorder (Senei, NEC, Japan) every week. Blood was collected from the comb for determination of erythrocyte and leukocyte numbers with a hemacytometer.

Morphological observation of the testes

After six weeks of treatment, the cocks were anesthetized with Nembutal anesthetic. Testes were removed and immediately fixed in 4% neutral buffered paraformaldehyde for at least 24 h. Tissue fragments were dehydrated in graded series of ethanol, embedded in paraffin and sectioned at 5 μm thickness for histological processing (hematoxylin and eosin staining) and examination by light microscopy.

Determination of serum testosterone (*T*)

Blood was collected from the heart after the cocks were anesthetized. Serum was separated and stored at –20 ºC until measurement of *T* by a radioimmunoassay kit in a single batch. *T* levels were determined in duplicate tubes with within-assay coefficient of variance 6.5%.

Results were expressed as mean±SEM. The differences between the groups of the experiment and control were statistically analyzed by Student *t*-test.

RESULTS

Effects of PCB on some physiological parameters

There was no significant difference between the control and PCB-treatment group in body weight, respiration rate, heart rate, body temperature, and the number of red and white blood cells in the adult cocks (Table 1).

Table 1 Effects of PCBs on body weight and some physiological parameters in adult cocks*

Parameter	Control	PCBs-treatment
Body weight (kg)	2.46±0.23	2.74±0.14
Heart rate (/min)	352.50±23.7	322.50±7.50
Respiration rate (/min)	14.75±1.25	14.50±0.29
Body temperature (ºC)	41.98±0.42	41.65±0.17
RBC number (10^6/mm^3)	3.63±0.22	3.81±0.07
WBC number (10^3/mm^3)	3.27±0.76	4.83±0.35

*Data were determined on the last day of treatment and expressed as the means±SEM (n=4)

Effects of PCB on testicular structure and serum *T* level

After PCB treatment, few sperm appeared in the artificially collected semen; testicular weight decreased markedly. The weight of the left testes decreased from 11.47±1.25 g in the control to 3.30 ±

0.72 g in the PCB group (P<0.01), and the right side from 11.89±1.33 g to 3.24±0.65 g (P<0.001). Morphological studies manifested that the normal testes were composed of a mass of convoluted seminiferous tubules which were lined with an epithelium of developing germ cells together with Sertoli cells. After PCB administration the structure of the seminiferous tubules were severely damaged (Fig.1) and the thickness of the tubular walls was reduced. The number of spermatogenic cells was greatly decreased and hyperchromatism nucleus appeared almost in all kinds of spermatogenic cells. Very few sperm were visible within the seminiferous tubules. Serum testosterone level also declined significantly from 0.47±0.01 ng/ml to 0.33±0.07 ng/ml (P<0.05) after PCB treatment.

Fig.1 Testicular structure of the normal and PCB-treated adult cocks
Upper panel: Testicular structure of the normal cock; (a) 200X; (b) 400X;
Lower panel: Testicular structure of the PCB-treated cock; (c) 200X; (d) 400X bar indicates 50 μm

DISCUSSION

It is well known that toxic effects of PCBs on chickens include a sudden drop in egg production of laying hens, a decrease of chicken hatchability, along with reduced weight gain, ascites, neurological disturbance (ataxial) and increased mortality of chicks (Bernard *et al*., 1999). Reproductive abnormalities caused by PCBs were observed in many vertebrates, such as inhibition of spermatogenesis in cods and newly hatched chickens (Sangalang *et al*., 1981; Fang *et al*., 2001). In the present study we found that PCBs had no significant effects on body weight and some physiological parameters including heart rate, respiration rate, body temperature and the number of red and white blood cells over a period of six weeks; but the reproductive function was severely damaged. These results indicated that the gonad was a sensitive target for disruption by PCB. Such disruption by PCBs was also observed in rats (Pflieger-Bruss *et al*., 1999). Male rats born to dams treated with 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) during gestation had various types of damage in their reproductive function and androgen pat-

tern, including decrease in epididymal sperm count and accessory sex organ weights, altered sexual behaviors and delayed puberty (Mably *et al*., 1992).

As xenoestrogens, PCBs were proved to possess estrogenic activity as well as toxic effect on many physiological functions. In vertebrate testes, many evidences indicated that estrogens perform critical functions. Estrogens are involved in Leydig cell development and regulation of spermatogenic progression (Hess *et al*., 1997). Male mice lacking functional estrogen receptors exhibit a wide variety of reproductive problems such as infertility, abnormal spermatogenesis, reduced testis size, and decreased sperm motility (Korach, 1994). However, estrogens also have negative effects on testicular functions by decreasing androgen production and inhibiting or damaging spermatogenesis (Bartke *et al*., 1977). In the present study, since spermatogenesis was disrupted and hyperchromatism nucleus appeared in almost all spermatogenic cells as a result of treatment by PCBs, it may be deduced that PCBs caused these adverse effects by both estrogenic and toxic actions.

Besides damage of spermatogenesis, PCBs also decreased the secretion of testosterone into serum of adult cocks. This inhibitory effect of PCBs was also reported in other animals. In rats, intraperitoneal injection of a PCB congener (3,3',4,4',5,5'-hexachlo-robiphenyl) produced a marked reduction in plasma testosterone concentration (Yeowell *et al*., 1987). Aroclor 1254, *o*,*p*'-dichloro (diphenyl) dichloroethene and other xenoestrogens could decreased 11-ketotestosterone production by damaging the estrogen membrane receptors in Atlantic croaker (Loomis and Thomas, 2000). Another xenoestrogen atrazine (a pesticide) could directly inhibit testosterone secretion by Leydig cells in rats (Friedmann, 2002). Aroclor 1248 down-regulated rat testicular steroidogenesis by an acute inhibition of 3β-HSD and P450 c17 (Andric *et al*., 2000). Reductions in androgen production had been previously reported after incubation of testicular tissue from rodents with estrogens (Bartke *et al*., 1977). Moreover, PCBs possess similar chemical structure to a pesticide DDT that could cause feminization of gull embryos via estrogenic activity (Fry and Toone, 1981). Based on these reports, PCBs may decrease testosterone secretion via estrogenic effects.

In conclusion, in the present study Aroclor 1254 (50 mg/kg BW) interfered with chicken spermatogenesis and reduced testosterone production without significantly changing physiological parameters including heart and respiration rates, body temperature, and the number of red and white blood cells. These data indicated that male reproductive functions of chickens are sensitive to PCBs disruption.

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