

Effects of 3,4-Dichloroaniline on Testicle Enzymes as Biological Markers in Rats¹

BO ZHANG² AND SEN LIN

Department of Biology, College of Applied Sciences and Humanities, Beijing Union University,
Beijing 100083, China

Objective To investigate the effects of 3,4-dichloroaniline (3,4-DCA) on activities of testicle enzymes as biological markers in rats. **Methods** Fifty male rats were randomly divided into 5 groups ($n=10$). One group was left untreated and used as a solvent control (administered orally by corn oil), while the other 4 groups were treated with 3, 4-DCA. Corn oil was used as a solvent, and 3,4-DCA was diluted into tested concentrations (39, 81, 170, and 357 mg/kg). All the groups orally administered 3,4-DCA or corn oil, once a day for 4 weeks. The testicle tissue was homogenized in a 0.1 mol/L potassium phosphate buffer (0.1 mol/L, pH 7.2). The crude homogenate was centrifuged at 6000 rpm for 5 min at 4 °C. The supernatant obtained was used as an enzyme extract for determination of the enzyme activities. **Results** Compared with the control, the activities of ALP, ACP, and SDH were increased significantly at a lower level of 3,4-DCA, and decreased at a higher level of 3, 4-DCA, whereas the activities of LDH, LDH-X, and G6PDH were inhibited significantly with the increased 3,4-DCA concentration. Organ coefficient "organ weight/total body weight $\times 100$ " of testis, liver, and spleen increased significantly with the increased 3,4-DCA concentration. These results suggest that 3,4-DCA toxicity to the male reproductive system was associated with the activities of testicular enzymes which are the sensitive biochemical endpoints reflecting 3,4-DCA toxicity to the male reproductive system. **Conclusion** 3,4-DCA has toxicity to the reproductive system in male rats.

Key words: 3, 4-dichloroaniline; Rat; Marker testicular enzymes

INTRODUCTION

Dichloroaniline is widely used as chemical intermediate in synthesis of 3,4-dichloroaniline (3,4-DCA), a precursor for synthesis and a degradation production of some herbicides (e.g. diuron, dinuron and propanil)^[1-2]. DCA is toxic to spleen, blood, and has immunotoxicity to mammals^[3-5] and induces acute effects on blood, kidney, liver, and bladder of rats. Studies *in vitro* and *in vivo* have shown that DCA has nephrotoxicity and splenotoxicity to mammals^[6-9].

Testicle enzymes as biochemical markers have been suggested as indicators of chemical exposure. Alterations in some testicle enzyme activities have been widely used as biomarkers to evaluate the function of organs due to their important role in energy production and biotransformation. Pandey *et al.*^[10] found that changes in activity of SDH, LDH, and gamma-GT may be responsible for the toxic effects of molybdenum on fertility of male rats. Pant *et al.*^[11] evaluated the effects of carbofuran on the reproductive system of male rats by measuring the

activities of SDH, LDH, gamma-GT, G6PDH, and beta-glucuronidase, and found that carbofuran is toxic to the reproductive system of male rats, suggesting that the activity of testicular-cell-specific enzymes induces damage to germ cells and Sertoli cells^[11-12].

3,4-DCA and chlorobenzene affect testosterone concentration and hepatic microsomal enzyme activities in crucian carp and DCA is toxic to many important organs and enzymes^[13-14]. Well-balanced testis enzyme activities are important conditions for the formation sperm cells. Enzyme activity assay is one of the important methods to evaluate the toxicity of xenobiotic. At present, no report is available on the effect of DCA on testis enzyme activities. This study was to investigate the effects of 3,4-DCA on testicular enzymes ALP, ACP, LDH, LDH-X, SDH, and G6PDH in Wistar rats.

MATERIALS AND METHODS

Technical grade 3,4-DCA (98% purity) was

¹This work was supported by the Natural Science Foundation of Beijing (No. 7063071).

²Correspondence should be addressed to Bo ZHANG, Tel: 86-10-62004564. Fax: 86-10-62004564. E-mail: zhangbo@ygi.edu.cn
Biographical note of the first author: BO ZHANG, female, born in 1962, professor, majoring in environmental toxicology.

purchased from Sigma-Aldrich (Germany). ALP, ACP, LDH, SDH, G6PDH, and Lowry protein diagnostic reagent kits were purchased from Nanjing Jiancheng Bioengineering Institute (China).

Adult male Wister rats weighing 180-220 g, provide by Beijing Weitong Lihua Laboratory Animal Limited Company, were housed under standard environmental conditions (20-22 °C, 40%-55% relative humidity, lights on 8:00-20:00) for 7 days before experiment with free access to water and standard commercial diet (Beijing Weitong Lihua Laboratory Animal Limited Company).

Fifty male rats were divided into 5 groups ($n=10$). One group was used as solvent control, while the other 4 groups were treated with 3,4-DCA. Corn oil was used as a solvent, and 3,4-DCA was diluted into tested concentrations (39, 81, 170, and 357 mg/kg). Four treatment groups orally administered 3,4-DCA at the concentration of 39, 81, 170, and 357 mg/kg, respectively. The solvent control group administered only corn oil. Rats were fasted for 24 h with free access to water prior to experiments. The treatment groups were intraperitoneally given 3,4-DCA and the control group was intraperitoneally given corn oil at 8:00, once a day for 4 weeks. After 4 weeks, the testis was

isolated from each rat, washed 3 times with potassium phosphate buffer (0.1 mol/L, pH 7.2), cut into small pieces and homogenized in potassium phosphate buffer (testis/total buffer is 1:10). The supernatant was obtained after the homogenate was centrifuged at 6 000 rpm for 5 min at 4 °C and used as an enzyme extract for determination of the enzyme activities .

Protein concentrations in the testis of rats were estimated using the folin-phenol reagent as previously described^[15]. ALP, ACP, LDH, LDH-X, SDH, and G6PDH activities in testis were determined with the diagnostic reagent kits (Nanjing Jiancheng Bioengineering Institute, China).

Results of different treatments were compared by one-way analysis of variance (ANOVA). $P<0.05$ was considered statistically significant. Statistical analysis was performed using SPSS 13.0 for Windows. Data were expressed as $\bar{x} \pm s$.

RESULTS

The changes in body weight and organ coefficient of rats 4 weeks after administration of 3,4-DCA are summarized in Table 1.

TABLE 1

Effects of 3,4-DCA on Body Weight, Testis Weight, Liver Weight, Spleen Weight, and Their Coefficient of Rats ($\bar{x} \pm s$)

3,4-DCA (mg/kg)	BW (g)	TW (g)	Testis Coefficient	LW (g)	Liver Coefficient	SW (g)	Spleen Coefficient
Control	427.50±14.87	3.22±0.25	0.75±0.06	15.64±1.21	3.99±0.41	1.45±0.09	0.34±0.02
39	448.74±16.97	3.39±0.34	0.75±0.08	17.93±1.90*	3.93±0.59	1.65±0.25	0.37±0.06
81	420.06±37.71	3.18±0.19	0.76±0.07	17.38±2.55	4.89±0.71*	1.52±0.23	0.37±0.07
170	409.43±19.16**	3.43±0.25	0.84±0.05*	17.97±1.90*	5.04±0.84**	1.69±0.18*	0.41±0.05*
357	378.90±42.08**	3.43±0.25	0.87±0.13*	22.71±3.05***	5.46±0.61***	1.74±0.27*	0.77±0.06***

Note. BW=body weight, TW=testis weight, LW=liver weight, SW=spleen weight. * $P<0.05$, ** $P<0.01$, *** $P<0.001$.

The results showed that body weight changed remarkably when the concentration of 3,4-DCA was equal or higher than 170 mg/kg. Testis weight did not change remarkably while testis coefficient changed remarkably when the concentration of 3,4-DCA was equal to or higher than 170 mg/kg. The liver weight and coefficient as well as spleen weight and coefficient changed with increased 3,4-DCA concentration.

After Wister rats were administered orally 3,4-DCA for 4 weeks, the changes in activities of ALP, ACP, LDH, LDH-X, SDH, and G6PDH in testis of rats 4 weeks after administration of 3,4-DCA are summarized in Table 2.

ALP activity changed with the concentration of 3,4-DCA and increased significantly at a lower

concentration of 3,4-DCA (81 mg/kg) and decreased evidently at a higher concentration of 3,4-DCA (375 mg/kg). The change in ACP and ALP activity was similar. ACP activity increased significantly at a lower concentrations of 3,4-DCA (39 to 81 mg/kg) and decreased significantly at a higher concentration of 3,4-DCA (375 mg/kg). The activities of ALP and ACP changed in a concentration-dependent manner.

The activity of LDH remained unchanged at a lower concentration of 3,4-DCA (39 mg/kg), and decreased significantly at a higher concentration (equal to or higher than 81 mg/kg) of 3,4DCA. The activity of LDH-X decreased dramatically when the concentration of 3,4-DCA was 39, 81, 170, and 357 mg/kg, respectively.

TABLE 2
Effects of 3,4-DCA on Activity of Testis Enzymes in Wister Rats ($\bar{x} \pm s$)

3,4-DCA Concentration (mg/kg)	ALP (μ /mg prot)	ACP (μ /mg prot)	LDH (μ /mg prot)	LDH-X (μ /mg prot)	SDH (μ /mg prot)	G6PDH (μ /mg prot)
Control	46.81 \pm 5.65	31.64 \pm 3.58	218.50 \pm 45.81	80.25 \pm 13.81	19.72 \pm 1.75	31.06 \pm 2.01
39	42.11 \pm 3.20	44.41 \pm 7.88*	186.34 \pm 29.01	34.29 \pm 16.23*	31.61 \pm 5.90*	32.05 \pm 6.61
81	64.69 \pm 11.61*	53.77 \pm 16.82**	123.94 \pm 24.21**	19.38 \pm 5.82**	10.41 \pm 2.05*	38.29 \pm 5.24
170	51.80 \pm 4.79	30.39 \pm 4.03	86.18 \pm 10.61**	12.74 \pm 4.61***	8.17 \pm 1.44*	18.71 \pm 6.53*
357	35.45 \pm 3.61*	21.17 \pm 2.48*	55.78 \pm 8.39***	3.39 \pm 1.25***	7.89 \pm 5.40*	10.56 \pm 4.41**

Note. * P <0.05, ** P <0.01, *** P <0.001.

The activity of SDH increased significantly at a lower concentration of 3,4-DCA (39 mg/kg), and decreased significantly at a higher concentration of 3,4-DCA (equal to or higher than 81 mg/kg).

The activity of G6PDH remained unchanged at a lower concentration of 3,4-DCA (39 and 81 mg/kg), and decreased significantly at a higher concentration of 3,4-DCA (equal to or higher than 170 mg/kg).

DISCUSSION

The results of our study indicate that 3,4-DCA is toxic to rats and the target organs are the kidney, liver, and urinary bladder^[16]. In this study, 3,4-DCA caused changes in testicular enzymes and affected the functions of the reproductive system of male rats. Testicular enzymes are sensitive biochemical endpoints and may be used as a marker reflecting 3,4-DCA toxicity to the reproductive system of male rats. The production and maturity are a complex process that is related to the activities of testicular enzymes. 3,4-DCA is a precursor for synthesis and a degradation product of some herbicides, and is commonly present in environment. It was reported that 3,4-DCA has acute effects on blood of Wister rats and also splenotoxicity and nephrotoxicity to mammals^[3, 6, 8]. In this study, 3,4-DCA was found to have effects on testis enzyme activities.

The activity of ALP is related to the mitosis of spermatogenic cells and glucose transport. ACP located in lysosome of leydig cells is involved in the protein synthesis by abduction of sex hormones. Changes in the activity of ALP and ACP may be used as an indicator of spermatogenesis function. In this study, ALP and ACP activities increased significantly at a lower concentration of 3,4-DCA and decreased significantly at a higher concentration of 3,4-DCA. Changes in the activity of testicular ALP and ACP of 3,4-DCA-treated rats also reflect testicular degeneration, which may be a consequence of suppressed testosterone and indicative of lytic activity^[17].

The activities of SDH and LDH in testicular tissue are associated with the maturation of the germinal epithelial layer of seminiferous tubule. SDH, mainly located in chondriosome of sertoli cells and spermatogenic cells, plays an important role in energy metabolism of sperms. SDH activity is related to fructose transferred into sorbic alcohol and glucose that provides energy to sperms. The SDH activity increases markedly throughout the maturation of germ cells and decreases during the depletion of germ cells^[18-19]. LDH, widely present in sertoli and spermatogenic cells, plays an important role in testis energy production and biotransformation. LDH-X is a special enzyme produced at the phase of primary spermatogenic cells. Since inhibition of LDH and LDH-X activities may induce denaturalization of spermatogenic cells^[20], LDH and LDH-X activities can be used in evaluating the function of spermatogenic cells. In this study, the activity of LDH and LDH-X reduced significantly at a concentrations equal to or higher than 81 mg/kg of 3,4-DCA and equal to or higher than 39 mg/kg of 3,4-DCA, respectively, indicating that the function of spermatogenic cells can be affected by 3,4-DCA, the LDH and LDH-X activity is sensitive to 3,4-DCA, and LDH-X activity is the sensitive endpoint to measure the toxicity of 3,4-DCA to testis.

In this study, the SDH activity increased dramatically at a lower concentration of 3,4-DCA (39 mg/kg), and decreased markedly at a concentration equal to or higher than 81 mg/kg of 3,4-DCA, suggesting that the movement of sperms may be affected by 3,4-DCA. The decreased activity of LDH, LDH-X, and SDH in the treated rats suggested a deterioration of germinal epithelium due to chemical exposure.

G6PDH, present in leydig, sertoli, and spermatogenic cells, is more active in leydig cells. The activity of G6PDH is associated with the function of leydig cells. In this study, the G6PDH activity decreased dramatically at a concentration equal to or higher than 170 mg/kg of 3,4-DCA,

suggesting that 3,4-DCA can injure the function of leydig cells.

In summary, the activities of ALP, ACP, LDH, LDH-X, SDH, and G6PDH are changed significantly after treatment with 3,4-DCA. The function of reproductive system in male rats may be impaired by 3,4-DCA. 3,4-DCA has potential toxicity to mammal reproduction system. The activities of ACP, SDH, LDH, and LDH-X are sensitive to 3,4-DCA and can be used as biomarkers to evaluate the function of testis.

REFERENCES

- Crossland N O (1990). A review of the fate and toxicity of 3, 4-dichloroaniline in aquatic environments. *Chemosphere* **21**(12), 1489-1497.
- Giacomazzi S, Cochet N (2004). Environmental impact of diuron transformation: a review. *Chemosphere* **56**(11), 1021-1032.
- Khan M F, Boor P J, Alock N W, *et al.* (1997). Oxidative stress in the splenotoxicity of aniline. *Fundam Appl Toxicol* **35**(1), 22-30.
- Guilhermino L, Soares A M, Carvalho A P, *et al.* (1998). Acute effects of 3,4-Dichloroaniline on blood of male Wister rats. *Chemosphere* **37**(4), 619-632.
- Barnett J B, Gandy J, Wilbourn D, *et al.* (1992). Comparison of the immunotoxicity of propanil and its metabolite, 3, 4-dichloroaniline, in C57B1/6 mice. *Fundam Appl Toxicol* **18**(4), 628-631.
- Bauchinger M, Kulka U, Schrnid E (1989). Cytogenetic effects of 3, 4-dichloroaniline in human lymphocytes and V79 Chinese haster cells. *Mutat Res* **226**(3), 197-202.
- Rankin G O, Yang D J, Teets V J, *et al.* (1986). 3, 5-Dichloroaniline-induced nephrotoxicity in the Sprague-Dawley rat. *Toxicology letters* **30**(2), 173-179.
- Hong S K, Anestis D K, Ball J G, *et al.* (2002). *In vitro* nephrotoxicity induced by chloronitrobenzenes in renal cortical slices from Fischer 344 rats. *Toxicology letters* **129**(1-2), 133-141.
- Avilova G G, Tkatcheva T A, Karpukhina E A, *et al.* (1998). Glucuronides, a possible urinary marker of nephrotoxicity and on indicator of exposure to 2, 6-dichloroaniline in rats. *Toxicology letters* **95**(Supplement 1), 189.
- Pandey R, Singh S P (2002). Effects of molybdenum on fertility of male rats. *Biometals* **15**(1), 65-72.
- Pant N, Prasad A K, Srivastava S P, *et al.* (1995). Effect of oral administration of carbofuran on male reproductive system of rat. *Hum Exp Toxicol* **14**(11), 889-894.
- Pant N, Srivastava S P (2003). Testicular and spermatotoxic effects of quinalphos in rats. *J Appl Toxicol* **23**(4), 271-274.
- Li W M, Yin D G, Zhou Y, *et al.* (2003). 3, 4-Dichloroaniline-induced oxidative stress in liver of crucian carp (*Carassius auratus*). *Ecotoxicol Environ Saf* **56**(2), 251-255.
- Qian Y, Yin D G, Li Y, *et al.* (2004). Effects of four chlorobenzenes on serum sex steroids and hepatic microsome enzyme activities in cracian carp. *Carassius auratus*. *Chemosphere* **57**(2), 127-133.
- Lowry O H, Rosenbrough N J (1951). Protein measurement with folin-phenol reagent. *Chem* **193**, 265-275.
- Valentovic M A, Yahia T, Ball J G, *et al.* (1997). 3, 4-Dichloroaniline acute toxicity in male Fischer 344 rats. *Toxicology* **124**(2), 125-134.
- Kaur R, Dhanuju C K, Kaur K (1999). Effect of dietary selenium on biochemical composition in rat testis. *Ind J Exp Biol* **37**(5), 509-511.
- Srivastava S, Sing G B, Srivastava S P, *et al.* (1990). Testicular toxicity of di-n-butyl phthalate in adult rats: effect on marker enzymes of spermatogenesis. *Ind J Exp Biol* **28**(1), 67-70.
- Pant N, Murty R C, Srivastava S P (2004). Male reproductive toxicity of sodium arsenite in mice. *Human Exp Toxicol* **23**(8), 399-403.
- Sinha N, Narayan R, Saxena D K (1997). Effect of endosulfan on the testis of growing rats. *Bull Environ Contam Toxicol* **58**(1), 79-86.

(Received May 16, 2008 Accepted December 2, 2008)