V. 10, n. 1, p. 07-10, jan - mar, 2014

UFCG - Universidade Federal de Campina Grande. Centro de Saúde e Tecnologia Rural – CSTR. Campus de Patos – PB. www.cstr.ufcg.edu.br

Revista ACSA:
http://www.cstr.ufcg.edu.br/acsa/

Revista ACSA – OJS:
http://150.165.111.246/ojs-patos/index.php/ACSA

Jurandy Mauro Penitente-Filho[1*]

Tarcízio Antônio Rego de Paula[2]

João Gabriel Silva Neves[2]

Sérgio Luís Pinto da Matta[3]

**Autor para correspondência*
Recebido para publicação em 15/01/2014. Aprovado em 01/04/2014.
[1] Departamento de Zootecnia, área de fisiologia e reprodução animal, Universidade Federal De Viçosa. E-mail: penitentefilho@yahoo.com.br;
[2] Departamento de Veterinária, Universidade Federal De Viçosa. E-mail: tarcizio@ufv.br; joaogabrielneves@yahoo.com.br;
[3] Departamento de Biologia Geral, Universidade Federal De Viçosa. E-mail: spermatta@yahoo.com.br;

AGROPECUÁRIA CIENTÍFICA NO SEMIÁRIDO – ISSN 1808-6845
Nota Técnica

INFLUENCE OF THE 2,4 DICHLOROPHENOXYACETIC ACID (TORDON®) ON WEIGHT OF THE ORGANS OF MALE ADULT WISTAR RATS

ABSTRACT

Thirty adult male Wistar rats were used in five groups. Two groups received oral treatment with aqueous solution of the Tordon® in different concentrations. Treatment O1: 2mL/L, (n=6); Treatment O2: 4mL/L, (n=6). Two groups received topical treatment in the cervical area: Treatment T1: 16.67mL/L (n=6), and Treatment T2: 400mL/L (n=6). A group control was used without the addition of the herbicide (n=6). Forty days after start of the experiment, the weight of the rats was assessed and they were euthanized; the internal organs (testis, liver, kidneys, spleen and intestine) were collected and their weights were assessed. There were no differences (P>0.05) in the body and organs weights among treatments. However, the proportional weight (%) of the liver in relation to body weight was higher in the O1 and O2 treatments (P<0.05) as well as the proportional weight of the kidneys in the O2 treatment. The T1 and T2 animals did not show harmful effects.

Keywords: Herbicide, 2,4-D, Internal organs, Rat.

INFLUÊNCIA DO 2,4 DICLOROFENOXIACÉTICO (TORDON ®) SOBRE O PESO DAS ÓRGÃOS DE RATOS WISTAR COM

RESUMO

Trinta ratos Wistar machos adultos foram usados em cinco grupos. Dois grupos receberam o tratamento por via oral com uma solução aquosa do Tordon ® em diferentes concentrações. Tratamento O1: 2 mL / L, (n = 6); Tratamento de O2: 4 mL / L (n = 6). Dois grupos receberam tratamento tópico na área cervical: Tratamento T1: 16.67mL / L (n = 6), e tratamento T2: 400 mL / L (n = 6). Um grupo controle foi utilizado sem a adição do herbicida (n = 6). Quarenta dias após o início da experiência, o peso dos ratos foi avaliado e foram sacrificados; os órgãos internos (testículos, fígado, rins, baço e intestino) foram recolhidos e os seus pesos foram avaliadas. Não houve diferença (P> 0,05) no corpo e órgãos pesos entre os tratamentos. No entanto, o peso proporcional

(%) do fígado em relação ao peso corporal foi mais elevado nos tratamentos O1 e O2 (P <0,05), bem como o peso proporcional dos rins no tratamento de O2. Os animais T1 e T2 não apresentaram efeitos prejudiciais.

Palavras-chave: Herbicidas, 2,4-D, Órgãos internos, Rato.

INTRODUCTION

Phenoxyacetic acids including 2,4-dichlorophenoxyacetic acid (2,4-D) are widely utilized organic acid herbicides that have undergone extensive toxicity and pharmacokinetic analyses **(**Timchalk, 2004). Tordon 2,4-D® has a similar formulation, in terms of active compounds, to the herbicide White Agent, extensively used in the Vietnam War (Oakes *et al*., 2002). This herbicide is basically composed by an association of two auxines 2,4-Dichlorophenoxyactic acid (2,4-D) and picloram and currently it is widely used to eradicate weeds in agriculture (Guerra et al. 1999). Oakes et al. (2002), reported a severe decrease in testicular mass with shrinkage of seminiferous tubules, with the use of Tordon 2,4-D® in adult rats, without interference, however, on the serum levels of testosterone.

Sauerhoff *et al*. (1977), showed that the pharmacokinetic profile of 2,4-D is defined in humans. Five male volunteers ingested a single dose of 5 $mg.Kg^{-1}$ of 2,4-D with no detectable clinical effects. Sauerhoff *et al*. (1977), also reported that essentially all 2,4-D is absorbed in the human gastrointestinal tract. According to Lachapelle *et al*. (2007) 2,4-dichlorophenoxyacetic acid blocks *in vitro* meiotic maturation and is therefore a potential environmental endocrine disrupter with reproductive effects.

MATERIAL AND METHODS

Animals and experimental design

Thirty wistar rats 60 days old, weighing around 318 grams, from the vivarium of the Nutrition and Health Department of Federal University of Viçosa were used. The animals were divided into control group, treated topically and orally with the association of 2,4 dichlorophenoxyacetic acid and picloram (Tordon®). The experimental period lasted for 40 days.

Aqueous solution of Tordon® was offered and it was obtained with the following dilutions:

O1 Treatment: 2 mL of Tordon®/ liter of water, orally (n = 6);

O2 Treatment: 4 mL of Tordon®/ liter of water, orally (n = 6).

The solution was offered *ad libitum* and the intake of Tordon® aqueous solution by the orally treated animals was measured by the difference of volume in the recipient.

Two groups also received daily topical treatment in the dorsal cervical region; it was used 0.5 mL solutions of Tordon® in the following concentrations:

Treatment T1: 16.67 mL of Tordon®/ liter of water (n = 6);

Treatment T2: 400 mL of Tordon®/ liter of water (n = 6).

A control group (n = 6) was used without addition of herbicide, and receiving saline solution in the dorsal cervical area.

Food was offered for all animals *ad libitum*.

Euthanasia and weight assessment

The body weight was assessed at the beginning and at the end of the experiment. Then, all animals were euthanized by overdose of sodium thiopental (Thionembutal).

Internal organs were collected and the weight of the liver, testes, kidneys, spleen and intestine were assessed by precision balance.

Based on body and organs weights, it was calculated the proportional weights (PW) of each collected organ by the following formula: PW (%) = OW / BW x 100, where OW = organ weight and BW = body weight.

Seminiferous tubules weight assessment

One of the testes was fragmented, fixed in 2.5% glutaraldehyde in 0.1 mol L^{-1} sodium phosphate buffer, pH 7.4, for 24 hours under refrigeration. The testicular fragments were then dehydrated in increasing concentrations of alcohol (50°, 70°, 80°, 90°, 95° and 100°) with exchanges every thirty minutes. After dehydration, the fragments were embedded in glycol methacrylate (Historesin Leica) and subsequently sectioned at 4μm thickness on a rotary microtome, equipped with glass knives. The sections were stained with 1% toluidine blue-sodium borate, mounted with Entellan (Merck) and examined under a light microscope.

By projecting a grid with 100 intersections (points) at 400X magnification, recording 10 fields randomly for each animal were counted on the matching points: seminiferous tubules and intertubular tissue. The volume of each testicular component was estimated from its percentage occupied in testis and volume of the testis. As the density of the testicle is around 1 (1.03 to 1.04; França, 1991), the testis weight was considered equal to its volume.

Statistical analysis

Body weight and body weight gain data were analyzed by analysis of variance (ANOVA) using initial body weight as covariate. The others variables were analyzed by analysis of variance using PROC ANOVA (SAS, 2002). Duncan's test was used to compare treatments. Evaluation of mean and standard error of mean were performed by using descriptive statistics. Significant level adopted was α=0.05.

RESULTS

For the orally treated animals, O1 and O2 treatments, the intake of Tordon® aqueous solution was 7.8 and 6.7 mL $animal^{-1}$ day^{-1}, respectively. In its composition, Tordon® has 240 g L^{-1} of 2,4-D. Thus, the doses for the orally treated animals were, respectively, 10.5 and 20.0 mg Kg^{-1} day^{-1}. For the topically treated

animals, T1 and T2 treatments, the doses were 5.7 and 126.1 mg Kg^{-1} day^{-1}, respectively.

Body weight, body weight gain and organs weights showed no differences among treatments (P>0.05; Table 01).

Nevertheless, when the organs weights were analyzed as a body weight ratio (Table 02), animals that received 2,4-D and picloram orally showed an increase (P<0.05) of the proportional hepatic weight. Besides, the animals that received orally the higher concentration (4 mL L^{-1}) of Tordon® also showed an increase of the proportional kidney weight.

Table 1. Body weight, body weight gain and weight of the organs of the male adult wistar rats exposed to association of 2,4 dichlorophenoxiacetic acid and picloram (Tordon®) orally or topically (Mean ± Standard error of mean):

Weight	Control	O1	O2	T1	T2
Initial body weight (g)	318.3 ± 12.8	315.0 ± 8.5	305.0 ± 19.8	323.3 ± 8.4	331.7 ± 15.1
Final body weight (g)	389.9 ± 17.9	396.9 ± 14.2	337.2 ± 26.1	379.1 ± 20.0	390.0 ± 21.7
Body weight gain (g)	71.6 ± 12.9	81.9 ± 16.1	32.2 ± 26.9	55.8 ± 25.6	58.4 ± 28.0
Liver (g)	13.8 ± 1.0	18.1 ± 1.3	17.0 ± 1.3	14.9 ± 1.4	15.3 ± 0.8
Testis (g)	2.7 ± 0.2	2.8 ± 0.1	2.6 ± 0.1	2.8 ± 0.1	2.8 ± 0.1
Seminiferous tubules (mg)	2.4 ± 0.2	2.5 ± 0.0	2.3 ± 0.1	2.5 ± 0.1	2.5 ± 0.1
Kidney (g)	3.4 ± 0.2	3.8 ± 0.3	3.8 ± 0.2	3.4 ± 0.2	3.9 ± 0.1
Spleen (g)	1.1 ± 0.1	1.2 ± 0.1	1.2 ± 0.1	1.1 ± 0.1	1.1 ± 0.0
Intestine (g)	38.2 ± 2.4	35.7 ± 2.4	40.8 ± 2.6	37.9 ± 6.1	38.2 ± 3.5

P>0.05; O1: 2 $mL.L^{-1}$, orally (n = 6); O2: 4 $mL.L^{-1}$, orally (n = 6); T1: 16.67 $mL.L^{-1}$, topically in the dorsal cervical area (n = 6); T2: 400 $mL.L^{-1}$, topically in the dorsal cervical area (n = 6); Control group: saline solution, topically, on the dorsal cervical region (n=6).

Table 2. Proportional weights (%) of the organs of the male adult wistar rats exposed to association of 2,4 dichlorophenoxiacetic acid and picloram (Tordon®) orally or topically (Mean ± Standard error of mean):

Organs weight / Body weight (%)	Control	O1	O2	T1	T2
Liver	3.5 ± 0.1^{c}	4.5 ± 0.2^{ab}	5.2 ± 0.6^{a}	3.9 ± 0.2^{bc}	3.9 ± 0.2^{bc}
Testis	0.7 ± 0.05^{a}	0.7 ± 0.02^{a}	0.8 ± 0.05^{a}	0.8 ± 0.05^{a}	0.7 ± 0.03^{a}
Seminiferous tubules	0.6 ± 0.04^{a}	0.6 ± 0.02^{a}	0.7 ± 0.05^{a}	0.7 ± 0.04^{a}	0.7 ± 0.02^{a}
Kidney	0.9 ± 0.03^{c}	0.9 ± 0.05^{bc}	1.2 ± 0.06^{a}	0.9 ± 0.03^{bc}	1.0 ± 0.05^{b}
Spleen	0.3 ± 0.02^{a}	0.3 ± 0.03^{a}	0.3 ± 0.01^{a}	0.3 ± 0.03^{a}	0.3 ± 0.02^{a}
Intestine	9.8 ± 0.4^{ab}	9.0 ± 0.5^{b}	12.5 ± 1.4^{a}	9.8 ± 1.3^{ab}	9.8 ± 0.8^{ab}

^{a,b,c} Different letters in the row are statistically different (P<0.05; Duncan's test); O1: 2 $mL.L^{-1}$, orally (n = 6); O2: 4 $mL.L^{-1}$, orally (n = 6); T1: 16.67 $mL.L^{-1}$, topically in the dorsal cervical area (n = 6); T2: 400 $mL.L^{-1}$, topically in the dorsal cervical area (n = 6); Control group: saline solution, topically, on the dorsal cervical region (n=6).

DISCUSSION

In the present study, body weight gain was not affected by Tordon®, however at the higher dose levels of 2,4-D (100 or 150 mg kg^{-1} day^{-1}) body weight gains of rats were decreased (Gorzinski et al., 1987). Besides, the liver weight also was not affected by Tordon® but the proportional liver weight was increased in both orally treated groups, dose levels of 100 or 150 mg kg^{-1} day^{-1} of 2,4-D can promote a slight elevation of liver weight by producing a swelling in the liver cells (Gorzinski et al., 1987). Therefore, Tordon® components can significantly decreased the respiratory control ratio of intact rat liver mitochondria (Oakes and Pollak, 1999).

An increase in the proportional kidney weight was observed in the orally treated animals at the higher dose. According to Gorzinski et al. (1987) 2,4-D can increase kidney weight and promote alterations in the renal proximal tubules.

Tordon® is one of the most widely used herbicide and its main form of application is by spraying. However, there are few studies related to skin absorption of the components of Tordon®, such as 2,4-D. The topically treated animals showed no harmful alterations in the organs weights or proportional organs weights. It suggest that 2,4-D is not significantly absorbed by skin, since it the highest concentration (400 mL L^{-1}) with a dose of 126.1 mg Kg^{-1} did not show any harmful effects

ACKNOWLEDGEMENTS

Authors thank to FAPEMIG (Foundation to Research Support of State of Minas Gerais) for financial support.

REFERENCES

FRANÇA, L.R. **Análise morfofuncional da espermatogênese de suínos adultos da raça Piau (Morphofunctional analyses of spermatogenesis of adult pigs of Piau breed).** Thesis (Master degree), Federal University of Minas Gerais. Belo Horizonte, Brazil. 1991

GUERRA, M.P.; TORRES, A.C.; TEIXEIRA, J.B. Embriogênese Somática e Sementes Sintéticas. In:

TORRES, A.C.; CALDAS, L.S.; BUSO, J.A. (Ed.). **Culturas de Tecidos e Transformação Genética de Plantas**. Brasília, Embrapa-CBAB. v.2. p. 533-568. 1999.

GORZINSKI, S.J., KOCIBA, R.J.; CAMPBELL, R.A.; SMITH, F.A.; NOLAN, R.J.; EISENBRANDT, D.L., Acute, pharmacokinetic, and subchronic toxicological studies of 2,4-dichlorophenoxyacetic acid. **Fund Appl Toxicol**, v.9, p.423-435, 1987.

LACHAPELLE, A.M.; RUYGROK, M.L.; TOOMER, M.; OOST, J.J.; MONNIE, M.L.; SWENSON, J.A.; COMPTON, A.A.; STEBBINS-BOAZ, B. The hormonal herbicide, 2,4-dichlorophenoxyacetic acid, inhibits *Xenopus* oocyte maturation by targeting translational and post-translational mechanisms. **Reprod. Toxicol**. v.23, p.20-31, 2007.

OAKES, D.J.; POLLAK, J.K. Effects of a herbicide formulation, Tordon 75D®, and its individual components on the oxidative functions of mitochondria. **Toxicology**, v.136, p.41-52, 1999.

OAKES, D.J.; WEBSTER, W.S.; BROWN-WOODMAN, P.D.C.; RITCHIE; H.E. Testicular changes induced by chronic exposure to the herbicide formulation, Tordon 75D® (2,4-dichlorophenoxyacetic acid and picloram) in rats. **Reprod. Toxicol.** v.16, p.281-289, 2002.

SAS Institute Inc. 2002: **SAS/STAT® 9.0** User's guide. Cary, NC: SAS Institute Inc.

SAUERHOFF, M.W.; BRAUN, W.H.; BLAUAND, G.E.; GEHRING, P.J. The fate of 2,4-dichlorophenoxyacetic acid (2,4-D) following oral administration to man. **Toxicology**, v.8, p.3-11, 1977.

TIMCHALK, C. Comparative inter-species pharmacokinetics of phenoxyacetic acid herbicides and related organic acids. **Toxicology**, v.200, p.1-19**,** 2004**.**