Preliminary results of testicular toxicity in the rat after chronic oral administration with the herbicide, Tordon 75D®

Oakes DJ, Webster WS, Woodman-Brown, PDC* and Ritchie, HE*

Department of Anatomy and Histology, Faculty of Medicine, University of Sydney,
Sydney. NSW 2006, AUSTRALIA.
*Department of Biomedical Sciences, Faculty of Health Sciences, University of Sydney, Lidcombe. NSW 2141, AUSTRALIA

Introduction

Toxicology of the male reproductive system has received increased interest in recent years partly as a result of suggestions that exposure of male army personnel and Vietnamese citizens during the Vietnam War to herbicide formulations may be associated with an increased incidence of reproductive problems (IOM,1996). The second most used herbicide in the Vietnam war was the formulation 'Agent White', which consisted of the active components 2,4-D and picloram (present as the triisopropanolamine salts). Tordon 75D®, the herbicide formulation chosen for this study, is similar in terms of its active components to the herbicide 'Agent White' and is also currently used by the agricultural industry in Australia.

This paper describes results from a study that involved dosing male rats for 9 weeks with Tordon 75D® followed by a 20 week recovery period. Preliminary results suggest some of the treated rats have persistent damage to their seminiferous tubules.

Methods

Eighteen male rats (Sprague-Dawley) weighing 300-400g were examined in the present study. Animals were gavaged 5 days per week for 9 weeks with either; 0.125, 0.25 or 0.5ml/kg Tordon 75D® or water (controls). The highest dose of Tordon 75D® corresponded to 150mg/kg 2,4-D acid equivalent and 37.5mg/kg picloram acid equivalent. Twenty weeks after termination of treatment the rats were killed and the testes were collected, weighed and fixed in 10% buffered neutral formalin. After routine processing, the testes were embedded in glycol methacrylate, and transverse 5μm sections were cut and stained with methylene blue. The herbicide formulation, Tordon 75D® was purchased from DowAgroSciences, Australia.

Results and Discussion

The results show that treatment with Tordon 75D® for 9 weeks followed by a 20 week recovery period was associated with severely reduced testes weight in two of the high dose animals and a dose-dependent reduction in testes weight overall (Fig. 1). The number of animals is small as this study is still in progress.

Although the data shows a dose response, it was not statistically significant due to the large variation of testes weight between animals. The histological studies showed that the small testes had large numbers of shrunken tubules with a generalised germ cell depletion. (Fig. 2a). Lesser number of affected tubules were seen in the moderately reduced testes from both the high and middle dose groups. Control testes did not show abnormal tubules (Fig. 2b).

Fig. 1

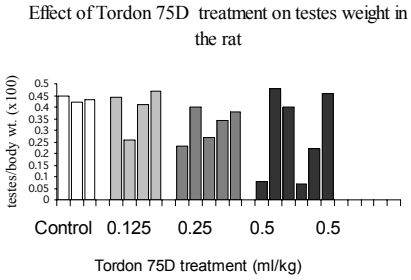
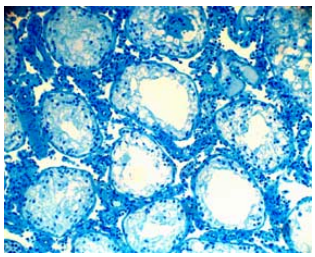
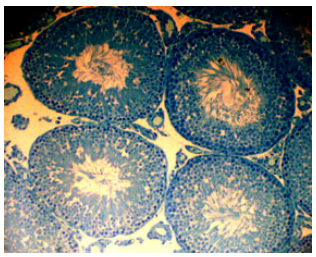


Fig. 2

(a) high dose Tordon 75D® testes



(b) Control testes



The high dose Tordon 75D® was associated with peak levels of 2,4-D (270 µg/ml serum) and picloram (26 µg/ml serum) within 30 minutes of dosing. These levels rapidly decreased and were almost undetectable by 24 hours. It is unlikely that the compounds accumulate with repeated dosing. The high dose Tordon 75® was associated with decreased body weight gain during the treatment period followed by catch-up growth in the recovery period.

2,4-D has been reported previously to cause reduced testicular weight in rats treated for 13 weeks with 300 mg/kg 2,4-D (pure compound) (Charles et al., 1989) with the 100 mg/kg dose having no effect. The considerably lower dose causing testicular toxicity in the present study suggests an additional effect due either to the picloram or other constituents of the Tordon 75D® formulation including a surfactant and defoaming agent.

Rats with reduced testicular weight seen in the study by Charles and colleagues also had increased thyroid weight and decreased T3 and T4 levels, suggesting that the testis toxicity caused by 2,4-D may be secondary to effects on the pituitary function. Further studies are now in progress to look at the temporal aspects of the testicular lesions caused by Tordon 75D® and their possible relationship to hormonal disturbances as opposed to direct testicular toxicity.

It has previously been shown that dioxin found in Agent Orange can cause testicular toxicity in rats (Chahoud *et al*., 1992). This present study suggests that the second most commonly used herbicide in Vietnam, Agent White, also has the potential to cause damage to the testes.

Acknowledgements
This research is being supported by a grant from the Australian Department of Veterans' Affairs.
The authors wish to thank Ann Korabelnikoff for her expert care and handling of animals.

References

1. Chahoud I, Hartmann J, Rune GM and Neubert D; *Arch. Toxicol.* **1992**,66,567
2. Charles J.M, Cunny H.C, Wilson R.D and Bus J.S; *Fund. Appl. Toxicol.* **1996**,33,161
3. Institute of Medicine (IOM), Veterans and Agent Orange – Update 1996. National Academy Press, USA.